Copper is an essential cofactor of various enzymes, but free copper is highly toxic to living cells. To maintain cellular metabolism at different ambient copper concentrations, bacteria have evolved specific copper homeostasis systems that mostly act as defence mechanisms. As well as under free-living conditions, copper defence is critical for virulence in pathogenic bacteria. Most bacteria synthesize P-type copper export ATPases as principal defence determinants when copper concentrations exceed favourable levels. In addition, many bacteria utilize resistance-nodulation-cell division (RND)-type efflux systems and multicopper oxidases to cope with excess copper. This review summarizes our current knowledge on copper-sensing transcriptional regulators, which we assign to nine different classes. Widespread one-component regulators are CueR, CopY and CsoR, which were initially identified in Escherichia coli, Enterococcus hirae and Mycobacterium tuberculosis, respectively. CueR activates homeostasis gene expression at elevated copper concentrations, while CopY and CsoR repress their target genes under copper-limiting conditions. Besides these one-component systems, which sense the cytoplasmic copper status, many Gram-negative bacteria utilize two-component systems, which sense periplasmic copper concentrations. In addition to these well-studied transcriptional factors, copper control mechanisms acting at the post-transcriptional and the post-translational levels will be discussed.

To cope with unfavourable copper concentrations, most bacteria utilize specific copper-induced defence mechanisms. Copper-transporting P-type ATPases are the principal copper homeostasis components across Gram-negative and Gram-positive bacteria. In addition, many bacteria synthesize multicompartment copper efflux systems belonging to the RND (resistance-nodulation-cell division) family or MCOs. Representative members of these copper homeostasis proteins are the copper-ATPase CopA, the RND system CusCFBA, and the MCO CueO of the Gram-negative model bacterium Escherichia coli (Rensing & Grass, 2003) (Fig. 1).

E. coli CopA exports excess Cu$^+$ from the cytoplasm to the periplasm, while CusCFBA extrudes Cu$^+$ from the periplasm (Franke et al., 2003; Long et al., 2010; Outten et al., 2001; Rensing et al., 2000). In addition, it has been reported that the Cus system excretes copper from the cytoplasm. CueO exhibits cuprous oxidase activity in vitro, suggesting that it converts periplasmic Cu$^{2+}$ to less toxic Cu$^{2+}$ in vivo (Singh et al., 2004). Besides, CueO oxidizes the siderophore enterobactin, which is primarily involved in iron acquisition (Grass et al., 2004). Oxidation of the siderophore prevents enterobactin-mediated reduction of Cu$^{2+}$ to Cu$^+$. The CopA–CueO and CusCFBA systems confer copper tolerance at moderate copper concentrations under aerobic conditions and at high copper concentrations under anaerobic conditions, respectively (Grass & Rensing, 2001; Outten et al., 2001). Both systems are required for full copper homeostasis to maintain metabolism and viability.
tolerance. In addition, some *E. coli* strains harbour the episomal *pcoABCDRSE* gene cluster, enabling these strains to survive at otherwise lethal copper concentrations (Brown et al., 1995). The central component of this system is the MCO PcoA, which can functionally substitute for CueO.

This review focuses on copper-sensing regulators and copper-responsive gene regulation in bacteria. We assigned copper-sensing transcriptional regulators to nine classes represented by the founding members from *E. coli* (classes 1–3: CueR, CusRS, ComR), other proteobacteria (classes 4 and 5: CopL, CorE), cyanobacteria (class 6: BxmR) and Gram-positive bacteria (classes 7–9: CopY, CsoR, YcnK) (Table 1). The majority of copper-responsive regulators described to date belong to class 1 (CueR), class 2 (CusRS), class 7 (CopY) and class 8 (CsoR). Regulators predicted by database searches only, whose functions have not been proven experimentally, have not been included in this

**Fig. 1.** Copper homeostasis in *E. coli*. Cu$^+$ and Cu$^{2+}$ ions enter the periplasm (shown in grey), probably via porins spanning the outer membrane. ComC lowers the permeability of the outer membrane for copper ions. It is unknown whether ComC interacts with porins. At low ambient copper concentrations, *comC* transcription is repressed by ComR (not shown). Cu$^+$ ions proceed from the periplasm into the cytoplasm by an unknown mechanism, while Cu$^{2+}$ ions do not cross the cytoplasmic membrane. Copper efflux involves the ATPase CopA and the multicomponent system CusCFBA. The multicopper oxidase CueO oxidizes periplasmic Cu$^+$ to Cu$^{2+}$. For further details, see text.

**Table 1.** Classification of copper-sensing transcriptional regulators

<table>
<thead>
<tr>
<th>Class</th>
<th>Founding member</th>
<th>Accession number</th>
<th>Size (aa)</th>
<th>Regulator family</th>
<th>Type of regulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>E. coli</em> CueR</td>
<td>EG13256</td>
<td>135</td>
<td>MerR</td>
<td>Activation (+ Cu)</td>
</tr>
<tr>
<td>2</td>
<td><em>E. coli</em> CusRS</td>
<td>EG13851</td>
<td>227</td>
<td>TCS</td>
<td>Activation (+ Cu)</td>
</tr>
<tr>
<td>3</td>
<td><em>E. coli</em> ComR</td>
<td>EG13435</td>
<td>210</td>
<td>TetR</td>
<td>Repression (− Cu)</td>
</tr>
<tr>
<td>4</td>
<td><em>X. axonopodis</em> CopL</td>
<td>AAR85972</td>
<td>122</td>
<td>None</td>
<td>Activation (+ Cu)</td>
</tr>
<tr>
<td>5</td>
<td><em>Myc. xanthus</em> CorE</td>
<td>MXAN_3426</td>
<td>211</td>
<td>ECF</td>
<td>Activation (+ Cu)</td>
</tr>
<tr>
<td>6</td>
<td><em>O. brevis</em> BxmR</td>
<td>BAD11074</td>
<td>136</td>
<td>SmtB/ArsR</td>
<td>Repression (− Cu)</td>
</tr>
<tr>
<td>7</td>
<td><em>Ent. hirae</em> CopY</td>
<td>CAA68635</td>
<td>145</td>
<td>HTH</td>
<td>Repression (− Cu)</td>
</tr>
<tr>
<td>8</td>
<td><em>Myc. tuberculosis</em> CsoR</td>
<td>Rv9067</td>
<td>119</td>
<td>DUF156</td>
<td>Repression (− Cu)</td>
</tr>
<tr>
<td>9</td>
<td><em>B. subtilis</em> YcnK</td>
<td>BSU03960</td>
<td>190</td>
<td>DeoR</td>
<td>Repression (+ Cu)</td>
</tr>
</tbody>
</table>
article. The last sections of this review briefly describe copper control mechanisms acting at the post-transcriptional and post-translational levels.

**Class 1: CueR-like one-component activators in proteobacteria**

*E. coli* CueR is the founding member of a well-studied one-component regulator class, which directly senses and responds to the cytoplasmic copper status (Outten et al., 2000) (Table 1). CueR belongs to the MerR family of transcriptional activators (Brown et al., 2003). MerR regulators exhibit a three-domain structure: an N-terminal DNA-binding domain (encompassing a helix-turn-helix (HTH) motif), a central dimerization domain, and a C-terminal effector-binding domain. Several members of the MerR family respond to metal ions such as Hg$^{2+}$ (MerR), Zn$^{2+}$ (ZntR), Pb$^{2+}$ (PbrR) and Cu$^{+}$ (CueR).

