Metal-responsive gene regulation and metal transport in *Helicobacter* species

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Abstract *Helicobacter* species are among the most successful colonizers of the mammalian gastrointestinal and hepatobiliary tract. Colonization is usually lifelong, indicating that *Helicobacter* species have evolved intricate mechanisms of dealing with stresses encountered during colonization of host tissues, like restriction of essential metal ions. The recent availability of genome sequences of the human gastric pathogen *Helicobacter pylori*, the murine enterohepatic pathogen *Helicobacter hepaticus* and the unannotated genome sequence of the ferret gastric pathogen *Helicobacter mustelae* has allowed for comparitive genome analyses. In this review we present such analyses for metal transporters, metal-storage and metal-responsive regulators in these three *Helicobacter* species, and discuss possible contributions of the differences in metal metabolism in adaptation to the gastric or enterohepatic niches occupied by *Helicobacter* species.

Keywords *Helicobacter* · Microbial pathogenesis · Gastrointestinal and hepatobiliary tract · Metal-responsive regulation · Metal transport

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Introduction

Metals play an important role in the metabolism of all organisms, and this is reflected by the wide variety of chemical reactions in which they are involved. Metals can be cofactors of enzymes, catalyzing basic functions like electron transport, redox reactions and energy metabolism, and are essential for maintaining the osmotic pressure of the cell (van Vliet et al. 2001). Since both metal limitation and metal overload delay growth and can cause cell death, metal homeostasis is of critical importance to all living organisms. In bacteria, metal homeostasis is achieved by balancing import, efflux, metabolism and storage (Fig. 1).

The genus *Helicobacter* belongs to the epsilon subdivision of the proteobacteria, order Campylobacterales, family of the *Helicobacteraceae*. The best known related species is *Campylobacter jejuni* (Eppinger et al. 2004). Members of the genus *Helicobacter* are all microaerophilic organisms, and in most cases catalase- and oxidase-positive, and many are also urease-positive. *Helicobacter* species colonize the mucosal surfaces of the gastrointestinal and hepatobiliary tract of mammals, including humans. These mucosal surfaces provide a challenging environment with continuous changes in environmental conditions, including the availability of metal ions (van Vliet et al. 2001).
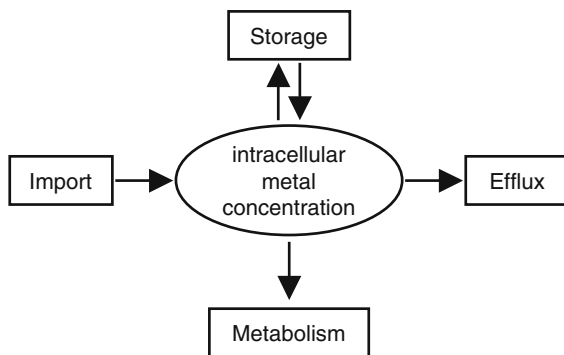
Fig. 1 Schematic representation of the mechanisms involved in maintaining homeostasic control of the intracellular metal concentration via concerted action of import, efflux, storage and metabolism

Colonization is usually lifelong, and often leads to the development of a wide range of inflammatory diseases (Blaser and Atherton 2004). Infection with *Helicobacter* species induces a strong proinflammatory response which includes the production of reactive oxygen species by the host immune system, and this necessitates the expression of systems detoxifying reactive oxygen species. Furthermore, the formation of reactive oxygen species is connected with iron metabolism, as oxygen radicals can be produced via the Fenton and Haber–Weiss reactions ($Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^-$ and $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet$). *Helicobacter* species are microaerophilic bacteria tolerating oxygen concentrations ranging from 2 to 10%. To combat oxidative stress, *Helicobacter* species expresses several key components of bacterial oxidative stress resistance, which are often metal-cofactored and controlled by metal-responsive regulatory systems (Baker et al. 2001; Seyler et al. 2001; Harris et al. 2002; Olczak et al. 2002; Wang et al. 2004; Ernst et al. 2005a; Hong et al. 2006). Many of the components of oxidative stress defense are essential for gastric colonization by *H. pylori* in animal models (Harris et al. 2003; Olczak et al. 2003; Barnard et al. 2004), and are likely to be of similar importance to colonization of other *Helicobacter* species.

