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TOPIC HIGHLIGHT

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Factors that mediate colonization of the human stomach by *Helicobacter pylori*

Ciara Dunne, Brendan Dolan, Marguerite Clyne

Ciara Dunne, Brendan Dolan, Marguerite Clyne, School of Medicine and Medical Science and Conway Institute of Biomedical and Biomolecular Science, University College Dublin, Belfield, Dublin 4, Ireland
Author contributions: Dunne C, Dolan B and Clyne M performed the research, analyzed the data, and wrote the paper.
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Correspondence to: Marguerite Clyne, PhD, School of Medicine and Medical Science and Conway Institute of Biomedical and Biomolecular Science, University College Dublin, Belfield, Dublin 4, Ireland. marguerite.clyne@ucd.ie
Telephone: +353-1-7166619 Fax: +353-1-7166619
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Abstract

Helicobacter pylori (*H. pylori*) colonizes the stomach of humans and causes chronic infection. The majority of bacteria live in the mucus layer overlying the gastric epithelial cells and only a small proportion of bacteria are found interacting with the epithelial cells. The bacteria living in the gastric mucus may act as a reservoir of infection for the underlying cells which is essential for the development of disease. Colonization of gastric mucus is likely to be key to the establishment of chronic infection. How *H. pylori* manages to colonise and survive in the hostile environment of the human stomach and avoid removal by mucus flow and killing by gastric acid is the subject of this review. We also discuss how bacterial and host factors may together go some way to explaining the susceptibility to colonization and the outcome of infection in different individuals. *H. pylori* infection of the gastric mucosa has become a paradigm for chronic infection. Understanding of why *H. pylori* is such a successful pathogen may help us understand how other bacterial species colonise mucosal surfaces and cause disease.

Key words: *Helicobacter pylori*; Colonization; Infection; Gastric mucosa; Urease; Flagella; Polymorphisms; Adhesins; CagA; Type Ⅳ secretion system

Core tip: Colonization of gastric mucus by *Helicobacter pylori* (*H. pylori*) key to the establishment of chronic infection. How *H. pylori* manages to colonise and survive in the hostile environment of the human stomach and avoid removal by "mucus flow" and killing by gastric acid is the subject of this review. We also discuss how bacterial and host factors may together go some way to explaining the susceptibility to colonization and the outcome of infection in different individuals. Understanding of how *H. pylori* causes chronic infection will likely serve as a valuable reference system for how other bacteria colonise mucosal surfaces.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram negative, microaerophillic, spiral shaped bacterium, which colonizes the human stomach. *H. pylori* is one of the most common infections in the world, persistently colonizing the gastric mucosa of over 50% of the global population.

Colonization with *H. pylori* induces a chronic gastritis in all infected individuals[1]. The majority of infections are asymptomatic, however long-term infection increases the risk of developing site-specific disease. 10%-15% of infections result in the development of peptic ulcer disease and *H. pylori* is associated with 95% of duodenal ulcers and 80% of gastric ulcers[2]. Infection with *H. pylori* is a significant risk factor for the development of gastric cancer and 1%-3% of infected individuals develop the disease[3,4]. As a result, *H. pylori* is classified as a group 1 carcinogen by the World Health Organisation[5]. Infection is associated in particular with intestinal-type (approximately 90% of patients) rather than diffuse-type gastric cancers (approximately 32% of patients)[6]. The risk of developing gastric cancer is reduced in patients with duodenal ulcers[7]. The gastric mucosa does not normally contain any mucosa-associated lymphoid tissue (MALT), however the pan-gastric inflammation induced by *H. pylori* infection results in the development of MALT. In < 0.1% of infected individuals this develops into B cell MALT lymphoma[3], however at early stages the lymphoma can be cured by eradication of *H. pylori*[8,9].

H. pylori exhibit a very strict tissue tropism. It colonizes the gastric mucosa of humans and is only found colonizing other sites in the body where gastric metaplasia occurs[10]. Within the gastric mucosa the majority of organisms are found living in gastric mucus[11] and we suggest that these organisms act as a reservoir of infection for the underlying epithelial cells. Only a small percentage of the organisms colonizing are found in association with epithelial cells but the interaction of the bacteria with the cells is essential for the development of disease. How *H. pylori* causes chronic infection, which can persist for the lifetime of the host, in the hostile acidic environment of the stomach while avoiding removal by both mucus, which is constantly "turning over", and the immune response is not completely understood. Evasion of the host innate immune response by *H. pylori* has recently been covered in two excellent reviews[12,13]. This review will focus on what we know about other specific bacterial and host factors that promote *H. pylori* survival in the stomach and colonization of the gastric mucosa, causing disease in some individuals but asymptomatic infection in others.

