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Nickel-binding and accessory proteins facilitating Ni-enzyme maturation in *Helicobacter pylori*

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Abstract

Helicobacter pylori colonizes the human gastric mucosa and this can lead to chronic gastritis, peptic and duodenal ulcers, and even gastric cancers. The bacterium colonizes over one-half of the worlds population. Nickel plays a major role in the bacteriums colonization and persistence attributes as two nickel enzyme sinks obligately contain the metal. Urease accounts for up to 10% of the total cellular protein made and is required for initial colonization processes, and the hydrogen oxidizing hydrogenase provides the bacterium a high-energy substrate yielding low potential electrons for energy generation. A battery of accessory proteins are needed for maturation or activation of each of the apoen-zymes. These include Ni-chaperones and GTPas-es, some of which are unique to each Ni-enzyme and others that are individually required for maturation of both the Ni-enzymes. *H. pylori's* need for some conventional hydrogenase maturation proteins playing roles in urease maturation may have to do with the poor nickel-sequestering ability of the UreE urease maturation protein compared to other systems. *H. pylori* also possesses a NixA nickel specific permease, a nickel dependent regulator (NikR), a recently identified nickel efflux system (CznABC), and a histidine-rich heat shock protein, HspA. Based on mutant analysis approaches all these proteins have roles in nickel homeostasis, in urease expression, and in host colonization. The His-rich putative nickel storage proteins Hpn and Hpn-like play roles in nickel detoxification and may influence the levels of Ni-activated urease that can be achieved.

Keywords

Nickel enzyme; Hyp protein; Hydrogenase; Urease; Gastric colonization; Ulcer; Gastric cancer; Nickel storage; Nickel regualtion; Histidine-rich

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Helicobacter pylori is a gram negative, motile, microaerophilic pathogen that colonizes the gastric mucosa of humans and causes chronic gastritis, peptic and duodenal ulcers, and even gastric cancers (Blaser 1990; Marshall and Warren 1984; Parsonnet et al. 1991; Parsonnet 1994; Parsonnet et al. 1994). The key aspect of its pathogenesis is its persistent nature (Israel and Peek 2006). The bacterium is characterized by its genetic diversity and its stringent adaptation to the human stomach, which includes ability to buffer the gastric acidity and adept motility attributes permitting transit of the stomach gastric contents or moving within the viscous mucous environment, respectively. Other important (virulence) characteristics are its ability to combat host-mediated oxidative stress and release of adhesions and cytotoxins that help maintain bacterial persistence (Mobley et al. 2001). *Helicobacter pylori* produces two final nickel sinks; they are the Ni-enzymes urease and hydrogenase (Evans et al. 1991; Maier et al. 1996). Much of the bacterium's metal metabolism is apparently centered on expression and maturation of these two enzymes. Urease accounts for up to 10% of the total cellular protein

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made and is essential for colonization and virulence (Bauerfeind et al. 1997; Hu and Mobley 1990; Tsuda et al. 1994a; Tsuda et al. 1994b), while the hydrogen utilizing hydrogenase provides the bacterium a compact and high energy non-carbon substrate for respiratory based energy generation (Maier et al. 1996; Olson and Maier 2002). In addition to these nickel enzymes, *H. pylori* also possesses a NixA nickel specific permease (Bauerfeind et al. 1996), accessory proteins UreIEFGH and HypABCDEF (some of which bind nickel) required for maturation or activation of the apoenzymes for the two nickel enzymes (Benoit et al. 2004; Mehta et al. 2003a; Olson et al. 2001; Voland et al. 2003), a nickel dependent regulator, NikR (Contreras et al. 2003; van Vliet et al. 2002), a recently identified nickel efflux system (CznABC) (Stahler et al. 2006) and a histidine-rich heat shock protein, HspA, a GroES homologue (Kansau et al. 1996; Kansau and Labigne 1996). Here we address the roles and characteristics of these proteins, with emphasis on the roles of the accessory proteins directly required for Ni-enzyme maturation, and on the His-rich putative nickel storage proteins Hpn and Hpn-like. *Helicobacter pylori* NikR is the subject of another manuscript (A. van Vliet) in this issue and will therefore not be covered here.