Helicobacter species can be subdivided into two major lineages (Solnick and Schauer 2001;

Fox 2002). The first lineage consists of the gastric *Helicobacter* species, which colonize the mucus layer overlaying the gastric epithelium. The best known example of a gastric *Helicobacter* species is *Helicobacter pylori*, the causative agent of gastritis, peptic ulcer disease and gastric adenocarcinoma (Blaser and Atherton 2004). However, many mammals are infected with gastric *Helicobacter* species, and they have been isolated from a wide variety of hosts, including dolphins, big cats and ferrets (Solnick and Schauer 2001; Fox 2002). The second lineage consists of the enteric or enterohepatic *Helicobacter* species, which colonize the mammalian intestinal and hepatobiliary tract, and have so far mostly been identified in rodents. The best characterized enteric *Helicobacter* species is *Helicobacter hepaticus*, which infects the intestines, bile ducts and liver of mice, and can cause hepatitis, hepatocellular carcinoma and possibly induce the formation of gallstones (Solnick and Schauer 2001; Fox 2002; Maurer et al. 2005).

The chronicity of infection suggests that *Helicobacter* species are well adapted to the conditions in their specific niches, and are more than capable of handling changes in these conditions (Blaser and Atherton 2004). *Helicobacter* species have a relatively small genome of 1.5–2.0 Mbp. The complete genome sequences of *H. pylori* (Tomb et al. 1997; Alm et al. 1999) and *H. hepaticus* (Suerbaum et al. 2003) have been published previously, whereas the almost finished, but unannotated genome sequence of the ferret gastric pathogen *Helicobacter mustelae* is available online (at http://www.sanger.ac.uk/Projects/H_mustelae). Together with the availability of the complete genome sequence of *C. jejuni* (Parkhill et al. 2000) this has allowed for comparative genomics, and here we will review the current knowledge on metal metabolism and metal-responsive gene regulation of *H. pylori* and *H. hepaticus*, focussing on the role of the metals iron, nickel and copper. We will also present hypotheses on metal-transport and metal-responsive regulation in *H. mustelae*, based on comparisons with *H. pylori* and *H. hepaticus*.

Metal-transport and storage of *Helicobacter* species

The gastric and enteric mucosa are niches with continuous changes in environmental conditions. For instance, the pH in the gastric mucosa is thought to range between 4 and 6.5, but acid shocks occasionally occur. Bioavailability of metals like iron and nickel is linked to the environmental pH, and thus *Helicobacter* species have to cope with both conditions of metal-restriction and metal-overload. Furthermore, the intestinal tract contains many bacterial species and thus enterohepatic *Helicobacter* species have to compete for metals and other nutrients. Metal metabolism has been studied intensively in *H. pylori*, but relatively little is known about metal metabolism of *H. mustelae* and *H. hepaticus*, and much of the information in this section is inferred from comparitive genomics using the *H. pylori* and *C. jejuni* genome sequences. In this section we will review the metal-transporter and metal-storage systems of the three selected *Helicobacter* species, of which an overview is given in Fig. 2.

Iron

Iron availability in the host tissues is mostly too low to support bacterial growth, as most iron is complexed into hemoglobin, or chelated by lactoferrin at mucosal surfaces (Andrews et al. 2003). Due to the insolubility of ferric iron, ferric iron transport requires an outer membrane receptor to transport the iron over the outer membrane, as well as a ABC transporter which transports the iron from the periplasm to the cytoplasm. In contrast, ferrous iron is soluble and only requires a inner membrane receptor.

Helicobacter pylori

Iron sources available in the gastric mucosa are lactoferrin, heme compounds, and iron derived from pepsin-degraded food. Iron is predicted to display increased solubility in the acidic conditions in the gastric mucosa, and eukaryotic iron-complexing proteins display lowered binding affinity at these conditions. *H. pylori* has been reported to be able to utilize heme compounds and ferric citrate as sole iron source (Dhaenens et al. 1999; Velayudhan et al. 2000), and it may be able to also use human lactoferrin, although this is still under debate (Bereswill et al. 1997). The *H. pylori* genome encodes eleven proteins predicted to be involved in iron transport and two proteins thought to function as iron storage proteins (Fig. 2).

H. pylori has three copies of the putative ferric citrate outer membrane receptor FecA, and three copies of the FrpB outer membrane receptor, which resembles a low-affinity enterochelin transporter in *Neisseria* species (Carson et al. 1999), for which in *H. pylori* the substrate is still unknown. There are two copies of the periplasmic binding protein CeuE, and finally a single inner membrane permease (FecD) and ATP-binding protein (FecE) (Fig. 2). Currently, mutants have only been described in the *fecDE* system and in one of the *fecA* genes (Velayudhan et al. 2000). Rather surprisingly, this did not affect iron transport. Thus the contribution of ferric iron uptake in *H. pylori* remains to be clarified. In the acidic, microaerobic gastric environment, ferrous iron (Fe^{2+}) is thought to constitute the main form of free iron, and this is transported by *H. pylori* via the FeoB protein (Velayudhan et al. 2000). FeoB-mediated iron acquisition is of major importance to *H. pylori*, as isogenic *feoB* mutants were unable to colonize the gastric mucosa of mice (Velayudhan et al. 2000).