UREASE AND MOTILITY: TWO KEY ESSENTIAL COLONIZATION FACTORS

Urease

H. pylori is not an acidophile and key to its ability to overcome the acidic conditions of the gastric lumen is the production of a very potent urease enzyme. The expression of urease[14] and its activation[15] is essential for colonization of the gastric mucosa. *H. pylori* survives at a pH range between 4.0 and 8.0 in the absence of urea[16]. However, in the presence of urea the organism can survive at a pH as low as 2.5. The urease enzyme of *H. pylori* hydrolyses urea to NH_3 and CO_2 and has a Km value for urea of 0.8 mmol/L[17], meaning that it displays a much higher affinity for its substrate than that of ureases produced by other bacterial species. This allows for the utilization of the limited amounts of urea (5 mmol/L) present in the human stomach. The generation of NH_3 provides both acid-neutralising and acid buffering capabilities, enabling *H. pylori* to raise the pH in its microenvironment and periplasm thus maintaining the proton motive force. The biosynthesis of urease is controlled by a seven gene cluster. The urease enzyme, estimated to be approximately 600 kDa in size[18], consists of a complex of 12 UreA and UreB subunits[19], which individually are 30 and 62 kDa in size[18]. The protein is originally produced as an immature apoenzyme, and activation takes place when four chaperone proteins, UreE, UreF, UreG and UreH assemble the catalytic site of the protein[20]. The insertion of 24 nickel ions into the enzyme is essential for complete activation of the protein, as well as GTP hydrolysis[19]. The genes encoding UreAB are located in one operon[21], while the *ureEFGH* genes are found on another[22]. Recent studies have shown that the *ureAB* operon can yield a 2.7 kB transcript that produces a functional enzyme and also a 1.4 kB transcript, the product of which exhibits much lower urease activity and is generated by cleavage of the 3' *ureB* region. The expression of this smaller transcript was shown to be influenced by pH, the presence of the histidine kinase ArsS and the phosphorylation state of the response regulator ArsR, illustrating the influence that pH has on the expression of an active urease[23]. Urease is found in both the cytoplasm of *H. pylori* and on the surface of the bacteria due to the lysis of some organisms[24-26]. Intracellular urease, regulated by external pH, acts to increase the pH of the periplasm and increase membrane potential thereby allowing protein synthesis at low pH[27,28]. A proton-gated channel, UreI, which regulates the uptake of urea[29], is only active at acidic pH and therefore does not allow for the transport of urea into the bacterial cell at neutral pH, thus preventing lethal alkalinisation of the cytoplasm[30]. Buffering of the periplasm also occurs through the conversion of CO_2 to HCO_3^- by the periplasmic α-carbonic anhydrase[31]. HCO_3^- acts in conjunction with NH_3 to buffer the periplasmic and cytoplasmic pH, generating neutral conditions. This acid acclimation feature of the bacteria, maintaining an intracellular neutral pH while the pH of the environment is acidic, is unique to *H. pylori*, and is critical to survival of the organism in the stomach. The data above is strong evidence that the urease enzyme of *H. pylori* is a factor absolutely essential for survival of the organism in the human stomach. Indeed it may be a factor that explains why *H. pylori* is not found readily at other sites in the body, as production of ammonia by the urease enzyme at sites that are not acidic could increase the local pH above pH 7.0 and it has been shown that *H. pylori* is sensitive to alkaline conditions[30].

Motility

Another key colonization factor shown to be absolutely essential for colonization is the possession of polar flagella which confer motility on *H. pylori*[32]. Non-motile mutants lacking flagella are unable to establish persistent infection in animal models[33,34]. Studies focusing on the motor protein MotB, have shown that it is the motility conferred on the organism by the flagella that contributes to colonization and the presence of flagella alone is not sufficient[35]. *H. pylori* possesses two to six sheathed unipolar flagella. The structures extend 3-5 μm from the bacterial surface, with bulb-like structures often seen at the tip of the filaments[32]. The sheath consists of both proteins and lipopolysaccharide, and is thought to be an extension of the bacterial outer membrane that protects the flagellar filaments from acid in the stomach[36]. Expression of the two major flagellar proteins, FlaA and FlaB, are required for full motility of the bacteria. FlaA mutants exhibit a greater decrease in motility than that of FlaB mutants[37]. Other components of the flagellar structure which have also been shown to be essential for motility and colonization include the hook protein FlgE[38] and FliD, which functions as a hook-associated protein[39].