NixA, the Nickel-specific permease

The *nixA* gene was first isolated in 1995 (as a gene enhancing urease activity) from a plasmid library composed of the *H. pylori* chromosome. It enhanced the Ni-enzyme activity when introduced into an *E. coli* strain carrying the urease structural genes (Mobley et al. 1995). Disruption of *nixA* in *H. pylori* led to a partial reduction of urease activity (up to 50% in some strains) (Bauerfeind et al. 1996; Nolan et al. 2002) which suggests the presence of additional nickel transporters in *H. pylori* yet to be characterized (Davis and Mobley 2005). Nevertheless, *nixA* mutants in the mouse-adapted strain SS1 failed to colonize the gastric mucosa, hence showing that NixA is required and that nickel uptake is of critical importance for the cell survival in the host (Nolan et al. 2002). NixA protein has been thoroughly characterized. It is an integral cytoplasmic membrane protein of approximately 37 kDa which belongs to the nickel-cobalt transporter family, found in a variety of microorganisms (Eitinger and Mandrand-Berthelot 2000; Saier et al. 1999). Members of this family usually have eight trans-membrane domains (Saier et al. 1999) and topology studies using *lacZ* and *phoA* gene fusions revealed this was indeed the case for *H. pylori* NixA (Fulkerson and Mobley 2000). Conserved Asp, Glu and His residues are located in the transmembrane domains and appear to be critical for nickel transport (Fulkerson et al. 1998); in addition, two essential domains have been characterized within helix II (GXXHAXDADH) and helix III (GX2FXXGHSSVV) of NixA (Fulkerson and Mobley 2000). Furthermore, other amino acids, including some with low in vitro affinity to nickel, have been shown to be also important for the function of NixA (Wolfram and Bauerfeind 2002). NixA has a high affinity for nickel, with an estimated Kd of 11.3 nM (Mobley et al. 1995), thus enabling *H. pylori* to efficiently scavenge the low concentrations of nickel found in the human body, which are estimated to be in the range of 2–11 nM (Sundermann 1993). Since high concentrations of nickel would be toxic to *H. pylori*, it is noteworthy that synthesis of NixA is repressed by nickel; this process is mediated by NikR (van Vliet et al. 2004; Wolfram et al. 2006).

The CznA BC metal efflux pump

A novel efflux pump with metal binding capacity and that influences urease activity in *H. pylori* was recently described (Stahler et al. 2006). The unique feature of the metal export apparatus is CznC, a nickel binding efflux protein. Mutations individually in any of the *czn A, B*, or *C* genes rendered the pathogen ineffective in animal stomach colonization, underscoring the importance of metal homeostasis to virulence. While the structural organization of the Czn complex is probably like the Czc-type trans-envelope transporters of *Ralstonia* (and some other bacteria) that play key roles in conferring metal resistance (Nies 2003), the *H. pylori* efflux

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pump was shown to have a unique physiological role, that is modulation of urease activity (Stahler et al. 2006). Urease activity was significantly enhanced in the *cznC* and *cznA* mutants, and the CznC protein was assigned a role of particular importance to nickel export. Recombinant forms of both CznC and CznB could bind nickel. The individual *cznA*, *cznB*, and *cznC* mutants were all more sensitive to cadmium, nickel, and zinc than the parent strain. It is proposed that urease activity is modulated by nickel efflux via CznABC to reduce Ni-activated urease levels, and that efflux of other metals such as cadmium and zinc prevents urease inhibition. The model is strengthened by the observations that considerable apourease (devoid of nickel) is oftentimes observed in *H. pylori*, so that intracellular nickel levels would be expected to rapidly affect urease activity via enzyme activation/inactivation (Stingl and De Reuse 2005). An example of the importance of metal cation discrimination to *H. pylori* physiology is highlighted by the unique properties described for this Czn pump complex.