CueR-type activators are widespread in proteobacteria (Liu et al., 2007) (Table 2). There is only one report on a CueR-like regulator (YhdB) in a non-proteobacterial species, the Gram-positive model bacterium *Bacillus subtilis* (Gaballa et al., 2003). In a later report, however, the same group revoked the contribution of YhdB in copper-responsive gene regulation (Smaldone & Helmann, 2007). In addition to *E. coli*, CueR-like activators have been analysed in the gamma-proteobacteria *Pseudomonas aeruginosa, Pseudomonas putida,* *Pseudomonas fluorescens* and *Salmonella* (Sal.) enterica, and the alphaproteobacteria *Agrobacterium tumefaciens* and *Rhodobacter sphaeroides* (Table 2).

**E. coli**

CueR forms dimers consisting of three functional domains (a DNA-binding, a dimerization and a metal-binding domain) characteristic of the MerR family, as revealed by crystal structure analyses of Cu$^{+}$-, Ag$^{+}$- and Au$^{+}$-bound forms of CueR (Changela et al., 2003). CueR coordinates one Cu$^{+}$ ion per monomer in a linear S–Cu$^{+}$–S centre encompassing two cysteine residues (Cys$^{112}$ and Cys$^{20}$) located at the dimer interface (Changela et al., 2003; Chen et al., 2003). By contrast, binding of Zn$^{2+}$ by ZntA requires three cysteines: two cysteines from one monomer (resembling Cys$^{112}$ and Cys$^{20}$ of CueR) and a third cysteine from the other monomer (resembling Ser$^{77}$ of CueR) (Changela et al., 2003).

Both the CueR holo-activator (carrying Cu$^{+}$) and the apo-activator (copper-free) bind to target promoters, but exclusively the holo-activator activates transcription (Andoy et al., 2009). Even at moderate copper concentrations, CueR activates transcription of the copper-ATPase gene *copA* and the MCO gene *cueO* (Outten et al., 2000; Stoyanov et al., 2001) (Fig. 1, Table 2).

CueR dimers bind to dyad-symmetrical sequences (CCTTCC-N$_{7}$-GGAAGG) in its target promoters (Stoyanov et al., 2001; Yamamoto & Ishihama, 2005). Typically, binding sites of transcriptional activators are located upstream of the RNA polymerase binding sites, while CueR binding sites overlap with the −35/−10 regions of its target promoters. Remarkably, CueR-dependent promoters are characterized by enlarged spacing (19–20 base pairs) between the −35 and −10 motifs.

The genome-wide transcriptional response to copper in *E. coli* has been determined by microarray studies (Kershaw et al., 2005; Yamamoto & Ishihama, 2005). Varying with ambient copper concentrations and time intervals between copper addition and RNA preparation, numerous differently transcribed genes have been identified. Among these were the above-described copper homeostasis genes *copA* and *cueO* as well as genes involved in flagellar biosynthesis, iron metabolism, energy metabolism and general stress response. In line with these findings, the *E. coli* chromosome contains 197 putative CueR-binding sites, most of which await experimental confirmation.

**P. aeruginosa**

*P. aeruginosa* is an opportunistic human pathogen. The genome-wide transcriptional response of copper-shocked and copper-adapted *P. aeruginosa* cultures has revealed a core set of genes comparably regulated under both conditions (Teitzel et al., 2006). This core set includes different transport genes, suggesting that copper tolerance is mainly achieved by copper efflux. A CueR-like regulator activates transcription of several target genes, including *cueA*, upon copper addition (Thaden et al., 2010). The *cueA* gene encodes a copper-ATPase, which is a major copper tolerance determinant in free-living cells and an important virulence factor in the mouse model (Schwan et al., 2005). In line with this finding, *cueR* transcription is activated by the global regulator LasR, which forms part of the LasR–LasI quorum-sensing system (Thaden et al., 2010). In addition to *cueA* (*copA1*), *P. aeruginosa* harbours a second copper-ATPase gene, *copA2* (González-Guerrero et al., 2010). CopA2 belongs to the FixI-like subclass of ATPases, whose members provide metals for extracytoplasmic enzymes (Argüello et al., 2011). Disruption of *copA2* reduces activity of membrane-associated cytochrome *c* oxidase. Both CopA1 and CopA2 excrete Cu$^{+}$ from the cytoplasm, but CopA2 exhibits lower activity, and consequently, CopA2 is dispensable for copper tolerance (González-Guerrero et al., 2010).

**P. fluorescens and P. putida**

*P. fluorescens* and *P. putida* are plant growth-promoting bacteria. Both strains contain a bicistronic operon, *cueAR*, encoding the copper-ATPase CueA and the autoregulated activator CueR (Adaikkalam & Swarup, 2002; Zhang & Rainey, 2008). In both strains, disruption of *cueAR* reduces tolerance to copper. Upon copper addition, CueR activates *cueAR* expression in both *P. fluorescens* and *P. putida*. Interestingly, expression of *P. fluorescens* *cueA* is also activated upon plant contact, suggesting that the copper-ATPase is important for the bacteria–plant interaction.
Table 2. Experimentally verified copper-responsive regulators

Abbreviations: MCO, multicopper oxidase; RND, resistance-nodulation-cell division-type Cu efflux system; TCS, two-component regulatory system.

