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Iron trafficking system in *Helicobacter pylori*

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Abstract *Helicobacter pylori* infections are closely associated with peptic ulcers, gastric malignancy and iron deficiency anemia. Iron is essential for almost all living organisms and the investigation of iron uptake and trafficking system is thus important to understand the pathological roles of *H. pylori*. Up to now, the iron trafficking system of *H. pylori* is not yet fully clear and merits further efforts in this regards. The available information about iron uptake and regulation has been discussed in this concise review, such as FeoB in ferrous transportation, FrpB2 in hemoglobin uptake, HugZ in heme processing, virulence factors (VacA and CagA) in transferrin utilization, Pfr and NapA in iron storage and Fur in iron regulation. The identified iron trafficking system will help us to understand the pathological roles of *H. pylori* in the various gastric diseases and iron deficiency anemia and stimulates further development of effective anti-bacterial drugs.

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Abbreviations used

ABC	ATP-binding protein cassette
Dps	DNA-protecting proteins
EMSA	electrophoretic mobility shift assay
Fur	ferric uptake regulator
H. pylori	*Helicobacter pylori*
IDA	iron deficiency anemia
sRNA	Small regulatory RNA

Introduction

Iron is essential for almost all living organisms and is closely involved in a wide range of metabolic and cellular pathways, such as electron transport, oxygen activation, peroxide reduction, amino acid and nucleoside synthesis and photosynthesis. Iron-containing cofactors, such as iron–sulfur clusters and heme groups, have been used as cofactors in over 100 enzymes in the primary and secondary metabolisms, where the reversible Fe^{2+}/Fe^{3+} redox pair is tuned through elegant regulations of the ligands and coordination environments to fulfill a broad spectrum of redox reactions. Bacteria normally require a high concentration (over 10^{-8} M) of iron for optional growth (Klebba et al. 1982). The free Fe^{3+} concentration in aqueous, neutral and aerobic environment is

quite limited, around 10^{-9}–10^{-18} M, as Fe^{3+} forms stable ferric oxide hydrate complexes ($Fe_2O_3{\cdot}nH_2O$) in this condition (Braun et al. 1998). Especially in the microenvironment of extracellular fluids and mucosal surfaces, iron is further limited through various mechanisms, including the blocked access to the interstitium by the epithelial barrier, transferrin binding to interstitial iron, sequestration of intracellular iron by ferritin and chelation of mucosal iron with lactoferrin (Payne 1993; Wooldridge and Williams 1993; Weinberg 2009). To overcome the limited availability of iron in the host environment, bacterial pathogens have developed at least two ways to satisfy its need for iron sources: (1) to avoid the need for iron by modify genetically as exemplified by *Borrelia burgdorferi*, using other cations such as copper to supplement the functions of iron-containing metalloproteins (Posey and Gherardini 2000); and (2) to develop high-affinity iron uptake systems with direct (uptake of iron from host iron proteins, including heme, ferritin, transferrin, lactoferrin, heme-containing hemoglobin and hemosiderin, and iron-containing enzymes) or indirect (employment of specialized secreted hemophores to acquire heme or the usage of siderophores as high affinity ferric chelators) interactions with the iron sources (Ratledge and Dover 2000). Bacteria normally extract iron from transferrin or lactoferrin before transporting the iron across the cell wall (Andrews et al. 2003). Similarly for heme-containing proteins, the entire heme group is removed from the protein and imported into the bacterium. The heme molecule is further degraded intracellularly to provide iron, prosthetic group or both (Genco and Dixon 2001).

As a Gram-negative, microaerophilic and spiral-shaped bacterium that colonizes in the gastric mucosa of humans (Marshall and Warren 1984), *Helicobacter pylori* (*H. pylori*) chronic infections are the major cause of atrophic gastritis, peptic ulcer disease, functional dyspepsia and gastric carcinomas (Blaser 1997). *H. pylori* strains have two major contact-dependent virulence factors, an active vacuolating toxin VacA (Cover 1996) and a 120–145 kDa cytotoxin-associated protein CagA (Backert and Selbach 2008). VacA is proposed to play roles in the early steps of bacterial gastric colonization by provoking cell vacuolation (Salama et al. 2001), and CagA is injected into host cells via type IV secretion system (Costa et al. 2009). Both proteins play important roles in enabling *H. pylori* to colonize in the epithelium (Tan et al. 2009).

