**Supplementary Table S1. Primers used.**

| name | sequence | comment |
|---|---|---|
| KO CorAII 1 AgeI 57° | AAAACCGGTGGTTTGCCCGCCTTCTCTTTA | knockout primer Cre-Lox CmcorA2 |
| KO CorAII 2 ApaI 58° | AAAGGGCCCCGGCCGTCCGACATTTGTT | knockout primer Cre-Lox CmcorA2 |
| KO CorAII 3 NcoI 59° | AAACCATGGCGGTTTCGTCGCCATGATCTC | knockout primer Cre-Lox CmcorA2 |
| KO CorAII 4 MunI 60° | AAACAATTGTCCATTCCATTACTGCCCCCTG | knockout primer Cre-Lox CmcorA2 |
| KO CorAIII 1 AgeI 60° | AAAACCGGTAGCAATCTGGGCAAGGGCAAC | knockout primer Cre-Lox CmcorA3 |
| KO CorAIII 2 ApaI 61° | AAAGGGCCCGCTCAGGCCTGCAGCAGGC | knockout primer Cre-Lox CmcorA3 |
| KO CorAIII 3 NcoI 60° | AAACCATGGATCGCTAGGACGCGGACCG | knockout primer Cre-Lox CmcorA3 |
| KO CorAIII 4 MunI 60° | AAACAATTGGCCGCTTTCCCATCTGAGTCTG | knockout primer Cre-Lox CmcorA3 |
| KO pitA AgeI 60°C | AAAACCGGTTGGAGGATGTTGCCGAGACGA | knockout primer Cre-Lox CmpitA |
| KO pitA ApaI 61,5 °C | AAAGGGCCCGCGCCTCAGGCGTTTTCCAG | knockout primer Cre-Lox CmpitA |
| KO pitA NotI 61 °C | AAAGCGGCCGCGGCAAGCGCGAGCGGAAT | knockout primer Cre-Lox CmpitA |
| KO pitA MunI 60°C | AAACAATTGGGCAATGTCCCTCGTCGGC | knockout primer Cre-Lox CmpitA |
| KO zntB1 MunI 61° | AAACAATTGGCGAACTGCGCAATGTGCTG | knockout primer Cre-Lox CmzntB |
| KO zntB2 NotI 61° | AAAGCGGCCGCACGTTAGCATGGCGCCGTGA | knockout primer Cre-Lox CmzntB |
| KO zntB3 ApaI 58° | AAAGGGCCCTACAACGACGAGGCGCTGAT | knockout primer Cre-Lox CmzntB |
| KO zntB4 AgeI 56° | AAAACCGGTTGATCCGCTTGATCACACCA | knockout primer Cre-Lox CmzntB |
| KO ZupT 1 NcoI | AAACCATGGCGGTGCTGGGTCTGTATGGCG | knockout primer Cre-Lox CmzupT |
| KO ZupT 2 NotI | AAAGCGGCCGCGCACCCCGACTGAAAAGCTCA | knockout primer Cre-Lox CmzupT |
| KO ZupT 1 AgeI | AAAACCGGTTCGAACTGATGTCGCTGGCGG | knockout primer Cre-Lox CmzupT |
| KO ZupT 1 SacI | AAAGAGCTCGTGAAGACCGTGGATGCCGAC | knockout primer Cre-Lox CmzupT |
| CorA1 Dis1 PstI 57°C | AAACTGCAGTCCACGATGAAGACCT | disruption primer pLO2-LacZ corA1 |
| CorA1 Dis2 XbaI 59°C | AAATCTAGAGCTTCGTCCTGCTGTT | disruption primer pLO2-LacZ corA1 |
| zupTRm 3´ Xba | AAATCTAGAAGTCGCCACCGGCAGCTAAGC | LacZ primer zupT |
| zupTRm 5´ PstI | AAACTGCAGCCGCACACGAAATCCCGCAGG | LacZ primer zupT |
| pitARm 3´ Xba | AAATCTAGACCTGGCTTGTCATTGGGATTC | LacZ primer pitA |
| pitARm 5´ PstI | AAACTGCAGGTTCGGTTGGCGGATTGT | LacZ primer pitA |
| JS RmcorA-Xba | AAATCTAGAGGGGACCAGGAAGGCTATCAG | LacZ primer corA1 |
| JS RmcorA-Pst | AAACTGCAGCGAACAGCAGGACGAAGCG | LacZ primer corA1 |
| JS corAIIXbaI | AAATCTAGACGAAACCGTCAGATCCAGCCC | LacZ primer corA2 |
| JS corAII 5´SphI | AAAGCATGCCCTGCGCAACGTGGTCTATCC | LacZ primer corA2 |

