Copper tolerance in bacteria requires the activation of multiple accessory pathways

Andrea Giachino | Kevin J. Waldron

INTRODUCTION

Copper is an essential micronutrient for bacteria, serving as an electron carrier and redox catalyst in various cuproenzymes (Rensing and Grass, 2003; Andreini et al., 2008; Festa and Thiele, 2011; Vest et al., 2013). Excess copper, however, is also cytotoxic. There is growing interest in utilizing copper for infection control owing to its antibacterial properties (Noyce et al., 2006; Warnes et al., 2012; Lemire et al., 2013). At the same time, copper tolerance pathways can constitute important virulence determinants, since the mammalian immune system utilizes copper excess and starvation in its arsenal against pathogenic bacteria (White et al., 2009; Djoko et al., 2015; Johnson et al., 2015; Ladomersky et al., 2017; Hyre et al., 2017; Stocks et al., 2019).

The mechanisms of intracellular copper handling, which regulate its acquisition, storage, detoxification and delivery to maturing cuproenzymes, have been studied over three decades in model organisms such as Escherichia coli (Grass and Rensing, 2001; Rensing and Grass, 2003; Dupont et al., 2011; Hodgkinson and Petris, 2012; Argüello et al., 2013; 2016; Nies and Herzberg, 2013). More recently, a body of evidence on copper-mediated cell death has accumulated for other Gram negative bacteria, particularly the pathogen Salmonella enterica. These studies suggested that the effects of copper toxicity and the general principles of copper resistance can be considered universal in the bacterial kingdom, with some copper homeostasis components being shared with archaea and eukaryotes as well (Andreini et al., 2008).

Abstract

Copper is a required micronutrient for bacteria and an essential cofactor for redox-active cuproenzymes. Yet, excess copper is extremely toxic, and is exploited as a bactericide in medical and biotechnological applications and also by the mammalian immune system. To evade copper toxicity, bacteria not only control intracellular copper homeostasis, but they must also repair the damage caused by excess copper. In this review, we summarize the bacterial cell-wide response to copper toxicity in Enterobacteria. Tapping into the abundant research data on two key organisms, Escherichia coli and Salmonella enterica, we show that copper resistance requires both the direct copper homeostatic response and also the indirect accessory pathways that deal with copper-induced damage. Since patterns of copper response are conserved through the Proteobacteria, we propose a cell-wide view of copper detoxification and copper tolerance that can be used to identify novel targets for copper-based antibacterial therapeutics.
In this review, we describe the mechanisms of copper toxicity in bacteria and the bespoke adaptation pathways that assuage it. We show that copper excess generates distinctive pleiotropic effects in the cell, and that different stress responses complement the copper homeostasis system in defending the cell against copper toxicity. Importantly, no single pathway can account for a cell’s copper resistance profile, and multiple layers of gene regulation are required for a stable adaptation to copper stress. We examine the case of two well-studied proteobacteria, E. coli and S. enterica, highlighting similarities and differences between the two species. Crucially, we comment on how pathogenic strains adapt to copper stress during infection, which can highlight future therapeutic targets. We also comment on the aspects of bacterial metal usage that require further investigation, hoping to stimulate future research.

2 | COPPER BIOCHEMISTRY IN BACTERIA

Bacterial copper homeostasis shows some distinctive features, as we have extensively reviewed elsewhere (Barwinska-Sendra and Waldron, 2017) (Figure 1). Briefly, copper exists in a chemical equilibrium between two forms, which are readily complexed by biological ligands (Beswick et al., 1976; Outten et al., 2001; Dupont et al., 2011): cupric ion, Cu(II), is favored in oxidizing compartments such as the Gram-negative periplasm, while cuprous ion, Cu(I), is prevalent during anaerobic growth, and in reducing compartments such as the cytoplasm. Cu(II) is more biologically inert, making it a relatively “safe” species for cells compared to Cu(I) (Rensing and Grass, 2003; Andreini et al., 2008). Intracellular reductants, including respiratory chain complexes, enterobactin, and cysteine, contribute to the generation of Cu(I) from Cu(II) (Rodriguez-Montelongo et al., 1993;...

**Figure 1** Copper fluxes in bacteria. In the extracellular environment, copper exists as a dynamic equilibrium between reduced Cu(I) (green) and oxidized Cu(II) (blue). The reduced species, Cu(I), can cross the bacterial outer membrane, although the entry route is unclear. Inside the bacterial periplasm, Cu(I) availability can be reduced by active efflux, oxidation to Cu(II), or sequestration by high-affinity ligands. Thus, the quota of bioavailable Cu(I) is lower than the total copper content. Bioavailable copper can be toxic to cells by catalyzing oxidative damage, displacing native metal cofactors in metalloenzymes, and can also enter other compartments. Similar mechanisms (efflux, oxidation, sequestration) can be employed in each bacterial compartment reducing the quota of bioavailable copper is reduced after crossing each membrane.
Rigo et al., 2004; Grass et al., 2004; Volentini et al., 2011), and more intracellular Cu(I) is generated enzymatically for incorporation into copper-containing cytochrome oxidases, at least in some organisms (Marckmann et al., 2019). Copper toxicity is enhanced during anaerobic growth (Outten et al., 2001; Espariz et al., 2007; Tan et al., 2017) and cells accumulate more copper under those conditions (Outten et al., 2001; Macomber et al., 2007).

For most bacteria, copper enters the cell via passive diffusion along its chemical gradient. Even though a handful of candidate bacterial copper importers have been suggested to date (Chillappagar et al., 2009; Balasubramanian et al., 2011; Ekici et al., 2014; Khalifaoui-Hassani et al., 2018; Han et al., 2019; Zhang et al., 2019), none of them seems to play this role in E. coli or S. enterica. In these organisms, a bespoke copper acquisition pathway has only been found in some pathogenic strains, which use the siderophore versiniabactin to combat copper starvation at the host-pathogen interface (Oelschlaeger et al., 2003; Henderson et al., 2009; Koh et al., 2017). The E. coli zinc importer ZupT was considered for some time to be a candidate for a copper importer, since ZupT-overexpressing cells display a slight copper phenotype (Lee et al., 2002). However, subsequent studies showed that this phenotype is likely attributed to imbalances in the iron supply chain and not increased copper import (Xu et al., 2019). Whether E. coli and S. enterica acquire enough copper to metalate their cuproenzymes via passive diffusion, or an unknown copper importer exists in these organisms, is currently speculative.

Since biological membranes are relatively impermeable to copper, the cytosol of most bacterial species constitutes a
copper-poor environment. In Gram negative bacteria, copper homeostasis is mainly played out in the periplasm, where most of the intracellular copper is located (Changela et al., 2003; Macomber et al., 2007; Parmar et al., 2018). Copper complexes enter the periplasm through porins, whose permeability may influence the rate of its acquisition (Egler et al., 2005). Notably, all known copper-requiring enzymes in Enterobacteria (copper-dependent superoxide dismutases, SODs, multi-copper oxidases, amine oxidases and terminal respiratory oxidases) are localized in the cell envelope (Figure 2). Periplasmic enzymes acquire their copper cofactor in the periplasm, from either a bioavailable pool or metallochaperones (Stolle et al., 2016; Stewart et al., 2019). Copper-containing respiratory oxidases can acquire their copper from the cytoplasm, as shown in other model organisms (Ekici et al., 2014; Khalfaoui-Hassani et al., 2018; Llases et al., 2019; Marckmann et al., 2019; Zhang et al., 2019).