Two iron-storage proteins have been characterized in *H. pylori*, the Pfr ferritin and HP-NAP (Dps) bacterioferritin (Fig. 2) (Bereswill et al. 1998b; Tonello et al. 1999). The Pfr ferritin serves as an intracellular iron deposit and protects *H. pylori* against intracellular iron toxicity and free iron-mediated oxidative stress (Waidner et al. 2002a). Iron stored in Pfr can be released and reused to support growth under iron-limited conditions (Waidner et al. 2002a). The HP-NAP protein is both homologous to bacterioferritins as well as to the DNA-binding proteins of the Dps family (Tonello et al. 1999). A role of HP-NAP in *H. pylori* iron storage, although suggested, is yet to be demonstrated.

Helicobacter mustelae

H. mustelae has been reported to be able to utilize heme compounds and iron chloride as sole iron source (Dhaenens et al. 1999). In addition, it is also capable of using ferric citrate (Stoof et al. 2005). This is partially reflected in the putative outer membrane iron-uptake proteins identified in *H. mustelae* (Fig. 2). There is a single ortholog of the outer membrane ferric citrate transporter FecA, and mutation of the *fecA* gene significantly reduces growth when ferric citrate is used as sole iron source (Stoof and van Vliet unpublished results). Furthermore *H. mustelae* contains two genes in tandem encoding orthologs of the FrpB outer membrane

receptor, and an ortholog of the *C. jejuni* CfrA outer membrane enterochelin receptor. Finally, there are three consecutive, > 80% identical genes encoding orthologs of the *H. hepaticus* HH0418 putative outer membrane protein (Fig. 2). The functions of the CfrA, FrpB and HH0418 orthologs are currently unclear. There is one ortholog of the periplasmic binding protein CeuE, and a single inner membrane FecDE ABC transporter system. Furthermore, there is an ortholog of the ferrous iron transporter FeoB present (Fig. 2). Finally, *H. mustelae* contains orthologs of the Pfr ferritin and HP-NAP (Dps) bacterioferritin, and it is likely that these have similar functions as the *H. pylori* counterparts.
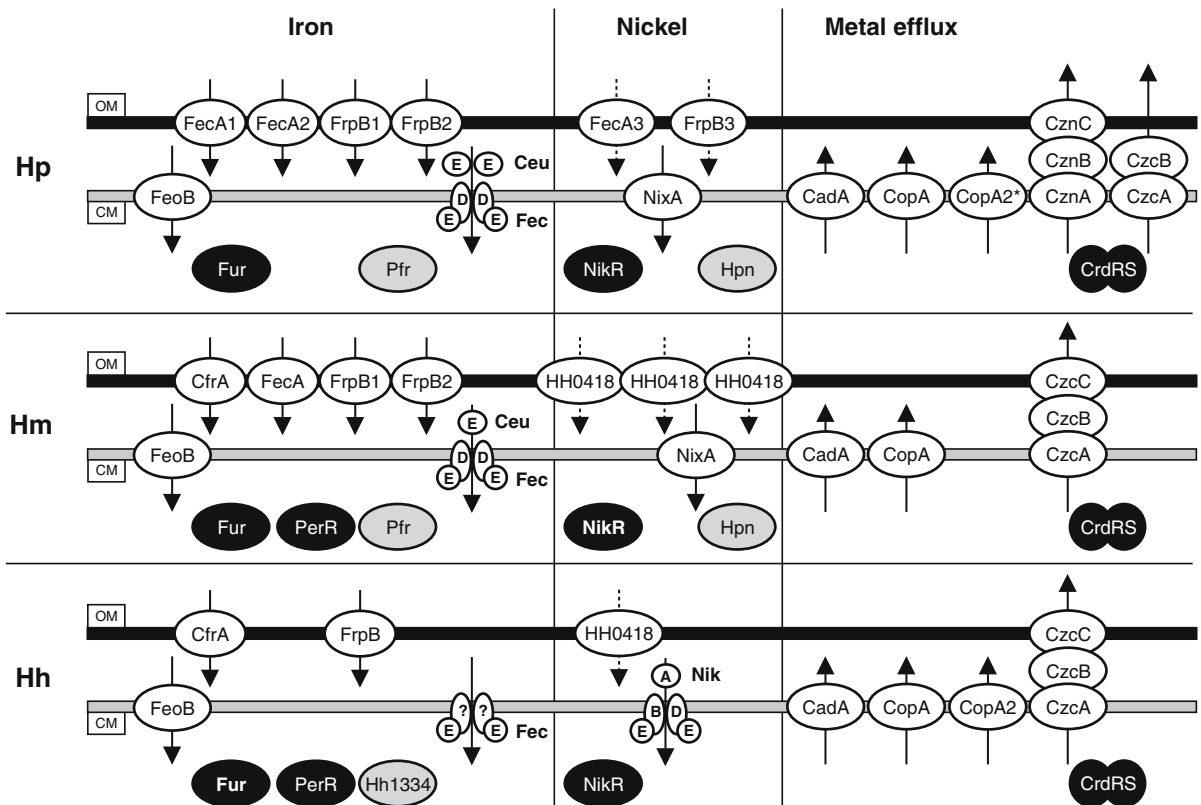

