Adv Exp Med Biol - Advances in Microbiology, Infectious Diseases and Public Health (2019) 1149: 57–75
https://doi.org/10.1007/5584_2019_359

Published online: 24 April 2019

Roles of Adhesion to Epithelial Cells in Gastric Colonization by *Helicobacter pylori*

Daniel A. Bonsor and Eric J. Sundberg

Abstract

Helicobacter pylori adherence to host epithelial cells is essential for its survival against the harsh conditions of the stomach and for successful colonization. Adherence of *H. pylori* is achieved through several related families of outer membrane proteins and proteins of a type IV secretion system (T4SS), which bridge *H. pylori* to host cells through protein-protein and other protein-ligand interactions. Local environmental conditions such as cell type, available host cell surface proteins and/or ligands, as well as responses by the host immune system force *H. pylori* to alter expression of these proteins to adapt quickly to the local environment in order to colonize and survive. Some of these host-pathogen interactions appear to function in a "catch-and-release" manner, regulated by reversible binding at varying pH and allowing *H. pylori* to detach itself from cells or debris sloughed off the gastric epithelial lining in order to return for subsequent productive interactions. Other interactions between bacterial adhesin proteins and host adhesion molecules, however, appear to function as a committed step in certain pathogenic processes, such as translocation of the CagA oncoprotein through the *H. pylori* T4SS and into host gastric epithelial cells. Understanding these adhesion interactions is critical for devising new therapeutic strategies, as they are responsible for the earliest stage of infection and its maintenance. This review will discuss the expression and regulation of several outer membrane proteins and CagL, how they engage their known host cell protein/ligand targets, and their effects on clinical outcome.

Keywords

Adhesion · Adhesin · Protein · Blood group antigen · Structure

D. A. Bonsor
Institute of Human Virology, University of Maryland School of Medicine, University of Maryland, Baltimore, MD, USA
e-mail: dbonsor@som.umaryland.edu

E. J. Sundberg (✉)
Institute of Human Virology, University of Maryland School of Medicine, University of Maryland, Baltimore, MD, USA

Department of Medicine, University of Maryland School of Medicine, University of Maryland, Baltimore, MD, USA

Department of Microbiology and Immunology, University of Maryland School of Medicine, University of Maryland, Baltimore, MD, USA
e-mail: esundberg@ihv.umaryland.edu

1 Introduction

Bacterial colonization of the stomach is fraught with danger. The constant production of hydrochloric acid and the resulting low pH, churning of the stomach, and the rapid turnover

of epithelial cells (on the order of every 2–3 days) makes the stomach an inhospitable place (Belanger and Leblond 1946; Lee 1985; Schreiber et al. 2004). However, *H. pylori* has evolved mechanisms to neutralize the acid (Langenberg et al. 1984; Mobley 1996) and to move towards the epithelial cell surface through the mucosal barrier (Beier et al. 1997; Croxen et al. 2006; Keilberg and Ottemann 2016) that protects the stomach lining, where the nearly neutral pH provides a much more tolerable environment. Once encountering the gastric epithelial cell layer, *H. pylori* must anchor themselves to host cell plasma membranes in order to prevent moving back into the lumen and expulsion from the stomach. Bacterial survival depends on these mechanisms of host cell adherence.

Once *H. pylori* bacteria have adhered to host cells, they will remain within the stomach and the host will remain asymptomatic in approximately 80% of infected individuals (Blaser et al. 1995; Israel et al. 2001; Parsonnet et al. 1997). However, the remaining 20% will go on to develop gastritis, peptic ulcer disease (PUD), mucosa-associated lymphoid tissue (MALT) lymphoma and/or gastric cancer (GC) during their lifetimes (Blaser et al. 1995; Israel et al. 2001; Parsonnet et al. 1997). The more virulent strains of *H. pylori* that can translocate the CagA protein are strongly associated with these diseases (Censini et al. 1996; Parsonnet et al. 1997). CagA is an oncoprotein that interferes with cell signaling pathways through its interactions with host factors such as E-cadherin, CRK, CSK, PAR-1, SHP-2, GRB-2 and ASPP-2 (Buti et al. 2011; Lu et al. 2008; Mimuro et al. 2002; Murata-Kamiya et al. 2007; Segal et al. 1999; Selbach et al. 2009; Tegtmeyer et al. 2011; Tsutsumi et al. 2003; Zhang et al. 2015), thereby perturbing cytoskeletal organization, motility, proliferation, cell-cell contact, mitogenic gene expression and apoptosis (compare Chap. 3 of this book). CagA is encoded by the cytotoxic-associated gene pathogenicity island (*cag*PAI), a region of the *H. pylori* genome that contains ~30 genes which encode for a T4SS that delivers CagA into host cells (Censini et al. 1996; Backert et al. 2015). CagL, another member of the *cag*PAI, forms part of the T4SS injection pilus and aids in adherence of *H. pylori* to host cells and the successful translocation of CagA (Posselt et al. 2013).

To achieve adherence, the *H. pylori* genome contains over 60 outer membrane protein (OMP) genes which can be divided into five paralogous gene families (Alm et al. 2000). The largest family consists of the Hop (*H. pylori* OMP) and Hor (Hop-related) genes, which encode 33 proteins. The second family is Hof (*Helicobacter* related) with eight genes, whilst the third, Hom (*Helicobacter* outer membrane) is the smallest with four genes (Alm et al. 2000). The remaining OMPs are contained within the iron-regulated and efflux pump OMP families. Driven by gene recombination and duplication, increase in mutational rate and the exchange of DNA between different strains (Didelot et al. 2013; Kennemann et al. 2011; Morelli et al. 2010), every genome of each strain of *H. pylori* differs in the OMPs that it possesses (Alm et al. 1999; Tomb et al. 1997). In addition to the genetic differences in the OMPs between strains, each strain also regulates expression of OMPs through several different mechanisms, including phase and allelic variation, gene conversion, gene duplication, and regulation in response to pH and salt. The combination of different OMPs and their regulation allows *H. pylori* to respond to the local environment in the stomach and host immune mechanisms in order to establish and maintain colonization (Kang and Blaser 2006; Odenbreit et al. 2009).

This review summarizes our current understanding of the bacterial adhesins, from both the OMP families and the *cag*PAI, that have been demonstrated to be involved in adherence of *H. pylori* as well as their pathogenic roles in the promotion of disease. An overview of adhesins and their known ligands is shown in Fig. 1.

2 OMP Domain Organization

To date, all *H. pylori* OMPs established as *bona fide* adhesins belong to the Hop, Hor and Hom

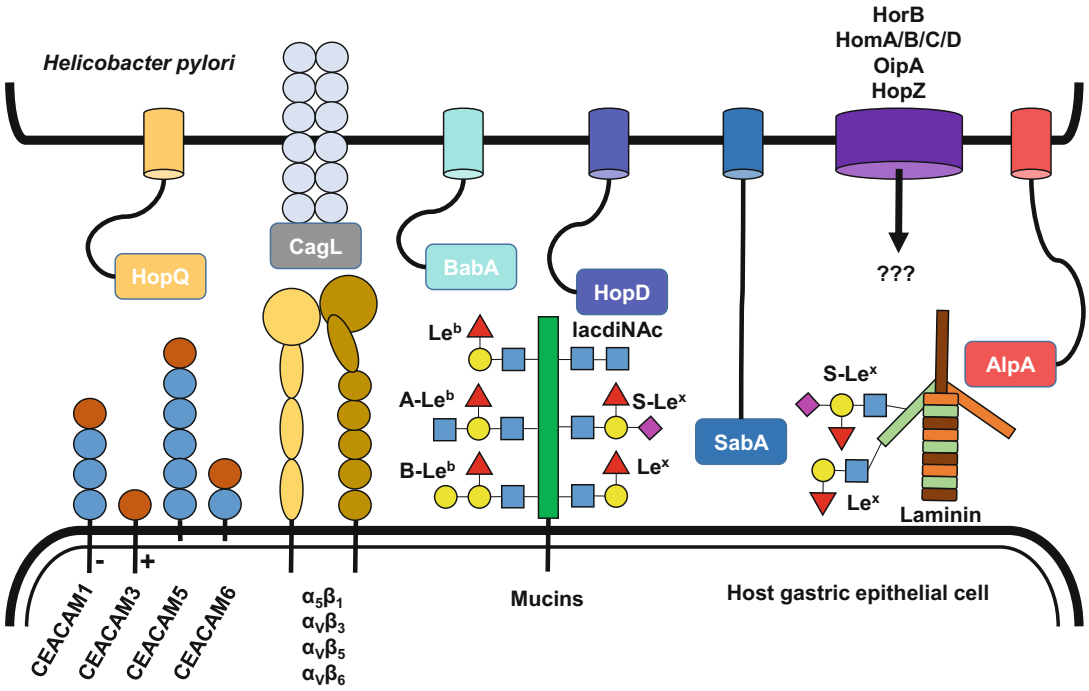

Fig. 1 Graphical representation of *H. pylori* adhesin interactions with host cell proteins. HopQ (yellow) interacts with CEACAM1, -3, -5 and -6 (IgV domain, brown, IgC2 domains, blue). CEACAM1 and CEACAM3 contain an immunoreceptor tyrosine-based inhibition (−) and activation (+) motifs, respectively. CagL (grey) is attached to the pilus tip (light grey circles) of the T4SS, where it can interact with the α5β1, αVβ3, αVβ5 and αVβ6 integrins. Mucins (green) are heavily glycosylated proteins and can be decorated with the blood group antigens (Le^b, A-Le^b and B-Le^B), both sialyated and asialyated Le^x, and lcadiNAc, which bind to BabA (cyan), SabA (blue) and HopD (purple), respectively. SabA also binds both sialyated and asialyated Le^x attached to Laminin. AlpA (pink) also binds Laminin. HorB, HomA/B/C/D, OipA and HopZ are outer membrane proteins of *H. pylori*. The host cell ligands of these proteins are currently unknown

families. An analysis of the genomic sequences of strains J99 and 26.695 grouped the OMPs into five separate families (Alm et al. 2000). Furthermore, comparison of the Hop and Hor families indicates that they share a common domain organization, including an N-terminal signal peptide of ~20–25 residues, a C-terminal β-barrel to anchor the protein to the outer membrane and a central domain that confers host protein specificity (Fig. 2a). The domain organization is reminiscent of autotransporters. Recently, however, several C-terminal β-barrels of the Hop proteins have been shown to be split, with a single strand found at the N-terminus, with the remaining strands found at the C-terminus (Coppens et al. 2018). Thus, the large extracellular domains are found to be inserted in the extracellular loops of the β-barrel (Fig. 2a).

3 Adhesins

Protein adhesins may recognize small host cell molecules (e.g., sugars) or larger ligands (e.g., proteins). Below, the known *H. pylori* adhesin proteins are discussed in detail, grouped according to the host cell binding partner to which they bind.