Regardless of the bacterial compartment, cells restrict the amount of copper that is available to interact with intracellular targets (Rensing and Grass, 2003). The possible strategies include controlling copper import, secreting excess copper, increasing the cell's buffering capacity and oxidizing Cu(I) to Cu(II) (Figure 1). Additional pathways are typically mobilized under stress conditions, to address the downstream effects of copper toxicity. In order to shed light on the different bacterial adaptations to copper, therefore, it is essential to understand the complex interplay between copper-induced damage and cellular responses.

3 | COPPER HOMEOSTASIS: THE EXAMPLE OF ENTEROBACTERIA

In *E. coli* and *S. enterica* (Figure 3), copper bioavailability in the cytoplasm is buffered to an exquisitely low concentration, equivalent to less than one atom per cell (Outten et al., 2000). Cytosolic copper concentration is under the control of the MerR-family transcriptional regulator, CueR (Outten et al., 2000), which has zeptomolar affinity for copper (Changela et al., 2003). The cue regulon includes the P-type ATPase CopA (Rensing et al., 2000), and the cuprochaperone CopZ (Meydan et al., 2017), both of which bind Cu(I) with attomolar affinity (Drees et al., 2015). The periplasmic multicopper oxidase CueO (CuiD in *S. enterica*) reduces the Cu(I) flux across the inner membrane by converting periplasmic Cu(I) to Cu(II) (Outten et al., 2001). The bidirectional metal-polyphosphate transporter, PitA, may also contribute to copper efflux (Keasling, 1997; Grillo-Puertas et al., 2014; Solioz, 2018).

The core cue regulon is functionally conserved in all proteobacteria (Andreini et al., 2008; Hernandez-Montes et al., 2012), and additional niche-specific adaptations can be seen in different species. For example, the anti-copper arsenal of *S. enterica* has expanded, likely as an adaptation to the host-pathogen interface in order to escape intracellular killing by macrophages (Achard et al., 2010; Ladomersky et al., 2017). In this organism, an additional periplasmic chaperone, CueP, acts as a copper sink (Pontel and Soncini, 2009; Osman et al., 2010) and supplies copper to...
FIGURE 4  Copper toxicity in the cell envelope. (a) Lipoprotein maturation is a complex process requiring the attachment of a lipid moiety to the maturing polypeptide and the removal of the signal peptide, which occurs on the periplasmic side of the inner membrane; outer membrane lipoproteins are further translocated to the outer membrane after maturation. Periplasmic Cu(I) (green) inhibits protein maturation by binding the conserved cysteine-1 residue (amber), where lipid modifications occur. Cells can reduce the effect of copper toxicity by overexpressing lipoprotein-maturation enzymes, which compete with Cu(I) for access to cysteine-1. (b) Periplasmic protein maturation often involves covalent linking of cysteine residues through disulfide bonds, chaperoned by disulfide oxidases. Copper can also catalyze disulfide bond formation, which often occur in a non-native fashion. Misfolded products must be resolved by a disulfide isomerase to avoid cell toxicity. (c) Peptidoglycan maturation is catalyzed by DD-transpeptidases (TPases), which cross-link different peptidoglycan layers, and LD-transpeptidases, which cross-link peptidoglycan and anchor it to outer membrane lipoproteins. LD-TPases have a cysteine residue in the active site (amber), which can be inhibited by Cu(I). During copper toxicity, Gram negative bacteria can still cross-link peptidoglycan using DD-TPases, but cannot attach it to the outer membrane, resulting in increased cell fragility. Moreover, DD-TPases can be inhibited by β-lactam antibiotics, which show a potent synergistic effect with copper. Protein names correspond to the Escherichia coli enzymes.
periplasmic SODs in conjunction with the P-type ATPases (Nies and Herzberg, 2013; Osman et al., 2013; Fenlon and Slauch, 2017). Moreover, most S. enterica serotypes (with the exception of the human-adapted serovars Typhi and Paratyphi A), carry a duplication of the entire cue system in the form of the partially redundant gol regulon (Osman et al., 2010; 2013). In these strains, the CueR homologue, GolS, regulates the expression of an extra CopA homologue, GolT (Espariz et al., 2007), and a putative cytosolic CopZ-like cuprochaperone, GolB (Checa et al., 2007). The pathogenic serovars take copper homeostasis a step further through the cytosolic copper storage protein, Csp3 (Vita et al., 2015), possibly as an adaptation to the intraphagosomal phase.

In contrast to S. enterica, E. coli takes a different approach to copper homeostasis to suit its surface- and biofilm-associated lifestyle. In E. coli, no additional cytosolic proteins are used on top of CopA and CopZ. Periplasmic copper homeostasis is controlled by the two-component sensory system CueSR (Gudipaty and McEvoy, 2014), which regulates the expression of the resistance, nodulation and division (RND) proton-cation antiporter CusCBA (Grass and Rensing, 2001), and the periplasmic cuprochaperone CusF (Franke et al., 2003). The cus regulon plays a role during anaerobic growth, when cells are especially susceptible to Cu(I) (Outtten et al., 2001), and is tuned to maintain periplasmic copper concentration at micromolar levels (Bagai et al., 2007; Yun et al., 2010; Gudipaty and McEvoy, 2014).

The principles exemplified in Enterobacteriae are conserved throughout the Proteobacteria clade: the copper-secreting ATPase CopA is the core component of copper homeostasis in most species, and additional systems, both chromosomal and plasmid-encoded, are apparently interchangeable based on the biological niche (Andrei et al., 2008; Hernandez-Montes et al., 2012). The copper-storage proteins, including metallothioneins, constitute an interesting example of niche-specific adaptation: these are primarily found in organisms that rely on cuproproteins for core metabolic processes, such as methanotrophs (Vita et al., 2015), but they may also contribute to the survival of intracellular pathogens within macrophages (Gold et al., 2008; Wolschendorf et al., 2011).

The evolutionary dynamism of copper homeostasis is especially evident in copper-rich environments, where horizontal gene transfer can blur the differences between species. This has particular relevance when considering copper as an antimicrobial, since copper-resistant strains may arise in a similar way as multidrug-resistant ones do. For example, some antibiotic-resistant strains of S. enterica have acquired a functional cut locus (Arai et al., 2019). The use of copper as a food supplement in the animal industry is associated with the spread of the pco locus in both E. coli and S. enterica (Tetaz and Luke, 1983; Brown et al., 1995; Qin et al., 2014; Fang et al., 2016; Billman-Jacobe et al., 2018; Chalmers et al., 2018). This genomic island provides enhanced copper oxidation through the multicopper oxidase PcoA (Lee et al., 2002), increased copper chelation through the periplasmic chaperones PcoC and PcoE (Djoko et al., 2008; Zimmermann et al., 2012), as well as an additional copper-sensing system, PcoSR (Rouch and Brown, 1997). Furthermore, many pathogenic E. coli and S. enterica strains have also acquired pathogenicity islands for the biosynthesis of yersiniabactin (Oelschlaeger et al., 2003; Henderson et al., 2009), which sequesters Cu(II) in the extracellular space (Chaturvedi et al., 2012) and scavenges nutrient copper during starvation (Koh et al., 2017).