Fig. 2 Comparison of iron-transporters, nickel-transporters, metal-efflux systems, metal-storage and metal-responsive regulatory systems of *Helicobacter pylori* (Hp, top), *Helicobacter mustelae* (Hm, middle) and *Helicobacter hepaticus* (Hh, bottom). The ion transported and the direction of transport are indicated. OM, outer membrane; CM, cytoplasmic membrane. Ion transporters are grouped depending on the metal (putatively) transported. Arrows denotes the predicted direction of transport, dashed arrows indicate that this transport function is speculative. Note that the subdivision of outer membrane transporters for nickel is also tentative and speculative. Regulatory proteins are given in black, storage proteins in gray

Helicobacter hepaticus

H. hepaticus has been reported to be able to utilize lactoferrin, transferrin, heme compounds and iron chloride (Dhaenens et al. 1999). *H. hepaticus* colonizes the intestinal and hepatobiliary tract, and this is reflected in differences in its repertoire of putative iron-acquisition systems when compared to *H. pylori* and *H. mustelae* (Fig. 2). The *H. hepaticus* genome contains a single ortholog of the FrpB outer membrane receptor, for which it is tempting to speculate that this functions as heme receptor. Interestingly, despite the relatively close phylogenetic relationship and similar ecological niche as *C. jejuni*, there are surprising differences in the iron acquisition systems present. *H. hepaticus* and *C. jejuni* share a CfrA ortholog, which in *C. jejuni* functions as enterochelin uptake system (Palyada et al. 2004). However, it is not known whether *H. hepaticus* is able to use enterochelin as iron source. An ortholog of the *C. jejuni* hemin transporter ChuA is absent in the *H. hepaticus* genome sequence (van Vliet et al. 1998). Next to predicted outer membrane iron-transporters, *H. hepaticus* also contains a gene annotated as *fecE*, the ATPase component of the Fec ABC transporter. However, an ortholog of the permease protein FecD is not present in the *H. hepaticus* genome (Fig. 2), although this function may be fulfilled by a protein lacking sufficient homology to FecD. There is also an ortholog of the ferrous iron transporter FeoB present. Finally, there is also a ferritin present in *H. hepaticus*, encoded by the HH1334 gene. The bacterioferritin (Dps) ortholog of *H. hepaticus* also binds iron, but is reported to function in DNA-protection against oxidative stress (Hong et al. 2006).

Nickel

Nickel plays an important role in metabolism of *Helicobacter* species and in pathogenesis of infection. This is mostly due to the role of nickel as cofactor for the urease and hydrogenase enzymes, which are both involved in acid resistance, mucosal colonization and metabolism. Nickel availability in human serum is very low

(2–11 nM) (Christensen et al. 1999), and the nickel concentration in ingested food varies significantly depending on the diet and on food sources (Sunderman 1993). Therefore *Helicobacter* species require efficient high-affinity transport of nickel to cater for the urease and hydrogenase enzymes.

Helicobacter pylori

The adaptation of *H. pylori* to life in the gastric mucosa seems to have led to a more pronounced role of nickel in its metabolism. The major nickel-transporter of *H. pylori* is the NixA protein, a 37 kDa protein of the HoxN-type of nickel/cobalt transporters (Mobley et al. 1995). Mutation of the *nixA* gene results in clear reduction of nickel transport and lowered urease activity (Bauerfeind et al. 1996), and mutation of *nixA* complemented nickel-sensitivity of a *H. pylori* nikR mutant (Ernst et al. 2005c). Absence of *nixA* also affected colonization efficiency in a mouse model of *H. pylori* infection, presumably due to the reduced urease activity (Nolan et al. 2002). A second putative nickel transport system is the *abcCD* locus, since *abcC nixA* double mutants showed only residual urease activity (Hendricks and Mobley 1997). However, nickel transport by the AbcCD system is yet to be demonstrated. A third system thought to be involved in nickel transport is the Dpp dipeptide permease, but mutation of this gene did not affect overall urease activity (Davis and Mobley 2005). Finally, two TonB-dependent outer membrane proteins (FecA3 [HP1400] and FrpB3 [HP1512, also called FrpB4]) have recently been shown to be regulated by nickel and the NikR-regulator (Ernst et al. 2006), and may thus represent a novel type of nickel-transporters.