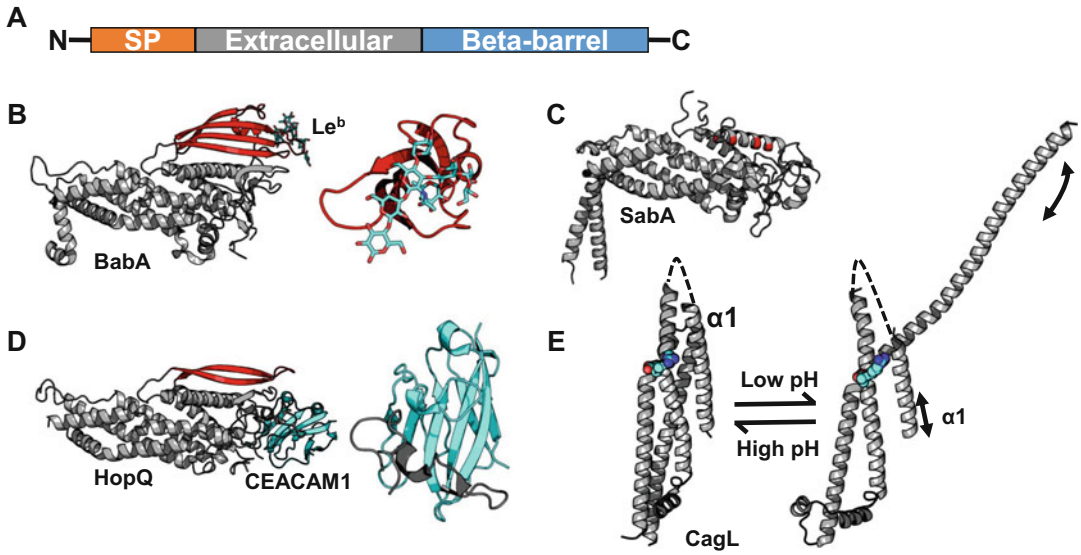

Fig. 2 Structures of *H. pylori* adhesins. (**a**) Domain organization of both the Hop and Hor proteins. *Top* – The original domain organization of Hop and Hor proteins consists of a N-terminal signal peptide (SP, orange), an extracellular domain (grey) and a C-terminal beta-barrel (blue), reminiscent of autotransporters. *Bottom* – The new domain organization of Hop and Hor proteins which show that part of the beta-barrel is formed from several residues of the N-terminus. (**b**) *Left* – Structure of BabA (PDB entry 5F9D, grey) interacting with the Lewis b blood group B heptasaccharide ligand (cyan sticks) through its insertion domain (red). *Right* – Closer inspection of the interaction. (**c**) Structure of SabA (PDB entry 4O5J, grey) with proposed s-Le^x binding site highlighted in red. (**d**) *Left* – Structure of the Type I HopQ-CEACAM1 complex (PDB entry 6AW2, grey and cyan, respectively). CEACAM1 does not contact the smaller insertion domain (red). (**e**) Conformational changes in CagL (grey) in response to pH exposes the RGD motif (cyan spheres). *Right* – At low pH, CagL exists in an extended state, with the α1 helix burying the arginine of the RGD motif (PDB entry 4X5U). *Left* – At high pH, CagL compacts and α1 moves exposing the RGD motif and allows recruitment of integrins (PDB entry 3ZCI)

3.1 Adhesins with Known Small Ligands

3.1.1 BabA/BabB/BabC

These adhesins are also known as HopS/HopT/HopU, respectively. The blood group antigen-binding adhesins (Bab) are approximately 80 kDa in size. The majority of research conducted on these proteins has been focused on BabA. BabA recognizes and can bind the mono-(H) or di-fucosylated (Lewis b, Le^b) blood group antigens from the O blood group (Boren et al. 1993; Ilver et al. 1998) and the A and B blood group antigens (A-Le^b and B-Le^b) (Aspholm-Hurtig et al. 2004), all of which are found on the surfaces of gastric epithelial cells and certain secreted mucins, including MUC1 and MUC5AC of the stomach (Linden et al. 2004; Sakamoto et al. 1989) and MUC5B in the salivary glands (Bosch et al. 2000; Veerman et al. 1997). Analysis of BabA binding to salivary proteins by matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) identified two proteins, which could bind BabA – proline-rich glycoprotein and gp-340 (Walz et al. 2009). BabB displays no Le^b binding activity, whilst the binding specificity of BabC is unknown (Kim et al. 2015; Saberi et al. 2016). BabA exists in two allele forms, *babA2*, which encodes for the full length protein, and *babA1*, which contains a 10-bp deletion in the signal peptide that results in a frame shift (Backstrom et al. 2004; Ilver et al. 1998). The location within the chromosome is highly variable. BabA and BabB are typically found at either locus A or B, with BabC commonly found in locus C (Armitano et al. 2013;

Colbeck et al. 2006; Hennig et al. 2006). Expression of BabA is regulated by phase variation in the signal peptide through a cytosine-thymine (CT) dinucleotide repeat (Ilver et al. 1998). The CT repeats can allow slipped strand mispairing (SSM) during replication and, thus, induce a reading frame shift (Colbeck et al. 2006; Ilver et al. 1998; Solnick et al. 2004; Styer et al. 2010). Regulation can also occur in response to host mucin expression (Skoog et al. 2012) and through gene conversion of *babA* with *babB*, creating BabA/B chimeras that have varying abilities to bind Le^b (Backstrom et al. 2004; Colbeck et al. 2006; Matteo et al. 2011; Pride and Blaser 2002; Solnick et al. 2004). In models of acute *H. pylori* infections in mice, gerbils and rhesus monkeys, BabA expression was found to be lost regularly during the early stages of infection due to phase variation or gene conversion (Hansen et al. 2017; Ohno et al. 2011; Solnick et al. 2004; Styer et al. 2010). This, however, appears to be a rare event in humans (Nell et al. 2014). However, these strains were isolated from chronically infected humans, for which mutation rates are approximately ten times slower compared to strains commonly found in acute infections (Nell et al. 2014). This surge of mutations observed in acute infections allows these *H. pylori* strains to respond quickly and adapt to the host environment (Linz et al. 2014).

The affinity of the extracellular domain of BabA for the blood group antigens is rather weak. Measured affinities include: Le^b antigen hexasaccharide, K_D = 250 μM; Le^b antigen pentasaccharide, K_D = 80 μM; H antigen pentasaccharide, K_D = 620 μM; B-Le^b septasaccharide, K_D = 40 μM; and A type 1 hexasaccharide (A6–1), K_D = 150 μM (Hage et al. 2015; Moonens et al. 2016). These are much weaker than Le^b antigen interaction with full length BabA as measured by surface plasmon resonance (SPR) analyses and cell binding assays, which exhibits an affinity of 390 pM (Aspholm-Hurtig et al. 2004; Ilver et al. 1998; Imberty et al. 2005; Moonens et al. 2016). Cross-linking of full length BabA indicates that it oligomerizes, primarily as trimers in *H. pylori* outer membranes (Moonens et al. 2016). The structure of the extracellular domain of BabA is a 4 + 3 helical bundle fold (Moonens et al. 2016) (Fig. 2b). An 80-residue insertion domain comprised of a four-stranded sheet with a helical loop crowning the beta-strands is located between helices 4 and 5. Several structures of BabA in complex with various blood group antigens have been determined by X-ray crystallography (Hage et al. 2015; Moonens et al. 2016). In these structures, all of the glycans bind to the insertion domain, specifically to two loops (Loop 1 and Loop 2) that connect the strands and the crowning helical loop (Moonens et al. 2016). The crowning helical loop is constrained by a disulfide that, upon reduction, prevents glycan binding (Moonens et al. 2016). DL1 and DL2 differ in sequence considerably across *H. pylori* strains and, consequently, *H. pylori* isolates exhibit distinct ABO preferences and Le^b affinities (Aspholm-Hurtig et al. 2004). Most strains produce BabA generalist adhesins that promote binding to each blood group glycan; however, several strains are Le^b-only specialists. These strains are found to have a shorter Loop 1, thereby preventing binding of the N-acetylgalactosamine or galactose sugar moieties in the larger A-Le^b and B-Le^b antigens, respectively (Aspholm-Hurtig et al. 2004).

As *H. pylori* resides in the stomach, it experiences a large pH gradient. The adherence of *H. pylori* in the gastric mucosa mediated by BabA-Le^b interactions display similar affinities between pH 4.0 and 6.0 (Bugaytsova et al. 2017). Further lowering the pH results in a 1000-fold reduction in adherence. Reconditioning of the *H. pylori* to a higher pH results in the recovery of binding to gastric mucosa, demonstrating that BabA displays a reversible pH sensitivity, which has been localized to residue 199 of the crowning helical loop (Bugaytsova et al. 2017). This residue resides in yet another region of BabA that is hypervariable in length and sequence amongst *H. pylori* strains. The pH_{50} of Le^b binding (the pH value at which BabA retains 50% of its Le^b binding) was determined for tens of strains and found to vary ~2.5 pH units, from 2.3 to 4.9. Deletion of residues 199–200 of BabA in strain

17.875 resulted in an increase of the pH_{50} from 3.3 to 3.9, mimicking strains where these residues are naturally missing (Bugaytsova et al. 2017). The presence of BabA can aid in the adherence of *H. pylori*, though it is not essential as many strains exist that lack the *babA* gene (Odenbreit et al. 2009) and strains that do contain *babA* have been observed to cause PUD or GC (Gerhard et al. 1999; Yamaoka et al. 2002c). This is particularly true if the *cag*PAI is also present (Azevedo et al. 2008).

3.1.2 SabA/SabB

These adhesins are also known as HopP and HopO, respectively. The sialic acid binding (Sab) proteins are slightly smaller than BabA with a molecular weight of ~70 kDa (Alm et al. 2000). SabA recognizes and binds sialylated glycans, whilst SabB does not (Mahdavi et al. 2002). The most studied ligand is the sialyl-Lewis x (s-Le^x) sugar found attached to O-glycans. Sialyated glycans are typically found in low concentrations in normal gastric musoca (Kobayashi et al. 2009). However, SabA can bind two minor gangliosides in the stomach, Neu5Acα3-neolactohexaosylceramide and Neu5Acα3-neolactooctaosylceramide, which can promote initial infection (Benktander et al. 2018). *H. pylori* most often cause gastric inflammation by triggering IL-8 induction in host cells responding to the infection. IL-8 activates *FUT3* and *B3GNT5*, two genes involved in the biosynthesis of s-Le^x (Magalhaes et al. 2015), leading to dramatic alterations in ganglioside sialyation profiles of the gastric mucosa and an enrichment of s-Le^x (Benktander et al. 2018; Magalhaes et al. 2015). SabA has been found to interact with several host cell glycoproteins that are sialyated including MUC5B, MUC7, laminin, carbonic anhydrase VI, zinc α2-glycoprotein, parotid secretory protein and the heavy chain of secretory IgA1 (Aspholm et al. 2006; Walz et al. 2005, 2009). SabA can also bind sialylated proteins on erythrocytes, which leads to hemagglutination (Unemo et al. 2005).

Regulation of SabA expression is complex. Phase variation is observed through two SSM mechanisms: one is observed in the CT dinucleotide repeat of the signal peptide as for *babA* and the second found within a polythymine repeat in the promoter region (Harvey et al. 2014; Kao et al. 2012; Yamaoka et al. 2002b, 2006). This can affect transcription of *sabA* through either altering regulatory protein interactions and/or RNA polymerase. The high sequence similarities between *sabA* and *sabB* also allow gene conversion between the two (Talarico et al. 2012). SabA expression is also regulated by the external pH, through the acid-responsive ArsRS two-component signal transduction system (Goodwin et al. 2008). At low pH (pH <5.0), SabA and SabB expression are repressed, whilst at higher pH they are upregulated. The type of mucins present in the mucosa can also regulate the expression of SabA (Skoog et al. 2012). Tumor mucosa from several patients was found to consist of different mucins and glycosylation patterns. These differences were found to have an effect on SabA expression (Skoog et al. 2012). High salt concentrations can upregulate SabA expression (Loh et al. 2018); several studies have revealed a link between high salt intake and an increase GC risk in humans.