| | | |
|---|---|---|
| JS corAIII 3´XbaI | AAATCTAGAGTCCGCGTCCTAGCGATCCAG | LacZ primer corA3 |
| JS corAIII 5´PstI | AAACTGCAGCGCCGAAGATGCCGAAGGTAT | LacZ primer corA3 |
| JS zntB 3´ XbaI | AAATCTAGATCGACTGCCAGCCTACTCGGC | LacZ primer zntB |
| JS zntB 5´SphI | AAAGCATGCTCCGTCTGCTGAACCGTCCGC | LacZ primer zntB |

**Suppl. Table S2. Comparison of the metal content of *E. coli* and *C. metallidurans* cells after incubation in the presence of one added metal only[a]**

| Concentration, (μM) | Co | Ni | Cu | Zn | Cd | Effect on other metals |
|---|---|---|---|---|---|---|
| *E. coli* W3110* | | | | | | |
| 0[b] | 1.13±,47 | 0.90±0.88 | 2.79±1.67 | 1.25±0.34 | 0.87±0.83 | |
| 10 | 17.8±10.9 | 4.28±0.53 | 2.75±0.86 | 2.15±0.20 | 73.1±43.0 | |
| 30 | 33.7±0.0 | 7.29±3.05 | 6.63±2.11 | 4.98±0.59 | 427±163* | Cd: 4-fold K, 3-fold Ca, 10-fold Mn |
| 100 | 554±123* | 52.5±32.0 | 19.86±11.5 | 11.9±2.2 | n.g. | Co: 4-fold Na, 3-fold Mg, 7-fold K, 7-fold Ca, 13-foldMn |
| *C. metallidurans* CH34 (0 μM: set 1 for each metal cation) | | | | | | |
| 10 | 7.65±2.28* | 4.38±1.22 | 5.43±4.21 | 1.47±0.21 | 9.25±0.31 | Co: 3-fold Zn |
| 30 | 14.8±1.2 | 9.67±3.63 | 6.47±4.58 | 1.94±0.39 | 31.1±1.5 | |
| 100 | 44.2±4.2 | 31.4±8.7 | 11.1±6.2 | 47.5±60.7 | 142±76 | |
| 300 | 139±22 | 109±41 | 36.2±20.5 | 193±262* | 383±347 | Zn: 5-fold Mn |
| 1000 | 412±202* | 312±93 | 115±11 | 1516±800* | n.g. | Co: 3-fold Cu; Zn: 17-fold Ca, 4-fold Mn, 3-fold Co |
| Strain AE104 | | | | | | |
| 0[b] | 1.89±1.16 | 0.48±0.09 | 0.59±0.23 | 0.95±0.06 | 0.84±0.34 | |
| 10 | 17.8±5.0 | 6.92±0.79* | 7.44±5.81* | 2.09±0.36 | 15.4±3.8 | Ni: 3-fold Co; Cu: 3-fold Co |
| 30 | 30.3±2.6 | 20.2±4.0 | 9.42±10.15* | 7.19±4.54* | 38.1±4.6 | Cu: 4-fold Co; Zn: 3-fold Co |
| 100 | 91.2±25.1* | 56.6±11.2* | 16.6±16.7* | 8.31±2.02 | n.g. | Co: 8-fold Cu; Ni: 3-fold Co; Cu: 4-fold Co |

[a]Cells were incubated after 10-fold dilution for 18 h with shaking at 30°C in TMM, additionally containing between 10 μM and 1 mM of the metal indicated (as divalent metal cation chlorides). The cfu was determined, and the metal content by ICP-MS. Listed is the content of the metal that was added in atoms per cell, for better comparison divided by the content of *C. metallidurans* CH34 cells grown in TMM without added metal (Table 2) with deviations shown. An effect on the content of a metal that was not added is indicated if the alteration was more the 2-fold and significant. In this case, a star in the respective cell indicates that another metal was being influenced and the effect was specified in the last row. Shaded cells indicate a linear dependence of the cellular metal content on the concentration of the metal added to the growth medium, boxes accelerated accumulation. [b]As in Table 2, n.g., no growth.