H. pylori infections have recently been associated with iron deficiency anemia (IDA) (Malfertheiner and Selgrad 2010). For example, *H. pylori* infection has been detected in up to 89.5% of children with IDA (Pacifico et al. 2010), and children with *H. pylori* eradicated exhibited a threefold increase in serum ferritin compared with infected children (Cardenas et al. 2011). Iron deficiency is characterized by lower levels of iron, ferritin and transferrin saturation, and higher levels of total iron-binding capacity, red cell zinc protoporphyrin and serum transferrin receptor (Goddard et al. 2011). To discover the molecular basis of the association between *H. pylori* infections with IDA and to identify possible targets during the treatment of IDA, it is important to understand the iron uptake and trafficking systems in this bacterium. *H. pylori* has been known to possess several iron uptake systems through genome-wide analysis (Tomb et al. 1997). First, *H. pylori* possesses homologues of a number of *fec* genes (encoding a Fe^{3+}-dicitrate transporter), despite the facts that the two regulatory proteins (FecR and FecI) are absent and the genes are not organized in a single operon. Additionally, *H. pylori* contains FeoB, a homologue of the *E. coli* Fe^{2+} transporter of FeoAB (Kammler et al. 1993) and the extracellular and cell-associated Fe^{3+}-reduction activity has been established (Worst et al. 1998). Thirdly, *H. pylori* contains NapA and Pfr, ferritin-like proteins for iron storage. Finally, the global ferric uptake regulator (Fur) is present in this bacterium and the Fur-binding boxes are found upstream of *fur* and the two *fecA* genes (Tomb et al. 1997). Unlike most other pathogens with siderophore-mediated iron uptake mechanism (Ge et al. 2009), *H. pylori* has not been shown to synthesize siderophores (Dhaenens et al. 1999; van Vliet et al. 2001). *H. pylori* is unable to compete for iron with mucus lactoferrin and interstitial transferrin in partially saturated forms (Senkovich et al. 2010); whereas the bacterium can use the two proteins once in fully saturated forms (Husson et al. 1993; Senkovich et al. 2010). This present concise review intends to cover the most recent reports and discoveries in the field of *Helicobacter* iron trafficking system, which may deepen our understanding of how this pathogenic bacterium adapts to the severe habitant environments in the host and causes gastric diseases and iron deficiency anemia.

Iron uptake in *H. pylori*

Ferrous iron uptake

Fe^{2+} is relatively soluble in aqueous solution under anaerobic, reducing or low pH conditions, and is hence the preferred form of iron used by bacteria under anaerobic/microaerophilic or low pH conditions. The bacterial Fe^{2+} transporters identified so far include FeoABC system in *E. coli* (Kammler et al. 1993), *Salmonella typhimurium* (Tsolis et al. 1996) and *H. pylori* (Velayudhan et al. 2000), *E. coli* EfeU (Grosse et al. 2006; Cao et al. 2007), *Synechocystis* 6803 FutA (Koropatkin et al. 2007), *Yersinia pestis* Yfe (Perry et al. 2007) and *Streptococcus pyogenes* MtsABC (Sun et al. 2008b, 2009). FeoB is a polytopic membrane protein generally composed of three domains: an N-terminal cytoplasmic GTPase domain, a helical domain and a C-terminal polytopic membrane-spanning domain. Transcription of the *feoB* gene is iron- and Fur-repressed (van Vliet et al. 2002). This protein is a high-affinity Fe^{2+} transporter (apparent $K_s \sim 0.54$ μM) and plays an important role in iron acquisition of *H. pylori* under iron-restricted conditions, as the *feoB* mutant strains were unable to colonize the gastric mucosa of mice (Fig. 1) (Velayudhan et al. 2000). Insertion mutation of the *feoB* gene resulted in a markedly reduced uptake ($\sim$10-fold) of Fe^{2+}- and Fe^{3+}-dicitrate; while complementation of the *feoB* mutation fully restored Fe uptake from Fe^{2+} and Fe^{3+}-dicitrate in the *feoB* mutant. Irrespective of the oxidation state of the Fe supplied, Fe is transported by FeoB into the cell in the reduced Fe^{2+} state, as extracellular and cell-associated Fe^{3+} reductase activity (up to 4.3 ± 0.7 nmol ferrozine-Fe^{2+} min^{-1} per 10^7 cells; ferrozine is a specific Fe^{2+} chelator) has been identified in the wild-type *H. pylori* (Worst et al. 1998; Velayudhan et al. 2000).