Several urease- and hydrogenase-associated systems have adapted to the central role of nickel. The accessory proteins involved in hydrogenase biogenesis also affect urease activity, and the HspA (GroES) chaperone contains a nickel-binding motif (Kansau et al. 1996). Next to the nickel-transporters and urease/hydrogenase accessory proteins, *H. pylori* also expresses one or two small, very histidine-rich proteins (Hpn) which show strong binding to nickel (Gilbert

et al. 1995; Ge et al. 2006). Mutation of the *hpn* gene rendered *H. pylori* sensitive to nickel (Mobley et al. 1999), which is suggestive of a role of Hpn in nickel storage or nickel sequestration, but this remains to be proven experimentally.

Helicobacter mustelae

Nickel transport in *H. mustelae* looks very similar to *H. pylori*. Both organisms have highly active urease and hydrogenase enzymes, and thus require efficient transport of nickel to cater for the high demand of nickel. *H. mustelae* contains a NixA ortholog, which probably represents the major nickel transporter. Interestingly, there are no clear orthologs of the FrpB3 and FecA3 proteins implicated in nickel transport of *H. pylori* (Ernst et al. 2006), but *H. mustelae* contains three tandem copies of the putative outer membrane receptor HH0418, which may fulfill a similar role as FecA3/FrpB3 in *H. pylori*. However, this awaits experimental validation. Finally, *H. mustelae* also expresses the histidine-rich Hpn protein (Gilbert et al. 1995), which is thought to function in nickel storage.

Helicobacter hepaticus

H. hepaticus expresses two nickel-cofactored enzymes, urease and the Ni/Fe hydrogenase (Maier et al. 2003; Mehta et al. 2005). This has necessitated the efficient transport of nickel, and this is likely to be mediated by the NikABDE ABC transporter system, which displays homology with the *E. coli* NikABCDE nickel transport system (De Pina et al. 1999). NikA is an ortholog of the *E. coli* nickel-binding periplasmic protein, and NikB is the predicted cytoplasmic membrane permease. NikD and NikE are predicted to function as ATPases which energize the transport (Navarro et al. 1993). There is no NixA ortholog present in *H. hepaticus*. The *H. hepaticus* genome contains a gene encoding a putative siderophore outer membrane receptor (HH0418), which is divergently orientated to the *nikABDE* operon encoding the predicted nickel-uptake ABC transporter system (Beckwith et al. 2001). It is therefore tempting to speculate that the HH0418

protein may function in nickel uptake, although this hypothesis may be farfetched and awaits rigorous experimental validation.

Copper

Copper is a cofactor for several proteins involved in electron transport, oxidases, and hydroxylases, but may also contribute to the formation of reactive oxygen species.

Helicobacter pylori

H. pylori expresses several proteins which are either involved in either copper transport or may act as copper chaperones. It is currently unclear whether there is a specific import system for copper, or whether it is transported by other metal transporters like FeoB or NixA (Mobley et al. 1999). However, *H. pylori* expresses several proteins involved in copper export, including the CopA and CopA2 P-type ATPases (Table 1), and the Crd copper resistance determinant (Melchers et al. 1998; Waidner et al. 2002b). Furthermore, *H. pylori* also expresses a small protein, CopP, that may function as copper chaperone (Beier et al. 1997; Bayle et al. 1998).

Helicobacter mustelae *and* Helicobacter hepaticus

Very little is known about copper metabolism of both *H. mustelae* and *H. hepaticus*. Both genomes contain orthologs of the CrdRS two-component regulatory system mediating copper-responsive induction of a Czc-like metal efflux system (Waidner et al. 2002b, 2005), but a role in copper metabolism is not yet established.

Metal efflux systems

Helicobacter pylori

Metal efflux is of great importance to *H. pylori*. The urease enzyme of *H. pylori* is sensitive to transition metals (Perez-Perez et al. 1994; Herrmann et al. 1999), and thus effective homeostasis of transition metals is necessary to prevent acid-sensitivity. *H. pylori* expresses three

Table 1 Metal-transport and metabolism-associated genes of selected *Helicobacter* species