4 | COPPER PREVENTS LIPOPROTEIN MATURATION IN THE CELL ENVELOPE

One important route of copper toxicity is the inhibition of lipoprotein maturation, leading to the accumulation of toxic precursors in the inner membrane (Figure 4a). Outer membrane lipoprotein maturation (reviewed in Okuda and Tokuda, 2011) involves the sequential acylation of the polypeptide chain, removal of the signal peptide, and translocation to the outer membrane. Copper binds to the acyl-accepting N-terminal cysteine residue, making it inaccessible to the enzymes in the pathway (Yakushi et al., 1997; May et al., 2019).

As a consequence, the adaptation to copper stress requires the activation of the envelope stress response (reviewed in Raivio, 2014), which in E. coli and S. enterica is coordinated by the two-component regulator CpxAR (Egler et al., 2005; Pontel et al., 2014). In E. coli, where this system has been characterized, CpxA senses the accumulation of the outer membrane lipoprotein NlpE (Yamamoto and Ishihama, 2005; 2006; Price and Raivio, 2009; May et al., 2019). Activated CpxR induces the overexpression of the two lipoprotein-acylating enzymes, Lgt and Lnt, and the inhibition of lipoprotein production through the small inhibitory RNA, MicL. All of these components contribute to copper tolerance (Rogers et al., 1991; Gupta et al., 1997; Egler et al., 2005; Yamamoto and Ishihama, 2005; Yamamoto and Ishihama, 2006; Riley et al., 2006; Price and Raivio, 2009; Guo et al., 2014; May et al., 2019). In fact, some of these genes are so important for the copper response that they were initially identified as copper tolerance determinants (nlpE as cutF, int as cutE, and micL as cutC) (Rouch et al., 1989; Rogers et al., 1991; Gupta et al., 1995; Armesano et al., 2003; Riley et al., 2006; Guo et al., 2014). In S. enterica, CpxR also contributes to copper homeostasis by regulating the expression of cueP (Pezza et al., 2016).

Overall, the cpx regulon represses the biosynthesis of copper-sensitive lipoproteins, and induces the production of maturation enzymes to remove the damage that was already dealt (Raivio, 2014; Mitchell and Silhavy, 2019). The cpx response also inhibits cell growth and proliferation, and promotes biofilm formation and cellular persistence. As a consequence, care must be taken to interpret experimental data on copper toxicity, since sub-toxic concentrations can lead to the formation of long-term persister cells (Grey and Steck, 2001).

5 | COPPER CAUSES PROTEIN MISFOLDING BY CATALYZING THE FORMATION OF NON-NATIVE DISULFIDE BONDS

The second route of copper toxicity in the bacterial envelope targets maturing polypeptides. Under aerobic conditions, copper can
oxidize exposed thiols catalyzing the formation of disulfide bonds (Figure 4b). In Enterobacteria, native folding of periplasmic polypeptides is normally mediated by two oxidoreductases, DsbA and DsbB (DsbL and Dsb in S. enterica) (Kadokura et al., 2003; Lin et al., 2009). DsbA/DsbL transfers its disulfide bond to maturing polypeptides, and is regenerated by DsbB/DsbL using reducing equivalents from the cytoplasm. When incorrect disulfide bonds are inserted by copper, however, an additional oxidoreductase pair is required to rearrange the misfolded peptides; this role is fulfilled by the E. coli DsbC and DsbD (also known as CutA) (Ito and Inaba, 2008), and the S. enterica DsbC and DsbD (Barchinger and Ades, 2013), which cooperates with CpxAR to fine-tune the envelope stress response. Interestingly, copper stress induces the RpoE (σ^E) regulon (reviewed in Barchinger and Ades, 2013), which cooperates with CpxAR to fine-tune the envelope stress response. Interestingly, copper stress induces the rpoE response in E. coli but not S. enterica (Egler et al., 2005; Yamamoto and Ishihama, 2006; Pontel et al., 2014), suggesting that S. enterica responds to copper-induced envelope damage exclusively through cpx.

In E. coli, the accumulation of misfolded proteins in the periplasm induces the RpoE (σ^E) regulon (reviewed in Barchinger and Ades, 2013), which cooperates with CpxAR to fine-tune the envelope stress response. Interestingly, copper stress induces the rpoE response in E. coli but not S. enterica (Egler et al., 2005; Yamamoto and Ishihama, 2006; Pontel et al., 2014), suggesting that S. enterica responds to copper-induced envelope damage exclusively through cpx.

6 | COPPER IMPAIRS PEPTIDOGLYCAN MATURATION

The last envelope-specific effect of copper toxicity is the weakening of peptidoglycan (Figure 4c). In Enterobacteria, peptidoglycan is assembled by two classes of transpeptidases (TPases): LD-TPases and DD-TPases. Cu(I) binds to the catalytic cysteine of LD-TPases, preventing them from cross-linking peptidoglycan and attach it to outer membrane lipoproteins (Mainardi et al., 2005; Peters et al., 2018). As a consequence, copper-stressed cells have a weaker cell envelope and are more sensitive to detergents and other membrane destabilizing compounds (Peters et al., 2018).

DD-TPases are copper-insensitive, however, they are inhibited by β-lactam and carbapenem antibiotics, resulting in a synergistic effect of these compounds with copper. The synergy with carbapenems is especially relevant, since copper inhibits metallo-β-lactamases with carbapenemase activity (Djoko et al., 2018).

7 | SOME ENVELOPE COMPONENTS CONTRIBUTE TO COPPER TOLERANCE IN UNKNOWN WAYS

In addition to the copper tolerance systems outlined above, additional envelope proteins have been implicated in the copper response. In E. coli, the porin OmpC and the outer membrane protein ComC (BshA) are induced upon copper stress (Kershaw et al., 2005; Mermod et al., 2012) and are required for full copper tolerance (Egler et al., 2005). Copper-dependent induction of ompC is mediated by RpoE, while that of comC occurs through a dedicated TetR-like transcriptional regulator, ComR (Mermod et al., 2012). The mechanism by which these proteins contribute to copper tolerance has not been elucidated, but they are known to respond to a number of envelope-targeting stressors (Richmond et al., 1999; Zheng et al., 2001; Egler et al., 2005; Maurer et al., 2005; Zhang et al., 2007). It has been proposed that outer membrane proteins may reduce copper entry to the periplasm by selecting against this ion on the cell surface (Egler et al., 2005), although such a model has not been thoroughly investigated to the best of our knowledge. These findings show that our knowledge of copper tolerance is not yet complete, and further studies are required to advance our understanding.