Transport via FeoB is highly specific for Fe^{2+} as some other divalent cations, including Co^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+} showed no significant competition for the transporter, albeit in concentrations of 100 times that of Fe^{2+} (Velayudhan et al. 2000). FeoB activity is independent of the ferric uptake energy transduction genes (*exbB*, *exbD*, *tonB*) (Schauer et al. 2008), indicating that Fe^{2+} uptake proceeds via a pathway distinct from that of Fe^{3+} (Velayudhan et al. 2000). Ferrous transport through *H. pylori* FeoB is supposed to rely on an active process energized by ATP, as the transport is inhibited by FCCP (a protonophore providing a route for the dissipation of the proton electrochemical gradient), DCCD (an inhibitor of ATP synthesis by binding to the proton-translocating ATP synthase) and orthovanadate (an inhibitor of ATP synthesis) (Velayudhan et al. 2000). However, further work indicated that ferrous transport is also dependent upon GTP hydrolysis (Eng et al. 2008), similar to the homologues in *Streptococcus thermophilus* (Ash et al. 2010), *Thermotoga maritime* (Hattori et al. 2009), *E. coli*, *Salmonella typhimurium* and *Vibrio cholerae* (Eng et al. 2008).

Heme uptake

Many pathogenic bacteria require heme for biosynthetic and energy utilization pathways. One major uptake method is via active ABC transporters which are composed of a heme-binding protein, a membrane permease and an ATPase (Davidson and Chen 2004). Generally, the heme-binding protein captures the heme molecule and transfers the molecule to the permease. The ATPase provides the energy for the subsequent transportation through the cell membrane. In the host, heme is often capped by a protein (hemoprotein) to increase solubility and hexacoordinated with four ligands from the protoporphyrin IX and two axial ligands from the side chains of hemoproteins. At least one heme/hemoglobin transporter is present in *H. pylori* since: (1) heme is found to be able to serve as the sole iron source for *H. pylori* growth (Worst et al. 1995); (2) in iron-deficient medium, the ability of *H. pylori* to extract iron from hemoglobin was observed (Senkovich et al. 2010); and (3) in a proteomic work performed by our group, hemoglobin from the culture medium was found to be uptaken into the *H. pylori* cells (Ge et al. 2007). For *H. pylori*, the possession of a heme uptake system has significant advantages: (1) desquamation of the epithelial cells results in the presence of heme on mucosal surfaces; (2) heme from epithelial cells and the bloodstream can be accessible by *H. pylori* at the intercellular junctions of epithelial cells; and (3) the production of hemolysin (Segal and Tompkins 1993) and cytotoxin (Leunk 1991) by *H. pylori* further contribute to the leakage of heme compounds. The mechanism of heme utilization in *H. pylori* is not yet completely clear, although several heme iron-repressible outer membrane proteins are supposed to be