Accessory proteins for hydrogenase maturation

Helicobacter pylori contains all the Hyp accessory proteins necessary for maturation of NiFe hydrogenases. These are HypABCDEF. While the genes for these are clustered together in one operon in most H_2 utilizing bacteria, they are instead separated into three different chromosomal locations on the *H. pylori* genome; *hypA* alone, *hypBCD* together, and *hypEF* together. In addition, the structural genes *hydABCD* are located at yet another area of the *H. pylori* chromosome. The most informative system for identifying the roles of these accessory proteins has been *Escherichia coli*, where the accessory proteins play roles in maturation of all three NiFe hydrogenases (the term hyp is derived from hydrogenase pleiotropic) and the currently-assigned roles are based primarily on the work of A.Böck and colleagues (Blokesch and Bock 2002; Blokesch et al. 2004a; Hube et al. 2002; Reissmann et al.2003). HypA (also known as HybF) is a Zn-containing protein (Atanassova and Zamble 2005)) but also binds stoichiometric amounts of nickel, and HypB is a GTPase (Blokesch et al. 2004a; Leach et al. 2005). The present model is that HypA serves as a nickel chaperone, and HypB as a regulator that thermodynamically controls the donation of the metal to the hydrogenase apoprotein or release of the nickel-free chaperone (Blokesch et al. 2004c; Leach et al. 2005; Reissmann et al. 2003). The present model for HypA/HypB function (Leach et al. 2005) involves a key metal binding site in HypB beyond the usual (Ni-binding) polyhistidine stretch. This high affinity site would serve as the nickel donor for hydrogenase. HypA would direct Ni(II)-bound HypB to the iron-loaded site of the hydrogenase large subunit, and facilitate the metal transfer. The model takes into account the weaker Ni-binding ability of HypA (compared to HypB), as important in facilitating the Ni-transfer step (Leach et al. 2005). The authors also suggested a feasible role for the low-affinity metal binding site of HypB; it may detect the nickel status of the enzyme in order to sense when to activate the GTPase. Such an activation step upon sensing proper nickel loading would be of benefit, as HypB's have notoriously sluggish GTPase activities when tested in vitro.

Two other Hyp proteins, HypF and HypE are involved in the synthesis of the cyanide ligand of the Fe metal center of hydrogenase (Reissmann et al. 2003). For this role, HypF is a carbamoyl-transferase, carbamoylating the HypE protein using carbamoyphosphate as substrate. The thio-cyanate product comes from an ATP-dependent dehydratase activity of HypE (Blokesch et al. 2004b). The origin of the other diatomic ligand (carbon monoxide) attached to the Fe center is not known. A HypC-HypD complex is likely involved in accepting the Fe-cyanide ligand and transferring the fully liganded Fe to the large subunit of hydrogenase. These maturation steps involve dynamic complexes between these accessory proteins and sometimes between these accessory proteins and the hydrogenase subunits (Blokesch et al. 2004a; Blokesch et al. 2004b). There is no reason to believe that the hydrogenase maturation steps performed by these proteins are different in *H. pylori*. As for mutants studied in other H_2 oxidizing bacteria, mutant strains in each of the *H. pylori hyp* genes yielded strains with a

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hydrogenase deficiency phenotype (Benoit et al. 2004; Mehta et al. 2003a; Olson et al. 2001). Similarly, the deficiency could be connected to nickel, as nickel supplementation to the medium of mutant strains partially restored the hydrogenase activity.

Accessory proteins for urease maturation

The gene products of the *ureIEFGH* operon, located downstream of the *ureAB* urease structural genes, are required for the production of active urease. Indeed, coexpression of the *ureI*, *ureE*, *ureF*, *ureG* and *ureH* genes was shown to be required along with structural genes *ureA* and *ureB* to yield fully active urease in *E. coli* (Cussac et al. 1992). More recently, Voland and coworkers have shown that *H. pylori ureE*, *ureF*, *ureG* and *ureH* mutants are severely deficient in urease activity (Voland et al. 2003). Among these four genes, the roles of *ureE* and *ureG* have drawn the most attention. While addition of excess nickel can restore urease activity in *hypA* or *hypB* mutants (see below), this is not the case for either the *ureE* or the *ureG* mutant (Benoit, Mehta Maier, unpublished data) which indicates that these genes are absolutely required for urease activity under any circumstance. However the UreE and the UreG protein do not appear to be involved in hydrogenase maturation, since mutants in either gene have hydrogenase activities comparable to those of wild type cells (Benoit and Maier 2003; Mehta et al. 2003a).