8 | COPPER DISPLACES OTHER METALS FROM PROTEIN BINDING SITES

Because of its strong affinity for thiol ligands, copper can mismetalate maturing proteins that require a molybdenum or iron cofactor. Mismetalation not only can occur on exposed metal-binding pockets, but also during protein maturation, as copper can mismetalate the metallochaperones supplying the metal cofactor to nascent polypeptides (Neumann and Leimkühler, 2008; Macomber and Imlay, 2009; Iobbi-Nivol and Leimkühler, 2013; Tan et al., 2014; 2017; Djoko et al., 2017). In some cases, cells can prevent mismetalation by expressing metallochaperones that have a higher specificity for their cognate partners. This is the case in E. coli, which switches from the copper-sensitive chaperone IscA to the copper-resistant SufA to survive copper stress (Macomber and Imlay, 2009; Fung et al., 2013; Tan et al., 2014).

Copper mismetalation affects cell metabolism in pleiotropic ways, since many biosynthetic enzymes are affected (Jo et al., 2008; Neumann and Leimkühler, 2008; Macomber and Imlay, 2009; Djoko et al., 2017; Tan et al., 2017). This can generate functional auxotrophies (Fung et al., 2013; Djoko et al., 2017), which the cell can adapt to by inducing alternative biosynthetic pathways. For example, E. coli responds to copper mismetalation of glutamate synthase by inducing an alternative route for glutamate biosynthesis from glutamine (Djoko et al., 2017). A better understanding of the biosynthetic alterations induced by copper, therefore, can inform future synergic therapies. Recently, it was proposed that a glutamate- and glutamine-deficient phenotype may make E. coli more sensitive to low pH under copper stress, although this has only been shown in a copA background (Djoko et al., 2017).

9 | COPPER, IRON AND ZINC ACT SYNERGISTICALLY IN CELLULAR KILLING

In addition to bypassing copper-sensitive enzymes, copper-stressed cells face the double challenge of supplying enough native cofactor
to nascent metalloenzymes while scavenging “free” metal ions liberated by copper. This is especially important in the case of iron, since it can generate ROS through Fenton/Haber-Weiss chemistry (Py and Barras, 2010). The master regulator of iron metabolism, Fur, is overexpressed under copper stress in both E. coli and S. enterica (Kershaw et al., 2005; Yamamoto and Ishihama, 2006; Price and Raivio, 2009; Pontel et al., 2014) and is required for copper resistance even at low copper concentrations (Grass et al., 2004). Moreover, many copper-responsive genes in the fur regulon are also regulated by CpxR and SoxS, the master regulator of the ROS response (Zheng et al., 1999; Kershaw et al., 2005; Große et al., 2006; Yamamoto and Ishihama, 2006; Cao et al., 2007; Price and Raivio, 2009; Fung et al., 2013; Pontel et al., 2014; Tan et al., 2014; 2017). These multiple layers of regulation are required to fine-tune the fur response, since copper- and iron-derived peroxide can damage the Fur protein (Varghese et al., 2007; Seo et al., 2014). Moreover, iron-generated ROS can damage CueR, resulting in reduced levels of copA and cueO transcripts (Xu et al., 2019). As a consequence, iron excess can increase copper sensitivity in the same way as copper excess can increase iron sensitivity (Grass et al., 2004; Chaturvedi et al., 2012; Koh et al., 2017).

Another ion that displays synergistic effects with copper is zinc (Grass et al., 2002). Even though copper and zinc do not appear to mismetlate each other’s partners, excess zinc also dysregulates Fur, increasing intracellular iron with the effects detailed above (Xu et al., 2019). Because the mammalian immune system exploits copper and zinc synergistically within macrophages (Kapetanovic et al., 2016), intraphagosomal pathogens have adapted to survive copper and zinc toxicity at the same time. For example, expression of the zinc-exporting ATPase, ZntA, is induced by copper in S. enterica (Kapetanovic et al., 2016) and strains lacking zntA are more sensitive to copper stress (Huang et al., 2017). Many other genes involved in the copper and zinc responses are activated by either ion (Pontel et al., 2014), which mediates pathway co-activation in a number of cases.
COPPER DISRUPTS CELLULAR REDOX POTENTIAL

Because of its facile redox cycle, copper can interact with many electron carriers, including molecular oxygen (Hiniker et al., 2005), peroxide (Gunther et al., 1995) and thiols (Rigo et al., 2004). Similarly to iron, copper can generate reactive oxygen species (ROS) via Fenton/Haber-Weiss chemistry in vitro (Macomber et al., 2007). However, the extent to which ROS contribute to copper toxicity in bacteria is still unclear.

In Enterobacteria, the response to redox stress is effected by the hpr and sox regulons (Figure 5), respectively, protecting the periplasm and cytoplasm (Kimura and Nishioka, 1997; Yamamoto and Ishihama, 2005; Urano et al., 2015). The sox response, in particular, is strongly upregulated under copper stress in both E. coli and S. enterica (Egler et al., 2005; Kershaw et al., 2005; Yamamoto and Ishihama, 2005; Urano et al., 2015) and is required for full copper resistance (Kershaw et al., 2005). The hpr regulon (also known as yed in E. coli and cop in S. enterica) is also involved in the copper response, at least in some conditions (Yamamoto and Ishihama, 2005; Espariz et al., 2007). The third regulon involved in the redox response, the cytosolic oxy system, does not seem to respond to copper in either organism (Yamamoto and Ishihama, 2005; Pontel et al., 2014).

One challenge in assessing whether ROS play a role in copper toxicity is that hpr and sox monitor the general redox state of the cell, responding to non-oxygen stressors such as nitric oxide, chlorine and various redox-cycling compounds (Nunoshiba et al., 1993; Dukan et al., 1996; Gu and Imlay, 2011; Liochev and Fridovich, 2011; Gennaris et al., 2015; Urano et al., 2015; 2017) in addition to peroxide and superoxide (Chiang and Schellhorn, 2012; Urano et al., 2017). Moreover, the two-component sensory system HprSR is a close homologue of CusSR, and cross-talk between the regulators has been demonstrated in E. coli (Yamamoto and Ishihama, 2005). The depletion of cellular NADPH levels, by contrast, can adventitiously activate the sox response by interfering with the regulator’s futile cycle (Liochev and Fridovich, 1992; Gaudu et al., 2000; Krapp et al., 2011). Therefore, the activation of hpr and sox does not necessarily demonstrate a role of ROS production in copper toxicity, as was assumed in the earliest models (Kimura and Nishioka, 1997).

One further challenge to the model of copper-induced ROS production is that copper toxicity is maximal under anaerobic conditions in bacteria (Outten et al., 2001; Espariz et al., 2007). Moreover, although copper has been shown to generate hydroxyl radicals and damage DNA in vitro (Cai et al., 1995; Moriwaki et al., 2008), direct DNA damage upon copper exposure has not been confirmed in vivo to the best of our knowledge, even in E. coli strains lacking all known copper-efflux genes (Macomber et al., 2007). Measurements of intracellular ROS showed that copper supplementation does not increase hydroxyl radical production and may even reduce superoxide levels (Park et al., 2012). Finally, the oxy regulon, which responds to cytosolic peroxide, does not seem to be upregulated under copper stress in either E. coli or S. enterica. Taken as a whole, these results suggest that ROS production may be one of many routes of copper toxicity, but not the main one.