In keeping with flagella being an essential colonization factor for *H. pylori* flagellar biogenesis is a very well regulated process, dysfunction of which can have a strong impact on motility and infection. For example, deletion of the regulator FlhA, which controls expression of *flaA, flaB* and *flgE*, leads to the generation of non-motile mutants[40]. Of interest FlhA also regulates urease expression, and deletion of this membrane protein leads to a decrease in colonization rates[41]. Thus flagellar biosynthesis and urease activity, two key essential colonization factors of *H. pylori*, are linked. The response regulator FlgR, which is part of the two-component FlgRS system, controls expression of RpoN-regulated genes, including *flgE* and *flaB*, and deletion of this regulator abolishes expression of its target genes[42]. *H. pylori* utilises the histidine kinase FlgS to detect changes in its environment which in turn activates FlgR to modify transcription of flagellar genes. Interestingly, it does so independently of pH, so there must be other environmental triggers that activate transcription of flagellar genes[43]. More recently, production of auto-inducer 2 (AI-2) protein, a product of the *luxS* gene, has been shown to regulate expression of flagellar genes. Auto-inducers play a role in quorum sensing, and so it is possible that *H. pylori* regulates expression of its flagellar genes in response to bacterial numbers and it's environment through this system[44,45]. This is supported by evidence that mutants deficient in *luxS* exhibit reduced motility and infectivity rates compared to the wild-type[46]. Thus efficient and controlled expression and synthesis of the flagellar components is essential for successful motility and colonization.

Modifications to the flagella that impact on motility and colonization can also occur at a post-transcriptional level. The glycosylation state of the structure has been shown to impact on both, with *H. pylori* mutants deficient in a deglycosylase HP0518, exhibiting increased levels of the pseudaminic acid on FlaA and displaying a hypermotile phenotype. Infection studies showed that the mutants are able to associate more closely with epithelial cells and induce rapid activation of NF-κB, and also exhibit increased *in vivo* colonization rates. Thus increased levels of flagellin glycosylation in turn seem to increase the ability of *H. pylori* to colonize[47]. Other genes, including HP0326B and HP0178, involved in the biosynthesis of the precursors to pseudaminic acid, have been shown to have a similar role to HP0518. Inactivation of these also affects motility and colonization[48]. As well as modifying sugars found directly on flagellin proteins, changes in bacterial peptidoglycan by transglycosylases affects the functioning of the flagella by disturbing the localisation of MotB, thus preventing the flagellum from moving[49]. Thus, like the urease enzyme, motility is an essential colonization factor for *H. pylori*. The structure of the flagella with an outer sheath to protect against the effect of acid, stringent regulation of flagellar biogenesis and post translational modifications to flagella which result in enhanced motility and ability to colonise all underline the pivotal role of motility and flagella in *H. pylori* colonization.

COLONIZATION OF GASTRIC MUCUS BY *H. PYLORI*

The mucus layer that overlies the epithelial cells in the gastrointestinal tract is a physical barrier which acts to prevent pathogens from colonizing and interacting with the underlying epithelium. Pathogens which infect mucosal surfaces share two main goals: (1) to overcome the mucus barrier; and (2) to interact with the underlying epithelial cells which results in disease. The majority (approximately 80%) of infecting *H. pylori* are found living in gastric mucus rather than in contact with the underlying epithelium[11].

Penetration of gastric mucus

The entire epithelial surface of the gastrointestinal tract is covered in a thick layer of secreted mucus[50]. The mucus layer in the stomach is approximately 300 μm thick[50], and *H. pylori* has to penetrate it in order to colonise and gain access to the underlying epithelium. A pH gradient exists across the gastric mucus layer with the pH being approximately neutral at the epithelium but very acidic (pH 1-2) close to the lumen. *H. pylori* can lose motility rapidly when it encounters acidic conditions[51], therefore it is imperative that it penetrates the gastric mucus quickly and establish persistent colonization in an area close to the epithelium[52].

H. pylori is able to alter mucus structure which may aid movement through the viscoelastic mucus gel layer. A thioredoxin system that specifically reduces interchain disulphide bonds of mucins has been identified[53]. This reduces the gel-forming capabilities of mucins and therefore the viscoelastic properties of mucus aid-

ing travel of the bacterium through mucus. In addition the rheology of gastric mucin exhibits a reversible pH-dependent transition. At a strongly acidic pH the viscoelastic properties of mucus increase whereas the mucus becomes less gel-like as the pH increases above approximately 4.0[54]. The ability of *H. pylori* to utilise urea to raise the pH in its microenvironment modifies mucus so that it is less gel-like, enabling the bacterium to move quickly through it[55]. *H. pylori* is usually found close to the gastric epithelium[56]. When the pH gradient that exists across the gastric mucus layer was disrupted in Mongolian gerbils *H. pylori* were no longer found close to the epithelium but were scattered throughout the mucus layer, suggesting that pH plays an important role in maintaining the particular localization of *H. pylori*[56]. The characteristic helical shape of *H. pylori* is also thought to play a role in mediating penetration of gastric mucus. Alterations in the cross linking of peptidoglycan in the outer membrane has been shown to modify the shape of *H. pylori.* Some cell shape mutants are unable to colonise as efficiently as helical shaped bacteria despite displaying similar motility to wildtype bacteria *in vitro*[57]. However mutants which exhibited the most dramatic changes in cell shape had reduced motility compared to that of the wild-type and other mutants, and they exhibited further reduced ability to colonise mice compared to mutants that retained wild type motility. This led to the hypothesis that the characteristic helical shape of *H. pylori* allows the bacteria to penetrate gastric mucus in a corkscrew like motion[58].