Helicobacter pylori UreE (19.4 kDa) is found as a dimer in solution and equilibrium dialysis studies show each dimer is able to bind only 1 Ni^{2+} ion (Benoit and Maier 2003). The protein contains one conserved His residue (His102), shown to be involved in nickel binding in other well studied UreE proteins, including *Klebsiella aerogenes* UreE (residue His96) (Colpas et al. 1999; Song et al. 2001) or *Bacillus pasteurii* UreE (residue His100) (Stola et al. 2006). By adding several His residues (His6-tag or His-rich tail of *K. aerogenes* UreE) to the *C*-terminus of the *H. pylori* UreE, it is possible to artificially increase the nickel binding capacity of the protein (as confirmed in vitro with purified proteins); introduction of any one of a number of engineered versions into the chromosome of mutants deficient in nickel sequestration led to an increase of urease activity. It was concluded that there is a correlation between the nickel-sequestering ability of UreE and the nickel activation steps yielding urease activity in *H. pylori* (Benoit and Maier 2003).

UreG (22 kDa) is thought to play a role in the urease maturation process as a GTPase (Mehta et al. 2003a); GTP hydrolysis is apparently required for a nickel transfer or a protein-protein interaction/release step. Although purified UreG showed negligible GTPase activity, the protein contains a conserved nucleotide-binding domain (GSGKT) known as a “P-loop” motif, and site-directed mutation in this conserved domain (Lys14 to Ala14) abolished the urease activity in *H. pylori* (Mehta et al. 2003a). The assignment of UreG as a GTPase required for the urease activation, as well as the critical role played by the P-loop motif have been reported in *K. aerogenes* (Moncrief and Hausinger 1997) or *B. pasteurii* (Zambelli et al. 2005).

Although UreF (28.6 kDa) and UreH (29.7 kDa) have not yet been characterized in *H. pylori*, their role as chaperone proteins can be anticipated by observation of their homologs in *K. aerogenes*. In this bacterium, UreD (homologous to *H. pylori* UreH) binds to apourease, thus inducing a conformational change required before the next steps (Park et al. 1994), which are (i) insertion of UreF to make a UreD-UreF apourease complex (Moncrief and Hausinger 1996) (ii) addition of UreG to yield a UreD-UreF-UreG apourease complex (Park and Hausinger 1995; Soriano and Hausinger 1999) and (iii) involvement of UreE with this complex (Soriano et al. 2000). This is in good agreement with a recent study from Park and coworkers, which suggests that the products of plasmid-borne *H. pylori ureFGH* genes expressed in *E. coli* cooperate together before interacting with UreE and UreI (Park et al. 2005).

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UreI (21.7 kDa) is a proton-gated inner membrane protein involved in urea transport as well as acid resistance (Rektorschek et al. 2000; Skouloubris et al. 1998). UreI possesses six membrane-spanning segments (Weeks et al. 2000) and conserved histidine residues, including His123, are essential for acid activation of the channel (Bury-Mone et al. 2001; Weeks et al. 2000; Weeks and Sachs 2001). The UreI protein is required for survival of the bacterium in the mouse or the gerbil gastric mucosa (Skouloubris et al. 1998) (Mollenhauer-Rektorschek et al. 2002).

Direct interactions among *H. pylori* urease accessory proteins have been reported, including UreF-UreH and UreG-UreE interactions, as well as interactions between the urease catalytic subunits UreA/B and UreI (Voland et al. 2003).