<table>
<thead>
<tr>
<th>Class</th>
<th>Regulator</th>
<th>Phylogenetic group</th>
<th>Target gene(s)</th>
<th>Gene product (function)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A. tumefaciens</td>
<td>Alphaproteobacteria</td>
<td>copARZ</td>
<td>CopA (ATPase), CopR (activator), CopZ (chaperone)</td>
<td>Nawapan et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>R. sphaeroides</td>
<td>Alphaproteobacteria</td>
<td>copA, copZ</td>
<td>CopA (ATPase), CopZ (chaperone)</td>
<td>Peuser et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>Gammaproteobacteria</td>
<td>copA, cueO</td>
<td>CopA (ATPase), CueO (MCO)</td>
<td>Outten et al. (2000)</td>
</tr>
<tr>
<td></td>
<td>P. aeruginosa</td>
<td>Gammaproteobacteria</td>
<td>cueA</td>
<td>CueA (ATPase)</td>
<td>Thaden et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>P. putida</td>
<td>Gammaproteobacteria</td>
<td>cueAR</td>
<td>CueA (ATPase), CueR (activator)</td>
<td>Adaiikkalam &amp; Swarup (2002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CopA (ATPase), CuiD (MCO), CueP (periplasmic Cu pool)</td>
<td>Pontel &amp; Soncini (2009)</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>Gammaproteobacteria</td>
<td>cusRS</td>
<td>cusCFBA, CusRS (TCS), CusCFBA (RND)</td>
<td>Outten et al. (2001)</td>
</tr>
<tr>
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<td>P. fluorescens</td>
<td>Gammaproteobacteria</td>
<td>copCD</td>
<td>CopA (ATPase), CopB (ATPase), CopCD (Cu import)</td>
<td>Mills et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>H. pylori</td>
<td>Epsilonproteobacteria</td>
<td>crdAB</td>
<td>CrdAB-CzcBA (RND)</td>
<td>Waidner et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Synechocystis</td>
<td>Cyanobacteria</td>
<td>copBAC</td>
<td>CopBAC (RND), CopRS (TCS)</td>
<td>Giner-Lamia et al. (2012)</td>
</tr>
<tr>
<td>2</td>
<td>E. coli</td>
<td>Gammaproteobacteria</td>
<td>comC</td>
<td>ComC (outer-membrane protein, Cu acquisition)</td>
<td>Mermord et al. (2012)</td>
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<td>X. axonopodis</td>
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<td>copA</td>
<td>CopA (MCO)</td>
<td>Voloudakis et al. (2005)</td>
</tr>
<tr>
<td></td>
<td>Myx. xanthus</td>
<td>Deltaproteobacteria</td>
<td>copB, cueB</td>
<td>CopB (ATPase), CuoB (MCO)</td>
<td>Gómez-Santos et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Ent. hirae</td>
<td>Gammaproteobacteria</td>
<td>copYZAB</td>
<td>CopY (repressor), CopZ (chaperone), CopA (ATPase), CopB (ATPase)</td>
<td>Oderrmatt &amp; Solizoz (1995)</td>
</tr>
<tr>
<td></td>
<td>Ent. faecalis</td>
<td>Gammaproteobacteria</td>
<td>copYZAB</td>
<td>CopY (repressor), CopZ (chaperone), CopA (ATPase), CopB (ATPase)</td>
<td>Reeyes-Jara et al. (2010)</td>
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<td>Gammaproteobacteria</td>
<td>copA</td>
<td>CopY (repressor), CopA (ATPase)</td>
<td>Vats &amp; Lee (2001)</td>
</tr>
<tr>
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<td>Gammaproteobacteria</td>
<td>copYAZ</td>
<td>CopY (repressor), CopZ (chaperone), CopA (ATPase)</td>
<td>Shafeeq et al. (2011)</td>
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<td>CopY</td>
<td>Gram-positive</td>
<td>copZA; ycnJ</td>
<td>CopZ (chaperone), CopA (ATPase), YcnJ (Cu import)</td>
<td>Schelder et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
<td>Gram-positive</td>
<td>ctpV-csoR</td>
<td>CtpV (ATPase), CsoR (repressor)</td>
<td>Corbett et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>C. glutamicum</td>
<td>Gram-positive</td>
<td>csoR-copAZ</td>
<td>CsoR (repressor), CopA (ATPase), CopZ (chaperone)</td>
<td>Liu et al. (2007)</td>
</tr>
<tr>
<td></td>
<td>Lis. monocytogenes</td>
<td>Gram-positive</td>
<td>csoR-copAZ</td>
<td>CsoR (repressor), CopA (ATPase), CopZ (chaperone)</td>
<td>Festa et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>Myx. tuberculosis</td>
<td>Gram-positive</td>
<td>csoR-rv0968-ctpV</td>
<td>MymT (Cu sequestration), LpqS (Cu efflux), Rv2963 (Cu efflux)</td>
<td>Grosselhohe et al. (2011)</td>
</tr>
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<td>Myx. tuberculosis</td>
<td>Gram-positive</td>
<td>mmyT, lpqS, rv2963</td>
<td>CopA (ATPase)</td>
<td>Dwarakanath et al. (2012)</td>
</tr>
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<td></td>
<td>Staph. aureus</td>
<td>Gram-positive</td>
<td>copA</td>
<td>CopA (ATPase), CsoR (repressor), CopZ (chaperone), CopA (ATPase)</td>
<td>Nakamoto et al. (2010)</td>
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<td>copZA</td>
<td>CopZ (chaperone), CsoR (repressor), CopA (ATPase)</td>
<td>Chilappagari et al. (2009)</td>
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<td>T. thermophilus</td>
<td>Deinococci</td>
<td>copZ-csoR-copA</td>
<td>CopZ (chaperone), CsoR (repressor), CopA (ATPase)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. subtilis</td>
<td>Gram-positive</td>
<td>ycnJ</td>
<td>YcnJ (Cu import)</td>
<td></td>
</tr>
</tbody>
</table>
**Sal. enterica**

*Sal. enterica* serovar Typhimurium tolerates higher copper concentrations than *E. coli* under anaerobic conditions, although it lacks a Cus copper efflux system (Pontel & Soncini, 2009). Instead, *Sal. enterica* synthesizes a periplasmic protein, CueP, which is essential for copper tolerance, particularly under anaerobic conditions, but in addition, contributes to copper tolerance under aerobic conditions. Copper-dependent *cuer* induction is strictly dependent on CueR, which binds a palindromic promoter sequence highly similar to the binding site defined for *E. coli* CueR. In addition to *cuer*, *Sal. enterica* CueR activates the copper-ATPase gene *copA* and the MCO gene *cuiD* (Espariz et al., 2007; Kim et al., 2002).

Besides CueR, *Sal. enterica* synthesizes another MerR-like regulator, GoIS, which is highly specific for Au⁺ ions but does not respond to Cu⁺ or Ag⁺ ions (Pontel et al., 2007). As mentioned above, *E. coli* CueR is not able to distinguish between these three metal ions (Changela et al., 2003).

**A. tumefaciens**

*A. tumefaciens* is a plant-pathogenic bacterium that induces tumour formation in dicotylic plants. *A. tumefaciens* contains the *copARZ* operon, which encodes the copper-ATPase CopA, the CueR-like activator CopR, and the putative copper chaperone CopZ (Nawapan et al., 2009). CopR induces expression of the *copARZ* operon in response to copper and silver by binding a palindromic promoter sequence similar to the motif recognized by *E. coli* CueR. Disruption of the *copARZ* operon reduces tolerance to copper but not to other metals. Unfortunately, we do not know whether a *copARZ* mutant is affected in tumour formation.

**R. sphaeroides**

The phototrophic purple bacterium *R. sphaeroides* contains two divergently transcribed genes, *copA* and *copZ*, which encode a copper-ATPase and a putative copper chaperone, respectively (Peuser et al., 2011). CueR activates expression of *copA* and *copZ*, which are preceded by palindromic sequences highly similar to the binding sites of *E. coli* CueR.

Surprisingly, deletion of *R. sphaeroides* *cuer* does not affect copper tolerance, while overexpression of *cuer* increases copper sensitivity. At present one may only speculate that another yet-to-be-identified copper defence system substitutes for CopA missing in the *cuer* deletion strain. Furthermore, it has to be clarified whether overexpression of CopA or another CueR target is responsible for copper sensitivity in the *cuer* overexpression strain.

**Class 2: CusRS-like two-component systems**

Two-component regulatory systems typically consist of a membrane-anchored sensor kinase and a cognate response regulator. Upon effector binding, the sensor kinase autophosphorylates at a conserved histidine residue prior to phosphotransfer to a conserved aspartate of the response regulator. In turn, the phosphorylated regulator activates transcription of its target genes.