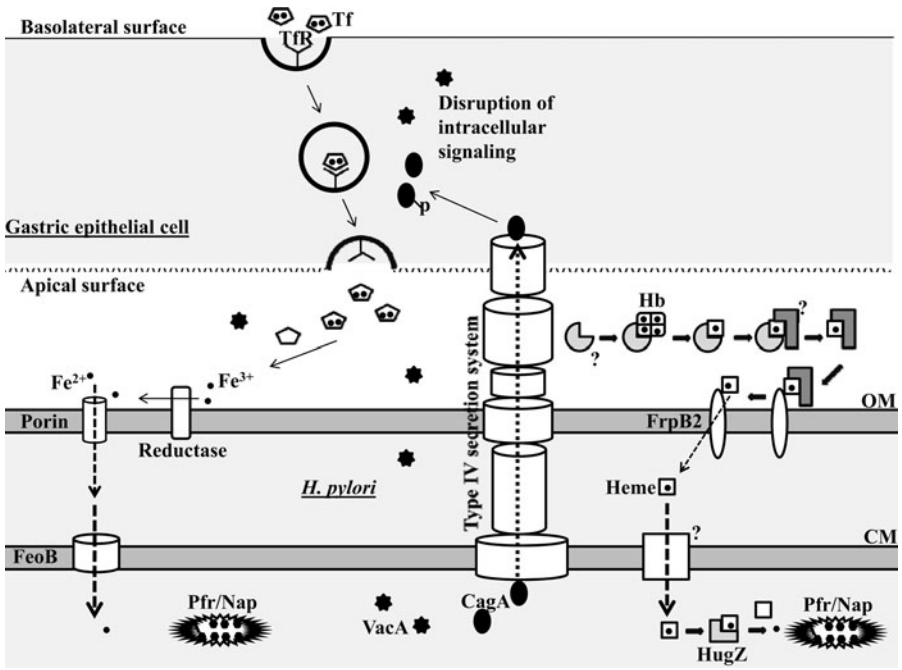

Fig. 1 A schematic overview of the major iron acquisition systems identified so far in *H. pylori*. CagA is injected into gastric epithelial cells via type IV secretion system. VacA and CagA cause disruption of intracellular signaling in the host cells. Both virulence factors contribute to the perturbation of transferrin recycling from endocytosis to transcytosis (apical mislocalization of the transferrin receptor to sites of bacterial attachment). Fe^{3+} (e.g. from Fe^{3+}-dicitrate) is reduced into Fe^{2+} by one kind of unidentified reductase. Fe^{2+} diffuses through porins of the outer membrane and is transported into the cytoplasm by FeoB, the process of which is energized by GTP or ATP hydrolysis. Heme from hemoglobin, extracted with a series of unidentified yet heme-binding proteins, is transported through the outer membrane with FrpB2 and through the cytoplasmic membrane with an unidentified transporter. Heme is then processed with HugZ, a heme oxygenase, to release iron. Free intracellular irons are stored in bacterial ferritins, NapA or Pfr. *Tf* transferrin; *TfR* transferrin receptor; *Hb* hemoglobin; *OM* outer membrane of *H. pylori*; *CM* cytoplasmic membrane of *H. pylori*

involved in heme binding and/or uptake (Worst et al. 1995, 1999). The *H. pylori* outer membrane protein FrpB2 is suggested to be a hemoglobin-binding protein, as this protein has the characteristic hemoglobin-binding motifs (FRAP and NPNL) and can maintain the *E. coli* cell growth only in the presence of hemoglobin (González-López and Olivares-Trejo 2009).

Upon uptake into the *H. pylori* cells, heme must be treated enzymatically to release the iron for subsequent cellular usage. Heme oxygenase plays key roles in the growth and colonization of the pathogenic bacteria such as *Campylobacter jejuni* and *Yersinia pestis* by catalyzing the cleavage of heme to biliverdin with the release of iron and carbon monoxide. Heme oxygenase mutants of *Corynebacterium diphtheria* and *Neisseria meningitides* were unable to utilize heme or hemoglobin as an iron source (Zhu et al. 2000; Kunkle and Schmitt 2007). HugZ, a proposed heme oxygenase in *H. pylori*, has been characterized biochemically (Guo et al. 2008). The heme oxygenase activity of HugZ is dependent on the presence of an electron donor, such as ascorbic acid or a NADPH-cytochrome P450 reductase system. HugZ cleaves the δ-meso carbon bridge of heme to give carbon monoxide and biliverdin IXδ (Guo et al. 2008). One microarray study reported that *hugZ* is induced by iron starvation (Merrell et al. 2003) and a hypothetical Fur box located before *hp0321* controls *hugZ* expression (Gancz et al. 2006). The crystallization work indicated that HugZ is distinct from other heme oxygenase by being a dimer in a split-barrel fold, a structure that is often found in flavin mononucleotide-binding proteins but has not been observed in hemoproteins (Hu et al. 2011). The heme is located in the dimer interface, and is bound to the side chain of His245 and an azide molecule provided from the crystallization buffer.

Iron uptake from host transferrin

Transferrin and lactoferrin in the holo form can support *H. pylori* growth as the sole iron source (Senkovich et al. 2010). However *H. pylori* favors binding of iron-free transferrin and lactoferrin, a hint of a relatively low ability of acquisition of iron from human serum which is not fully iron saturated (Senkovich et al. 2010). On the other hand this finding may represent a novel strategy of *H. pylori* to permit limited growth in host tissue during persistent colonization while to prevent excessive injury and iron depletion. This is further supported by the finding that the two virulence factors VacA and CagA carefully control their coordinative activities to prevent harsh damage to the gastric mucosa: CagA diminishing VacA-induced vacuolation and VacA reducing CagA-induced hummingbird phenotype (Argent et al. 2008). As the two most studied virulence factors in *H. pylori*, the functions of CagA and VacA have been extensively reviewed (Atherton and Blaser 2009; Dorer et al. 2009; Wen and Moss 2009; Basso et al. 2010). The major roles of CagA and VacA include: cell scattering, cell–cell dissociation, pro-inflammatory and mitogenic effects for CagA; cytoplasmic vacuolization in cultured cell, increasing permeability of polarized epithelial cells and apoptosis for VacA. In a recent elegant study by Tan et al. (Tan et al. 2011), a newly discovered concurrent CagA/VacA activity for micronutrient acquisition and host tissue colonization was reported, which has been extensively discussed in a priority paper evaluation in Future Microbiology (Boyanova 2011). The most important results of the work include (Tan et al. 2011): (1) VacA induces apical mis-localization of transferrin receptors to regions of *H. pylori* attachment and both factors coordinate together on polarized uptake and recycling of transferrin; (2) CagA is essential in the uptake of iron in vivo as *cag*-deficient mutants showed reduced gastric colonization in iron-deficient Mongolian gerbil model; (3) CagA EPIYA motifs are important for host internalization of transferrin; and (4) both factors promote iron acquisition from an enhanced apical colonization of the host cells. However, some questions remain to be answered. Holo-transferrin from the gastric epithelial cells is transported to the apical *H. pylori* microcolonies through transcytosis. What is the form utilized directly by *H. pylori*, the holo-transferrin or the released iron? In either case, what is the transporter system on the *H. pylori* surface to be involved in the transportation? As *H. pylori* cannot compete with the eukaryotic cells for the holo-transferrin due to its high affinity for the transferrin receptor (Young et al. 1984), it would be beneficial to the bacteria that the iron is released, either enzymatically or acidically, from transferrin into the extracellular space. Upon iron utilization from transferrin, it would be necessary for the bacteria to deal with the apo- or partially saturated transferrin, since the partially saturated transferrin is toxic to *H. pylori* (Senkovich et al. 2010).