How exactly copper activates hpr and sox, if not through ROS, is currently unclear. One possible explanation for hpr activation is that the sensor HprS may monitor the redox state of the inner membrane (Urano et al., 2017). Since ionic copper interacts with respiratory complexes (Volentini et al., 2011), it is possible that HprS responds to alterations in bacterial respiration under copper stress. In organisms possessing both the hpr and cus systems, cross-talk between the regulators could be an additional source of hpr activation. The recruitment of the sox response may occur by direct copper-catalyzed reduction of the regulator, or indirectly, through alterations in the NADPH pool.

In any case, the pattern of copper damage through ROS seems to be less universal than generally assumed. It must be noted that efficient oxidation of periplasmic Cu(I) and a copper-poor cytoplasm are key features of enterobacterial copper homeostasis. In organisms lacking multicopper oxidases, or with an increased cytosolic copper quota, Fenton/Haber-Weiss chemistry may play a bigger role. Therefore, more research on the subject is required to inform possible therapeutic approaches.

ADDITIONAL FACTORS CONTRIBUTE TO COPPER TOLERANCE DURING ANAEROBIC GROWTH

Anaerobic conditions pose a specific challenge to copper homeostasis, especially in Enterobacteria. On the one hand, multicopper oxidases such as CueO/CuII are inactive without dioxygen (Outten et al., 2001). On the other, the lack of oxygen shifts the chemical equilibrium toward the more toxic Cu(I) (Beswick et al., 1976). It has also been observed that bacterial cells accumulate more copper under anoxic conditions (Outten et al., 2001; Macomber et al., 2007), although the mechanism causing this is unclear. In any case, bacterial cells are more sensitive to copper during anaerobic growth (Outten et al., 2001; Espariz et al., 2007).

Some components of the copper response are specifically mobilized during anaerobic growth. Glutathione, for example, may contribute to the E. coli copper tolerance by chelating copper and restoring the cellular redox balance during anaerobic (Macomber and Imlay, 2009) but not aerobic growth (Helbig et al., 2008). The DNA-binding mini-ferritin, Dps, also protects E. coli from copper damage during anerobiosis (Thieme and Grass, 2010) but is dispensable during aerobicosis (Große et al., 2014). Accordingly, many copper homeostasis genes are upregulated during the transition to anaerobic growth. This includes the cus operon as well as copA, which is regulated by the oxygen sensor FNR in addition to CueR (Partridge et al., 2007). Interestingly, it has been proposed that the S. enterica CueO homologue contributes to copper tolerance even under anaerobic conditions (Espariz et al., 2007), but the mechanism of this contribution is unclear since the lack of oxygen should preclude oxidase activity.

Taken as a whole, the available evidence suggests that strict or facultative anaerobes must face additional challenges when adapting
to copper toxicity under anoxic conditions. This shows promise for therapeutic approaches, since reducing agents should feature a powerful synergy with copper treatment.

12 | CONCLUSIONS AND PERSPECTIVES

In this review, we provided a holistic view of the bacterial response to copper toxicity, focusing on two intensively studied organisms, *E. coli* and *S. enterica*. Many studies have focused on elucidating copper homeostasis in diverse bacteria. The direct response to copper excess is highly conserved and generally involves: (a) sensing of the increased copper availability by sensors located in the different bacterial compartments; (b) activation of bespoke transcriptional networks; (c) overproduction of copper efflux pumps that secrete copper from the copper-poisoned compartment; and (d) recruitment of copper-binding and copper-oxidizing proteins, such as metallothioneins, copper oxidases, copper storage proteins, and/or copper-chaperones that prevent copper from interacting with cellular components.

The copper homeostasis system, however, is insufficient to protect cells from copper toxicity. Additional bacterial pathways are needed to minimize and repair copper-induced damage, enabling cellular survival under copper stress. The collective data on enterobacterial systems suggests that these systems are mainly responsible for: (a) protecting the integrity and function of the cell envelope; (b) maintaining the cell’s redox balance and detoxifying any ROS eventually generated by copper excess; and (c) ensuring the homeostasis of other metals, especially iron. Importantly, understanding the copper response can shed light on the mechanisms of copper toxicity. For example, we notice that copper adaptation is most often explored under aerobic conditions, whereas oxygen availability should be considered an important variable in studies of copper toxicity.

A full understanding of the bacterial response to copper toxicity is not just of academic interest. Emerging evidence demonstrates that copper plays a role in the mammalian immune system, potentially making components of the bacterial copper response suitable targets for future drug development. The “contact-killing” toxicity of solid copper-alloy surfaces has also received attention in recent years, owing to their potential use to reduce the spread of surface-associated pathogens in the clinic (Noyce et al., 2006; Warnes et al., 2012). Copper-based compounds and materials have a long history of such applications in agriculture and in medicine, and are appealing due to a wide-spectrum antimicrobial activity, low cost, and what seems to be a relatively low risk of resistance emerging among bacteria (Lemire et al., 2013). Moreover, metal ions promise to be potent synergistic drugs to enhance or complement the effectiveness of existing and future antibacterials (Peters et al., 2018). As a consequence, a better understanding of bacterial copper responses can inform novel therapeutic approaches, and will be required to fully unlock the potential of copper as an antimicrobial of the future.

ACKNOWLEDGMENTS

We wish to thank Francesca Focarelli for input into this manuscript in relation to Salmonella copper homeostasis. K JW was supported by the Biotechnology and Biological Sciences Research Council (BB/S006818/1).

AUTHOR CONTRIBUTIONS

AG conceived the concept for review and drafted the manuscript, with writing input and supervision from K JW.

ORCID

Kevin J. Waldron https://orcid.org/0000-0002-5577-7357

REFERENCES

Achard, M.E., Tree, J.J., Holden, J.A., Simpfendorfer, K.R., Wijburg, O.L., Strugnell, R.A., et al (2010). The multi-copper-oxidase CueO of *Salmonella enterica* serovar Typhimurium is required for systemic virulence. *Infection and Immunity*, 78, 2312–2319.

Andreini, C., Banci, L., Bertini, I. and Rosato, A. (2008). Occurrence of copper proteins through the three domains of life: a bioinformatic approach. *Journal of Proteome Research*, 7, 209–216.

Arai, N., Sekizuka, T., Tamamura, Y., Kusumoto, M., Hinenoya, A., Yamasaki, S., et al (2019) Salmonella genomic island 3 is an integrative and conjugative element and contributes to copper and arsenic tolerance of *Salmonella enterica*. *Antimicrobial Agents and Chemotherapy*, 63, e00429-19.

Argiello, J.M., Patel, S.J. and Quintana, J. (2016). Bacterial Cu(+)–ATPases: models for molecular structure-function studies. *Metallomics*, 8, 906–914.

Argiello, J.M., Raimunda, D. and Padilla-Benavides, T. (2013). Mechanisms of copper homeostasis in bacteria. *Frontiers in Cellular and Infection Microbiology*, 3, 73.

Arnesano, F., Banci, L., Benvenuti, M., Bertini, I., Calderone, V., Mangani, S., et al (2003). The evolutionarily conserved trimeric structure of CutA1 proteins suggests a role in signal transduction. *Journal of Biological Chemistry*, 278, 45999–46006.