Accessory proteins common to both Ni-enzyme maturation systems

Helicobacter pylori has all the known accessory proteins needed for the individual maturation of hydrogenase and urease. Nevertheless, when mutant strains in each of the hydrogenase genes were assayed for urease activity an unexpected phenotype was obtained for two mutants, *hypA* or *hypB* (see table 1). These two strains were almost devoid of urease activity, and full urease activity could be achieved by supplementing the cultures with 5 μM Ni (Olson et al. 2001) The mutant strains synthesized normal levels of urease apoproteins, but the urease pool was lacking in nickel content (Olson et al. 2001). Also, polar affects of the mutation were ruled out as the cause of this phenotype as chromosomal complementation of the *hypA* strain (to create strain hypA:kanHA) with the wild type version of the gene restored both hydrogenase and urease activities (see table 1); complementation of the *hypB* strain was unnecessary as the next open reading frame was *hypC*, and a *hypC* mutant was normal in urease activity. It seems that *H. pylori* is unique in that the two Hyp proteins are involved in maturation of both Ni-enzymes. Gene targeted mutant strains in *HypA* and *HypB* in which key residues were targeted (i.e. the Ni-binding His2 for HypA, and the GTP binding Lys59 of HypB) resulted in the conclusion that these functions (Ni binding and GTPase, respectively) are involved in the Ni-dependent maturation of both hydrogenase and urease (Mehta et al. 2003a,b). *H. pylori* HypA and HypB interact with each other in a 1:1 molar ratio based on cross-linking and immunoblotting approaches, and this interaction did not depend on added nickel or GTP. *H. pylori* HypA and HypB must have the unique ability to recognize Ni-dependent maturation machinery for two different Ni-enzymes, and these two Ni-enzymes have completely different nickel centers. It therefore seems likely that HypA/B would interact with other accessory proteins at relatively early steps in the sequential Ni-enzyme maturation process. Preliminary studies involving mixing of pure *H. pylori* Ure accessory proteins and HypA/B, then capturing the intimate contacts by crosslinking have indicated that HypA can recognize accessory proteins from the heterologous system (Benoit, Mehta, Maier, unpublished data). Most likely, the HypA/B complex is able to donate/mobilize Ni to a UreE/G complex to facilitate Ni donation ultimately to urease.

The *Helicobacter pylori* Ni-enzyme maturation summary is shown in Fig. 1. It relies heavily on the best-studied systems *E. coli* (for hydrogenase) and *K. aerogenes* (for urease) for function of many components, but also incorporates the unique roles of hypA/B, and their preliminarily-defined interactions with UreE. The reason for the additional complexity in maturation components (use HypA/B when all the other Ure accessory components should suffice) required for *H. pylori* urease maturation may be related to the high nickel demand of the gastric pathogen for abundant urease expression. While *K. aerogenes* UreE can bind 5–6 nickel ions per dimer, the *H. pylori* UreE dimer can bind only one (see table 2). At the same time the Ni-enzyme (urease) that depends on UreE for nickel is present as only about 0.1% of the total cell protein (*K. aerogenes*) compared to up to 10% (*H. pylori*) (see table 2 legend). It seems likely that *H. pylori* may have incorporated an already needed (for hydrogenase) Ni-sequestering

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protein to aid its nickel sequestering capacity for urease maturation, as its UreE is relatively poor in total Ni-binding capacity. In support of this, it was observed that introduction of engineered versions of UreE containing additional His residues into *H. pylori hypA* or *hypB* strains resulted in 5–10 fold greater urease activities than the mutant strains (see accessory proteins for urease maturation section above).

The Ni-binding chaperone-homologue HspA

An interesting *H. pylori* heat-shock protein in the GroES family was characterized (Kansau et al. 1996; Kansau and Labigne 1996). The *N*-terminal portion is homologous to other GroES proteins, but HspA uniquely contains 27 additional residues at the His-rich *C*-terminus that are involved in nickel binding. The *C*-terminus is rich in His residues. Among a series of divalent cations tested, HspA showed the greatest specificity and affinity for nickel; it bound about two nickel ions per HspA molecule and with a Kd of 1.8 μM. Another Ni-binding domain but with lower metal affinity was identified by use of nickel levels above 30 μM. With its two identified and distinct functional domains, HspA is proposed to play a role as a nickel carrier (domain B), and as a classical GroES chaperonin (the A domain). Among many clinical isolates tested, the A domain is highly conserved, whereas the B domain encompasses two variant type sequences. The different domains elicit distinct host immunological responses (Kansau et al. 1996). HspA may play a role in urease maturation as expression of HspA in *E. coli* cells containing urease-encoding genes resulted in a fourfold increase in urease activity. It would be interesting to assess the urease expression with truncated (domain-specific) or site-changed versions of the HspA.