*E. coli* possesses 30 sensor kinases and 34 response regulators, most of which belong to cognate two-component systems, including the copper-responsive CusRS system (Yamamoto et al., 2005). In addition to *E. coli*, copper-responsive two-component systems have been characterized in the proteobacteria *P. fluorescens*, *P. putida*, *Pseudomonas syringae*, and *Helicobacter pylori*, the cyano bacterium *Synechocystis* sp. PCC 6803, and the Gram-positive bacterium *Corynebacterium glutamicum* (Table 2).

Membrane-bound CusS is thought to sense the periplasmic copper status. Upon binding of Cu⁺, CusS is expected to autophosphorylate and donate the phosphoryl group to CusR, which in turn, activates transcription of the *cusCFBA* system and the *cuxRS* operons (Franke et al., 2003; Gudipaty et al., 2012; Outten et al., 2001, 2003). The two operons share a single palindromic binding site for CusR (AAAATGACAA-N₂-TTGTCAATTT) flanked by the −35/−10 motifs of the *cusi* and *cuxr* promoters (Yamamoto & Ishihama, 2005). While the *E. coli* genome contains 197 putative CueR-binding sites, no further CusR-binding sites have been detected.

In addition to CusRS, two further two-component regulatory systems, CpxRA and YedWV, are related to copper homeostasis (Yamamoto & Ishihama, 2005, 2006). In contrast to CusS, however, the sensor kinase CpxA does not directly sense the periplasmic copper status but instead responds to copper-induced protein misfolding. Its cognate response regulator CpxR controls expression of genes involved in motility, chemotaxis and envelope stress response, as well as two protease genes, encoding the periplasmic protease DegP and the membrane protease HtpX. The role of the YedWV system in copper homeostasis is unclear, but the sensor kinase YedV serves as phosphoryl donor not only to its cognate response regulator YedW but also to CusR (Yamamoto et al., 2005).

Some *E. coli* strains harbour the episomal *pcoABCDRSE* gene cluster, which is similar to the *P. syringae* *pcoABCDRS* cluster, enabling these strains to survive at otherwise lethal copper concentrations (Brown et al., 1995; Mills et al., 1993). The central component of this system is the MCO PcoA, which can functionally substitute for CueO. Copper-responsive...
activation of the pco operon is mediated by the PcoRS two-component system.

**P. fluorescens**

*P. fluorescens* synthesizes the putative copper uptake system CopCD, when copper becomes limiting (Zhang & Rainey, 2008). In contrast to *P. putida* and *P. syringae*, which contain copper-inducible copABCD operons, the copper export genes copAB are absent in *P. fluorescens*. Upon copper addition, the two-component system CopRS inhibits copCD expression by a yet-undefined mechanism. Mutants lacking copCD or copS tolerate higher copper concentrations than the wild-type.

**P. putida**

*P. putida* contains the copper-induced cinAQ operon (Quaranta et al., 2009). CinA belongs to the plastocyanin–azurin family. Plastocyanin is a copper enzyme, and its expression is copper-regulated in the cyanobacterium *Synechocystis* sp. PCC 6803 (Zhang et al., 1992). However, its role in copper homeostasis remains to be established in *P. putida*. Upon copper exposure, cinAQ transcription is activated by the two-component system CinRS. Histidine residues His37 and His147 located in the periplasmic loop of the sensor kinase CinS are likely candidates to coordinate copper.

**P. syringae**

*P. syringae* carries the plasmid-borne copper tolerance genes copABCDRS, which are similar to the *E. coli* pcoABCDRS genes (Brown et al., 1995; Mills et al., 1993). Expression of the copABCD operon depends on the two-component system CopRS. The periplasmic protein CopC and the inner-membrane protein CopD are believed to form a copper uptake system, since *P. syringae* strains overexpressing the copCD genes are hypersensitive to copper and accumulate larger amounts of copper than the parental strain (Cha & Cooksey, 1993). Cotranscription of copper export genes, copAB, and copper uptake genes, copCD, indicates additional regulation at the post-transcriptional or post-translational level.

**H. pylori**

*H. pylori*, which plays an important role in stomach ulcers, induces expression of the copper-ATPase CopA and the CrdA-CrdB-CzxB-CzxA system, which exhibits similarity to the *E. coli* Cus system, upon copper exposure (Waidner et al., 2005). The two-component system CrdRS is essential for copper-responsive activation of the crdA promoter. Mutants defective for either CrdR or CrdS are copper-sensitive. Although CrdR exhibits clear similarity to CusR of *E. coli*, CrdR apparently binds a mirror repeat sequence (Yamamoto & Ishihama, 2005).

**Synechocystis sp. PCC 6803**

The unicellular cyanobacterium *Synechocystis* 6803 synthesizes a plasmid-borne RND-type copper efflux system encoded by the copBAC operon to cope with excess copper (Giner-Lamia et al., 2012). Either of two genetically distinct two-component CopRS systems (encoded by the chromosomal copMRS and the episomal pcopMRS operons) is sufficient to induce copBAC expression. Double mutants lacking both regulatory systems exhibit a copper sensitive phenotype, while single mutants are as copper tolerant as the wild-type. In addition to copBAC, CopRS activates transcription of the copMRS operon, and thus appears to be autoregulatory.

The isolated periplasmic domain of the sensor kinase CopS binds Cu²⁺ but not Zn²⁺, Ni²⁺ or Co²⁺, suggesting that CopS specifically responds to copper (Giner-Lamia et al., 2012). Direct repeat sequences (TTTCAT-N₅-TTTCAT) upstream of the copBAC and copMRS transcription start sites are thought to act as binding sites of the response regulator CopR.

*Synechocystis* 6803 performs oxygenic photosynthesis. Electron transport between cytochrome bf and photosystem I is mediated by the cuproenzyme plastocyanin (PetE), which can be replaced by cytochrome c₅₅₃ (PetF) when copper becomes limiting (Zhang et al., 1992). Neither the petE nor the petF gene is regulated by CopRS.

**C. glutamicum**

The soil bacterium *C. glutamicum* excretes large quantities of l-glutamate and l-lysine. Upon exposure to copper, *C. glutamicum* strongly induces expression of the divergently transcribed copy-copS-cg3283-cg3282-copB and cg3286-copO-cg3288-cg3289 operons, which encode the two-component system CopRS, the copper-ATPase CopB, and the extracellular MCO CopO (Schelder et al., 2011). In contrast to its *E. coli* counterpart, CusR, which binds a dyad-symmetrical sequence, CopR binds the direct repeat sequence TGAAGATTT-N₅-TGAAGATTT within the copy-cg3286 intergenic region, which is the only CopR target in the entire *C. glutamicum* genome. The copy region is characterized by an extremely high G+C content, suggesting that this region has only recently been acquired by lateral gene transfer.

**Class 3: ComR, a TetR-like regulator, controls copper acquisition in *E. coli***

Copper ions (Cu⁺ and Cu²⁺) pass the outer membrane of *E. coli* and enter the periplasm probably via porins, while only Cu⁺ is able to cross the inner membrane and reach the cytoplasm by a currently unknown mechanism (Outten et al., 2001; Rensing & Grass, 2003) (Fig. 1). The outer-membrane protein ComC reduces the permeability of the outer membrane to copper (Mermod et al., 2012). It remains to be elucidated whether ComC limits copper acquisition by affecting porin activity or by another mechanism. Under
copper-limiting conditions, \textit{comC} transcription is repressed by the TetR-like regulator ComR. Upon copper binding, ComR is released from the \textit{comC} promoter and transcription is relieved.