The affinity of the extracellular domain of SabA for s-Le^x is slightly tighter than BabA is for Le^b, with an affinity of 20 μM as determined by SPR (Pang et al. 2014). The same study indicated that SabA can also bind non-sialyated Lewis x (Le^x), albeit weaker with an affinity of 50 μM (Pang et al. 2014). No binding was observed between SabA and Lewis A, Le^b or Lewis Y glycans. Structurally, SabA is similar to BabA, as they share the 4 + 3 helical bundle fold (Fig. 2c). However, the insertion domain of SabA differs in sequence, is 50–70 residues shorter and some of the residues appear to be conformationally dynamic, as they are not resolved in the X-ray crystal structure. Although no high-resolution structure of a SabA-s-Le^x exists, an alanine scan of conserved residues, from a multiple sequence alignment of BabA and SabA sequences and a ligand binding site prediction program, identified a potential ligand binding pocket on the surface of SabA (Pang et al. 2014) (Fig. 2c). Two mutations (Y148A and

K152A) that had no effect on s-Le^x binding were found to weaken binding to Le^x. The Q159A mutation inhibited SabA binding to both s-Le^x and Le^x, whereas the Q162A mutation only inhibited binding to Le^x (Pang et al. 2014). This binding pocket is distinct from the insertion domain of BabA which binds blood group antigens and the carcinoembryonic antigen-related cell adhesion molecules (CEACAM) binding loop of HopQ (see below).

SabA$^+$ strains appear to be associated with GC as observed in a diverse cohort of patients (Yamaoka et al. 2006). However, another study restricted to Taiwanese patients failed to identify any significant differences with patients infected with *sabA*$^+$ and *sabA*$^-$ strains and the prevalence of gastric atrophy (Sheu et al. 2006). A similar observation is seen in a Japanese study restricted to Japanese patients (Yanai et al. 2007), suggesting that there may be geographical and environmental factors confounding the link between SabA and disease incidence and severity.

3.1.3 HopD

This GalNAcβ1-4GlcNAc glycan motif (N,N-′-diacetyllactosediamine or lacdiNAc) -binding adhesin (LabA) is a protein with a molecular weight of 77 kDa (Alm et al. 2000). LacdiNAc is only observed as an O-linked glycan on MUC5AC expressed on the superficial and foveolar epithelium of the stomach (Rossez et al. 2014). The lacdiNAc motifs comprise ~7% of human adult gastric mucin O-glycans (Kenny et al. 2012; Rossez et al. 2014). Several different strains of *H. pylori* were shown to adhere to lacdiNAc, with strain 26.695 showing the strongest adherence. *H. pylori* lysate from strain 26.695 was incubated with gastric mucins in the presence and absence of soluble lacdiNAc as a competitive binder. The supernatants were compared by SDS-PAGE analysis and revealed a prominent band in the competition experiment. Proteomic analysis identified this protein as HopD (Rossez et al. 2014). No structure has been reported for HopD, though it probably has a structure grossly similar to BabA and SabA.

3.2 Adhesins with Known Proteins

3.2.1 HopQ

HopQ is a 68 kDa protein (Alm et al. 2000) found in two allelic forms, Type I and Type II (Cao and Cover 2002). These two forms share approximately 70% sequence identity at the protein level. Both HopQ types have a significant association with GC and gastritis, with Type I HopQ also found to be associated with an increased risk of PUD (Leylabadlo et al. 2016; Ohno et al. 2009). Furthermore, HopQ Type I is found significantly more often in *cag*PAI$^+$ versus *cag*PAI$^-$ strains (Loh et al. 2008). *hopQ* was the first gene identified that is located outside of the *cag*PAI and is also essential for the translocation of the CagA oncoprotein through the T4SS (Belogolova et al. 2013; Jimenez-Soto et al. 2013). HopQ expression is regulated by salt concentrations like SabA, with higher amounts of salt leading to HopQ upregulation (Loh et al. 2018).

HopQ binds CEACAM receptors on host cell surfaces (Javaheri et al. 2016; Königer et al. 2016). Twelve CEACAMs are found in humans (Tchoupa et al. 2014) and display distinct expression patterns, with certain CEACAMs only expressed in specific tissue types (Hammarstrom 1999; Zebhauser et al. 2005). Various CEACAM members are found to possess a similar domain architecture: they are comprised of a single N-terminal IgV domain, which predominately homodimerizes, though a few can also heterodimerize; followed by a variable number of IgC2 domains and a C-terminal transmembrane helix or a glycosylphosphatidylinositol anchor (Gray-Owen and Blumberg 2006; Tchoupa et al. 2014). HopQ is found to bind the N-terminal dimerization domains of only CEACAM1, -3, -5 and -6 as determined by flow cytometry with CEACAM6 binding the weakest (Javaheri et al. 2016; Königer et al. 2016). A humanized mouse model that expresses CEACAM5 results in a significant difference in gastritis activity upon *H. pylori* infection compared to control mice (Königer et al. 2016). Isolation of *H. pylori* from these humanized mice after 6 weeks of infection display a more active T4SS

as observed by the amount of CagA translocated in AGS cells *in vitro*. This is followed by a less active T4SS and lower IL-8 induction in CEACAM5 mice after 3 and 12 months of infection compared to wild-type mice (Königer et al. 2016). It is thought that to prevent clearance of the infection, *H. pylori* responds to the inflammation by lowering the activity of T4SS (i.e., a rheostat model) (Barrozo et al. 2013; Königer et al. 2016). HopQ binds one CEACAM monomer (Bonsor et al. 2018; Javaheri et al. 2016; Königer et al. 2016; Moonens et al. 2018; Tegtmeyer et al. 2019), with CEACAM1 and CEACAM3 binding affinities of ~200 and ~400 nM, respectively (Bonsor et al. 2018). CEACAM1 binding to the Type II HopQ allele is ~6-fold tighter (Moonens et al. 2018). The structure of HopQ, like those of BabA and SabA, is comprised of the common 4 + 3 helical bundle, with an insertion domain that is longer and better resolved than that of SabA, but shorter than that of BabA (Javaheri et al. 2016) (Fig. 2d). Crystal structures of the Type I HopQ in complex with CEACAM1 and CEACAM3 clearly show that CEACAMs interact with a disordered loop (in the unbound HopQ structure) of 13 residues that folds across the CEACAM dimerization interface and extends the CEACAM beta-sheet, such that Type I HopQ recognizes the monomeric form of CEACAMs and disrupts their dimerization (Bonsor et al. 2018; Moonens et al. 2018). Alanine mutagenesis of the Type I HopQ loop or the CEACAM dimerization interface failed to identify a critical residue that was important for binding (Bonsor et al. 2018), though larger substitutions in Type I HopQ such as L150N and V156N resulted in significant reduction in *H. pylori* binding to CEACAM1 expressing MKN28 cells (Moonens et al. 2018). Shortening of the loop weakened binding, whereas swapping the loop with that of BabA inactivates binding of CEACAMs and impairs translocation of CagA (Bonsor et al. 2018). The crystal structure of the Type II HopQ CEACAM1 complex is very similar to the Type I HopQ structure with two major differences. First, the disordered loop is shorter in Type II HopQ and as such does not extend the CEACAM beta-sheet. Second, the loop is more hydrophobic and results in less hydrogen bonds across the dimerization interface (Moonens et al. 2018).

The role of pH, disulfide bonds and glycans on the HopQ-CEACAM interaction have also been investigated. BabA binding to Le^b was both pH sensitive and reversible (Bugaytsova et al. 2017), whereas HopQ could still bind CEACAM1 at pH 4.0, but at lower pH values binding was neither detectable nor reversible (Bonsor et al. 2018). The CEACAM binding loop of HopQ, unlike the Le^b binding site on BabA, is not constrained by a disulfide bond, however, a disulfide exists preceding the loop and other loops proximal to the CEACAM binding site contain disulfides. Reduction of these disulfide bonds had little impact on the affinity of HopQ for CEACAM1 (Bonsor et al. 2018). CEACAMs are heavily glycosylated proteins. CEACAM1 decorated with high mannose type glycans or no glycans bind with a similar affinity to HopQ compared with the aglycosylated N-terminal domain of CEACAM1. However, CEACAM1 glycosylated with complex type glycans are found to bind eightfold tighter to HopQ, suggesting that glycans may have a role in HopQ binding through some unknown mechanism (Bonsor et al. 2018).

3.2.2 AlpA/AlpB

Also known as HopC and HopB, respectively, AlpA and AlpB share 45% sequence identity with a molecular weight of ~56–57 kDa (Alm et al. 2000). AlpA and AlpB appear to be co-expressed in all clinical strains of *H. pylori*, suggesting an essential or important function for these proteins (Odenbreit et al. 2009), in contrast with other OMPs. AlpA expression is upregulated in response to oxidative stress (Huang and Chiou 2011). Deletion of these genes results in the failed infection of guinea pigs and gerbils, the inability to adhere to human gastric tissue sections (de Jonge et al. 2004a; Senkovich et al. 2011; Sugimoto et al. 2011), and failed to stimulate secretion of IL-8 and IL-6, suggesting that these proteins are pro-inflammatory (Lu et al. 2007; Selbach et al. 2002). Stimulation of IL-8 expression may be specific to the geographic location of the strain, as deletion of AlpA and AlpB only

reduced IL-8 secretion with East Asian strains (Lu et al. 2007). All strains that possess AlpA and AlpB can perturb host cell signaling pathways such as ERKs, c-Fos and cAMP-responsive element binding protein, whereas activation of Jun N-terminal Kinase, c-Jun and NF-κB signaling were specific to East Asian strains (Lu et al. 2007).

A solution of semi-purified human extracellular matrix causes aggregation of *H. pylori* (Williams et al. 2008), which is significantly reduced in a Δ*alpA/B* strain (Senkovich et al. 2011). This effect was also observed in Matrigel, a mixture of predominately collagen IV and laminin from Engelbreth-Holm-Swarm mouse sarcoma cells (Senkovich et al. 2011). Experiments with purified mouse laminin show clear binding of AlpA and AlpB by flow cytometry (Senkovich et al. 2011). Expression of AlpA and AlpB in *Escherichia coli* causes a gain of function, allowing these bacteria to adhere to mouse laminin (Senkovich et al. 2011).

3.2.3 CagL

CagL is a 25 kDa protein that forms part of the *H. pylori* T4SS, responsible for translocation of CagA into host gastric epithelial cells (Fischer et al. 2001; Kwok et al. 2007). CagL is thus strongly associated with an increased risk of GC. CagL is expressed and attached to the surfaces of the T4SS pili that form when *H. pylori* physically contacts gastric epithelial cells (Shaffer et al. 2011). CagL is essential for the translocation of CagA as deletion of CagL causes the failure of the formation of the pili (Fischer et al. 2001; Kwok et al. 2007; Shaffer et al. 2011). CagL contains an Arg-Gly-Asp (RGD) motif, a known integrin binding ligand. Various studies have demonstrated that CagL can bind to α5β1, αVβ3, αvβ5 and αvβ6 through the RGD motif (Barden and Niemann 2015; Conradi et al. 2012a, b; Kwok et al. 2007; Wiedemann et al. 2012), although three studies have shown that CagA can still be translocated in an RGD-independent manner – deletion of CagL in the P12 strain had no effect on CagA translocation nor IL-8 secretion (Jimenez-Soto et al. 2009), deletion of the RGD motif in the SU2 strain resulted in a weakened interaction between CagL and the α5β1 and αvβ5 integrins and no observed binding to αvβ6 (Bonig et al. 2016) and the CagL^{RGA} mutation only weakened the interaction with αvβ5 (Wiedemann et al. 2012). CagL binds to integrins with an affinity of ~100–200 nM (Koelblen et al. 2017; Kwok et al. 2007; Wiedemann et al. 2012), independent of whether the integrin is in an extended closed or open state (Koelblen et al. 2017). This interaction is pH sensitive, with maximal binding occurring at pH 6.5 (Bonsor et al. 2015).