Hpn and Hpn-like proteins

An *H. pylori* protein rich in histidine residues (47% histidine residues, TIGR annotation HP1427, see Fig. 2) was identified almost 10 years ago. It was named 'Hpn' because it was first identified in *H. p ylori* and had affinity for nickel ions (Gilbert et al. 1995). Hpn is also present in the ferret and cat gastric *Helicobacter* pathogens, but it is absent from the mouse (liver) pathogen *H. hepaticus*. *H. pylori* mutants lacking *hpn* were more sensitive to nickel and bismuth than the parent strain, but a *nixA* strain was not more sensitive; this indicates that Hpn may sequester metals that accumulate internally via a passive equilibrium mechanism (from a high external metals environment) (Mobley et al. 1999). As *nixA* itself is regulated by nickel levels (through NikR) , it would seem to be of benefit to *H. pylori* to have Hpn proteins available to detoxify rapid fluxes in metal levels. Recombinant Hpn was recently purified, characterized and analyzed for metal content; it exists mainly as a range of multimeric forms in solution. In the presence of Ni^{2+} and imidazole, a high molecular weight aggregate (> 500 kDa) of Hpn is observed, but with addition of DTT, the amount of the high MW form decreases greatly and the predominant form is a 136 kDa species. This size corresponds to a 20-mer, and it was suggested that this is the physiologically relevant form in vivo (Ge et al.2006). Still, the 136 kDa form is apparently in equilibrium with the other multimers; subjection of the pure (from gel filtration chromatography) 136 kDa form to gel filtration again yields not only the 136 kDa form but the other multimers observed in the original sample as well. Each Hpn monomer of 7 kDa reversibly binds 5 nickel ions at pH of 7.4. Although it is presumed the bulk of the nickel sequestering occurs via the multiple imidazole groups, Ni^{2+} -bound Hpn was very low in free thiolates, indicating the four Cys residues also take part in Ni-binding. The moderate Kd of 7.1 μM for nickel means the protein could facilitate nickel transfer to *H. pylori* Ni-chaperones with lower Kd's, such as HypA (Kd of 1.3 μM) or HspA (Kd of 1.8 μM). Such an intimate interaction and perhaps Ni transfer steps could be investigated by cross-linking approaches.

Nickel can be released from the Hpn protein by decreasing the pH or by adding nickel chelating agents such as EDTA (Ge et al. 2006). The former observation may be highly relevant to *H.*

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pylori nickel physiology and Ni-enzyme expression, as the bacterium can survive a wide pH range and can alter its pH environment. Also, if Hpn serves a (nickel) storage role it would be expected to use a facile mechanism for release of stored nickel. Nickel levels in *E. coli* BL21 cells expressing *H. pylori* Hpn from an inducible plasmid was higher than in uninduced cells or those lacking the (pET-hpn) plasmid (Ge et al. 2006). The level of nickel in the *H. pylori* strain 26695 cytoplasm was observed to be slightly higher than in an *hpn* deletion mutant. All the above is consistent with a proposed Ni-storage and detoxification role for the His-rich protein. Additionally, Hpn represents 2% of the total protein of *H. pylori* 26695, so it would be expected to play a large role in the cells nickel budget and Ni-homeostasis. No differences in the urease activities in the *hpn* mutant compared to the wild-type were reported in the two published studies in which *hpn* mutants were described (Ge et al. 2006; Gilbert et al. 1995).

Another *H. pylori* His-rich protein is annotated as histidine and glutamine rich protein (HP1432, see Fig 2). The *N*-terminal sequence (46 residues) of HP1432 shows 56% identity to Hpn and the protein was thus termed Hpn-like. It has not been purified, but has been preliminarily studied by a targeted mutagenesis approach (Seshadri and Maier, abstract D21, 105th general meeting ASM, 2005). Sequence analysis indicates that 25% of the amino acids are histidine residues including a stretch of six consecutive histidine residues while 30 of the 72 amino acid residues are glutamine residues (42%) (Fig. 2). The His residues can predictably be assigned metal binding roles, but the role of the glutamines is a mystery. Additionally, Hpn-like has an Met-Ala-His motif at the *N*-terminus which is similar to Asp-Ala-His motif involved in nickel binding in human serum albumin (Callan and Sunderman 1973;Gilbert et al. 1995;Tabata and Sarkar 1992).