Gene	*H. pylori*[a]	*H. hepaticus*[b]	*H. mustelae*	Proposed function
fecA1	0686	–	Present	Outer membrane receptor
fecA2	0807	–	–	Outer membrane receptor
fecA3	1400	–	–	Outer membrane receptor
frpB1	0876	1847	Present	Outer membrane receptor
frpB2	0916/0915	–	Present	Outer membrane receptor
frpB3[c]	1512	–	–	Outer membrane receptor
cfrA	–	0721	Present	Outer membrane receptor
hh0418	–	0418	3 copies	Outer membrane receptor
fecD	0889	?	Present	Inner membrane permease
fecE	0888	1759	Present	ATPase
ceuE1	1562	?	Present	Periplasmic binding protein
ceuE2	1561	–	–	Periplasmic binding protein
feoB	0687	0033	Present	Ferrous iron transporter
pfr	0653	1334	Present	Ferritin
dps[d]	0243	0210	Present	Bacterioferritin/Dps ortholog
nixA	1077	–	Present	Inner membrane nickel transporter
nikABDE	–	0417-0414	–	Inner membrane nickel ABC transporter
cadA	0791	0586	Present	P-type ATPase
copA	1072	0682	Present	P-type ATPase
copA2	1502	1022	–	P-type ATPase
cznABC	0969-0971	0625-0623	Present	Cobalt–zinc–nickel resistance determinant
crdAB	1326-1327	–	–	Copper resistance determinant
fur	1027	0893	Present	Ferric uptake regulator
perR	–	0942	Present	Peroxide stress regulator
nikR	1338	0352	Present	Nickel-responsive regulator
crdRS	1365/1364	1656-1657?	?	Two-component copper-regulatory system

[a] Gene numbers from the genome sequence of *H. pylori* strain 26695 (Tomb et al. 1997)

[b] Gene numbers from the genome sequence of *H. hepaticus* strain ATCC51449 (Suerbaum et al. 2003)

[c] Alternative designation *frpB4*

[d] The *H. pylori* Dps protein is also known as Neutrophil Activating Protein (HP-NAP)

P-type ATPases, which can function in efflux of toxic metals. Two of these P-type ATPases are suggested to be CopA homologs which are involved in copper metabolism (see previous section). The third P-type ATPase is the *H. pylori* CadA protein, which is homologous to bacterial cadmium and copper P-type ATPases, and contains eight transmembrane domains (Melchers et al. 1996). Mutational studies demonstrated that CadA is involved in resistance to cadmium, zinc and cobalt, but not to copper or nickel. This indicates that the CadA exporter is not specific for a single metal. Mutation of *cadA* also reduced urease activity and nickel accumulation in some but not all *cadA* mutants (Herrmann et al. 1999). The other type of metal-efflux systems in *H. pylori* consists of the Czc system. These are cation-proton antiporters, and usually contain an cytoplasmic membrane transporter (CzcA), a periplasm-spanning protein (CzcB) and an OM protein thought to be involved in transport over the OM (CzcC). The *H. pylori* genome contains two Czc-like systems, the Crd copper resistance determinant (see previous section), and a cobalt–zinc–nickel (Czn) resistance determinant (Table 1) (Stähler et al. 2006). The latter system is also implicated in acid-resistance (possibly via nickel efflux) (Stähler et al. 2006) and efflux of metronidazole (van Amsterdam et al. 2005).

Helicobacter mustelae *and* Helicobacter hepaticus

The *H. mustelae* and *H. hepaticus* genomes contains, respectively, two and three genes encoding P-type ATPase orthologs, which are tentatively named CadA, CopA and CopA2, like in *H. pylori*. Similarly, there is a single CzcABC

metal efflux system present. The function of these systems has not been investigated yet.

Metal-responsive regulatory systems of *Helicobacter* species

Rapid responses to stressful changes in environmental conditions are often mediated via changes in transcription of sets of genes, that encode some factor involved in the dealing with these stresses. Examples of this are the expression of oxidative stress defense genes in response to oxidative stress. In many bacteria, such stress-responsive systems are often encoded by genes organized in an operon, and the transcription is regulated by one or two regulatory proteins.