Several X-ray crystal structures of CagL have been determined. The first two structures revealed a four helix bundle (Barden et al. 2013; Choi et al. 2015). The RGD motif is located on a helix, which is currently the only known RGD motif not found in a loop (Fig. 2e). The following two structures identified that CagL could undergo a large conformational change, where two of the antiparallel helices become fused to form a single longer helix (Fig. 2e) and then dimerized through a domain swapped dimer mechanism (Barden et al. 2014; Bonsor et al. 2015). This conformational change is a result of low pH in both crystals and solution, though it is found not to be important for adhesion, IL-8 secretion nor CagA translocation (Bonsor et al. 2015). However, subtle conformational changes are apparent in the first helix (α1), which packs against the RGD motif (Barden et al. 2013; Bonsor et al. 2015). At low pH, α1 buries the arginine of the RGD preventing adhesion to host cells, whereas at higher pH α1 undergoes a registry shift, exposing the RGD motif and thus allows adherence, cell spreading, focal adhesion formation and heparin-binding epidermal growth factor activation (Bonsor et al. 2015; Saha et al. 2010; Tegtmeyer et al. 2010).

Several polymorphisms exist in the α1-α2 loop (residues 58–62), which may affect disease outcome in *H. pylori* infections. Worldwide, E59/I60 polymorphisms are associated significantly with GC-associated *H. pylori* isolates (Gorrell et al. 2016). Several studies indicated specific polymorphisms in local populations: in Iranian patients, the D58 polymorphism is typically observed in PUD, whereas the N58 polymorphism is associated with GC (Cherati et al.

2017); whilst in Taiwanese patients, the Y58/E59 mutations were over-represented in GC patients (Yeh et al. 2011). The role of Y58/E59 mutations is, however, debated as it has produced conflicting data. Replacement of the Y58/E59 mutation with D58/K59 resulted in a strain with a less active T4SS, lower CagA translocation and IL-8 secretion (Yeh et al. 2013). However, mutation of CagL in strain 26,695 (N58Y/E59E) inhibited CagA translocation in another study (Tegtmeyer et al. 2014). A final report investigated a larger group of polymorphisms (Y58/E59, D58/K59, D58/E59, N58/E59 and N58/K59) in both the P12 and 26.695 strains of *H. pylori* and found no significant changes in CagA translocation or IL-8 secretion (Tafreshi et al. 2015).

3.3 Adhesins with Unknown Ligands or Proteins

3.3.1 HorB

HorB or HP0127 is a 30 kDa protein. While very little is known about this protein, it is predicted to have a C-terminal β barrel domain and an N-terminal signal peptide, as well as a domain architecture similar to the Hop proteins (Snelling et al. 2007). Deletion of the gene results in a *H. pylori* strain that has a twofold reduction in adhesion, a lower production of LPS O-chains and thus the Lewis X and Y antigens attached to it. In mouse infection assays, colonization was reduced for Δ*horB* strains (Snelling et al. 2007).

3.3.2 HomA/HomB/HomC/HomD

These four proteins form the smallest OMP family and are each approximately 75 kDa in size (Oleastro et al. 2008). HomA and HomB share 90% sequence identity, and are 50% identical to HomC and HomD (Alm et al. 2000). HomC exists in three allelic forms (Kim et al. 2016). HomA and HomB occupy two defined loci within the bacterial chromosome (Alm et al. 2000). HomB can exchange positions with HomA and may have resulted from gene duplication (Alm et al. 1999, 2000; Oh et al. 2006; Tomb et al. 1997). Deletion of HomB results in *H. pylori* strains that exhibit less adherence to gastric epithelial cells and cause lower IL-8 secretion (Oleastro et al. 2008). One strain tested had two copies of HomB. Sequential deletion of the genes led to a further decrease in adherence (Oleastro et al. 2008). All four proteins are predicted to be 24-stranded β barrels (Servetas et al. 2018). The sequence variation between HomA and HomB is predicted to occur within the extracellular loops. Similar variance is observed in HomC (Servetas et al. 2018).

HomB is strongly associated with PUD (Oleastro et al. 2008). It also correlates with the presence of the *cagA*, *babA*, *hopQ* and *oipA* genes (Oleastro et al. 2008). Correlation is also observed with one of the *homC* alleles and the presence of *babA* at locus A (see BabA), suggesting that HomC may play a similar role in disease as BabA (Kim et al. 2016).

3.3.3 OipA

Outer inflammatory protein A, or HopH, is relatively small compared to the other abovementioned OMP adhesins, with a molecular weight of ~34 kDa (Alm et al. 2000). No structural data exists for this protein, but like the other Hop proteins is predicted to have a C-terminal β barrel and an N-terminal signal peptide (Alm et al. 2000). Therefore, the extracellular domain would be small, consisting of approximately 75 residues. *OipA* regulation is achieved through phase variation in the CT nucleotide repeat of the signal peptide and is found either in an *oipA*-off or *oipA*-on state (Saunders et al. 1998; Yamaoka et al. 2000). OipA is believed to stimulate IL-8 secretion from host cells and cause inflammation, hence its name, though the evidence for this is conflicting. Several studies showed that *oipA* mutants did not alter IL-8 secretion *in vitro* or inflammation in gerbils (Dossumbekova et al. 2006; Franco et al. 2008). However, other studies clearly show that OipA does in fact cause IL-8 secretion and promotes inflammation (Yamaoka et al. 2000, 2002a). Furthermore, it has been shown that IL-8 secretion by OipA is regulated through PI3K/Akt activation and inactivation of FoxO1/3a (Tabassam et al. 2012). This conflict is thought to be compounded by the fact that OipA

is strongly associated with the *cag*PAI, which stimulates IL-8 secretion. Indeed, two studies have shown that greater than 95% of *cag*PAI$^+$ strains contain an *oipA*-on allele, whilst no *cag*PAI$^-$ strain has been found with an *oipA*-on allele (Ando et al. 2002; Farzi et al. 2018; Odenbreit et al. 2009). Mutation of the *H. pylori* J68 strain (*cag*PAI$^-$/ *oipA*-off) to produce an *oipA*-on strain, results in a bacterium with increased adherence, but did not alter IL-8 secretions of host cells (Horridge et al. 2017). Mutation of the 26.695 strain (*cag*PAI$^+$/*oipA*-on) to produce an *oipA*-off strain failed to stimulate IL-8 secretion, demonstrating that OipA is essential, but insufficient for IL-8 secretion (Horridge et al. 2017). The 26.695 *cag*PAI$^+$/*oipA*-off mutant strain was also found incapable of CagA translocation (Horridge et al. 2017). This is the second adhesin gene that does not reside with the *cag*PAI locus (HopQ was the first, see above), which has been shown to be essential for a functional T4SS. As the *oipA*-on allele correlates so strongly with *cag*PAI$^+$ strains, OipA is found to be associated with PUD, GC and MALT lymphoma (Dabiri et al. 2009). Purified OipA is found to trigger apoptosis of gastric epithelial cell lines through increasing Bax and cleaved Caspase 3 concentrations and lowering of Bcl-2 (Teymournejad et al. 2017).

3.3.4 HopZ

HopZ is a ~74 kDa protein (Alm et al. 2000; Peck et al. 1999). Like *hopQ*, its closest homologue (Oleastro and Menard 2013), *hopZ* exists as two alleles, which can undergo recombination (Kennemann et al. 2011; Peck et al. 1999). HopZ expression is regulated through SSM within the CT dinucleotide repeats in the signal peptide. The switch from off-to-on is influenced by colonization density and during infection (Kennemann et al. 2011, 2012; Peck et al. 1999; Yamaoka et al. 2002b). Deletion of *hopZ* results in lower adherence of *H. pylori* to gastric epithelial cell lines (Yamaoka et al. 2002b), while mutation of the gene does not affect colonization of guinea pig stomachs (de Jonge et al. 2004a). However, in germ-free transgenic mice, *hopZ* deletion reduces *H. pylori* survival in their stomachs (Giannakis et al. 2009). HopZ does not appear to be associated with gastric disease, except for MALT lymphoma where lower expression levels of HopZ are found (Chiarini et al. 2009; de Jonge et al. 2004b; Kennemann et al. 2012). Indeed, the *hopZ*-off state is found to be associated with MALT lymphoma (Lehours et al. 2004).

4 Conclusions

H. pylori has successfully adapted to life in the human stomach. The ever changing local environment (churning of the stomach, acidic environment, constant shedding of epithelial cells) in the stomach has placed substantial selective pressure on *H. pylori* to escape the acidic lumen on the stomach and swim towards the gastric epithelial lining, where it can use a repertoire of over 60 outer membrane proteins to achieve adherence to host cells. This large selection of adhesion molecules allows *H. pylori* to rapidly change the proteins that it presents on its cell surface to adhere the host proteins and/or ligands that are present in the local environment. Furthermore, altering its outer membrane proteins aids in lessening of the immune response by the host and, thus, its elimination. However, some of the proteins are associated with a more severe clinical outcome. This review has discussed several important adhesion-host protein/ligand interactions which are important in *H. pylori* colonization, survival and disease. These data could provide druggable or vaccine targets for the eradication of *H. pylori*.

References

Alm RA, Ling LS, Moir DT, King BL, Brown ED, Doig PC, Smith DR, Noonan B, Guild BC, de Jonge BL, Carmel G, Tummino PJ, Caruso A, Uria-Nickelsen M, Mills DM, Ives C, Gibson R, Merberg D, Mills SD, Jiang Q, Taylor DE, Vovis GF, Trust TJ (1999) Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature 397:176–180. https://doi.org/10.1038/16495

Alm RA, Bina J, Andrews BM, Doig P, Hancock RE, Trust TJ (2000) Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. Infect Immun 68:4155–4168. https://doi.org/10.1128/IAI.68.7.4155-4168.2000

Ando T, Peek RM, Pride D, Levine SM, Takata T, Lee YC, Kusugami K, van der Ende A, Kuipers EJ, Kusters JG, Blaser MJ (2002) Polymorphisms of *Helicobacter pylori* HP0638 reflect geographic origin and correlate with cagA status. J Clin Microbiol 40:239–246. https://doi.org/10.1128/JCM.40.1.239-246.2002

Armitano RI, Matteo MJ, Goldman C, Wonaga A, Viola LA, De Palma GZ, Catalano M (2013) *Helicobacter pylori* heterogeneity in patients with gastritis and peptic ulcer disease. Infect Genet Evol 16:377–385. https://doi.org/10.1016/j.meegid.2013.02.024

Aspholm M, Olfat FO, Norden J, Sonden B, Lundberg C, Sjostrom R, Altraja S, Odenbreit S, Haas R, Wadstrom T, Engstrand L, Semino-Mora C, Liu H, Dubois A, Teneberg S, Arnqvist A, Boren T (2006) SabA is the *H. pylori* hemagglutinin and is polymorphic in binding to sialylated glycans. PLoS Pathog 2: e110. https://doi.org/10.1371/journal.ppat.0020110

Aspholm-Hurtig M, Dailide G, Lahmann M, Kalia A, Ilver D, Roche N, Vikstrom S, Sjostrom R, Linden S, Backstrom A, Lundberg C, Arnqvist A, Mahdavi J, Nilsson UJ, Velapatino B, Gilman RH, Gerhard M, Alarcon T, Lopez-Brea M, Nakazawa T, Fox JG, Correa P, Dominguez-Bello MG, Perez-Perez GI, Blaser MJ, Normark S, Carlstedt I, Oscarson S, Teneberg S, Berg DE, Boren T (2004) Functional adaptation of BabA, the *H. pylori* ABO blood group antigen binding adhesin. Science 305:519–522. https://doi.org/10.1126/science.1098801

Azevedo M, Eriksson S, Mendes N, Serpa J, Figueiredo C, Resende LP, Ruvoen-Clouet N, Haas R, Boren T, Le Pendu J, David L (2008) Infection by *Helicobacter pylori* expressing the BabA adhesin is influenced by the secretor phenotype. J Pathol 215:308–316. https://doi.org/10.1002/path.2363