**Suppl. Table S3. Effect of multiple metal incubation on *C. metallidurans* strain CH34 wild type[a]**

| Concentration, ($\mu$M) | Co | Ni | Cu | Zn | Cd | Effect on other metals |
|---|---|---|---|---|---|---|
| 10 | 32±2% (30%) | 33±2% (28%) | 20±1% (78%) | 54±2% (21%) | 54±2% (3%) | |
| 30 | 27±0% (8%) | 30±0% (38%) | 44±1% (71%) | 101±0% (29%) | 29±0% (5%) | |
| 100 | 631±51% (10%) | 227±18% (28%) | 301±17% (55%) | 202±17% (128%) | 396±34% (53%) | 2-fold Ca, 4-fold Mn |
| 300 | | | n.g. | | | |

[a]Cells were incubated after 10-fold dilution for 18 h with shaking at 30°C in TMM, additionally containing the indicated concentration of all metals in a ratio Co:Ni:Cu:Zn:Cd = 1:1:1:1:1. The cfu was determined, and the metal content by ICP-MS. Listed is the content of a metal in atoms per cell, divided by the metal content of CH34 cells cultivated in TMM with only this particular metal added at the same concentration (Suppl. Table 2). The number in parenthesizes is the deviation of the denominator used (content of a metal in a single metal situation). An effect on the content of a metal that was not added is indicated if the alteration was more than 2-fold and significant. n.g., no growth

**Suppl. Table S4. Minimal inhibitory concentrations of various *C. metallidurans* mutant strains[a].**

| Mutant strain | $Zn^{2+}$, ($\mu$M) | $Co^{2+}$, ($\mu$M) | $Ni^{2+}$, ($\mu$M) | $Cd^{2+}$, ($\mu$M) | $Cu^{2+}$, (mM) | EDTA, ($\mu$M) |
|---|---|---|---|---|---|---|
| AE104 | 127±43 | 500±100 | 360±83 | 150±39 | 1.5±0.2 | 1513±234 |
| Δ*zupT* | 133±58 | 600±0 | 350±50 | 160±7 | 1.4±0.1 | 275±50 |
| Δ*pitA* | 120±35 | 400±0 | 380±84 | 140±35 | 1.3±0.1 | 1375±144 |
| Δ*corA*$_1$*::Kan* | 113±42 | 400±0 | 480±150 | 100±56 | 1.3±0 | 388±246 |
| Δ*corA*$_2$ | 120±35 | 400±0 | 340±55 | 150±34 | 1.5±0 | 1375±144 |
| Δ*corA*$_3$ | 113±42 | 400±0 | 320±84 | 150±34 | 1.5±0.1 | 1375±144 |
| Δ*zupT* Δ*pitA* | 130±14 | 253±271 | 300±82 | 140±20 | 1.4±0.1 | 450±100 |

[a] Strains were grown for 48h in TMM, diluted 1:100 and streaked on TMM agar plates with increasing metal concentrations. Growth was monitored after 5 days at 30°C. Results were confirmed by at least three independent experiments.

**Suppl. Table S5. Minimal inhibitory concentrations of various *C. metallidurans* mutant strains in metal-EDTA complexes[a].**