Hpn-like is upregulated at pH 5.0 (compared to pH 7.0) by the two-component system ArsRS (HP166–HP165) (Bury-Mone et al. 2004; Pflock et al. 2004; Pflock et al. 2006). Both Hpn and Hpn-like are activated in the presence of nickel by the nickel sensor NikR (Contreras et al. 2003).The regulation studies thus far suggest that the Hpn proteins would be expressed at a time when nickel detoxification and/or nickel storage would be an advantage. To further explore the roles of these (*hpn* and *hpn-like*) genes, individual gene-disruption mutant strains were created, and a double mutant lacking genes for both these histidine rich proteins was also studied (Seshadri, Benoit, Maier, unpublished data, ASM Abstract 2005). Previous results showed that an *hpn* mutant is more sensitive to nickel than is wild-type (Mobley et al. 1999). Recent preliminary studies (S. Seshadri and R. Maier, abstract D21, 105th general meeting ASM, 2005) indicate the Hpn-like protein also may play a role in nickel detoxification. While Hpn is a predominant protein in *H. pylori*, we have no information on the relative amount of Hpn-like that is synthesized.

Only a minor percentage of *H. pylori* urease is Ni-activated (Stingl and De Reuse, 2005) and that nickel can be a factor sometimes limiting urease activity rather than activity being limited by the amount of urease protein (van Vliet et al. 2001). It was observed that addition of just 1 μM nickel to the growth medium increased the urease activities but did not cause an increase in urease expression levels (van Vliet et al. 2002) and disrupting *H. pylori* genes encoding a metal efflux pump (*cznABC*) led to an increase in urease activity (Stahler et al. 2006), presumably by making cytosolic nickel available.

H. pylori apparently synthesizes more urease apo-enzyme than it can (Ni) activate in some lab conditions, but such low nickel levels would also be expected in the host environment. In some low Ni conditions the Hpn and Hpn-like mutant strains contain significantly more urease activity than the parent strain (Seshadri and Maier, abstract D21, 105th general meeting ASM, 2005). It seems likely that these Hpn proteins compete with Ni-dependent urease maturation enzymes in nickel deficient conditions. This may not be a negative situation for *H. pylori* even though it relies on active urease to transit the stomach; the successful gastric pathogen would

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be expected to accomplish transit rapidly and spend the vast majority of its life residing in the mucin layer or adjacent to gastric epithelia in a highly nickel-restricted environment. As such the bacterium would need to conserve its scarce nickel reserves for H_2 oxidation (Maier et al. 1996) or for later stage urease production (Tanahashi et al. 2000). In addition, synthesis of apo-urease (lacking metal, but still extruded from the cell) may have advantages to *H pylori* in vivo existence. For example, non-ureolytic (inactive) urease has been shown to play roles in pathogenesis and (for *H. pylori*) to significantly alter inflammatory responses of gastric cells (Suzuki et al. 2002; Tanahashi et al. 2000). It is clear we have much to learn about the roles of *H. pylori's* nickel metabolism that allows the bacterium overwhelming success in colonizing and persisting in the human stomach.