In contrast to many other copper-responsive regulators, ComR does not regulate its own expression (Mermod \textit{et al.}, 2012). It remains unknown to date whether ComR regulates target genes other than \textit{comC}. Although ComC-like proteins are widespread in Gram-negative bacteria, one can only speculate whether expression of \textit{comC} orthologues is generally copper-regulated and whether such regulation involves TetR-like regulators.

\textbf{Class 4: CopL-type regulators are unique to \textit{Xanthomonas} species}

The plant pathogen \textit{Xanthomonas axonopodis pv. vesicatoria} (formerly \textit{Xanthomonas campestris pv. vesicatoria}) contains a plasmid-borne MCO gene that confers copper resistance on this bacterium (Voloudakis \textit{et al.}, 2005). Confusingly, the MCO gene has been designated \textit{copA}, a name otherwise used for copper-ATPase genes (Table 2). Copper-inducible \textit{copA} expression strictly depends on the upstream gene \textit{copL}, as shown by nonpolar insertion of a kanamycin-resistance gene in \textit{copL}. Remarkably, \textit{copA} expression cannot be restored by \textit{in trans} complementation.

The CopL protein is rich in cysteines and histidines, suggesting that CopL binds copper ions and thus directly senses the cellular copper status (Voloudakis \textit{et al.}, 2005). Upon copper binding, CopL is thought to bind the \textit{copA} promoter and activate \textit{copA} transcription. In contrast to \textit{copA}, the \textit{copL} gene is constitutively transcribed.

Apparently, CopL homologues are unique to \textit{Xanthomonas} species, and CopL does not exhibit similarity to any known regulator. Unfortunately, promoter binding by CopL has not been experimentally demonstrated yet.

\textbf{Class 5: CorE, a copper-responsive ECF sigma factor in \textit{Myxococcus (Myx.) xanthus}}

The deltaproteobacterium \textit{Myx. xanthus} is a model bacterium to study cell differentiation and fruiting body formation. Recently, an ECF (extracytoplasmic function) sigma factor, CorE, was shown to activate expression of the \textit{cuoB} and \textit{copB} genes, which encode a MCO and a copper-ATPase, respectively (Gómez-Santos \textit{et al.}, 2011). CorE binds Cu$^{2+}$ and Cu$^{+}$ ions by its cysteine-rich C-terminal extension, which is lacking in other sigma factors. CorE–Cu$^{2+}$ is the active form, which binds and activates its target promoters, while CorE–Cu$^{+}$ does not bind to DNA. At least four cysteine residues in the C-terminal domain are involved in copper coordination as shown by site-directed mutagenesis. The cysteines at positions 184 and 189 are most important for binding of Cu$^{+}$ and Cu$^{2+}$, respectively.

CorE-like ECF sigma factors have been predicted for only 21 other species, including some non-proteobacterial strains (Gómez-Santos \textit{et al.}, 2011). However, 10 of these sigma factors lack Cys$^{189}$, which is essential for CorE activity, putting their function as copper-responsive regulators into question. On the other hand, Cys$^{181}$, which is dispensable for CorE function, is conserved in all 21 candidates.

\textbf{Class 6: BxmR, a SmtB/ArsR-like regulator in cyanobacteria}

The filamentous cyanobacterium \textit{Oscillatoria brevis} synthesizes the SmtB/ArsR-like regulator BxmR, which represses transcription of the \textit{bxa}1 and \textit{bmtA} genes encoding a copper-ATPase and a cysteine-rich metallothionein, respectively, at low ambient copper concentrations (Liu \textit{et al.}, 2004, 2008). In its copper-free form, BxmR binds to conserved inverted repeat sequences (TGAA-N$_6$-TTCA) in the \textit{bxa}1 and \textit{bmtA} promoters \textit{in vitro}. Upon Cu$^{+}$ addition, these BxmR–promoter complexes dissociate.

In its dimeric form, BxmR binds four Cu$^{+}$ ions (Liu \textit{et al.}, 2008). BxmR encompasses two distinct metal-binding sites, an N-terminal (z3N) Cu$^{+}$-binding site and a C-terminal (z5C) Zn$^{2+}$–binding site. The z3N and z5C sites are characterized by Cys-X$_2$-Cys-X$_4$+3-Cys-X-Cys and Asp-X-His-X$_{10}$-His-X$_2$-Glu motifs, respectively.

SmtB/ArsR-type regulators from different cyanobacteria have been shown to coordinate different metal ions (Busenlehner \textit{et al.}, 2003; Liu \textit{et al.}, 2008). With the exception of BxmR, however, no other member of this repressor family has yet been shown to respond to copper. Apparently, few mutations are sufficient to change the metal specificity of members of the SmtB/ArsR family, suggesting that copper sensing by BxmR is a very recent adaptation.

\textbf{Class 7: CopY-like repressors in Gram-positive bacteria}

\textit{Enterococcus (Ent.) hirae} CopY is the founding member of a copper-responsive repressor family belonging to the superfamily of HTH regulators (Odermatt & Solioz, 1995; Solioz \textit{et al.}, 2010) (Table 1). CopY-type regulators are widespread in Gram-positive bacteria, but apparently absent in Gram-negative species (Liu \textit{et al.}, 2007) (Table 2). In addition to \textit{Ent. hirae}, a role of CopY repressors in copper homeostasis has been demonstrated for \textit{Enterococcus (Ent.) faecalis}, \textit{Lactococcus (Lac.) lactis}, \textit{Streptococcus mutans}, and \textit{Streptococcus pneumoniae} (Table 2).

\textit{Ent. hirae} The intestinal bacterium \textit{Ent. hirae} (formerly \textit{Streptococcus faecalis}) harbours the \textit{copYZAB} operon encoding the repressor CopY, the copper chaperone CopZ, and two copper-ATPases, CopA and CopB (Odermatt & Solioz, 1995) (Fig. 2). Like \textit{E. coli} CopA, \textit{Ent. hirae} CopB effectively extrudes excess Cu$^{+}$ ions from the cytoplasm. Originally, \textit{Ent. hirae} CopA was discussed as importing Cu$^{+}$ ions under copper-limiting conditions, because \textit{copA} mutants grow poorly upon copper
starvation (Solioz & Stoyanov, 2003). As shown recently, CopA belongs to the FixI subclass of copper-ATPases, and thus most likely acts as a low-activity exporter providing copper for extracytoplasmic enzymes (Argu¨ello et al., 2011; González-Guerrero et al., 2010; Lübben et al., 2009). The copper chaperone CopZ delivers Cu\(^{+}\) to the repressor CopY by direct interaction (Cobine et al., 1999). In addition, CopZ specifically interacts with CopA (Multhaup et al., 2001), although copper transfer from CopZ to CopA has not been demonstrated yet.