Bagai, I., Liu, W., Rensing, C., Blackburn, N.J. and McEvoy, M.M. (2007). Substrate-linked conformational change in the periplasmic component of a Cu(I)/Ag(I) efflux system. *Journal of Biological Chemistry*, 282, 35695–35702.

Balasubramanian, R., Kenney, G.E. and Rosenzweig, A.C. (2011). Dual pathways for copper uptake by methanotrophic bacteria. *The Journal of Biological chemistry*, 286, 37313–37319.

Barchinger, S.E. and Ades, S.E. (2013). Regulated proteolysis: control of the Escherichia coli mE-dependent cell envelope stress response. In: D. Dougan (Ed.) Regulated Proteolysis in Microorganisms, *Subcellular Biochemistry*. Dordrecht, Netherlands: Springer, Vol. 66, pp. 129–160.

Barwinska-Sendra, A. and Waldron, K.J. (2017). The role of intermetal competition and mis-metalation in metal toxicity. *Advances in Microbial Physiology*, 70, 315–379.

Beswick, P.H., Hall, G.H., Hook, A.J., Little, K., McBrien, D.C. and Lott, K.A. (1976). Copper toxicity: evidence for the conversion of cupric to cuprous copper in vivo under anaerobic conditions. *Chemico-Biological Interactions*, 14, 347–356.

Billman-Jacobe, H., Liu, Y., Haites, R., Weaver, T., Robinson, L., Marenda, M., et al (2018). pSTM6-275, a conjugative IncHI2 plasmid of *Salmonella enterica* that confers antibiotic and heavy-metal resistance under changing physiological conditions. *Antimicrobial agents and chemotherapy*, 62, e02357-17.

Brown, N.L., Barrett, S.R., Camakaris, J., Lee, B.T.O. and Rouch, D.A. (1995). Molecular genetics and transport analysis of the...
copper-resistance determinant (pcO) from Escherichia coli plasmid pR1004. *Molecular Microbiology*, 17, 1153–1166.

Cai, L., Koropatnick, J. and Cherian, M.G. (1995). Metallothionein protects DNA from copper-induced but not iron-induced cleavage in vitro. *Chemico-Biological Interactions*, 96, 143–155.

Cao, J., Woodhall, M.R., Alvarez, J., Cartron, M.L. and Andrews, S.C. (2007). EfeUOB (YcdNOB) is a tripartite, acid-induced and CpxAR-regulated, low-pH Fe2+ transporter that is cryptic in *Escherichia coli* K-12 but functional in *E. coli* O157:H7. *Molecular Microbiology*, 65, 857–875.

Chalmers, G., Rozas, K.M., Machawachadi, R.G., Scott, H.M., Norman, K.N., Nagaraja, T.G., et al. (2018). Distribution of the pco Gene cluster and associated genetic determinants among swine *Escherichia coli* from a controlled feeding trial. *Genes*, 9, 504.

Changela, A., Chen, K., Xue, Y., Holschen, J., Outten, C.E., O’Halloran, E.T., Chaturvedi, K.S., Hung, C.S., Crowley, J.R., Stapleton, A.E. and others (2012). Regulators of oxidative stress response to copper. *Journal of Bacteriology*, 194, 2362–2370.

Chiang, S.M. and Schellhorn, H.E. (2012). Copper toxicity and acid stress in copper and zinc toxicity in innate immune defense against bacterial pathogens. *Molecular Microbiology*, 53, 161–169.

Chiang, S.M. and Schellhorn, H.E. (2012). Regulators of oxidative stress response genes in *Escherichia coli* and their functional conservation in bacteria. *Archives of Biochemistry and Biophysics*, 525, 1307–1318.

Chillappagar, S., Miethke, M., Trip, H., Kuipers, O.P. and Marahiel, M.A. (2009). Copper acquisition is mediated by Ycn and Regulated by YcnK and CsoR in *Bacillus subtilis*. *Journal of Bacteriology*, 191, 2362–2370.

Djoko, K.Y., Achard, M.E.S., Phan, M.D., Lo, A.W., Miraula, M., Promhbul, S., et al. (2018) Copper ions and coordination complexes as novel carbapenem adjuvants. *Antimicrobial Agents and Chemotherapy*, 62, e02280-17.

Djoko, K.Y., Ong, C.L., Walker, M.J. and McEwan, A.G. (2015). The role of copper and zinc toxicity in innate immune defense against bacterial pathogens. *The Journal of Biological Chemistry*, 290, 18954–18961.

Djoko, K.Y., Phan, M.D., Peters, K.M., Walker, M.J., Schembri, M.A. and McEwan, A.G. (2017). Interplay between tolerance mechanisms to copper and acid stress in *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 114, 6818–6823.

Djoko, K.Y., Xiao, Z. and Wedd, A.G. (2008). Copper resistance in *E. coli*: the multicopper oxidase PcoA catalyzes oxidation of copper(I) in *Cu(I)Cu(II)-PcoC*. *Chembiochem*, 9, 1579–1582.

Drees, S.L., Beyer, D.F., Lenders-Lomshcer, C. and Lubben, M. (2015). Distinct functions of serial metal-binding domains in the *Escherichia coli* plasmid pBETaseCopA. *Molecular Microbiology*, 97, 423–438.

Dukan, S., Dadon, S., Smulski, D.R. and Belkin, S. (1996). Hypochlorous acid activates the heat shock and acid activates the heat shock and acid resistance of *Escherichia coli* K-12. *Journal of Bacteriology*, 178, 2297–2307.

Ekiç, S., Turkerslan, S., Pawlik, G., Dancis, A., Baliga, N.S., Koch, H.G., et al. (2014). Intracytoplasmic copper homeostasis controls cytochrome c oxidase production. *mBio*, 5, e01055-13.

Eslariz, M., Checa, S.K., Audero, M.E.P., Pontel, L.B. and Soncini, F.C. (2007). Dissecting the *Salmonella* response to copper. *Microbiology*, 153, 2989–2997.

Fang, L., Li, X., Li, L., Li, S., Liao, X., Sun, J., et al. (2016). Co-spread of metal and antibiotic resistance within ST3-INch12 plasmids from *E. coli* isolates of food-producing animals. *Scientific Reports*, 6, 25312.
Neumann, M. and Leimkühler, S. (2008). Heavy metal ions inhibit mol-lybdoenzyme activity by binding to the dithiolene moiety of molybdo-pterin in Escherichia coli. The FEBS Journal, 275, 5678–5689.

Nies, D.H. and Herzberg, M. (2013). A fresh view of the cell biology of copper in enterobacteria. Molecular Microbiology, 87, 447–454.

Noyce, J.O., Michels, H. and Keviil, C.W. (2006). Potential use of copper surfaces to reduce survival of epidemic meticillin-resistant Staphylococcus aureus in the healthcare environment. Journal of Hospital Infection, 63, 289–297.

Nunoshita, T., deRojas-Walker, T., Wishnok, J.S., Tannenbaum, S.R. and Demple, B. (1993). Activation by nitric oxide of an oxidative-stress response that defends Escherichia coli against activated macrophages. Proceedings of the National Academy of Sciences of the United States of America, 90, 9993–9997.