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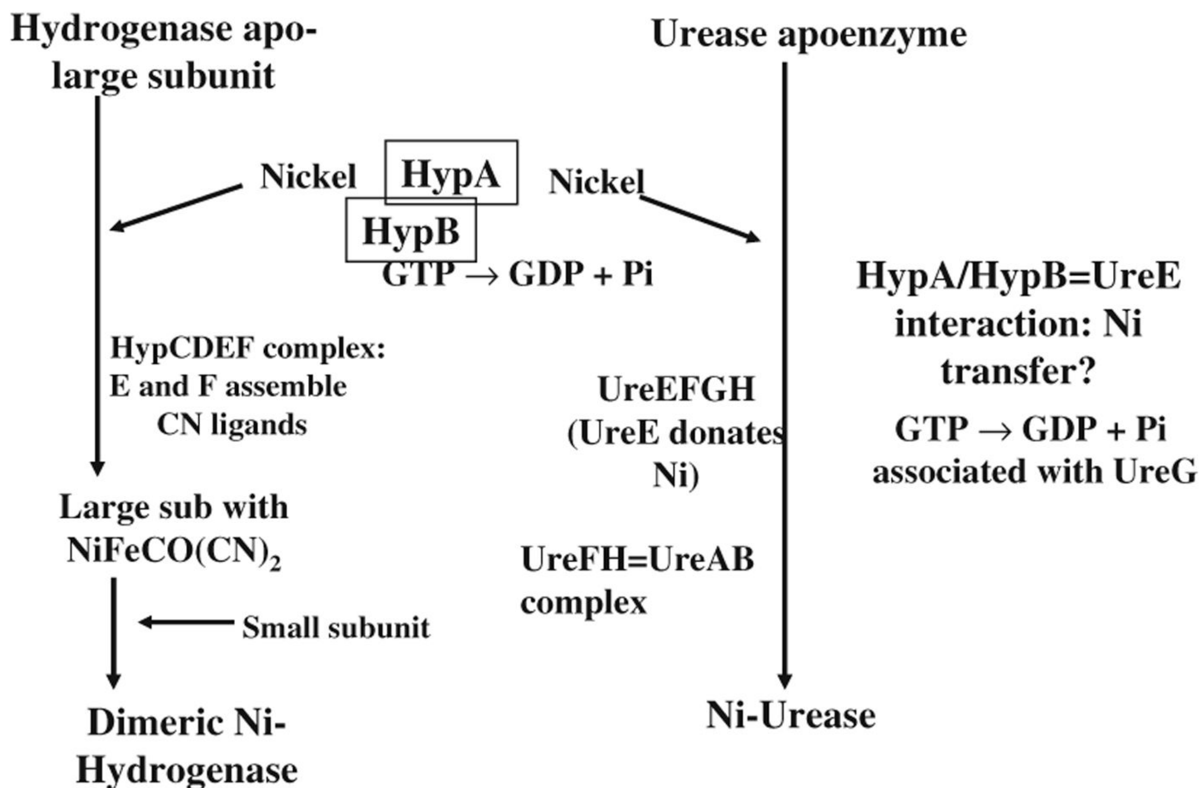

Fig. 1.
Helicobacter Ni-Enzyme Maturation

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Hpn

HP1427, JHP1320; Histidine (H) rich, metal binding protein; proposed storage role (Ge et al, Biochem J. 2006)

MAHHEEQHGGHHHHHHHTHHHHYHGGEHHHHHHSSHHEEGC
CSTSDSHHQEEGCCHGHHE

Hpn-like

HP1432, JHP1321; Histidine (H) and glutamine (Q) rich polypeptide

MAHHEQQQQQANSQHHHHHHAHHHHYYGGEHHHHNAQQHA
EQQAEQQAQQQQQQQAHQQQQQKAQQQNQQY

Fig. 2.
Histidine rich proteins of *H. pylori*

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Table 1

Ni-enzyme activities of *Helicobacter pylori* accessory protein mutants

Strain	Hydrogenase activity[a]	Urease activity[b]
Wild type	1.4 ± 0.3	64 ± 23
HypA	<0.01	0.3 ± 0.1
HypB	<0.01	1.6 ± 0.5
HypC	<0.01	62 ± 19
HypD	<0.01	71 ± 11
HypE	<0.01	76 ± 17
HypF	<0.01	78 ± 14
hypA:kanHA	1.2 ± 0.3	62 ± 8

[a] nmol H_2 oxid per min per 10^8 cells

[b] μmole urea hydrolyzed per min per mg protein

Urease activity of *hypA* and *hypB strains* was fully restored by adding 5 μM nickel. Data is from Olson et al. 2001, Mehta et al. 2003a and Benoit et al. 2004

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Table 2

Comparisons of UreE from two sources

Bacterium	Ni-binding residues	Ni-binding abilities (per homodimeter)	Urease made[a]
K. aerogenes	H-96, H-110, H-112	5–6	0.1%
H. pylori	H-102	1	10%

[a]percent of total cell protein, for Ka from R. Hausinger (pers. commun); for Hp, from Bauer and Mobley (1997).