Backert S, Tegtmeyer N, Fischer W (2015) Composition, structure and function of the *Helicobacter pylori* cag pathogenicity island encoded type IV secretion system. Future Microbiol 10(6):955–965. https://doi.org/10.2217/fmb.15.32

Backstrom A, Lundberg C, Kersulyte D, Berg DE, Boren T, Arnqvist A (2004) Metastability of *Helicobacter pylori bab* adhesin genes and dynamics in Lewis b antigen binding. Proc Natl Acad Sci U S A 101:16923–16928. https://doi.org/10.1073/pnas.0404817101

Barden S, Niemann HH (2015) Adhesion of several cell lines to *Helicobacter pylori* CagL is mediated by integrin alphaVbeta6 via an RGDLXXL motif. J Mol Biol 427:1304–1315. https://doi.org/10.1016/j.jmb.2015.01.006

Barden S, Lange S, Tegtmeyer N, Conradi J, Sewald N, Backert S, Niemann HH (2013) A helical RGD motif promoting cell adhesion: crystal structures of the *Helicobacter pylori* type IV secretion system pilus protein CagL. Structure 21:1931–1941. https://doi.org/10.1016/j.str.2013.08.018

Barden S, Schomburg B, Conradi J, Backert S, Sewald N, Niemann HH (2014) Structure of a three-dimensional domain-swapped dimer of the *Helicobacter pylori* type IV secretion system pilus protein CagL. Acta Crystallogr D Biol Crystallogr 70:1391–1400. https://doi.org/10.1107/S1399004714003150

Barrozo RM, Cooke CL, Hansen LM, Lam AM, Gaddy JA, Johnson EM, Cariaga TA, Suarez G, Peek RM Jr, Cover TL, Solnick JV (2013) Functional plasticity in the type IV secretion system of *Helicobacter pylori*. PLoS Pathog 9:e1003189. https://doi.org/10.1371/journal.ppat.1003189

Beier D, Spohn G, Rappuoli R, Scarlato V (1997) Identification and characterization of an operon of *Helicobacter pylori* that is involved in motility and stress adaptation. J Bacteriol 179:4676–4683. https://doi.org/10.1128/jb.179.15.4676-4683.1997

Belanger LF, Leblond CP (1946) A method for locating radioactive elements in tissues by covering histological sections with a photographic emulsion. Endocrinology 39:8–13. https://doi.org/10.1210/endo-39-1-8

Belogolova E, Bauer B, Pompaiah M, Asakura H, Brinkman V, Ertl C, Bartfeld S, Nechitaylo TY, Haas R, Machuy N, Salama N, Churin Y, Meyer TF (2013) *Helicobacter pylori* outer membrane protein HopQ identified as a novel T4SS-associated virulence factor. Cell Microbiol 15:1896–1912. https://doi.org/10.1111/cmi.12158

Benktander J, Barone A, Johansson MM, Teneberg S (2018) *Helicobacter pylori* SabA binding gangliosides of human stomach. Virulence 9:738–751. https://doi.org/10.1080/21505594.2018.1440171

Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A (1995) Infection with *Helicobacter pylori* strains possessing *cagA* is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res 55:2111–2115

Bonig T, Olbermann P, Bats SH, Fischer W, Josenhans C (2016) Systematic site-directed mutagenesis of the *Helicobacter pylori* CagL protein of the Cag type IV secretion system identifies novel functional domains. Sci Rep 6:38101. https://doi.org/10.1038/srep38101

Bonsor DA, Pham KT, Beadenkopf R, Diederichs K, Haas R, Beckett D, Fischer W, Sundberg EJ (2015) Integrin engagement by the helical RGD motif of the *Helicobacter pylori* CagL protein is regulated by pH-induced displacement of a neighboring helix. J Biol Chem 290:12929–12940. https://doi.org/10.1074/jbc.M115.641829

Bonsor DA, Zhao Q, Schmidinger B, Weiss E, Wang J, Deredge D, Beadenkopf R, Dow B, Fischer W, Beckett D, Wintrode PL, Haas R, Sundberg EJ (2018) The *Helicobacter pylori* adhesin protein HopQ exploits the dimer interface of human CEACAMs to

facilitate translocation of the oncoprotein CagA. EMBO J. https://doi.org/10.15252/embj.201798664

Boren T, Falk P, Roth KA, Larson G, Normark S (1993) Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. Science 262:1892–1895. https://doi.org/10.1126/science.8018146

Bosch JA, de Geus EJ, Ligtenberg TJ, Nazmi K, Veerman EC, Hoogstraten J, Amerongen AV (2000) Salivary MUC5B-mediated adherence (ex vivo) of *Helicobacter pylori* during acute stress. Psychosom Med 62:40–49

Bugaytsova JA, Bjornham O, Chernov YA, Gideonsson P, Henriksson S, Mendez M, Sjostrom R, Mahdavi J, Shevtsova A, Ilver D, Moonens K, Quintana-Hayashi MP, Moskalenko R, Aisenbrey C, Bylund G, Schmidt A, Aberg A, Brannstrom K, Koniger V, Vikstrom S, Rakhimova L, Hofer A, Ogren J, Liu H, Goldman MD, Whitmire JM, Aden J, Younson J, Kelly CG, Gilman RH, Chowdhury A, Mukhopadhyay AK, Nair GB, Papadakos KS, Martinez-Gonzalez B, Sgouras DN, Engstrand L, Unemo M, Danielsson D, Suerbaum S, Oscarson S, Morozova-Roche LA, Olofsson A, Grobner G, Holgersson J, Esberg A, Stromberg N, Landstrom M, Eldridge AM, Chromy BA, Hansen LM, Solnick JV, Linden SK, Haas R, Dubois A, Merrell DS, Schedin S, Remaut H, Arnqvist A, Berg DE, Boren T (2017) *Helicobacter pylori* adapts to chronic infection and gastric disease via pH-responsive BabA-mediated adherence. Cell Host Microbe 21:376–389. https://doi.org/10.1016/j.chom.2017.02.013

Buti L, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL (2011) *Helicobacter pylori* cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. Proc Natl Acad Sci U S A 108:9238–9243. https://doi.org/10.1073/pnas.1106200108

Cao P, Cover TL (2002) Two different families of *hopQ* alleles in *Helicobacter pylori*. J Clin Microbiol 40:4504–4511. https://doi.org/10.1128/JCM.40.12.4504-4511.2002

Censini S, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A (1996) *cag*, a pathogenicity island of *Helicobacter pylori*, encodes type I-specific and disease-associated virulence factors. Proc Natl Acad Sci U S A 93:14648–14653. https://doi.org/10.1073/pnas.93.25.14648

Cherati MR, Shokri-Shirvani J, Karkhah A, Rajabnia R, Nouri HR (2017) Helicobacter pylori cagL amino acid polymorphism D58E59 pave the way toward peptic ulcer disease while N58E59 is associated with gastric cancer in north of Iran. Microb Pathog 107:413–418. https://doi.org/10.1016/j.micpath.2017.04.025

Chiarini A, Cala C, Bonura C, Gullo A, Giuliana G, Peralta S, D'Arpa F, Giammanco A (2009) Prevalence of virulence-associated genotypes of *Helicobacter pylori* and correlation with severity of gastric pathology in patients from western Sicily, Italy. Eur J Clin Microbiol Infect Dis 28:437–446. https://doi.org/10.1007/s10096-008-0644-x

Choi JM, Choi YH, Sudhanva MS, Devakumar S, Lee KH, Cha JH, Lee SH (2015) Crystal structure of CagL from *Helicobacter pylori* K74 strain. Biochem Biophys Res Commun 460:964–970. https://doi.org/10.1016/j.bbrc.2015.03.135

Colbeck JC, Hansen LM, Fong JM, Solnick JV (2006) Genotypic profile of the outer membrane proteins BabA and BabB in clinical isolates of *Helicobacter pylori*. Infect Immun 74:4375–4378. https://doi.org/10.1128/IAI.00485-06

Conradi J, Huber S, Gaus K, Mertink F, Royo Gracia S, Strijowski U, Backert S, Sewald N (2012a) Cyclic RGD peptides interfere with binding of the *Helicobacter pylori* protein CagL to integrins alphaVbeta3 and alpha5beta1. Amino Acids 43:219–232. https://doi.org/10.1007/s00726-011-1066-0

Conradi J, Tegtmeyer N, Woźna M, Wissbrock M, Michalek C et al (2012b) An RGD helper sequence in CagL of *Helicobacter pylori* assists in interactions with integrins and injection of CagA. Front Cell Infect Microbiol 2(70). https://doi.org/10.3389/fcimb.2012.00070

Coppens F, Castaldo G, Debraekeleer A, Subedi S, Moonens K, Lo A, Remaut H (2018) Hop-family *Helicobacter* outer membrane adhesins form a novel class of type 5-like secretion proteins with an interrupted beta-barrel domain. Mol Microbiol. https://doi.org/10.1111/mmi.14075

Croxen MA, Sisson G, Melano R, Hoffman PS (2006) The *Helicobacter pylori* chemotaxis receptor TlpB (HP0103) is required for pH taxis and for colonization of the gastric mucosa. J Bacteriol 188:2656–2665. https://doi.org/10.1128/JB.188.7.2656-2665.2006

Dabiri H, Maleknejad P, Yamaoka Y, Feizabadi MM, Jafari F, Rezadehbashi M, Nakhjavani FA, Mirsalehian A, Zali MR (2009) Distribution of *Helicobacter pylori cagA*, *cagE*, *oipA* and *vacA* in different major ethnic groups in Tehran, Iran. J Gastroenterol Hepatol 24:1380–1386. https://doi.org/10.1111/j.1440-1746.2009.05876.x

de Jonge R, Durrani Z, Rijpkema SG, Kuipers EJ, van Vliet AH, Kusters JG (2004a) Role of the *Helicobacter pylori* outer-membrane proteins AlpA and AlpB in colonization of the guinea pig stomach. J Med Microbiol 53:375–379. https://doi.org/10.1099/jmm.0.45551-0

de Jonge R, Pot RG, Loffeld RJ, van Vliet AH, Kuipers EJ, Kusters JG (2004b) The functional status of the *Helicobacter pylori* sabB adhesin gene as a putative marker for disease outcome. Helicobacter 9:158–164. https://doi.org/10.1111/j.1083-4389.2004.00213.x

Didelot X, Nell S, Yang I, Woltemate S, van der Merwe S, Suerbaum S (2013) Genomic evolution and transmission of *Helicobacter pylori* in two South African families. Proc Natl Acad Sci U S A

110:13880–13885. https://doi.org/10.1073/pnas.1304681110

Dossumbekova A, Prinz C, Mages J, Lang R, Kusters JG, Van Vliet AH, Reindl W, Backert S, Saur D, Schmid RM, Rad R (2006) *Helicobacter pylori* HopH (OipA) and bacterial pathogenicity: genetic and functional genomic analysis of *hopH* gene polymorphisms. J Infect Dis 194:1346–1355. https://doi.org/10.1086/508426

Farzi N, Yadegar A, Aghdaei HA, Yamaoka Y, Zali MR (2018) Genetic diversity and functional analysis of *oipA* gene in association with other virulence factors among *Helicobacter pylori* isolates from Iranian patients with different gastric diseases. Infect Genet Evol 60:26–34. https://doi.org/10.1016/j.meegid.2018.02.017

Fischer W, Puls J, Buhrdorf R, Gebert B, Odenbreit S, Haas R (2001) Systematic mutagenesis of the *Helicobacter pylori cag* pathogenicity island: essential genes for CagA translocation in host cells and induction of interleukin-8. Mol Microbiol 42:1337–1348. https://doi.org/10.1046/j.1365-2958.2001.02714.x