Oelschlaeger, T.A., Zhang, D., Schubert, S., Carniel, E., Rabsch, W., Karch, H., et al (2003). The high-pathogenicity island is absent in human pathogens of Salmonella enterica subspecies I but present in isolates of subspecies III and VI. Journal of Bacteriology, 185, 1107–1111.

Okuda, S. and Tokuda, H. (2011). Lipoprotein sorting in bacteria. Annual Review of Microbiology, 65, 239–259.

Osmann, D., Patterson, C.J., Bailey, K., Fisher, K., Robinson, N.J., Rigby, S.E., et al (2013). The copper supply pathway to a Salmonella Cu, Zn-superoxide dismutase (SodCII) involves P(1b)-type ATPase copper efflux and periplasmic CueP. Molecular Microbiology, 87, 466–477.

Osman, D., Waldron, K.J., Denton, H., Taylor, C.M., Grant, A.J., Mastroeni, P., et al (2010). Copper homeostasis in Salmonella is atypical and copper-CueP is a major periplasmic metal complex. The Journal of Biological Chemistry, 285, 25259–25268.

Outten, F.W., Huffman, D.L., Hale, J.A. and O’Halloran, T.V. (2001). The independent cue and cus systems confer copper tolerance during aerobic and anaerobic growth in Escherichia coli. The Journal of Biological Chemistry, 276, 30670–30677.

Outten, F.W., Outten, C.E., Hale, J. and O’Halloran, T.V. (2000). Transcriptional activation of an Escherichia coli copper efflux regulon by the chromosomal MerR homologue, cueR. The Journal of Biological Chemistry, 275, 31024–31029.

Park, H.-J., Nguyen, T.T.M., Yoon, J. and Lee, C. (2012). Role of reactive oxygen species in Escherichia coli inactivation by cupric ion. Environmental Science & Technology, 46, 11299–11304.

Parmar, J.H., Quintana, J., Ramirez, D., Laubenbacher, R., Arguiello, J.M. and Mendes, P. (2018). An important role for periplasmic storage in Pseudomonas aeruginosa copper homeostasis revealed by a combined experimental and computational modeling study. Molecular Microbiology, 110, 357–369.

Partridge, J.D., Sanguinetti, G., Dibden, D.P., Roberts, R.E., Poole, R.K. and Green, J. (2007). Transition of Escherichia coli from aerobic to micro-aerobic conditions involves fast and slow reacting regulatory components. Journal of Biological Chemistry, 282, 11230–11237.

Peters, K., Pazos, M., Edoo, Z., Hugonnet, J.-E., Martorana, A.M., Polissi, A., et al (2018). Copper inhibits peptidoglycan LD-transpeptidases suppressing β-lactam resistance due to bypass of penicillin-binding proteins. Proceedings of the National Academy of Sciences of the United States of America, 115, 10786–10791.

Pezza, A., Pontel, L.B., López, C. and Soncini, F.C. (2016). Compartment and signal-specific codependence in the transcriptional control of Salmonella periplasmic copper homeostasis. Proceedings of the National Academy of Sciences of the United States of America, 113, 11573–11578.

Pontel, L.B., Scampoli, N.L., Porwollik, S., Checa, S.K., McClelland, M. and Soncini, F.C. (2014). Identification of a Salmonella ancillary copper detoxification mechanism by a comparative analysis of the genome-wide transcriptional response to copper and zinc excess. Microbiology, 160, 1659–1669.

Pontel, L.B. and Soncini, F.C. (2009). Alternative periplasmic copper-resistance mechanisms in Gram negative bacteria. Molecular Microbiology, 73, 212–225.

Price, N.L. and Raivo, T.L. (2009). Characterization of the Cpx regulon in Escherichia coli strain MC4100. Journal of Bacteriology, 191, 1798–1815.

Py, B. and Barras, F. (2010). Building Fe–S proteins: bacterial strategies. Nature Reviews Microbiology, 8, 436.

Qin, Y., Hasman, H., Aarestrup, F.M., Alwathnani, H.A. and Rensing, C. (2014). Genome sequences of three highly copper-resistant Salmonella enterica subspp. I serovar typhimurium strains isolated from pigs in Denmark. Genome Announcements, 2, e01334–14.

Raivo, T.L. (2014). Everything old is new again: an update on current re-search on the Cpx envelope stress response. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1843, 1529–1541.

Rensing, C., Fan, B., Sharma, R., Mitra, B. and Rosen, B.P. (2000). CopA: an Escherichia coli Cuf1(translocating P-type ATPase. Proceedings of the National Academy of Sciences of the United States of America, 97, 652–656.

Rensing, C. and Grass, G. (2003). Escherichia coli mechanisms of copper homeostasis in a changing environment. FEMS microbiology reviews, 27, 197–213.

Richmond, C.S., Glasner, J.D., Mau, R., Jin, H. and Blattner, F.R. (1999). Genome-wide expression profiling in Escherichia coli K-12. Nucleic acids research, 27, 3821–3835.

Rietsch, A., Belin, D., Martin, N. and Beckwith, J. (1996). An in vivo path-way for disulfide bond isomerization in Escherichia coli. Proceedings of the National Academy of Sciences of the United States of America, 93, 13048–13053.

Rigo, A., Corazza, A., Luisa di Paolo, M., Rossetto, M., Ugolini, R. and Scarp, M. (2004). Interaction of copper with cysteine: stability of cuprous complexes and catalytic role of cupric ions in anaerobic thiol oxidation. Journal of Inorganic Biochemistry, 98, 1495–1501.

Riley, M., Abe, T., Arnaud, M.B., Berlyn, M.K., Blattner, F.R., Chaudhuri, R.R., et al (2006). Escherichia coli K-12: a cooperatively developed annotation snapshot-2005. Nucleic Acids Research, 34, 1–9.

Rodriguez-Montelongo, L., de la Cruz-Rodriguez, L.C., Farias, R.N. and Massa, E.M. (1993). Membrane-associated redox cycling of copper mediates hydroperoxide toxicity in Escherichia coli. Biochimica et Biophysica Acta, 1144, 77–84.

Rogers, S.D., Bhave, M.R., Mercer, J.F., Camakaris, J. and Lee, B.T. (1991). Cloning and characterization of cutE, a gene involved in copper transport in Escherichia coli. Journal of Bacteriology, 173, 6742–6748.

Rouch, D., Camakaris, J. and Lee, B. (1989). Copper transport in Escherichia coli. In: Winge, D. and Hamer, D. (Eds.) Metal Ion Homeostasis: Molecular Biology and Chemistry. New York: Alan R Liss, pp. 469–477.

Rouch, D.A. and Brown, N.L. (1997). Copper-inducible transcriptional regulation at two promoters in the Escherichia coli copper resistance determinant pco. Microbiology, 143(4 Pt 4), 1191–1202.

Seo, S.W., Kim, D., Latif, H., O’Brien, E.J., Szubin, R. and Palsson, B.O. (2014). Deciphering Fur transcriptional regulatory network highlights its complex role beyond iron metabolism in Escherichia coli. Nature Communications, 5, 4910–4910.