Interaction of H. pylori with gastric mucus

H. pylori has been shown to form microcolonies within the mucus secreted by the surface epithelium of the gastric mucosa[59]. MUC5AC is the predominant secreted gel forming mucin expressed by gastric surface epithelial cells and *H. pylori* has been shown to co-localise with MUC5AC *in vivo*[60]. Lower mucus neck cells found in the antral glands produce MUC6. α1,4-linked N-acetyl-glucosamine capped O-linked glycans expressed on the surface of MUC6 have anti-microbial activity towards *H. pylori,* inhibiting the synthesis of cholesteryl-α-D-glucopyranoside a vital cell wall component[61]. Aberrant expression of MUC2 occurs occasionally in the stomach in areas of intestinal metaplasia, however *H. pylori* is not found in association with areas of complete intestinal metaplasia, although it is sometimes found in areas of incomplete metaplasia[62,63]. This suggests that MUC5AC or a molecule co-expressed with it may explain the preference of *H. pylori* for gastric mucus.

H. pylori has been shown to interact with the Lewis^b blood group antigen structure found on the surface of MUC5AC in gastric mucus and this interaction, which has been extensively studied, is mediated through the bacterial outer membrane protein BabA[64]. *H. pylori* can also interact with sialylated structures found on mucins *via* the sialic-acid binding adhesin (SabA). This 66 kDa outer membrane protein is a major adhesin of *H. pylori* and mediates binding to sialyl-Lewis^x and sialyl-Lewis^a glycoconjugates[65]. During chronic infection by *H. pylori* there is an increase in the proportion of sialylated structures present in the gastric mucosa, which is attributed to mucosal inflammation and transformation[66], and so SabA is thought to play a role in promoting chronic infection.

H. pylori can also interact with the membrane bound mucin MUC1 and this has been shown to be mediated *via* both BabA and SabA outer membrane adhesins. A Δ*babA* strain of *H. pylori* displayed significantly reduced adherence to MUC1 from two independent sources[67]. In addition, mutants lacking SabA, also displayed some reduction in binding suggesting that binding to sialylated structures play a role in the interaction of *H. pylori* with MUC1 *in vivo*[67]. MUC1 has been shown to be important in limiting infection by Helicobacter in a mouse model of infection[68]. Over expression of MUC1 was shown to counter regulate *H. pylori* induced gastric inflammation[69]. The interaction of *H. pylori* with MUC1 blocks *H. pylori* stimulated β-catenin nuclear translocation and attenuates IL-8 production and neutrophil induced gastric inflammation[70]. Speculation that augmented expression of MUC1 could be used for treatment of *H. pylori* gastritis highlights the need for model systems that express adherent mucus layers and appropriate mucins in order to test such hypotheses. Using an *in vitro* infection model we recently showed a clear interaction between *H. pylori* and the membrane bound mucin MUC1 present on the apical surface of the mucus secreting cell line HT29-MTX-E12[71] suggesting that this might be a useful model system to study the interaction of *H. pylori* with mucus and mucins.

Infection of mice with *H. pylori* demonstrated a reduction in the rate of mucin turnover and decreased levels of MUC1 in infected animals, thus creating a more stable niche for the bacteria to colonise and increasing the ability of the organisms to interact with the epithelial cells[72]. The bacterial factors which signal to the cells to modulate mucin synthesis and turnover have not yet been elucidated.

Gastric mucins have been shown to promote the proliferation of *H. pylori* and to alter gene expression[73]. We looked at the interaction of *H. pylori* with purified native mucins from different animals that were printed on a microarray slide. Three strains of *H. pylori* were examined, strains J99, G27 and 26695. Strain 26695 is known not to express active BabA or SabA proteins and is therefore unable to bind to Lewis^b or Sialyl Lewis^x. Surprisingly all three strains bound to porcine gastric mucins equally well. This finding suggests that in addition to BabA and SabA other bacterial adhesins must exist which can mediate the binding of *H. pylori* strains such as strain 26695 to mucin.