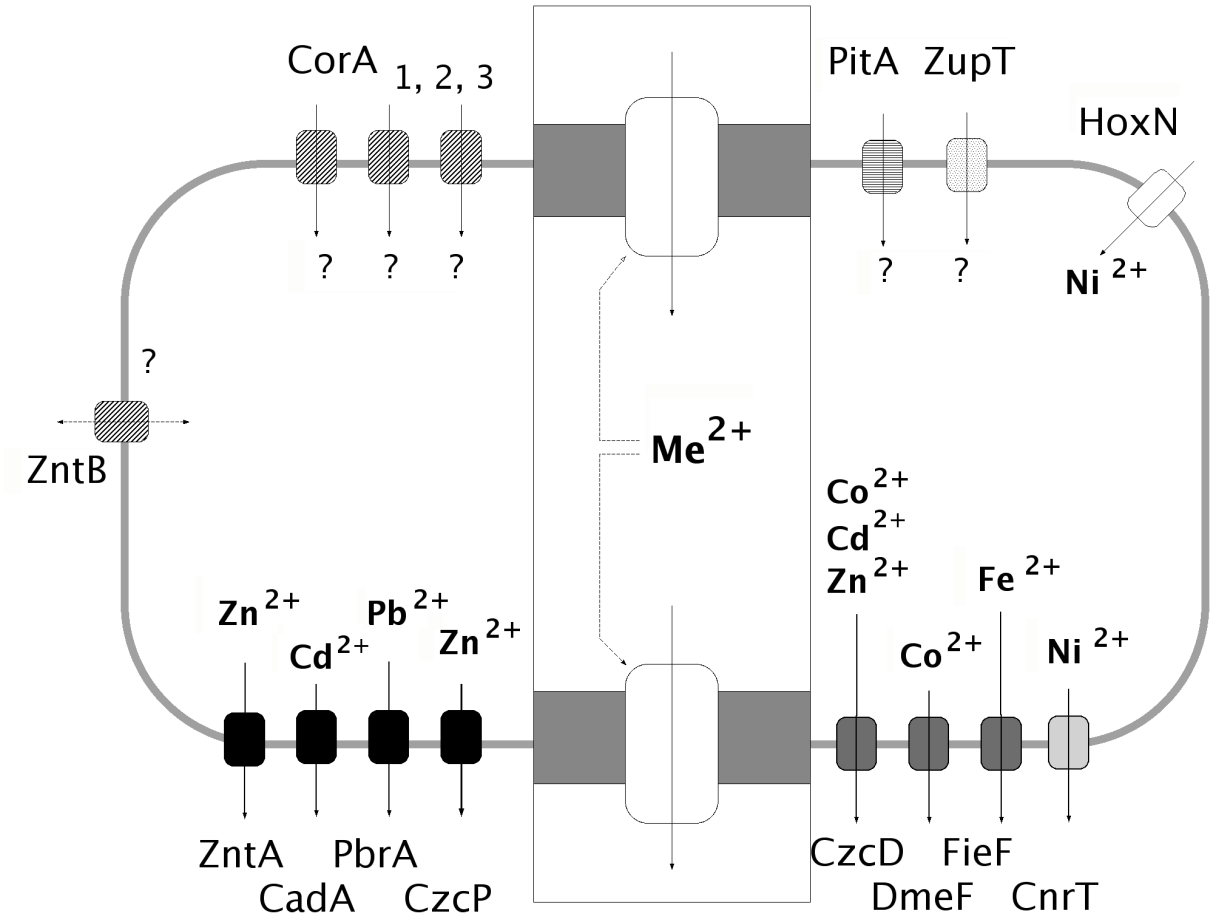
| Mutant strain | MIC of an EDTA:metal cation complex in a 2:1 ratio, (mM EDTA) | | | | | | |
|---|---|---|---|---|---|---|---|
| | $Mg^{2+}$ | $Zn^{2+}$ | $Cu^{2+}$ | $Ni^{2+}$ | $Cd^{2+}$ | $Co^{2+}$ | none[b] |
| AE104 | 1.5±0.2 | 1.7±0.2 | 1.8±0.2 | 1.7±0.1 | 1.8±0.2 | 1.7±0.2 | 1.5±0.2 |
| Δ*zupT* | 0.6±0.1 | 1.6±0.2 | 0.9±0.4 | 0.7±0 | 0.9±0.4 | 1.5±0 | 0.3±0 |
| Δ*pitA* | 1.5±0 | 1.6±0.2 | 1.8±0.2 | 1.7±0.1 | 1.8±0.3 | 1.5±0 | 1.4±0.1 |
| Δ*corA*$_1$*::kan* | 1.0±0.3 | 1.6±0.2 | 1.8±0.2 | 1.7±0.1 | 1.7±0 | 2.0±0 | 0.4±0.2 |
| Δ*zupT* Δ*pitA* | 0.6±0.1 | 2.7±0 | 0.7±0.5 | 0.7±0 | 0.5±0 | 0.7±0 | 0.5±0.1 |

[a] Cells were incubated on solidified Tris-buffered mineral salts medium containing SL6 and 2 g/l sodium gluconate in the presence of the indicated concentration of EDTA:metal cation complexes in a 2:1 molar ratio (concentration of EDTA indicated) for 5 days at 30°C. At least three independent experiments, deviations indicated. [b]as in Table 3.