Franco AT, Johnston E, Krishna U, Yamaoka Y, Israel DA, Nagy TA, Wroblewski LE, Piazuelo MB, Correa P, Peek RM Jr (2008) Regulation of gastric carcinogenesis by *Helicobacter pylori* virulence factors. Cancer Res 68:379–387. https://doi.org/10.1158/0008-5472.CAN-07-0824

Gerhard M, Lehn N, Neumayer N, Boren T, Rad R, Schepp W, Miehlke S, Classen M, Prinz C (1999) Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. Proc Natl Acad Sci U S A 96:12778–12783. https://doi.org/10.1073/pnas.96.22.12778

Giannakis M, Backhed HK, Chen SL, Faith JJ, Wu M, Guruge JL, Engstrand L, Gordon JI (2009) Response of gastric epithelial progenitors to *Helicobacter pylori* isolates obtained from Swedish patients with chronic atrophic gastritis. J Biol Chem 284:30383–30394. https://doi.org/10.1074/jbc.M109.052738

Goodwin AC, Weinberger DM, Ford CB, Nelson JC, Snider JD, Hall JD, Paules CI, Peek RM Jr, Forsyth MH (2008) Expression of the *Helicobacter pylori* adhesin SabA is controlled via phase variation and the ArsRS signal transduction system. Microbiology 154:2231–2240. https://doi.org/10.1099/mic.0.2007/016055-0

Gorrell RJ, Zwickel N, Reynolds J, Bulach D, Kwok T (2016) *Helicobacter pylori* CagL Hypervariable motif: a global analysis of geographical diversity and association with gastric cancer. J Infect Dis 213:1927–1931. https://doi.org/10.1093/infdis/jiw060

Gray-Owen SD, Blumberg RS (2006) CEACAM1: contact-dependent control of immunity. Nat Rev Immunol 6:433–446. https://doi.org/10.1038/nri1864

Hage N, Howard T, Phillips C, Brassington C, Overman R, Debreczeni J, Gellert P, Stolnik S, Winkler GS, Falcone FH (2015) Structural basis of Lewis^b antigen binding by the *Helicobacter pylori* adhesin BabA. Sci Adv 1:e1500315. https://doi.org/10.1126/sciadv.1500315

Hammarstrom S (1999) The carcinoembryonic antigen (CEA) family: structures, suggested functions and expression in normal and malignant tissues. Semin Cancer Biol 9:67–81. https://doi.org/10.1006/scbi.1998.0119

Hansen LM, Gideonsson P, Canfield DR, Boren T, Solnick JV (2017) Dynamic expression of the BabA Adhesin and its BabB Paralog during *Helicobacter pylori* infection in rhesus macaques. Infect Immun 85. https://doi.org/10.1128/IAI.00094-17

Harvey VC, Acio CR, Bredehoft AK, Zhu L, Hallinger DR, Quinlivan-Repasi V, Harvey SE, Forsyth MH (2014) Repetitive sequence variations in the promoter region of the adhesin-encoding gene sabA of helicobacter pylori affect transcription. J Bacteriol 196:3421–3429. https://doi.org/10.1128/JB.01956-14

Hennig EE, Allen JM, Cover TL (2006) Multiple chromosomal loci for the *babA* gene in *Helicobacter pylori*. Infect Immun 74:3046–3051. https://doi.org/10.1128/IAI.74.5.3046-3051.2006

Horridge DN, Begley AA, Kim J, Aravindan N, Fan K, Forsyth MH (2017) Outer inflammatory protein a (OipA) of *Helicobacter pylori* is regulated by host cell contact and mediates CagA translocation and interleukin-8 response only in the presence of a functional cag pathogenicity island type IV secretion system. Pathog Dis 75. https://doi.org/10.1093/femspd/ftx113

Huang CH, Chiou SH (2011) Proteomic analysis of upregulated proteins in *Helicobacter pylori* under oxidative stress induced by hydrogen peroxide. Kaohsiung J Med Sci 27:544–553. https://doi.org/10.1016/j.kjms.2011.06.019

Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T (1998) *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. Science 279:373–377. https://doi.org/10.1126/science.279.5349.373

Imberty A, Mitchell EP, Wimmerova M (2005) Structural basis of high-affinity glycan recognition by bacterial and fungal lectins. Curr Opin Struct Biol 15:525–534. https://doi.org/10.1016/j.sbi.2005.08.003

Israel DA, Salama N, Arnold CN, Moss SF, Ando T, Wirth HP, Tham KT, Camorlinga M, Blaser MJ, Falkow S, Peek RM Jr (2001) *Helicobacter pylori* strain-specific differences in genetic content, identified by microarray, influence host inflammatory responses. J Clin Invest 107:611–620. https://doi.org/10.1172/JCI11450

Javaheri A, Kruse T, Moonens K, Mejias-Luque R, Debraekeleer A, Asche CI, Tegtmeyer N, Kalali B, Bach NC, Sieber SA, Hill DJ, Koniger V, Hauck CR, Moskalenko R, Haas R, Busch DH, Klaile E, Slevogt H, Schmidt A, Backert S, Remaut H, Singer BB, Gerhard M (2016) Helicobacter pylori adhesin HopQ engages in a virulence-enhancing interaction

with human CEACAMs. Nat Microbiol 2:16189. https://doi.org/10.1038/nmicrobiol.2016.189

Jimenez-Soto LF, Kutter S, Sewald X, Ertl C, Weiss E, Kapp U, Rohde M, Pirch T, Jung K, Retta SF, Terradot L, Fischer W, Haas R (2009) *Helicobacter pylori* type IV secretion apparatus exploits beta1 integrin in a novel RGD-independent manner. PLoS Pathog 5:e1000684. https://doi.org/10.1371/journal.ppat.1000684

Jimenez-Soto LF, Clausen S, Sprenger A, Ertl C, Haas R (2013) Dynamics of the Cag-type IV secretion system of *Helicobacter pylori* as studied by bacterial co-infections. Cell Microbiol 15:1924–1937. https://doi.org/10.1111/cmi.12166

Kang J, Blaser MJ (2006) Bacterial populations as perfect gases: genomic integrity and diversification tensions in *Helicobacter pylori*. Nat Rev Microbiol 4:826–836. https://doi.org/10.1038/nrmicro1528

Kao CY, Sheu BS, Sheu SM, Yang HB, Chang WL, Cheng HC, Wu JJ (2012) Higher motility enhances bacterial density and inflammatory response in dyspeptic patients infected with *Helicobacter pylori*. Helicobacter 17:411–416. https://doi.org/10.1111/j.1523-5378.2012.00974.x

Keilberg D, Ottemann KM (2016) How *Helicobacter pylori* senses, targets and interacts with the gastric epithelium. Environ Microbiol 18:791–806. https://doi.org/10.1111/1462-2920.13222

Kennemann L, Didelot X, Aebischer T, Kuhn S, Drescher B, Droege M, Reinhardt R, Correa P, Meyer TF, Josenhans C, Falush D, Suerbaum S (2011) *Helicobacter pylori* genome evolution during human infection. Proc Natl Acad Sci U S A 108:5033–5038. https://doi.org/10.1073/pnas.1018444108

Kennemann L, Brenneke B, Andres S, Engstrand L, Meyer TF, Aebischer T, Josenhans C, Suerbaum S (2012) In vivo sequence variation in HopZ, a phase-variable outer membrane protein of *Helicobacter pylori*. Infect Immun 80:4364–4373. https://doi.org/10.1128/IAI.00977-12

Kenny DT, Skoog EC, Linden SK, Struwe WB, Rudd PM, Karlsson NG (2012) Presence of terminal N-acetylgalactosaminebeta1-4N-acetylglucosamine residues on O-linked oligosaccharides from gastric MUC5AC: involvement in *Helicobacter pylori* colonization? Glycobiology 22:1077–1085. https://doi.org/10.1093/glycob/cws076

Kim A, Servetas SL, Kang J, Kim J, Jang S, Cha HJ, Lee WJ, Kim J, Romero-Gallo J, Peek RM Jr, Merrell DS, Cha JH (2015) *Helicobacter pylori bab* Paralog distribution and association with *cagA*, *vacA*, and *homA/B* genotypes in American and South Korean clinical isolates. PLoS One 10:e0137078. https://doi.org/10.1371/journal.pone.0137078

Kim A, Servetas SL, Kang J, Kim J, Jang S, Choi YH, Su H, Jeon YE, Hong YA, Yoo YJ, Merrell DS, Cha JH (2016) *Helicobacter pylori* outer membrane protein, HomC, shows geographic dependent polymorphism that is influenced by the Bab family. J Microbiol 54:846–852. https://doi.org/10.1007/s12275-016-6434-8

Kobayashi M, Lee H, Nakayama J, Fukuda M (2009) Roles of gastric mucin-type O-glycans in the pathogenesis of *Helicobacter pylori* infection. Glycobiology 19:453–461. https://doi.org/10.1093/glycob/cwp004

Koelblen T, Berge C, Cherrier MV, Brillet K, Jimenez-Soto L, Ballut L, Takagi J, Montserret R, Rousselle P, Fischer W, Haas R, Fronzes R, Terradot L (2017) Molecular dissection of protein-protein interactions between integrin alpha5beta1 and the *Helicobacter pylori* Cag type IV secretion system. FEBS J 284:4143–4157. https://doi.org/10.1111/febs.14299

Königer V, Holsten L, Harrison U, Busch B, Loell E, Zhao Q, Bonsor DA, Roth A, Kengmo-Tchoupa A, Smith SI, Mueller S, Sundberg EJ, Zimmermann W, Fischer W, Hauck CR, Haas R (2016) Helicobacter pylori exploits human CEACAMs via HopQ for adherence and translocation of CagA. Nat Microbiol 2:16188. https://doi.org/10.1038/nmicrobiol.2016.188

Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, Konig W, Backert S (2007) *Helicobacter* exploits integrin for type IV secretion and kinase activation. Nature 449:862–866. https://doi.org/10.1038/nature06187

Langenberg ML, Tytgat GN, Schipper MEI (1984) *Campylobacter*-like organisms in the stomach of patients and healthy individuals. Lancet 1:1348–1349. https://doi.org/10.1016/S0140-6736(84)91836-1

Lee ER (1985) Dynamic histology of the antral epithelium in the mouse stomach: III. Ultrastructure and renewal of pit cells. Am J Anat 172:225–240. https://doi.org/10.1002/aja.1001720305

Lehours P, Menard A, Dupouy S, Bergey B, Richy F, Zerbib F, Ruskone-Fourmestraux A, Delchier JC, Megraud F (2004) Evaluation of the association of nine *Helicobacter pylori* virulence factors with strains involved in low-grade gastric mucosa-associated lymphoid tissue lymphoma. Infect Immun 72:880–888. https://doi.org/10.1128/IAI.72.2.880-888.2004

Leylabadlo HE, Yekani M, Ghotaslou R (2016) *Helicobacter pylori hopQ* alleles (type I and II) in gastric cancer. Biomed Rep 4:601–604. https://doi.org/10.3892/br.2016.634

Linden S, Mahdavi J, Hedenbro J, Boren T, Carlstedt I (2004) Effects of pH on *Helicobacter pylori* binding to human gastric mucins: identification of binding to non-MUC5AC mucins. Biochem J 384:263–270. https://doi.org/10.1042/BJ20040402

Linz B, Windsor HM, McGraw JJ, Hansen LM, Gajewski JP, Tomsho LP, Hake CM, Solnick JV, Schuster SC, Marshall BJ (2014) A mutation burst during the acute phase of *Helicobacter pylori* infection in humans and rhesus macaques. Nat Commun 5:4165. https://doi.org/10.1038/ncomms5165