While the interaction of *H. pylori* with gastric mucins has been well characterized the interaction of the organism with other non-mucin components of mucus has not received as much attention. In addition to mucin *H.*

pylori has recently been shown to interact with mucus bound glycolipids[74] and we have previously shown that *H. pylori* interacts directly with TFF1, a member of the trefoil peptide family of proteins which is co-expressed with MUC5AC in the stomach, and this interaction is mediated by the core oligosaccharide portion of *H. pylori* LPS[75,76]. The optimum pH for *H. pylori* binding to TFF1 was 5.0-6.0[76]. The pH dependence of this interaction indicates that binding of *H. pylori* to TFF1 in the stomach could promote colonization of the mucus layer adjacent to the gastric epithelial surface. The interaction of *H. pylori* with TFF1 may explain the distinct tropism that the organism exhibits for gastric tissue and specifically for the gastric mucin MUC5AC. A role for TFF1 in colonization of mucosal surfaces by *H. pylori* was confirmed in a study which showed that *H. pylori* mutants expressing a truncated core oligosaccharide unable to interact with TFF1 have a reduced ability to colonise the mucus layer produced by the mucus secreting HT29-MTX-E12 cell line compared to wildtype strains[71]. In summary *H. pylori* interacts with both secreted and membrane bound mucins in the intestinal tract. These interactions are mediated *via* the well described outer membrane adhesins BabA and SabA interacting with fucosylated and sialylated glycans found on mucins and possibly other as yet undescribed adhesins. *H. pylori* has also been shown to interact with TFF1 a small protein found co-expressed with MUC5AC in the stomach. This suggests that the interaction of bacteria with non mucin components of mucus warrants further investigation.

INTERACTION OF *H. PYLORI* WITH THE GASTRIC EPITHELIAL CELLS

While the majority of *H. pylori* organisms live in gastric mucus and only a small percentage of infecting organisms interact with gastric epithelial cells, it is widely accepted that the organisms in contact with the epithelial cells cause disease. A number of bacterial outer membrane proteins have been identified that can act as adhesins. Following binding of *H. pylori* to epithelial cells the organism signals to the cells to subvert host cell function and this results in the development of pathology.

BabA

BabA together with SabA, both already mentioned above, are the two best characterized adhesins of *H. pylori*. Binding of *H. pylori* to fucosylated structures, including the H-1 type and Lewis^b blood group antigen[77] is mediated *via* BabA, a 78 kDa outer membrane protein[64]. Two genes encode for a BabA protein, *babA1* and *babA2*. Only the protein encoded by *babA2* is functionally active, due to the presence of a 10 bp insert in the gene that encodes for a translational initiation codon[64].

A high level of heterogeneity is found in the BabA protein amongst strains, with various polymorphisms being identified, and different levels of Lewis^b binding observed[78]. There is also a high level of allelic variation in *bab* genes. *H. pylori* possesses a closely related gene to *babA*, *babB*, and both proteins are highly similar in their N- and C-terminus regions, but vary quite significantly in their central region. The BabB protein does not bind Lewis^b, indicating that the central region of the two proteins confers unique functions[79].

Geographic location can also influence the binding specificities of BabA, with strains from specific regions often exhibiting a specialist phenotype in which they only bind to certain blood group antigens, whereas strains from other regions are more generalised in their BabA-mediated binding and interact with numerous different blood-group antigens. This differential binding is thought to be a result of a selective pressure that led to evolution of the BabA protein, with the predominant blood group of a specific region influencing the binding specificity of the BabA adhesin[80]. Furthermore, strains have been identified which possess an active *babA2* gene, but do not produce a functional protein[81].

BabA expression is extremely dynamic *in vivo*. Experimental infection of Rhesus macaques showed a loss of babA expression post-infection, caused by either a change in the number of CT dinucleotide repeats in the 5' region of *babA*, or replacement of *babA* by the uncharacterised *babB*. Both events yielded a non-functional BabA protein that exhibited no Lewis^b binding[82]. Another study showed loss of BabA expression six months after infection of Mongolian gerbils, and this was attributed to nucleotide changes that introduced a stop codon into the sequence, which in turn yielded a truncated BabA protein[83]. When these results were elaborated on, experiments showed that modification in six amino acids eliminated binding of *H. pylori* BabA to Lewis^b following infection of Rhesus macaques. The *babA2* gene underwent gene conversion in which a portion of the gene was replaced by a portion of the non-functioning *babA1* gene. While the strains still expressed BabA, these six amino acid changes were enough to eliminate BabA binding[84]. A recent study reported inter-micro-niche variation in *H. pylori* infected patients, with isolates of the same strain exhibiting differences in *babA* and *babB* copy number and gene location, as well as BabA/B chimeras[85]. BabA is thus a highly variable protein that is easily susceptible to change at both a genetic and protein level.

SabA

H. pylori binds to sialylated structures present on gastric mucin and on epithelial cells *via* the sialic-acid binding adhesin, SabA. SabA also binds to sialylated receptors on neutrophils, which leads to nonopsonic activation of the neutrophils, phagocytosis of the bacteria and induction of the oxidative burst response[86]. Furthermore, the adhesin exhibits haemagglutinating activity, binding to gangliosides on erythrocytes in mucosal blood vessels. Differences between strains in their ability to bind to sialylated carbohydrates has been seen, and such differences may allow for the pathogen to adapt to changing

glycosylation patterns in the host during infection[87]. Similar to BabA there is a high degree of genetic diversity in SabA. The gene has a number of poly-T tracts in its promoter region and a stretch of CT dinucleotide repeats in its coding region[88]. Phase variation can occur at these sites, and leads to allelic variation in the SabA locus.