The ion-responsive regulatory systems of bacteria usually consist of a single regulatory protein, that combines sensor and effector functions in one molecule (Escolar et al. 1999). It senses the cytoplasmic ion concentration and, when activated, can induce or repress transcription of the genes encoding the corresponding uptake, efflux and/or storage systems (Andrews et al. 2003). Four types of metal-responsive proteins have been identified in *Helicobacter* species: the iron-responsive regulator Fur (Bereswill et al. 1998a), the peroxide-regulatory protein PerR (Suerbaum et al. 2003; Belzer et al. 2005b), the nickel-responsive regulator NikR (van Vliet et al. 2002a) and the copper-responsive two-component regulatory system CrdRS (Waidner et al. 2005). Comparison of the three *Helicobacter* genome sequences with that of *C. jejuni* has indicated an interesting distribution of metal-responsive regulators (Fig. 3A). The Fur regulator is present in all *Helicobacter* species and in *C. jejuni*, although its functions and regulons differ (see below). The PerR regulator is present in *H. mustelae*, *H. hepaticus* and *C. jejuni*, but absent in *H. pylori* (Fig. 3A). This is rather surprising since the main proteins regulated by PerR are catalase and AhpC (Bsat et al. 1998; van Vliet et al. 1999), and these are present in all four species. The NikR regulator is present in all three *Helicobacter* species, but is absent in *C. jejuni*. This is likely to be linked to the presence of the nickel-cofactored urease system in the investigated *Helicobacter*
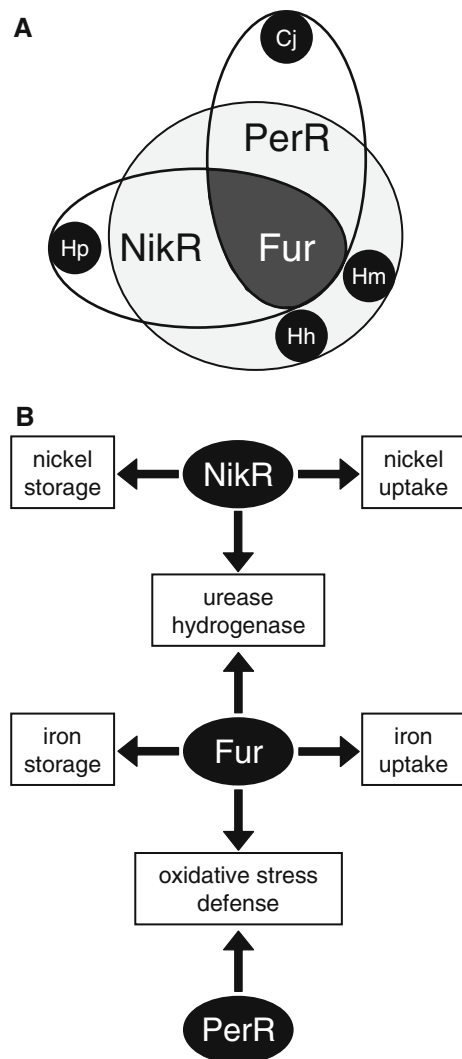

Fig. 3 Distribution and overlapping functions of the Fur, NikR and PerR regulatory systems in *Helicobacter* and *Campylobacter* species. (**A**) Distribution of the regulators in *Helicobacter pylori* (Hp), *Helicobacter mustelae* (Hm), *Helicobacter hepaticus* (Hh) and *Campylobacter jejuni* (Cj). The Fur protein is ubiquitous in all four species (indicated by the dark gray area), whereas the PerR and NikR proteins are only present in 3/4 species (indicated in light gray). (**B**) Overlapping functions of the Fur, NikR and PerR regulatory systems in *Helicobacter*

species, and the absence of this enzyme in *C. jejuni*. Finally, the distribution of the CrdRS is difficult to predict, since two-component regulatory systems share significant sequence homology both for the response regulator and the sensory histidine kinase, and this makes predictions based on homology searches risky.

Fur

The Fur protein is primarily known for its role as repressor of iron acquisition systems (Hantke 2001). It fullfils this classical function in all three *Helicobacter* species as well, as the *H. pylori* Fur mediates regulation of iron acquisition outer membrane proteins (Delany et al. 2001a; van Vliet et al. 2002b). However, the Fur protein also regulates part of the nitrogen metabolism of *H. pylori*, via iron-responsive repression of the AmiE aliphatic amidase (van Vliet et al. 2003). Interestingly, the *H. pylori* Fur protein is bifunctional, since it also functions in its iron-free (apo)form. Iron-free Fur represses the expression of the Pfr ferritin and the iron-cofactored superoxide dismutase SodB (Delany et al. 2001b; Ernst et al. 2005a). This form of regulation may compensate for the absence of iron-responsive regulation via small RNAs like RyhB (Masse and Gottesman 2002), which is of great importance in several pathogenic bacteria, but has so far not been reported for *H. pylori*.

In *H. hepaticus*, Fur co-regulates expression of the peroxide stress defense genes AhpC and KatA with PerR (see below) (Belzer et al. 2005b), and also mediates iron-responsive regulation of the urease system (Belzer et al. 2007). The rationale behind Fur-regulation of urease expression is not directly apparent, but may be linked to Fur functioning as a sensory system for iron-restricted conditions encountered in the natural niche of *H. hepaticus*.

PerR

The peroxide stress regulator PerR is a Fur homolog and is responsible for iron- and manganese-dependent regulation of *ahpC* and *katA* in *Bacillus subtilis* and *C. jejuni* (Bsat et al. 1998; van Vliet et al. 1999). The *H. hepaticus* and *H. mustelae* genomes contain a gene encoding a PerR homolog, and this may serve as a replacement system for the OxyR regulator, which is absent in *Helicobacter* species and *C. jejuni*. However, PerR is not present in *H. pylori*, and this raises the interesting question whether the PerR system was either lost by *H. pylori* or gained by the other species.