Iron storage in *H. pylori*

Ferritin plays a significant role in iron homeostasis: to catalyze the oxidation of iron into a ferric oxide core within the protein shell (ferroxidase activity), thus avoiding the deleterious effects of excessive intracellular iron (Waidner et al. 2002); and to provide cells with iron which can be released by acidic pH or reactive oxidative species inside the cells. Similar observations have been made by a proposed nickel storage protein Hpn (Ge et al. 2006a, b). *H. pylori* ferritin (Pfr) enables *H. pylori* to multiply under severe iron starvation and protects the bacteria from acid-amplified iron toxicity (Waidner et al. 2002), functions essential for the survival of *H. pylori* in the gastric mucosa due to the inability of the *pfr* mutants to colonize in a Mongolian gerbil animal model (Waidner et al. 2002). Crystal structures of Pfr in apo, low-iron-bound (79 irons per 24-mer), intermediate and high-iron-bound (4,165 irons per 24-mer) states indicated that significant conformational changes are apparent at the BC loop and the entrance of the fourfold symmetry channel in the intermediate and high-iron-bound states, with little changes in the apo and low-iron-bound states (Cho et al. 2009). The fourfold channel is dominated with negative electrostatic potential around the entry hole and within the interior, indicating a potential for metal binding. The imidazole rings of His149 are facing the inside of the pore, and Glu147 and Asn148 residues are directed outside. The imidazole rings of His149 at the channel entrance undergo conformational changes (imidazole rings, 5.4–5.9 Å apart in the apo and low-iron-bound states; 3.0–3.2 Å in the intermediate and high-iron-bound states) to facilitate iron binding. Fe ions are

0.3–2.5 Å away from the slightly distorted tetra-imidazole planes depending on the iron-bound state: 2.5, 2.3 and 0.3 Å in the low-, intermediate- and high-iron states, respectively. One water molecule is located axially above the iron and is anchored by Asn148 residues in a six-coordinate arrangement, showing the possibility of replacing the water with another iron here. The induced conformational change upon Fe loading makes it possible for the imidazole nitrogen atoms to exert charge repulsion, thus facilitating the translocation of Fe ions through the channel (Cho et al. 2009). The ferroxidation catalyzed by Pfr is quite efficient, with K_m and k_{cat} values to be 32.31 mM and 0.49 s^{-1}, respectively (Cho et al. 2009). His149 contributes much to the catalytic efficiency as H149L mutant exhibits kinetic parameters of 24.37 mM and 0.18 s^{-1}, corresponding to 36.7% of the efficiency of the wild-type. The ferroxidation dominantly occurs in the apo and low-iron states, whereas the mineral-surface auto-oxidation is mainly in the intermediate and high-iron states (Cho et al. 2009). One mechanism of iron biomineralization in Pfr has been proposed (Cho et al. 2009): (1) Fe^{2+} ions are coordinated to the residues Glu17, Glu50, His53, Glu94, Gln127 and Glu130, and enter the ferroxidase center via a solvent molecule (Wat1, hydrogen-bound to Glu50 and Ser20), where they are oxidized to Fe^{3+}; (2) Fe^{3+} ions migrate to the inner nucleation site via Wat2 (hydrogen-bound to Glu130 and Glu49; the later one is further hydrogen bound to His53) by the cluster of glutamate residues (Glu49, Gln126 and Glu130); (3) iron nucleation takes place and ferrihydrite mineral cores are formed; and (4) iron oxidation changes from protein-dominated to mineral-surface-dominated and irons enter directly into the core where they are oxidized on the growing crystalline surface.