Differences in the length of the CT dinucleotide repeats found in the coding regions are often seen, giving rise to various alleles. The number of repeats differs among strains, and was originally thought to determine the functionality of the *sabA* gene, with seven repeats yielding a functional gene, while six or eight repeats leads to the gene being turned off[89]. However, later findings speculated that other sequences in the gene rather than the number of repeats were playing a role in the functionality of SabA. The sequence following the CT repeat region has been shown to play a role in expression, with strains possessing seven CT repeats having both in frame and out of frame *sabA* genes depending on the nucleotide sequence following the repeat region[88].

The poly-T tracts which are found upstream of the *sabA* gene also undergo phase variation. Recently it was shown that the length of these tracts can vary between strains, and that the length can influence the promoter activity of the *sabA* gene. This variation also arises through slip-strand mispairing, similar to the CT repeats[90]. This variation could be very beneficial to *H. pylori*, for example when it is exposed to acidic conditions. SabA is regulated by the acid-responsive two-component ArsRS system[91]. Expression of the gene correlates inversely with the acid secretion in the stomach[92]. Therefore, when acidic conditions prevail, there is low SabA expression. The ArsRS system itself appears to repress expression of SabA, with a major increase in adherence dependent on SabA seen in mutants lacking the histidine kinase ArsS. However, this repressive effect on SabA expression only occurs in strains that have an in-frame SabA allele[93]. This highlights the role that phase variation plays in infection, as it may allow *H. pylori* to alter its adhesin expression in response to a change in environmental conditions.

Another mechanism that *H. pylori* utilises to modify *SabA* expression is gene duplication. It was recently shown that an increase in the copy number of *sabA* leads to greater production of the protein, which in turn leads to greater adherence by the bacteria *in vitro*. It is thought this gene conversion event occurs due to natural uptake of the DNA by competent bacteria[94]. The *sabA* locus is therefore a highly heterogenic one, enabling *H. pylori* to alter its adhesin profile depending on the microenvironment and what host defences it comes in contact with.

AlpA and AlpB

AlpA and AlpB are two closely related proteins carried on the same operon in the *H. pylori* genome[95]. Loss of these proteins was found to influence the ability of *H. pylori* to colonise the guinea pig stomach[96] and to bind to gastric tissue, indicating a role in adhesion of the bacteria[97]. Virtually all strains express AlpA and AlpB, indicating they have an essential function[98]. Recently, these two proteins were shown to contribute to the ability of *H. pylori* to bind host laminin[99]. While their role in colonization is established, the effect AlpA/B has on infection remains unclear.

HopZ

Helicobacter expresses up to 33 outer membrane proteins (OMPs) or Hops (Helicobacter outer membrane porins). HopZ was identified as an OMP of *H. pylori* that plays a role in colonization[100]. Two allelic variants of the *hopZ* gene were identified, with a 20 amino acid region present in only one allele. The sequence of *hopZ* consists of a number of dinucleotide repeats in the signal peptide. These have also been found in other outer membrane proteins of *H. pylori*, and it is thought that they allow for slip-strand mispairing, which in turn generates phenotypic variation between strains. The number of repeats varies among strains, and determines functionality of the protein, with 7 or 10 dinucleotides allowing for expression of an intact protein[100]. The role of HopZ in infection has been largely unexplored. There is strong selection *in vivo* for HopZ expression as *hopZ* ON variants were recovered from volunteers challenged with a *hopZ* OFF strain, BCS 100[101]. Transmission of *H. pylori* within families has also been associated with a status change of *hopZ*. In contrast, *hopZ* sequences obtained from 26 sets of sequential isolates from chronically infected individuals showed no changes of status, suggesting that the *hopZ* status selected during early infection is subsequently stable[101].

CagA and cag PAI

Bacterial factors that increase the virulence of certain strains are responsible in part for the outcome of infection with *H. pylori*. The best characterized virulence factor is the product of the cytotoxin-associated gene A, CagA[102]. Strains expressing CagA are associated with more severe forms of disease and expression is closely related to that of the vacuolating cytotoxin, VacA[103]. Following binding of *H. pylori* to epithelial cells strains that express the CagA protein can inject it into the cells *via* a Type IV secretion system (T4SS) encoded by genes present on a pathogenicity island termed the *cag*PAI. Upon translocation into host cells CagA can be phosphorylated by host cell kinases and act to subvert host cell signaling mechanisms[104].