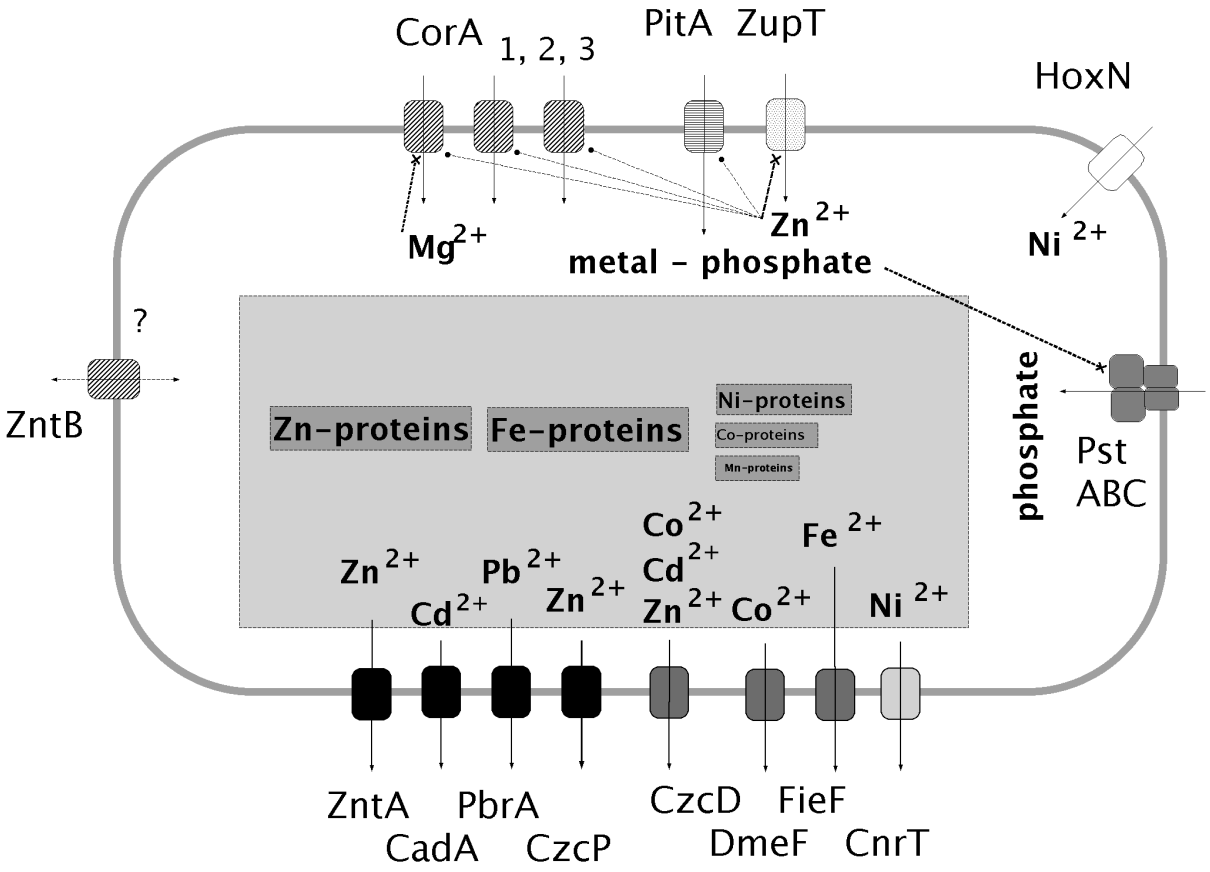
**Suppl. Table S6. Effect of multiple metal incubation on mutant strains of *C. metallidurans*[a]**