Loh JT, Torres VJ, Algood HM, McClain MS, Cover TL (2008) *Helicobacter pylori* HopQ outer membrane protein attenuates bacterial adherence to gastric

epithelial cells. FEMS Microbiol Lett 289:53–58. https://doi.org/10.1111/j.1574-6968.2008.01368.x

Loh JT, Beckett AC, Scholz MB, Cover TL (2018) High-salt conditions alter transcription of *Helicobacter pylori* genes encoding outer membrane proteins. Infect Immun 86. https://doi.org/10.1128/IAI.00626-17

Lu H, Wu JY, Beswick EJ, Ohno T, Odenbreit S, Haas R, Reyes VE, Kita M, Graham DY, Yamaoka Y (2007) Functional and intracellular signaling differences associated with the *Helicobacter pylori* AlpAB adhesin from Western and East Asian strains. J Biol Chem 282:6242–6254. https://doi.org/10.1074/jbc.M611178200

Lu HS, Saito Y, Umeda M, Murata-Kamiya N, Zhang HM, Higashi H, Hatakeyama M (2008) Structural and functional diversity in the PAR1b/MARK2-binding region of *Helicobacter pylori* CagA. Cancer Sci 99:2004–2011. https://doi.org/10.1111/j.1349-7006.2008.00950.x

Magalhaes A, Marcos-Pinto R, Nairn AV, Dela Rosa M, Ferreira RM, Junqueira-Neto S, Freitas D, Gomes J, Oliveira P, Santos MR, Marcos NT, Xiaogang W, Figueiredo C, Oliveira C, Dinis-Ribeiro M, Carneiro F, Moremen KW, David L, Reis CA (2015) *Helicobacter pylori* chronic infection and mucosal inflammation switches the human gastric glycosylation pathways. Biochim Biophys Acta 1852:1928–1939. https://doi.org/10.1016/j.bbadis.2015.07.001

Mahdavi J, Sonden B, Hurtig M, Olfat FO, Forsberg L, Roche N, Angstrom J, Larsson T, Teneberg S, Karlsson KA, Altraja S, Wadstrom T, Kersulyte D, Berg DE, Dubois A, Petersson C, Magnusson KE, Norberg T, Lindh F, Lundskog BB, Arnqvist A, Hammarstrom L, Boren T (2002) *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. Science 297:573–578. https://doi.org/10.1126/science.1069076

Matteo MJ, Armitano RI, Romeo M, Wonaga A, Olmos M, Catalano M (2011) *Helicobacter pylori bab* genes during chronic colonization. Int J Mol Epidemiol Genet 2:286–291

Mimuro H, Suzuki T, Tanaka J, Asahi M, Haas R, Sasakawa C (2002) Grb2 is a key mediator of *helicobacter pylori* CagA protein activities. Mol Cell 10:745–755. https://doi.org/10.1016/S1097-2765(02)00681-0

Mobley HL (1996) The role of *Helicobacter pylori* urease in the pathogenesis of gastritis and peptic ulceration. Aliment Pharmacol Ther 10(Suppl 1):57–64. https://doi.org/10.1046/j.1365-2036.1996.22164006.x

Moonens K, Gideonsson P, Subedi S, Bugaytsova J, Romao E, Mendez M, Norden J, Fallah M, Rakhimova L, Shevtsova A, Lahmann M, Castaldo G, Brannstrom K, Coppens F, Lo AW, Ny T, Solnick JV, Vandenbussche G, Oscarson S, Hammarstrom L, Arnqvist A, Berg DE, Muyldermans S, Boren T, Remaut H (2016) Structural insights into polymorphic ABO glycan binding by *Helicobacter pylori*. Cell Host Microbe 19:55–66. https://doi.org/10.1016/j.chom.2015.12.004

Moonens K, Hamway Y, Neddermann M, Reschke M, Tegtmeyer N, Kruse T, Kammerer R, Mejias-Luque R, Singer BB, Backert S, Gerhard M, Remaut H (2018) *Helicobacter pylori* adhesin HopQ disrupts trans dimerization in human CEACAMs. EMBO J. https://doi.org/10.15252/embj.201798665

Morelli G, Didelot X, Kusecek B, Schwarz S, Bahlawane C, Falush D, Suerbaum S, Achtman M (2010) Microevolution of *Helicobacter pylori* during prolonged infection of single hosts and within families. PLoS Genet 6:e1001036. https://doi.org/10.1371/journal.pgen.1001036

Murata-Kamiya N, Kurashima Y, Teishikata Y, Yamahashi Y, Saito Y, Higashi H, Aburatani H, Akiyama T, Peek RM Jr, Azuma T, Hatakeyama M (2007) *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the beta-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. Oncogene 26:4617–4626. https://doi.org/10.1038/sj.onc.1210251

Nell S, Kennemann L, Schwarz S, Josenhans C, Suerbaum S (2014) Dynamics of Lewis^b binding and sequence variation of the *babA* adhesin gene during chronic *Helicobacter pylori* infection in humans. MBio 5. https://doi.org/10.1128/mBio.02281-14

Odenbreit S, Swoboda K, Barwig I, Ruhl S, Boren T, Koletzko S, Haas R (2009) Outer membrane protein expression profile in *Helicobacter pylori* clinical isolates. Infect Immun 77:3782–3790. https://doi.org/10.1128/IAI.00364-09

Oh JD, Kling-Backhed H, Giannakis M, Xu J, Fulton RS, Fulton LA, Cordum HS, Wang C, Elliott G, Edwards J, Mardis ER, Engstrand LG, Gordon JI (2006) The complete genome sequence of a chronic atrophic gastritis *Helicobacter pylori* strain: evolution during disease progression. Proc Natl Acad Sci U S A 103:9999–10004. https://doi.org/10.1073/pnas.0603784103

Ohno T, Sugimoto M, Nagashima A, Ogiwara H, Vilaichone RK, Mahachai V, Graham DY, Yamaoka Y (2009) Relationship between *Helicobacter pylori hopQ* genotype and clinical outcome in Asian and Western populations. J Gastroenterol Hepatol 24:462–468. https://doi.org/10.1111/j.1440-1746.2008.05762.x

Ohno T, Vallstrom A, Rugge M, Ota H, Graham DY, Arnqvist A, Yamaoka Y (2011) Effects of blood group antigen-binding adhesin expression during *Helicobacter pylori* infection of Mongolian gerbils. J Infect Dis 203:726–735. https://doi.org/10.1093/infdis/jiq090

Oleastro M, Menard A (2013) The role of *Helicobacter pylori* outer membrane proteins in adherence and pathogenesis. Biology (Basel) 2:1110–1134. https://doi.org/10.3390/biology2031110

Oleastro M, Cordeiro R, Ferrand J, Nunes B, Lehours P, Carvalho-Oliveira I, Mendes AI, Penque D,

Monteiro L, Megraud F, Menard A (2008) Evaluation of the clinical significance of *homB*, a novel candidate marker of *Helicobacter pylori* strains associated with peptic ulcer disease. J Infect Dis 198:1379–1387. https://doi.org/10.1086/592166

Pang SS, Nguyen ST, Perry AJ, Day CJ, Panjikar S, Tiralongo J, Whisstock JC, Kwok T (2014) The three-dimensional structure of the extracellular adhesion domain of the sialic acid-binding adhesin SabA from *Helicobacter pylori*. J Biol Chem 289:6332–6340. https://doi.org/10.1074/jbc.M113.513135

Parsonnet J, Friedman GD, Orentreich N, Vogelman H (1997) Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. Gut 40:297–301. https://doi.org/10.1136/gut.40.3.297

Peck B, Ortkamp M, Diehl KD, Hundt E, Knapp B (1999) Conservation, localization and expression of HopZ, a protein involved in adhesion of *Helicobacter pylori*. Nucleic Acids Res 27:3325–3333. https://doi.org/10.1093/nar/27.16.3325

Posselt G, Backert S, Wessler S (2013) The functional interplay of *Helicobacter pylori* factors with gastric epithelial cells induces a multi-step process in pathogenesis. Cell Commun Signal 11:77. https://doi.org/10.1186/1478-811X-11-77

Pride DT, Blaser MJ (2002) Concerted evolution between duplicated genetic elements in *Helicobacter pylori*. J Mol Biol 316:629–642. https://doi.org/10.1006/jmbi.2001.5311

Rossez Y, Gosset P, Boneca IG, Magalhaes A, Ecobichon C, Reis CA, Cieniewski-Bernard C, Joncquel Chevalier Curt M, Leonard R, Maes E, Sperandio B, Slomianny C, Sansonetti PJ, Michalski JC, Robbe-Masselot C (2014) The lacdiNAc-specific adhesin LabA mediates adhesion of *Helicobacter pylori* to human gastric mucosa. J Infect Dis 210:1286–1295. https://doi.org/10.1093/infdis/jiu239

Saberi S, Schmidt A, Eybpoosh S, Esmaili M, Talebkhan Y, Mohajerani N, Oghalaie A, Eshagh Hosseini M, Mohagheghi MA, Bugaytova J, Boren T, Mohammadi M (2016) *Helicobacter pylori* strains from duodenal ulcer patients exhibit mixed *babA/B* genotypes with low levels of BabA adhesin and Lewis^{b} binding. Dig Dis Sci 61:2868–2877. https://doi.org/10.1007/s10620-016-4217-z

Saha A, Backert S, Hammond CE, Gooz M, Smolka AJ (2010) *Helicobacter pylori* CagL activates ADAM17 to induce repression of the gastric H, K-ATPase alpha subunit. Gastroenterology 139:239–248. https://doi.org/10.1053/j.gastro.2010.03.036

Sakamoto S, Watanabe T, Tokumaru T, Takagi H, Nakazato H, Lloyd KO (1989) Expression of Lewis^{a}, Lewis^{b}, Lewis^{x}, Lewis^{y}, siayl-Lewis^{a}, and sialyl-Lewis^{x} blood group antigens in human gastric carcinoma and in normal gastric tissue. Cancer Res 49:745–752

Saunders NJ, Peden JF, Hood DW, Moxon ER (1998) Simple sequence repeats in the *Helicobacter pylori* genome. Mol Microbiol 27:1091–1098. https://doi.org/10.1046/j.1365-2958.1998.00768.x

Schreiber S, Konradt M, Groll C, Scheid P, Hanauer G, Werling HO, Josenhans C, Suerbaum S (2004) The spatial orientation of *Helicobacter pylori* in the gastric mucus. Proc Natl Acad Sci U S A 101:5024–5029. https://doi.org/10.1073/pnas.0308386101

Segal ED, Cha J, Lo J, Falkow S, Tompkins LS (1999) Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *Helicobacter pylori*. Proc Natl Acad Sci U S A 96:14559–14564. https://doi.org/10.1073/pnas.96.25.14559

Selbach M, Moese S, Meyer TF, Backert S (2002) Functional analysis of the *Helicobacter pylori cag* pathogenicity island reveals both VirD4-CagA-dependent and VirD4-CagA-independent mechanisms. Infect Immun 70:665–671. https://doi.org/10.1128/IAI.70.2.665-671.2002

Selbach M, Paul FE, Brandt S, Guye P, Daumke O, Backert S, Dehio C, Mann M (2009) Host cell interactome of tyrosine-phosphorylated bacterial proteins. Cell Host Microbe 5:397–403. https://doi.org/10.1016/j.chom.2009.03.004

Senkovich OA, Yin J, Ekshyyan V, Conant C, Traylor J, Adegboyega P, McGee DJ, Rhoads RE, Slepenkov S, Testerman TL (2011) *Helicobacter pylori* AlpA and AlpB bind host laminin and influence gastric inflammation in gerbils. Infect Immun 79:3106–3116. https://doi.org/10.1128/IAI.01275-10

Servetas SL, Kim A, Su H, Cha JH, Merrell DS (2018) Comparative analysis of the Hom family of outer membrane proteins in isolates from two geographically distinct regions: the United States and South Korea. Helicobacter 23:e12461. https://doi.org/10.1111/hel.12461

Shaffer CL, Gaddy JA, Loh JT, Johnson EM, Hill S, Hennig EE, McClain MS, McDonald WH, Cover TL (2011) *Helicobacter pylori* exploits a unique repertoire of type IV secretion system components for pilus assembly at the bacteria-host cell interface. PLoS Pathog 7:e1002237. https://doi.org/10.1371/journal.ppat.1002237

Sheu BS, Odenbreit S, Hung KH, Liu CP, Sheu SM, Yang HB, Wu JJ (2006) Interaction between host gastric Sialyl-Lewis X and *H. pylori* SabA enhances *H. pylori* density in patients lacking gastric Lewis B antigen. Am J Gastroenterol 101:36–44. https://doi.org/10.1111/j.1572-0241.2006.00358.x

Skoog EC, Sjoling A, Navabi N, Holgersson J, Lundin SB, Linden SK (2012) Human gastric mucins differently regulate *Helicobacter pylori* proliferation, gene expression and interactions with host cells. PLoS One 7:e36378. https://doi.org/10.1371/journal.pone.0036378

Snelling WJ, Moran AP, Ryan KA, Scully P, McGourty K, Cooney JC, Annuk H, O'Toole PW (2007) HorB (HP0127) is a gastric epithelial cell adhesin.