The *cagPAI* contains approximately 30 genes, many of which are involved in synthesis of the T4SS[105]. CagL is a highly conserved protein amongst *H. pylori* strains that forms the tip of the pilus of the T4SS, allowing for CagA translocation into host cells. It possesses an arginine-glycine-aspartate (RGD) motif, which allows the bacteria to bind to the $\alpha_5\beta_1$ integrin on the surface of target cells[106]. A closely related protein, CagI, has also

been shown to be an essential part of the T4SS, and the expression of both proteins is influenced by the expression of other *cag*PAI products, indicating that their expression requires partial assembly of the T4SS[107]. CagI however is not required for transport of CagA from the cytoplasm to the bacterial membrane[108]. The recently annotated transmembrane spanning CagM protein also appears to contribute to CagA translocation, with mutants deficient in the protein exhibiting little to no CagA translocation upon contact with host cells[109]. Non-CagPAI proteins have also been shown to be involved in the translocation process. For example the outer membrane protein HopQ is required for CagA injection as well as the intracellular responses induced by CagA[110]. While not essential, the iron-regulator Fur was also shown to influence CagA expression and cellular phenotypic changes induced upon CagA injection using adherence assays with AGS cells[111]. CagA positive strains of *H. pylori* which possess a functional BabA along with VacA are thought to be more virulent in the disease type they induce[112]. BabA-mediated adherence contributes to the pathogenesis of disease by inducing proinflammatory cytokines and precancerous related factors (such as CDX2), a phenotype which is dependent upon expression of the T4SS[113]. While BabA appears to promote CagA mediated pathogenesis, in contrast, binding of *H. pylori via* the AlpAB outer membrane proteins may signal to restrict the amount of CagA protein injected into the host cell[114]. AlpAB also seems to influence signalling cascades and immune responses upon infection, with differences seen between strains of different origins[115].

EPIYA motifs present on CagA are phosphorylated and the 145 kDa phosphorylated protein induces a strong immune response[116]. The number of motifs varies amongst strains, and strains with a higher number of motifs are more biologically active and hence more virulent, potentially playing a role in the development of gastric carcinoma[117]. Each EPIYA motif can be classified into a certain type, namely EPIYA-A,-B,-C and -D motifs[118]. Studies have shown that host kinases can be specific in the EPIYA motif that they target, with c-Src kinases exhibiting preference for EPIYA-C and EPIYA-D motifs, while c-Abl is more general and phosphorylates all four motifs[119].

CagA also exhibits phosphorylation-independent effects, many of which remain elusive. A conserved-motif in the C-terminus of the non-phosphorylated protein has recently been identified, and was shown to interact with the host hepatocyte growth factor receptor Met, which contributes to cellular proliferation and inflammation *via* the Akt signalling pathway, which activates NF-κB and β-catenin[120]. CagA also disrupts Par1b kinase activation, a protein involved in maintaining cell polarity and adherens junctions, independently of tyrosine phosphorylation[121]. The complex formed between E-cadherin and β-catenin in the adherens junctions of cells is also targeted by *H. pylori* in a CagA phosphorylation-independent manner. A physical interaction between CagA and E-cadherin disrupts the complex, and leads to a cytoplasmic accumulation of β-catenin, a protein which, when deregulated, has been shown to contribute to carcinogenesis[122]. Taken together the above suggests that binding of *H. pylori* to epithelial cells is a dynamic process and bacteria can alter the expression of adhesins depending on the availability of receptors. Close association of the bacteria with epithelial cells enables the translocation of the bacterial CagA protein across the cell membrane where it can be phosphorylated and lead to subversion of cell signaling. CagA can also cause disruption of epithelial cell junctions in a phosphorylation independent manner. CagA is thus a key virulence factor of *H. pylori*. Figure 1 illustrates *H. pylori* colonization of gastric mucus, the interaction of bacteria with host cells and the events triggered by that interaction.

HOST FACTORS THAT PLAY A ROLE IN THE DEVELOPMENT OF *H. PYLORI* ASSOCIATED DISEASE

The ability of *H. pylori* to cause disease depends not just on bacterial factors but also on environmental and host factors. A number of host gene polymorphisms have been identified which are thought to increase *H. pylori* colonization and increase susceptibility to disease. Some polymorphisms have been identified as risk factors for *H. pylori* associated gastric cancer. The *IL-1* gene cluster located on chromosome 2q is composed of three genes which encode the pro-inflammatory cytokines IL-1α and IL-1β as well as their endogenous receptor antagonist IL-1ra. IL-1β is a potent inhibitor of gastric acid secretion *in vivo*[123,124]. *H. pylori* infection results in an upregulation of IL-1β, which plays an important role in initiating and amplifying the immune response[125,126]. Three diallelic polymorphisms in the *IL-1β* gene have been identified all of which are C-T substitutions at positions -511, -31 and +3954 from the transcriptional start site. Polymorphisms at position -511 and -31 are associated with increased IL-1β secretion and subsequent hypochlorhydria in the presence of *H. pylori*[127], symptoms which have been implicated in the development of gastric cancer. A recent study in Venezuela has reported that infection with the more virulent *cagA*+ *H. pylori* strains is associated with individuals harbouring the +3954 polymorphism[128].