In *C. jejuni*, *perR* mutants were shown to express very high levels of both catalase and alkyl hydroperoxide reductase and to be highly resistant to peroxide stress (van Vliet et al. 1999). Like Fur repressors, PerR repressors also recognize a specific sequence upstream of their target genes although the consensus sequence has only been confirmed in *B. subtilis* (Herbig and Helmann 2001). Identification of PerR-binding sites is complicated by their similarity to Fur-binding sequences. Although PerR responds to iron, it has been suggested that PerR may use manganese as its metal cofactor (Herbig and Helmann 2001). Preliminary analysis of Fur and PerR expression in *H. hepaticus* suggests that Fur and PerR co-regulate expression of catalase and AhpC, in a similar level to what was described for *C. jejuni* (Belzer et al. 2005b).

NikR

The NikR regulator belongs to the class of ribbon-helix-helix (RHH) proteins. It was first described in *E. coli* as repressor of the nickel uptake operon *nikABCDE* (De Pina et al. 1999). Binding of nickel-cofactored NikR to a 5′-GTATGA-N_{16}-TCATAC-3′ sequence in the *nikA* promoter region results in repression of transcription (Chivers and Sauer 2000), and this allows NikR to maintain intracellular nickel homeostasis in a similar manner as Fur and iron. NikR orthologs are present in all the *Helicobacter* genomes sequenced thusfar, but has only been studied in *H. pylori* (van Vliet et al. 2004a). NikR was shown to function as a repressor as well as an inducer of transcription, since nickel-dependent binding of NikR to 5′-TATWATT-N_{11}-AATW-ATA-3′ sequences in the *ureA* and *nixA* promoters resulted in induction and repression of transcription, respectively (Delany et al. 2005; Ernst et al. 2005c). Furthermore, NikR and Fur display overlapping regulons and each regulates the expression of the other protein and the corresponding regulon (Bury-Mone et al. 2004; van Vliet et al. 2004b), allowing intricate regulation of gene expression in different environmental conditions.

In *H. pylori* NikR controls utilization of nickel, by mediating nickel-responsive regulation of

urease expression (van Vliet et al. 2002a; Ernst et al. 2005c). A similar regulation of urease expression has been observed in *H. mustelae* (Stoof and van Vliet, unpublished results). Interestingly, the urease system of *H. hepaticus* is not nickel-responsive at the transcriptional level, but only at the post-translational level (Belzer et al. 2005a). This indicates that NikR may not be involved in controlling urease expression in *H. hepaticus*, and thus this suggests that NikR-controlled nickel-responsive regulation of urease expression represents a specific adaptation to the gastric lifestyle.

CrdRS

The CrdRS system was identified in *H. pylori* as the two-component regulatory system required for copper-responsive induction of the copper-resistance determinant CrdAB-CzcBA (Waidner et al. 2002b, 2005). It is not yet known how the CrdRS system senses copper, but absence of either CrdR or CrdS disturbs regulation of Crd (Waidner et al. 2005). The CrdRS system plays an important role in gastric colonization in a mouse infection model for *H. pylori* (Panthel et al. 2003) and is also implicated in acid resistance of *H. pylori* (Loh and Cover 2006). Whether *H. mustelae* and *H. hepaticus* contain orthologs of CrdRS is not known, although both genomes contain two-component regulatory systems with sequence homology to CrdRS.

Conclusions

Although they are considered to be fastidious, *Helicobacter* species are well adapted to their ecological niche. From genomic comparisons it is apparent that while clustered in a single species, there are considerable differences in genomic content (Eppinger et al. 2004) which are also reflected in the components of metal-transport systems as reviewed here. There seems to be no specific evolution of metal-transporters equipped for either the gastric or enteric environment, with possibly the exception of the NixA vs. the Nik-ABDE nickel transporters, the Hpn nickel-binding protein and possibly the presence of a ferric

citrate outer membrane transporter, which are only present in the gastric *Helicobacter* species. Therefore differences in niche adaptation may well be more at the level of gene regulation and biochemical properties of the regulated proteins.

The considerable differences between *H. pylori* and *H. mustelae* also need to be taken into account when using the *H. mustelae* ferret infection model, which has been proposed as suitable animal model for *H. pylori* infection. Although pathogenesis of *H. mustelae* infection does mimic that of *H. pylori* infection of humans, there are still many differences between *H. pylori* and *H. mustelae* as well as between man and ferret that need to be taken into account, and will make direct application of this model difficult. Similarly, the differences between host, niche and genetics of *H. hepaticus* and *H. pylori* make direct comparisons very difficult.

In conclusion, the availability of genome sequences of different *Helicobacter* species has allowed for comparative genomic analyses of metal transport systems, and this shows the significant diversity in these systems within a single genus. It also displays the versatility of these bacteria to adapt to the diversity of conditions encountered in their natural gastric or enteric niches in mammals.

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