Helicobacter 12:200–209. https://doi.org/10.1111/j.1523-5378.2007.00499.x

Solnick JV, Hansen LM, Salama NR, Boonjakuakul JK, Syvanen M (2004) Modification of *Helicobacter pylori* outer membrane protein expression during experimental infection of rhesus macaques. Proc Natl Acad Sci U S A 101:2106–2111. https://doi.org/10.1073/pnas.0308573100

Styer CM, Hansen LM, Cooke CL, Gundersen AM, Choi SS, Berg DE, Benghezal M, Marshall BJ, Peek RM Jr, Boren T, Solnick JV (2010) Expression of the BabA adhesin during experimental infection with *Helicobacter pylori*. Infect Immun 78:1593–1600. https://doi.org/10.1128/IAI.01297-09

Sugimoto M, Ohno T, Graham DY, Yamaoka Y (2011) *Helicobacter pylori* outer membrane proteins on gastric mucosal interleukin 6 and 11 expression in Mongolian gerbils. J Gastroenterol Hepatol 26:1677–1684. https://doi.org/10.1111/j.1440-1746.2011.06817.x

Tabassam FH, Graham DY, Yamaoka Y (2012) *Helicobacter pylori*-associated regulation of forkhead transcription factors FoxO1/3a in human gastric cells. Helicobacter 17:193–202. https://doi.org/10.1111/j.1523-5378.2012.00939.x

Tafreshi M, Zwickel N, Gorrell RJ, Kwok T (2015) Preservation of *Helicobacter pylori* CagA translocation and host cell proinflammatory responses in the face of CagL Hypervariability at amino acid residues 58/59. PLoS One 10:e0133531. https://doi.org/10.1371/journal.pone.0133531

Talarico S, Whitefield SE, Fero J, Haas R, Salama NR (2012) Regulation of *Helicobacter pylori* adherence by gene conversion. Mol Microbiol 84:1050–1061. https://doi.org/10.1111/j.1365-2958.2012.08073.x

Tchoupa AK, Schuhmacher T, Hauck CR (2014) Signaling by epithelial members of the CEACAM family – mucosal docking sites for pathogenic bacteria. Cell Commun Signal 12:27. https://doi.org/10.1186/1478-811X-12-27

Tegtmeyer N, Hartig R, Delahay RM, Rohde M, Brandt S, Conradi J, Takahashi S, Smolka AJ, Sewald N, Backert S (2010) A small fibronectin-mimicking protein from bacteria induces cell spreading and focal adhesion formation. J Biol Chem 285:23515–23526. https://doi.org/10.1074/jbc.M109.096214

Tegtmeyer N, Wessler S, Backert S (2011) Role of the *cag*-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. FEBS J 278:1190–1202. https://doi.org/10.1111/j.1742-4658.2011.08035.x

Tegtmeyer N, Lind J, Schmid B, Backert S (2014) *Helicobacter pylori* CagL Y58/E59 mutation turns-off type IV secretion-dependent delivery of CagA into host cells. PLoS One 9:e97782. https://doi.org/10.1371/journal.pone.0097782

Tegtmeyer N, Harrer A, Schmitt V, Singer BB, Backert S (2019) Expression of CEACAM1 or CEACAM5 in AZ-521 cells restores the type IV secretion deficiency for translocation of CagA by *Helicobacter pylori*. Cell Microbiol 21:e12965. https://doi.org/10.1111/cmi.12965

Teymournejad O, Mobarez AM, Hassan ZM, Talebi Bezmin Abadi A (2017) Binding of the *Helicobacter pylori* OipA causes apoptosis of host cells via modulation of Bax/Bcl-2 levels. Sci Rep 7:8036. https://doi.org/10.1038/s41598-017-08176-7

Tomb JF, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, Ketchum KA, Klenk HP, Gill S, Dougherty BA, Nelson K, Quackenbush J, Zhou L, Kirkness EF, Peterson S, Loftus B, Richardson D, Dodson R, Khalak HG, Glodek A, McKenney K, Fitzegerald LM, Lee N, Adams MD, Hickey EK, Berg DE, Gocayne JD, Utterback TR, Peterson JD, Kelley JM, Cotton MD, Weidman JM, Fujii C, Bowman C, Watthey L, Wallin E, Hayes WS, Borodovsky M, Karp PD, Smith HO, Fraser CM, Venter JC (1997) The complete genome sequence of the gastric pathogen *Helicobacter pylori*. Nature 388:539–547. https://doi.org/10.1038/41483

Tsutsumi R, Higashi H, Higuchi M, Okada M, Hatakeyama M (2003) Attenuation of *Helicobacter pylori* CagA x SHP-2 signaling by interaction between CagA and C-terminal Src kinase. J Biol Chem 278:3664–3670. https://doi.org/10.1074/jbc.M208155200

Unemo M, Aspholm-Hurtig M, Ilver D, Bergstrom J, Boren T, Danielsson D, Teneberg S (2005) The sialic acid binding SabA adhesin of *Helicobacter pylori* is essential for nonopsonic activation of human neutrophils. J Biol Chem 280:15390–15397. https://doi.org/10.1074/jbc.M412725200

Veerman EC, Bolscher JG, Appelmelk BJ, Bloemena E, van den Berg TK, Nieuw Amerongen AV (1997) A monoclonal antibody directed against high M (r) salivary mucins recognizes the SO3-3Gal beta 1-3GlcNAc moiety of sulfo-Lewis[a]: a histochemical survey of human and rat tissue. Glycobiology 7:37–43. https://doi.org/10.1093/glycob/7.1.37

Walz A, Odenbreit S, Mahdavi J, Boren T, Ruhl S (2005) Identification and characterization of binding properties of *Helicobacter pylori* by glycoconjugate arrays. Glycobiology 15:700–708. https://doi.org/10.1093/glycob/cwi049

Walz A, Odenbreit S, Stuhler K, Wattenberg A, Meyer HE, Mahdavi J, Boren T, Ruhl S (2009) Identification of glycoprotein receptors within the human salivary proteome for the lectin-like BabA and SabA adhesins of *Helicobacter pylori* by fluorescence-based 2-D bacterial overlay. Proteomics 9:1582–1592. https://doi.org/10.1002/pmic.200700808

Wiedemann T, Hofbaur S, Tegtmeyer N, Huber S, Sewald N, Wessler S, Backert S, Rieder G (2012) *Helicobacter pylori* CagL dependent induction of gastrin expression via a novel alphavbeta5-integrin-integrin linked kinase signalling complex. Gut 61:986–996. https://doi.org/10.1136/gutjnl-2011-300525

Williams JC, McInnis KA, Testerman TL (2008) Adherence of *Helicobacter pylori* to abiotic surfaces is influenced by serum. Appl Environ Microbiol 74:1255–1258. https://doi.org/10.1128/AEM.01958-07

Yamaoka Y, Kwon DH, Graham DY (2000) A M (r) 34,000 proinflammatory outer membrane protein (oipA) of *Helicobacter pylori*. Proc Natl Acad Sci U S A 97:7533–7538. https://doi.org/10.1073/pnas.130079797

Yamaoka Y, Kikuchi S, el-Zimaity HM, Gutierrez O, Osato MS, Graham DY (2002a) Importance of *Helicobacter pylori oipA* in clinical presentation, gastric inflammation, and mucosal interleukin 8 production. Gastroenterology 123:414–424. https://doi.org/10.1053/gast.2002.34781

Yamaoka Y, Kita M, Kodama T, Imamura S, Ohno T, Sawai N, Ishimaru A, Imanishi J, Graham DY (2002b) *Helicobacter pylori* infection in mice: role of outer membrane proteins in colonization and inflammation. Gastroenterology 123:1992–2004. https://doi.org/10.1053/gast.2002.37074

Yamaoka Y, Souchek J, Odenbreit S, Haas R, Arnqvist A, Boren T, Kodama T, Osato MS, Gutierrez O, Kim JG, Graham DY (2002c) Discrimination between cases of duodenal ulcer and gastritis on the basis of putative virulence factors of *Helicobacter pylori*. J Clin Microbiol 40:2244–2246. https://doi.org/10.1128/JCM.40.6.2244-2246.2002

Yamaoka Y, Ojo O, Fujimoto S, Odenbreit S, Haas R, Gutierrez O, El-Zimaity HM, Reddy R, Arnqvist A, Graham DY (2006) *Helicobacter pylori* outer membrane proteins and gastroduodenal disease. Gut 55:775–781. https://doi.org/10.1136/gut.2005.083014

Yanai A, Maeda S, Hikiba Y, Shibata W, Ohmae T, Hirata Y, Ogura K, Yoshida H, Omata M (2007) Clinical relevance of *Helicobacter pylori sabA* genotype in Japanese clinical isolates. J Gastroenterol Hepatol 22:2228–2232. https://doi.org/10.1111/j.1440-1746.2007.04831.x

Yeh YC, Chang WL, Yang HB, Cheng HC, Wu JJ, Sheu BS (2011) H. pylori cagL amino acid sequence polymorphism Y58E59 induces a corpus shift of gastric integrin alpha5beta1 related with gastric carcinogenesis. Mol Carcinog 50:751–759. https://doi.org/10.1002/mc.20753

Yeh YC, Cheng HC, Yang HB, Chang WL, Sheu BS (2013) H. pylori CagL-Y58/E59 prime higher integrin alpha5beta1 in adverse pH condition to enhance hypochlorhydria vicious cycle for gastric carcinogenesis. PLoS One 8:e72735. https://doi.org/10.1371/journal.pone.0072735

Zebhauser R, Kammerer R, Eisenried A, McLellan A, Moore T, Zimmermann W (2005) Identification of a novel group of evolutionarily conserved members within the rapidly diverging murine Cea family. Genomics 86:566–580. https://doi.org/10.1016/j.ygeno.2005.07.008

Zhang XS, Tegtmeyer N, Traube L, Jindal S, Perez-Perez-G, Sticht H, Backert S, Blaser MJ (2015) A specific A/T polymorphism in Western tyrosine phosphorylation B-motifs regulates *Helicobacter pylori* CagA epithelial cell interactions. PLoS Pathog 11(2):e1004621. https://doi.org/10.1371/journal.ppat.1004621