Solliz, M. (2018). Copper homeostasis in gram-negative bacteria. In: Copper and Bacteria: Evolution, Homeostasis and Toxicity. Cham: Springer International Publishing, pp. 49–80.

Stewart, L.J., Thaqi, D., Kobe, B., McEwan, A.G., Waldron, K.J. and Djoko, K.Y. (2019). Handling of nutrient copper in the bacterial envelope. Metallomics, 11, 50–63.

Stocks, C.J., Phan, M.-D., Achard, M.E.S., Nhu, N.T.K., Condon, N.D., Gawthorne, J.A., et al (2019). Uropathogenic Escherichia coli employs both evasion and resistance to subvert innate immune-mediated zinc toxicity for dissemination. Proceedings of the National Academy of Sciences of the United States of America, 116, 6341–6350.
Stolle, P., Hou, B. and Bruser, T. (2016). The Tat Substrate CueO Is Transported in an Incomplete Folding State. The Journal of biological chemistry, 291, 13520–13528.

Subedi, P., Paxman, J.J., Wang, G., Ukuwela, A.A., Xiao, Z. and Heras, B. (2019). The Scs disulfide reductase system cooperates with the metallochaperone CueP in Salmonella copper resistance. The Journal of Biological Chemistry, 294, 15876–15888.

Tan, G., Cheng, Z., Pang, Y., Landry, A.P., Li, J., Lu, J., et al (2014). Copper binding in IscA inhibits iron-sulphur cluster assembly in Escherichia coli. Molecular Microbiology, 93, 629–644.

Tan, G., Yang, J., Li, T., Zhao, J., Sun, S., Li, X., et al (2017) Anaerobic copper toxicity and iron-sulfur cluster biogenesis in Escherichia coli. Applied and Environmental Microbiology, 83, e00867-17.

Tetaz, T.J. and Luke, R.K. (1983). Plasmid-controlled resistance to copper of Mycobacterium tuberculosis. Molecular Microbiology, 154, 1263–1268.

Thieme, D. and Grass, G. (2010). The Dps protein of Escherichia coli is involved in copper homeostasis. Microbiological Research, 165, 108–115.

Urano, H., Umezawa, Y., Yamamoto, K., Ishihama, A. and Ogasawara, H. (2015). Cooperative regulation of the common target genes between H2O2-sensing YedVW and Cu2+-sensing CusSR in Escherichia coli. Microbiology, 161, 729–738.

Urano, H., Yoshida, M., Ogawa, A., Yamamoto, K., Ishihama, A. and Ogasawara, H. (2017). Cross-regulation between two common ancestral response regulators, HprR and CusR, in Escherichia coli. Microbiology, 163, 243–252.

Varghese, S., Wu, A., Park, S., Imlay, K.R.C. and Imlay, J.A. (2007). Submicromolar hydrogen peroxide disrupts the ability of Fur protein to control free-iron levels in Escherichia coli. Molecular Microbiology, 64, 822–830.

Vest, K.E., Hashemi, H.F. and Cobine, P.A. (2013). The copper metallome in eukaryotic cells. Metal Ions in Life Sciences, 12, 451–478.

Vita, N., Platsaki, S., Basle, A., Allen, S.J., Paterson, N.G., Crombie, A.T., et al (2015). A four-helix bundle stores copper for methane oxidation. Nature, 525, 140–143.

Volentini, S.I., Farias, R.N., Rodriguez-Montelongo, L. and Rapisarda, V.A. (2011). Cu(I) reduction by Escherichia coli cells is dependent on respiratory chain components. BioMetals, 24, 827–835.

Warnes, S.L., Highmore, C.J. and Keever, C.W. (2012) Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: implications for public health. mBio, 3, e00489-12.

White, C., Lee, J., Kambe, T., Fritsche, K. and Petris, M.J. (2009). A role for the ATP7A copper-transporting ATPase in macrophage bactericidal activity. The Journal of Biological Chemistry, 284, 33949–33956.

Wolschendorf, F., Ackart, D., Shrestha, T.B., Hascall-Dove, L., Nolan, S., Lamichhane, G., et al (2011). Copper resistance is essential for virulence of Mycobacterium tuberculosis. Proceedings of the National Academy of Sciences of the United States of America, 108, 1621–1626.

Xu, Z., Wang, P., Wang, H., Yu, Z.H., Au-Yeung, H.Y., Hirayama, T., et al (2019). Zinc excess increases cellular demand for iron and decreases tolerance to copper in Escherichia coli. Journal of Biological Chemistry.

Yakushi, T., Tajima, T., Matsuyama, S. and Tokuda, H. (1997). Lethality of the covalent linkage between mislocalized major outer membrane lipoprotein and the peptidoglycan of Escherichia coli. Journal of Bacteriology, 179, 2857–2862.

Yamamoto, K. and Ishihama, A. (2005). Transcriptional response of Escherichia coli to external copper. Molecular Microbiology, 56, 215–227.

Yamamoto, K. and Ishihama, A. (2006). Characterization of copper-inducible promoters regulated by CpxA/CpxR in Escherichia coli. Bioscience, Biotechnology, and Biochemistry, 70, 1688–1695.

Yucel, B., Robinson, G.K. and Shepherd, M. (2020). The copper-responsive ScsC protein of Salmonella promotes intramacrophage survival and interacts with the arginine sensor Artl. The FEBS Journal.

Yun, B.Y., Xu, Y., Piao, S., Kim, N., Yoon, J.H., Cho, H.S., et al (2010). Periplasmic domain of CusA in an Escherichia coli Cu+/Ag+ transporter has metal binding sites. Journal of Microbiology, 48, 829–835.

Zhang, X.-S., García-Conterras, R. and Wood, T.K. (2007). YcfR (BhsA) influences Escherichia coli biofilm formation through stress response and surface hydrophobicity. Journal of Bacteriology, 189, 3051–3062.

Zhang, Y., Blaby-Haas, C.E., Steimle, S., Verissimo, A.F., Garcia-Angulo, V.A., Koch, H.G., et al (2019). Cu Transport by the extended family of CcoA-like transporters (CalT) in proteobacteria. Scientific Reports, 9, 1208.

Zheng, M., Doan, B., Schneider, T.D. and Storz, G. (1999). OxyR and SoxRS regulation of fur. Journal of Bacteriology, 181, 4639–4643.

Zheng, M., Wang, X., Templeton, L.J., Smulski, D.R., LaRossi, R.A. and Storz, G. (2001). DNA microarray-mediated transcriptional profiling of the Escherichia coli response to hydrogen peroxide. Journal of Bacteriology, 183, 4562–4570.

Zimmermann, M., Udagedara, S.R., Sze, C.M., Ryan, T.M., Howlett, G.J., Xiao, Z., et al (2012). PcoE - A metal sponge expressed to the periplasm of copper resistance Escherichia coli. Implication of its function role in copper resistance. Journal of Inorganic Biochemistry, 115, 186–197.

How to cite this article: Giachino A, Waldron KJ. Copper tolerance in bacteria requires the activation of multiple accessory pathways. Mol Microbiol. 2020;114:377–390. https://doi.org/10.1111/mmi.14522