NapA, a neutrophil-activating protein in *H. pylori*, is able to mediate neutrophil adhesion to endothelial cells (Yoshida et al. 1993) and to bind to mucin, a highly glycosylated protein that is the main component of the mucous layer and to neutrophil glycophingolipids (Teneberg et al. 1997; Namavar et al. 1998). Sequence similarity comparison indicates that NapA is among the families of DNA-protecting proteins (Dps) and ferritin. This protein forms a 150 kDa dodecamer in a structure of hexagonal rings of 9–10 nm diameter with a hollow central core (Tonello et al. 1999) as seen in other Dps proteins, which is quite different from the 12 nm icositetrameric ferritins. On the other hand, NapA is similar to the ferritin proteins in the resistance to thermal and chemical denaturation (Tonello et al. 1999). The expression of NapA has been shown to be positively regulated by iron, repressed by Fur (Cooksley et al. 2003), and to be unaffected by copper, nickel or zinc (Dundon et al. 2001), suggesting a role for NapA in the iron homeostasis. Moreover, the expression is upregulated under acid conditions, e.g. ~2.4-fold at pH 4.5 compared with the neutral conditions (Wen et al. 2003). NapA is capable of iron binding (up to 500 Fe ions per oligomer), besides bismuth and nickel bindings (Tonello et al. 1999; Ge et al. 2007; Sun et al. 2008a). Similar to Pfr, the high capacity of iron binding of NapA is attributed to its ferroxidase activity: to bind and oxidize Fe^{2+} at the highly conserved inter-subunit ferroxidase center of the Dps family proteins and carry out ferroxidation by hydrogen peroxide (Chiancone et al. 2004), which is so efficient that the production of the toxic hydroxyl radicals through Fenton chemistry is drastically minimized (Stadtman and Berlett 1991). Besides as iron storage proteins, both Pfr and NapA have a DNA-binding/protection function under acidic environment (Ceci et al. 2007; Huang et al. 2010), indicating a significant urease-independent role in the acid adaption of *H. pylori* under physiological conditions (Table 1).

Iron regulation in *H. pylori*

Bacteria have developed complex strategies to maintain intracellular iron homeostasis: to satisfy its own requirement for the iron in a variety of metabolic functions and to prevent the toxic effects owing to the hydroxyl radicals produced by Fenton reaction in the presence of excessive iron (Stadtman and Berlett 1991). The major bacterial metal-dependent transcription regulator is known as ferric uptake regulator (Fur), a global regulator in the control of diverse functions such as acid tolerance, oxidative stress response, metabolic pathways and virulence factors (Andrews et al. 2003). The topic of this regulator has been extensively covered in some other excellent reviews, such as Carpenter et al. (2009), Danielli and Scarlato (2010), Wang et al. (2006), Fischer et al. (2009), and so on. This concise review intends to cover only some basic points of *H. pylori* Fur and the new

Table 1 Proposed iron trafficking proteins in *H. pylori* (Tomb et al. 1997)

Gene No.	Protein	Function
hp0687	FeoB	Iron(II) transport protein
hp1561	CeuE	Iron(III) ABC transporter, periplasmic iron-binding protein
hp1562	CeuE	Iron(III) ABC transporter, periplasmic iron-binding protein
hp0888	FecE	Iron(III) dicitrate ABC transporter, ATP-binding protein
hp0889	FecD	Iron(III) dicitrate ABC transporter, permease protein
hp0686	FecA	Iron(III) dicitrate transport protein
hp0807	FecA	Iron(III) dicitrate transport protein
hp1400	FecA	Iron(III) dicitrate transport protein
hp0653	Pfr	Non-heme iron-containing ferritin
hp1341	TonB	Siderophore-mediated iron transport protein
hp0243	NapA	Neutrophil activating protein (bacterioferritin)
hp0136	Bcp	Bacterioferritin comigratory protein
hp1129	ExbD	Biopolymer transport protein
hp1130	ExbB	Biopolymer transport protein
hp1339	ExbB	Biopolymer transport protein
hp1340	ExbD	Biopolymer transport protein
hp1445	ExbB	Biopolymer transport protein
hp1446	ExbD	Biopolymer transport protein
hp0876	FrpB	Iron-regulated outer membrane protein
hp1512	FrpB	Iron-regulated outer membrane protein
hp0915	FrpB	Iron-regulated outer membrane protein
hp0916	FrpB	Iron-regulated outer membrane protein
hp0318	HugZ	Heme oxygenase
hp0642		NAD(P)H-flavin oxidoreductase

findings about this metallo-regulator. Readers may refer to the above-mentioned reviews for further information.