CopY encompasses an N-terminal DNA-binding domain and a C-terminal metal-binding domain with a Cys-X-Cys-X\(_4\)-Cys-X-Cys motif (Solioz & Stoyanov, 2003; Strausak & Solioz, 1997). CopY coordinates one Zn\(^{2+}\) ion per monomer at low copper concentrations, which is replaced by two Cu\(^{+}\) ions at elevated copper concentrations. In its zinc-loaded dimeric form, CopY represses transcription of the \(\text{copYZAB}\) operon by binding to a dyad-symmetrical sequence (TACA-N\(_2\)-TGTA) in the target promoter (Portmann et al., 2006). In its copper-loaded form, CopY is released from the promoter, allowing \(\text{copYZAB}\) transcription to proceed.

**Ent. faecalis**

*Ent. faecalis* is a very close relative of *Ent. hirae*. The genome-wide transcriptional response to copper has been determined for *Ent. faecalis* wild-type and *copY* mutant strains (Reyes-Jara et al., 2010). Several hundred genes are differentially expressed upon copper exposure in both strains. Remarkably, expression patterns of wild-type and *copY* mutant strains are largely similar, suggesting that relatively few genes belong to the CopY regulon. The main target of CopY with respect to copper homeostasis is the *cop* operon. Additional transcription factors responding directly or indirectly to copper may be involved in copper homeostasis. Indeed, transcription of Rrf2-, Cro/CI- and SorC/DeoR-like regulatory genes is induced shortly after copper exposure, but experimental evidence for their roles in copper homeostasis is lacking to date.

**Lac. lactis**

The intestinal bacterium *Lac. lactis*, which is widely used for fermentation of dairy products, synthesizes the copper-responsive repressor CopR, which is structurally and functionally similar to *Ent. hirae* CopY (Magnani et al., 2008). The *copR* gene forms part of the *copRZA* operon encoding CopR, the copper chaperone CopZ, and the copper-ATPase CopA. Besides the *copRZA* operon, CopR represses transcription of five additional operons including the monocistronic *copB* operon encoding a second copper-ATPase, CopB. Surprisingly, a mutant lacking both copper-ATPases, CopA and CopB, is almost as copper tolerant as the wild-type. However, the *Lac. lactis* *copA* gene complements the copper-sensitive phenotype of an *E. coli* *copA* mutant, supporting the role of *Lac. lactis* CopA in copper export. All promoters of CopR-repressed operons contain a conserved palindromic sequence (TACA-N\(_2\)-TGTA) thought to act as a CopR-binding site. Addition of Cu\(^{+}\) and Ag\(^{+}\) ions releases CopR from the *copRZA* promoter, while Zn\(^{2+}\), Ni\(^{2+}\) and Cd\(^{2+}\) have no effect.

**Streptococcus mutans and Streptococcus pneumoniae**

In the oral bacterium *Streptococcus mutans*, known for its ability to cause caries, CopY controls copper-dependent transcription of the *copYZAB* operon, which confers copper tolerance on this bacterium (Vats & Lee, 2001). The *copYZAB* operon in another oral bacterium, *Streptococcus gordonii*, is involved in biofilm detachment but is dispensable for biofilm formation (Mittrakul et al., 2004).

The pathogenic bacterium *Streptococcus pneumoniae* contains the operon *copY-cupA-copA*, which encodes the repressor CopY, a protein of unknown function (CupA), and the copper-ATPase CopA (Shafeeq et al., 2011). Transcription of the *cop* operon is induced by copper and repressed by zinc, suggesting that the two metals compete for the same ligands in CopY. CopA is the major copper tolerance determinant in *Streptococcus pneumoniae*, while CupA is less important for copper defence. In addition to its role in copper homeostasis under free-living conditions, the *cop* operon is important for virulence (Shafeeq et al., 2011). Expression of the *cop* operon

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**Fig. 2.** Copper homeostasis in *Ent. hirae*. Cu\(^{+}\) ions are exported by the ATPases CopA and CopB, the latter ATPase being the more active transporter. The chaperone CopZ delivers Cu\(^{+}\) ions to CopY. For further details, see text.
is induced during respiratory infection of mice, and CopA is critical for survival in the nasopharynx.

Class 8: CsoR-like repressors are widespread in prokaryotes

*Mycobacterium* (*Myc.*) *tuberculosis* CsoR is the founding member of a widespread copper-responsive repressor family (Liu et al., 2007) (Table 1). In addition to *Myc. tuberculosis*, CsoR-type regulators have been shown to repress copper homeostasis genes in the Gram-positive bacteria *B. subtilis*, *Listeria* (*List.*-) *monocytogenes*, *Staphylococcus* (*Staph.*-) *aureus*, and *Streptomyces lividans* (Table 2). CsoR-type regulators have been predicted for many proteobacteria, cyanobacteria and deinococci (Liu et al., 2007). Experimental evidence for copper-responsive gene regulation by CsoR in proteobacteria and cyanobacteria is lacking to date. However, CsoR has been shown to control copper homeostasis in *Thermus thermophilus* belonging to the phylum *Deinococcus–Thermus*, which is only distantly related to proteobacteria and Gram-positive species (Sakamoto et al., 2010). Apparently, CsoR homologues are the primary one-component copper-responsive regulators in prokaryotes lacking CueR (Liu et al., 2007).

Crystal structures have been solved for CsoR from *Myc. tuberculosis*, *Streptomyces lividans* and *T. thermophilus* (Dwarakanath et al., 2012; Liu et al., 2007; Sakamoto et al., 2010). Mt–CsoR forms homodimers, while Sl–CsoR and Tt–CsoR form tetramers. The core structures of the three regulators are very similar. These regulators do not contain any known DNA-binding motif, but antiparallel four-helix bundles have been suggested to act as a DNA-binding fold. All three regulators bind two Cu\(^{+}\) ions per dimer. Each copper ion is coordinated by one residue of the first monomer and two residues of the second monomer. C–H–C motifs coordinate Cu\(^{+}\) in Mt–CsoR (Cys\(^{36}\), His\(^{61}\), Cys\(^{65}\)) and Sl–CsoR (Cys\(^{75}\), His\(^{100'}\), Cys\(^{104'}\)), while copper ion binding involves a C–H–H motif in Tt–CsoR (Cys\(^{41}\), His\(^{70'}\), and His\(^{66'}\)).

*Myc. tuberculosis*

The Gram-positive human-pathogenic bacterium *Myc. tuberculosis* harbours the *csoR-rv0968-ctpV* operon encoding the repressor CsoR, a conserved hypothetical protein (DUF1490), and the copper-ATPase CtpV (Liu et al., 2007). Transcription of this operon is induced by copper, consistent with the predicted role of CtpV in excretion of excess copper under free-living conditions. Additionally, expression of *ctpV* is induced during infection of mouse lungs, suggesting a role of the copper-ATPase in virulence (Ward et al., 2010).

At low ambient copper concentrations, copper-free CsoR represses transcription of the *csoR-rv0968-ctpV* operon by binding a palindromic sequence (GTAGCCCAACCC-N\(^{10}\)-GGGTTGGGATAC) overlapping the −35/−10 region (Liu et al., 2007). Upon Cu\(^{+}\) ion binding, CsoR no longer binds to DNA, leading to derepression of the *csoR-rv0968-ctpV* operon.