| Concentration, ($\mu$M) | Co | Ni | Cu | Zn | Cd | Effect on other metals |
|---|---|---|---|---|---|---|
| Strain AE104 | | | | | | |
| 10 | 3.18±0.03 | 2.94±0.02 | 0.89±0.04 | 1.44±0.00 | 1.06±0.02 | |
| 30 | 6.20±0.17 | 7.02±0.04 | 3.53±0.03 | 3.10±0.07 | 2.72±0.19 | 3-fold Na |
| 100 | | | n.g. | | | |
| Strain AE104 Δz*upT* | | | | | | |
| 10 | 3.87±0.18 | 5.12±0.19 | 1.84±0.05 | 1.69±0.06 | 1.10±0.05 | 4-fold Na |
| 30 | | | n.g. | | | |
| Strain AE104 Δ*zupT* Δ*pitA* | | | | | | |
| 10 | 5.63±0.21 | 6.77±0.33 | 2.15±0.23 | 2.00±0.05 | 1.36±0.04 | 5-fold Na, 3-fold Mg |
| 30 | | | n.g. | | | |

[a]Cells were incubated after 10-fold dilution for 18 h with shaking at 30°C in TMM, additionally containing the indicated concentration of all metals in a ratio Co:Ni:Cu:Zn:Cd = 1:1:1:1:1. The cfu was determined, and the metal content by ICP-MS. Listed is the content of a metal in atoms per cell, divided by the metal content of *C. metallidurans* CH34 wild type cells cultivated under the same multi metal conditions (Suppl. Table 2). An effect on the content of a metal that was not added is indicated if the alteration was more the 2-fold and significant. n.g., no growth




**Suppl. Figure S1. Metal homeostasis in *C. metallidurans*.** Neglecting binding processes, metal homeostasis in bacteria can be described as a kinetical flow equilibrium of uptake and efflux processes with the cytoplasmic metal concentration possibly controlling synthesis and activity of the uptake and efflux proteins (inlay). The efflux systems in *C. metallidurans* have been extensively studied. Although they transport chemically related metal cations, the four P-type ATPases (black, bottom) ZntA, CadA, PbrA and CzcP could be mainly assigned to export of zinc, cadmium, lead and zinc, respectively. Likewise, the CDF proteins (dark grey, bottom) CzcD, DmeF and FieF export cobalt/zinc/cadmium, cobalt, and ferrous iron, and CnrT (light grey) nickel. While ZntA, CadA, PbrA, DmeF and FieF maintain a kind of basic resistance, CzcP, CzcD and CnrT enhance export of their substrates across the inner membrane for further export across the outer membrane by CzcCBA and CnrCBA (not shown). In contrast, knowledge of the import systems is meager. Substrates of three CorA importers, of PitA and ZupT are a matter of speculation. HoxN is encoded as part of the hydrogenase biosynthesis cluster and may import nickel cations as cofactors of these enzymes. It is unknown if ZntB is an importer or exporter. Nevertheless, importer and exporter may line up in a kind of shunt to maintain cellular homeostasis of a single metal cation independently from the others by regulated uptake and efflux (inlay). Alternatively, the cells take up more or less what they can get and sort their cytoplasmic metal bouquet later by regulated efflux ("worry later"). Phosphate, magnesium, calcium, copper and iron siderophore import are not shown in this overview (19, 38, 52, 58, 59).




**Suppl. Figure S2. No metal homeostasis shunts but "worry later".** The efflux systems are shown as in Suppl. Fig. 1, again without CzcCBA and CnrCBA. All five uptake systems shown on top were down-regulated by zinc, *zupT* was strongly up-regulated by zinc and *corA*$_1$ by magnesium starvation (dashed lines). In the presence of phosphate, divalent metals should be mainly present as cation-phosphate complexes, which are taken up by PitA. ZupT is able to extract and import zinc bound to complexes such as phosphate or EDTA. As shown by the effect of a Δ*pitA* deletion, phosphate accumulation of the cells was enhanced, probably due to the expression of a PstABC uptake system (dashed line). Otherwise, none of the five systems studied was essential for uptake of a single metal. They may overlap in their substrate specificity and be able to substitute for each other. The efflux pumps keep these values in the correct range.