Helicobacter pylori Fur (*Hp*Fur) is a protein of 17 kDa with 150 amino acids, and is able to complement an *E. coli fur* knockout mutant strain (Bereswill et al. 1998, 1999). *H. pylori* Fur assumes a homodimeric tertiary structure (Dian et al. 2011) and is able to form oligomers in the absence of target DNA (Vitale et al. 2009). Each monomer is composed of a DNA-binding domain (residues 1–92; containing a winged Helix–Turn–Helix motif, $\alpha2$–$\alpha4$ and $\beta1$–$\beta2$), a dimerization domain (98–105; containing three anti-parallel β-strands, $\beta3$, $\beta4$ and $\beta5$, and two α-helices, $\alpha5$ and $\alpha6$) and a short hinge region (93–97) (Dian et al. 2011). The dimerization of *Hp*Fur is mediated by $\beta5$–$\beta5$ interactions resulting in a six-stranded anti-parallel β-sheet between the two *Hp*Fur dimerization domains. Crystal structure indicates that *Hp*Fur contains three metal-binding sites: a structural zinc-binding site S1, a regulatory binding site S2 and a co-regulatory binding site S3 (Dian et al. 2011). The structural site S1 (tetra-coordination to two CxxC motifs) is required for the in vitro formation of *Hp*Fur dimers (Vitale et al. 2009), as zinc-binding in S1 helps to stabilize the $\beta3$–$\beta4$–$\beta5$ sheet. The metal-binding environment of S2 is different for the two subunits: tetrahedral (Oε1 of Glu90 and three Nε2 from His42, His97 and His99) in chain B and distorted octahedral (Oε1 and Oε2 of Glu90, Oε2 of Glu110 and three Nε2 from His42, His97 and His99) in chain A (Dian et al. 2011). Metallation of S2 triggers a conformational change in *Hp*Fur as S2 always contains a His from the loop between $\alpha2$ and $\alpha3$ in the DNA-binding domain. The differential geometries in the S2 sites suggest a flexible manner in the control of DNA binding. The S3 sites from both chains are similar in geometry: tetrahedral with the four ligands to be Oε2 of Glu117, Oδ2 of Asp98 and two Nε2 from His96 and

His134. This site is not essential for DNA binding; whereas metal binding in S3 affects the strength of *Hp*Fur response to excess metal. The flexible metal-binding and regulation properties of S2 and S3 sites may potentiate their coordination in a delicate way, albeit undiscovered yet, to determine the differential apo- and holo-Fur regulations as discussed below.

In the classical Fe^{2+}-Fur repression paradigm (Fig. 2), iron binding leads to an increased affinity of Fur to the AT-rich Fur-boxes. Fur binding to the Fur-boxes blocks the binding of RNA polymerase, thus resulting in transcriptional repression of target genes (Baichoo and Helmann 2002). The targets regulated in this way include genes involved in iron uptake and trafficking (Delany et al. 2001a, b; van Vliet et al. 2002; Danielli et al. 2009), such as *frpB* and *exbB*, and some other genes in acid resistance, like *amiE* (van Vliet et al. 2003). *Hp*Fur regulations go far beyond the classical paradigm, such as in the utilization of apo-Fur as a repressor, the so-called apo-Fur repression. The two well-known apo-Fur repression targets are *pfr* (Bereswill et al. 2000; Delany et al. 2001a, b) and *sodB* (Ernst et al. 2005). The *pfr* expression is repressed under iron-limited conditions and is constitutively expressed in a *fur* mutant (Bereswill et al. 2000). Apo-Fur has three binding regions in *pfr*, with the highest binding affinity for Fur in the transcriptional start site and the other two in the further upstream (Delany et al. 2001a, b). DNase I footprinting analysis and electrophoretic mobility shift assays (EMSA) indicated that there is one Fur binding region in *sodB* (Ernst et al. 2005), despite with little homology with the known iron-bound *Hp*Fur box and the *E. coli* consensus Fur binding sites (Delany et al. 2001a, b; Ernst et al. 2005).