In addition to CsoR, *Myc. tuberculosis* synthesizes a second CsoR-like repressor, designated RicR (Festa et al., 2011). In contrast to CsoR, which controls a single operon (*csoR-rv0968-ctpV*), RicR regulates a five-locus regulon including the *ricR* gene itself. Other genes of this regulon encode the MymT protein, which may be involved in intracellular sequestration or transport of copper, and the membrane-associated proteins LpqS and Rv2963, thought to excrete excess copper. All five loci are preceded by the palindromic sequence TACCC-N\(^{5}\)-GGGTA, which most likely serves as a RicR-binding site under copper-limiting conditions. At elevated copper concentrations, RicR is released from its target promoters, and, in turn, transcription is derepressed. A *ricR* mutant constitutively expresses the entire RicR regulon, suggesting that CsoR does not recognize the RicR-binding sites, which differ in space from the CsoR-binding site (see above). Alternatively, the number of CsoR proteins per cell may be too low to saturate all RicR-binding sites. Remarkably, *mymT* and other genes of the RicR regulon are unique to pathogenic mycobacteria, suggesting that this regulon is relevant for virulence.

*B. subtilis*

In *B. subtilis*, the monocistronic *csoR* gene is localized directly upstream of the *copZA* operon, which encodes a copper chaperone and a copper-ATPase (Smaldone & Helmann, 2007). At low copper concentrations, CsoR represses *copZA* transcription by binding a palindromic sequence (TACCCTAC-N\(^{5}\)-GTATGGTA) overlapping the *copZ* promoter. At elevated copper concentrations, CsoR no longer binds the promoter and transcription is relieved. In a mutant lacking the CsoR repressor, the *copZA* operon is constitutively (copper-independently) transcribed.

*Lis. monocytogenes*

The human intracellular pathogen *Lis. monocytogenes* contains a *csoR-copAZ* operon encoding the repressor CsoR, the copper-ATPase CopA, and the copper chaperone CopZ (Corbett et al., 2011). Mutants defective for *copA* are copper-sensitive under free-living conditions, but are as virulent as the wild-type in orally infected mice. Unlike other pathogenic bacteria, *Lis. monocytogenes* may circumvent copper damage in phagosomes by rapid movement into the cytoplasm.

CsoR represses *csoR-copAZ* transcription under copper-limiting conditions, while transcription is derepressed at elevated copper concentrations (Corbett et al., 2011). Like other CsoR regulators, *Lis. monocytogenes* CsoR contains a conserved C–H–C motif (Cys\(^{32}\), His\(^{87}\), Cys\(^{71}\)) essential for Cu\(^{+}\) ion coordination. As mentioned above, *Ent. hirae* CopZ delivers Cu\(^{+}\) ions to the CopY repressor (Cobine et al., 1999). In contrast, *Lis. monocytogenes* CopZ is not involved in copper transfer to the CsoR repressor but
instead may serve as a cytoplasmic copper buffer. Only when CopZ is saturated is CsoR able to sense excess copper.

**Staph. aureus**

The opportunistic human pathogen *Staph. aureus* synthesizes two CsoR-like regulators designated CsoR and CstR (Grosseohme et al., 2011). In its copper-free form, CsoR represses transcription of the copper-ATPase gene *copA*. Upon copper binding, CsoR is released from the *copA* promoter and transcription is derepressed. Like other CsoR-type regulators, *Staph. aureus* CsoR coordinates one Cu⁺ ion per monomer by a conserved C-H-C motif (Cys⁴¹, His⁶⁶, Cys⁷⁰). Disruption of the *csoR* gene leads to constitutive *copA* expression, suggesting that CstR does not functionally substitute for CsoR.

In CstR, the residue corresponding to His⁶⁶ of CsoR is replaced by asparagine, and consequently CstR does not act as a copper sensor (Grosseohme et al., 2011). Unlike CsoR, CstR forms disulfide cross-linked dimers upon anaerobic incubation with sulfite. CstR represses transcription of the divergent *cstR-tauE* and *cstA-cstB-sqr* operons, which presumably are required for sulfur assimilation from thiosulfate.

Many other bacteria have the capacity to synthesize two or more CsoR-like proteins (Liu et al., 2007). Like CstR, several of these putative regulators lack the histidine involved in copper binding, suggesting that these proteins control physiological processes other than copper homeostasis.

*Streptomyces lividans*

The filamentous soil bacterium *Streptomyces lividans* forms vegetative mycelia, aerial hyphae and spores. Interestingly, copper is crucial for morphological differentiation, but not vegetative growth (Keijser et al., 2000). Because of its genetic accessibility, *Streptomyces lividans* is widely used in biotechnology to produce secondary metabolites and secreted proteins.

Two tetramers of *Streptomyces lividans* CsoR associate with its target promoters upstream of the *csoR* and *copZA* genes, leading to repression of transcription (Dwarakanath et al., 2012). Apparently, both the chaperone CopZ and the regulator CsoR buffer cytoplasmic copper at low concentrations. Only at higher copper concentrations, is transcription of *csoR* and *copZA* derepressed. Remarkably, transcription of more than 400 genes is significantly enhanced in a *csoR* deletion strain as compared with the wild-type. In contrast to *csoR* and *copZA* promoters, however, their promoters lack putative CsoR-binding sequences, suggesting that these genes are not direct targets of CsoR repression. Instead, a *csoR* deletion may mimic copper overload.

*T. thermophilus*

The thermophilic bacterium *T. thermophilus* belongs to the phylum *Deinococcus–Thermus*, and thus, is only distantly related to all the other bacteria mentioned above. *T. thermophilus* harbours the *copZ-csoR-copA* operon, which is repressed by CsoR under copper-limiting conditions (Sakamoto et al., 2010). In *vivo*, *T. thermophilus* CsoR is very promiscuous and binds various metal ions, including Cu⁺, Cu²⁺, Zn²⁺, Cd²⁺, Ag⁺ and Ni²⁺, all of which release CsoR from the *copZ* promoter. In *vivo*, copper and zinc ions significantly increase *copZ-csoR-copA* expression, suggesting that the response of CsoR to various metal ions is physiologically relevant. Besides the *copZ* promoter, there are no other CsoR-binding sites within the entire *T. thermophilus* genome. It remains to be elucidated whether CopA, in addition to copper, extrudes other metal ions.

**Class 9: YcnK, a DeoR-like repressor in *B. subtilis***

Besides CsoR (see above), *B. subtilis* synthesizes the copper-responsive repressor YcnK, which belongs to the DeoR family of transcription regulators (Chillappagari et al., 2009). YcnK consists of an N-terminal DNA-binding domain with an HTH motif and a C-terminal copper-sensing domain similar to NosL. In contrast to all the other copper-responsive repressors described above, which repress their target genes under copper-limiting conditions, YcnK represses its target gene, *ycnJ*, under conditions of copper excess.

The *ycnJ* gene encodes a putative copper importer encompassing two domains similar to CopC and CopD proteins of *P. syringae* (Chillappagari et al., 2009). Deletion of *ycnJ* leads to reduced cellular copper contents and growth defects under copper-limiting conditions. Repression of the *ycnJ* gene by copper-loaded YcnK limits synthesis of the importer to copper-limiting conditions. In addition to YcnK, CsoR is involved in *ycnJ* regulation, as maximum *ycnJ* expression is only observed in strains lacking both repressors.