For the Fur activation genes, apo- or holo-Fur binds far upstream from the transcriptional start site and facilitates transcription possible by enabling optional binding of RNA polymerase (Carpenter et al. 2009). The positive transcriptional regulation targets include proteins important in chemotaxis and motility. One example of positive regulation by iron-bound Fur is *nifS*, encoding a Fe–S cluster synthesis protein (Alamuri et al. 2006). Fur binds to the *nifs* promoter in the presence of the Fe^{2+} substitute Mn^{2+} by EMSA analysis, and *nifS* expression is upregulated in the presence of Fe^{2+}. Two Fur boxes for *nifS* are predicted to be located far upstream of the transcriptional start site. For some bacteria, Fur activation is indirect and mediated by the presence of small regulatory RNAs (sRNA) which acts post-transcriptionally on the decay and translation of target mRNAs (Massé et al. 2007; Gripenland et al. 2010). sRNAs are also present in *H. pylori* (Xiao et al. 2009a, b; Sharama et al. 2010). For example, two sRNA are complementary to *frpB* (encoding an iron-regulated outer membrane protein)

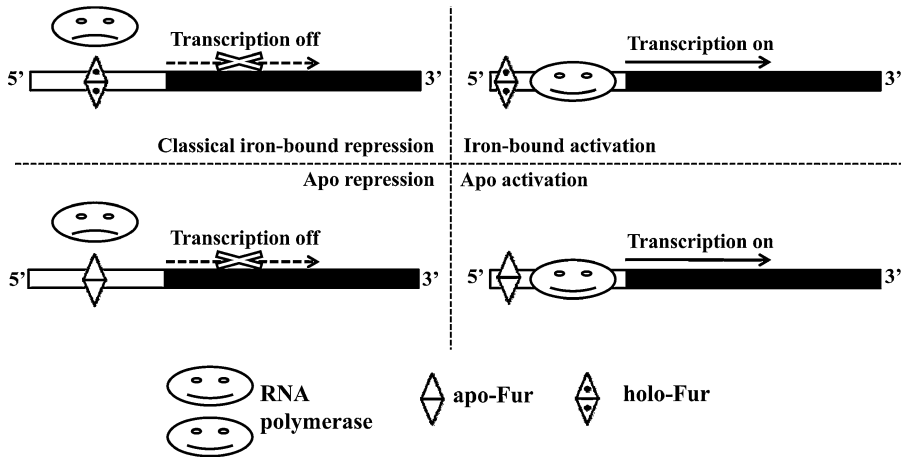

Fig. 2 A schematic overview of the functions of *Hp*Fur. In the classical iron-bound and apo repression, holo- or apo-Fur recognizes the Fur box consensus sequence upstream of the promoter region of the regulated genes and inhibits transcription by blocking the movement of RNA polymerase. In the positive regulation, holo- or apo-Fur recognizes the Fur box consensus sequence far upstream of the transcriptional start site and promotes transcription by increasing the binding affinity of RNA polymerase to the promoter region

and *ceuE* (a periplasmic iron-binding protein), both of which are regulation targets of Fur (Xiao et al. 2009a, b).

Conclusions and perspectives

Helicobacter pylori infections have been closely associated with various gastric diseases and IDA. As iron is essential for almost all living organisms, the investigation of the iron uptake and trafficking system is important to understand the pathological roles of *H. pylori* and the relevant proteins may be the future targets of antibiotics or other antibacterial treatments (Whitmire et al. 2007). The available information about iron uptake and regulation has been discussed in this concise review. Mechanisms for iron uptake should be further studied and evaluated, especially considering that most *H. pylori* are free-swimming, instead of fixed colonization in the stomach. This could contribute much to the better understanding of the association between *H. pylori* infections and IDA or gastric diseases.

Ferrous ion is diffused though the outer membrane by some kinds of porin proteins and transported through the cytoplasmic membrane by FeoB. It is not yet clear which reductase(s) is responsible for the reduction of ferric into ferrous ions in extracellular space. FrpB2 is capable of hemoglobin usage; whereas it is not known whether hemoglobin as a full molecule or the extracted heme group is transported into the cytoplasm of *H. pylori*. During the transportation by FrpB2, which system will provide the energy, an ATPase/GTPase domain in the FrpB2 or an independent system, such as the ExbBD-TonB? While there is a strong support of CagA and VacA in iron (transferrin) acquisition from the host, the mechanism is not known: what kinds of signal pathways and transporters are involved? The association between *H. pylori* infection and IDA is established, but how about the mechanism: does the bacterium directly remove iron from the host or is the severe inflammation the source of the iron loss? Therefore further work is needed to fully understand the iron trafficking system in *H. pylori*.

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