**Post-transcriptional copper-responsive gene regulation in Rhodobacter capsulatus**

All copper-responsive regulatory mechanisms described above control copper homeostasis at the level of transcription initiation. A post-transcriptional copper control mechanism has recently been described for the photosynthetic alphaproteobacterium *R. capsulatus* (Rademacher et al., 2012) (Fig. 3). Upon copper exposure, *R. capsulatus* induces expression of the MCO CutO, conferring copper tolerance (Wiethaus et al., 2006). The cutO gene (RCAP_rcc02110) forms part of the tricistronic orf635-cutO-cutR operon. Transcription of this operon is strictly dependent on a copper-independent (constitutive) promoter upstream of orf635 (Rademacher et al., 2012). While orf635 expression is not affected by the cellular copper status, cutO and cutR are expressed only at elevated copper concentrations. Differential expression is based on the orf635–cutO intergenic mRNA, which forms a stem–loop structure. Site-specific mutations destabilizing this structure abolish copper regulation, most likely by preventing
mRNA degradation at low copper concentrations. Remarkably, compensatory mutations restoring stem–loop formation also restore copper-responsive regulation, suggesting that mRNA stability depends on structure formation rather than sequence conservation.

Copper regulation is not transferable to *E. coli*, as shown by a reporter fusion to the *orf635-cuto-cutR* (RCAP_rcc02111-RCAP_rcc02110-RCAP_rcc02109) operon strictly dependent on a constitutive promoter (P<sub>const</sub>). In the wild-type, the *orf635-cuto-cutR* mRNA is specifically degraded at low copper concentrations by a mechanism based on a stem–loop structure formed by the *orf635-cuto* intergenic mRNA. Mutations destabilizing this structure abolish mRNA degradation under copper-limiting conditions. Compensatory mutations, which stabilize the stem–loop structure, restore copper control of mRNA stability.

**Post-translational regulation by copper-responsive proteolysis in *Ent. hirae***

Growth of *Ent. hirae* at ambient copper concentrations up to 5 mM depends on enhanced production of the copper-ATPase CopB, which is achieved by copper induction of *copYZAB* transcription (Lu & Solioz, 2001; Odermatt et al., 1994). The steady-state levels of the copper chaperone CopZ rise with increasing copper concentrations up to 0.5 mM, but clearly decrease above 0.5 mM to hardly detectable at 5 mM (Lu & Solioz, 2001). The lability of CopZ at high copper concentrations is due to proteolytic degradation, as shown with crude cell extracts (Lu & Solioz, 2001; Solioz, 2002) (Fig. 2). Copper-induced CopZ degradation is mediated by a serine protease, as implicated by preincubation of extracts with different protease inhibitors. Copper-loaded CopZ protein is more rapidly degraded than copper-free CopZ.

Copper-responsive proteolysis of CopZ is likely to affect CopY activity, as CopZ delivers Cu<sup>2+</sup> ions to the repressor CopY, which in its copper-loaded form is released from the *copYZAB* promoter (Cobine et al., 1999, 2002). Thus, CopZ directly downregulates the activity of the repressor. As mentioned above, CopZ plays a different role in *Lis. monocytogenes* as it limits the access of copper to the CsoR regulator (Corbett et al., 2011). Interestingly, the affinity of *Ent. hirae* CopY for DNA is also reduced at high copper concentrations, when CopZ levels are low, suggesting that CopY receives copper by another as-yet-unknown mechanism.

Three observations suggest that copper-induced proteolysis is not limited to *Ent. hirae* but also occurs in other bacteria. Firstly, CopZ-like proteins as likely protease targets are widespread in bacteria. Secondly, CopZ-like domains form part of many copper-ATPases, suggesting that these ATPases might also be regulated by targeted proteolysis. Such regulation might be especially important when ambient copper concentrations decrease. Thirdly, *E. coli* induces expression of two proteases, DegP and HtpX, upon copper exposure (Yamamoto & Ishihama, 2006). It remains to be elucidated whether these proteases are specifically involved in degradation of copper homeostasis proteins.

**Conclusions and future perspectives**

Both Gram-negative and Gram-positive bacteria utilize copper-ATPases as principal defence determinants to excrete excess copper from the cytoplasm. Upon copper addition, all bacteria examined so far induce ATPase expression, but different species utilize structurally and functionally different regulators to control ATPase gene transcription (Table 2). As a general rule, Gram-negative bacteria activate ATPase gene transcription with increasing copper concentrations, while Gram-positive bacteria repress transcription under copper-limiting conditions.

Most Gram-negative species activate ATPase expression by CueR-like one-component regulators or by CusRS-like two-component systems, while Gram-positive bacteria repress ATPase gene transcription by CopY- and CsoR-like regulators. CsoR homologues have been proposed to be the primary copper-responsive regulators in prokaryotes lacking CueR and CopY homologues (Liu et al., 2007). Indeed, a CsoR homologue controls ATPase expression in *T. thermophilus*, which is only distantly related to Gram-positive species and
proteobacteria (Sakamoto et al., 2010). Future studies are required to clarify the roles of predicted CsoR homologues in proteobacteria.

Many Gram-negative bacteria synthesize MCOs as additional copper defence determinants (Table 2). In these species, MCO expression is activated by either CueR or CusRS homologues. Although MCOs function in the periplasm, there is no apparent preference for CusRS systems, which sense periplasmic copper concentrations, over CueR sensors, which respond to the cytoplasmic copper status. It is worth noting that the copper-inducible MCO in the Gram-positive bacterium C. glutamicum contains a cysteine residue, thought to serve as a lipid anchor, immediately downstream of the signal peptide cleavage site (Palmer & Berks, 2012; Schelder et al., 2011).

Besides copper-ATPases and MCOs, many Gram-negative bacteria synthesize RND-type multicomponent copper efflux systems, which span both the cytoplasmic and the outer membrane (Table 2). Primarily, these RND systems secrete copper from the periplasm, and may be less important for copper export from the cytoplasm (Rensing & Grass, 2003). Apparently, expression of RND systems is exclusively controlled by two-component systems (Table 2). One might thus speculate that copper excretion from the periplasm has to be coupled to direct sensing of periplasmic copper concentrations by CusS homologues. As one would expect for bacteria lacking an outer membrane, copper-induced RND systems are absent in Gram-positive species.

For each of the four major classes of copper-responsive regulators (represented by CueR, CusRS, CopY and CsoR), several members from different species have been characterized (Table 2). By contrast, the remaining five classes are based on single examples only. ComR and YcnK control copper import in E. coli and B. subtilis, respectively, while copper defence systems (efflux ATPases, MCOs and RND systems) are regulated by the major copper sensors in these species. ComR and YcnK belong to the widespread TetR and DeoR families, respectively, suggesting that copper import in other bacteria may also be under the regime of as-yet-unrecognized ComR or YcnK homologues. Multiple copper-responsive regulators in the same species may be advantageous to fine-tune copper homeostasis in bacteria.

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References