Polymorphisms in *IFN-γ* were associated with infection by *cag* positive strains and polymorphism in *TNF-α* were associated with development of peptic ulcer disease while polymorphisms in *IL-10* favoured the development of intestinal metaplasia and non-cardia gastric cancer[129]. Polymorphisms which effect cytokine function may explain the highly variable outcomes of *H. pylori* infection, highlighting the importance of genetic background of the host in disease progression and susceptibility to infection by specific *H. pylori* strains.

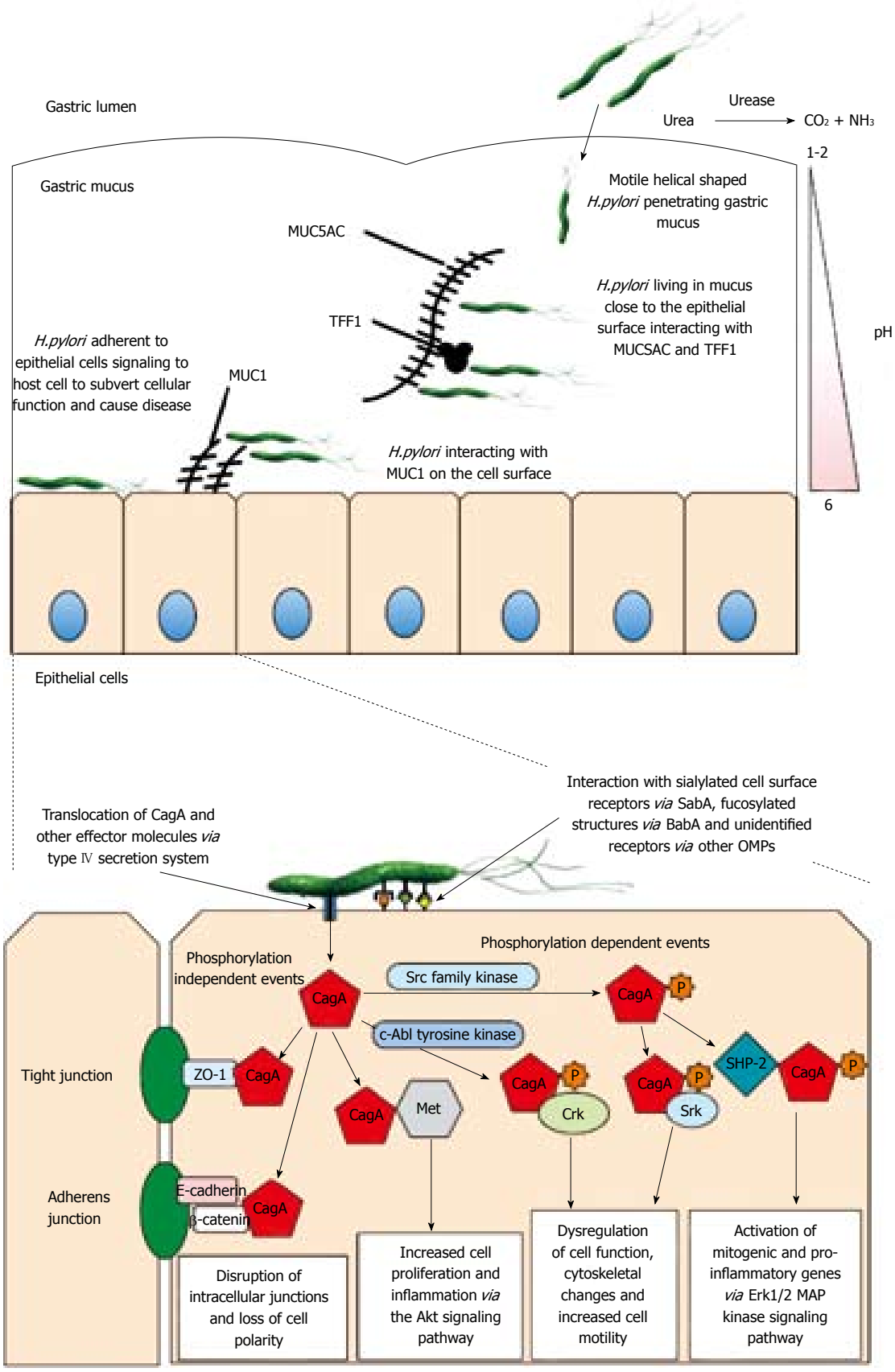

Figure 1 Colonization and infection of the gastric mucosa by *Helicobacter pylori*. The majority of bacteria live in gastric mucus close to the epithelial surface. Motile, helical shaped bacteria can penetrate gastric mucus and escape the acidic conditions of the gastric lumen. Urease acts to generate ammonia in the presence of urea thus raising the pH and protecting the transiting bacteria from the effects of gastric acid. A subset of bacteria interact with gastric epithelial cells. Translocation of cytotoxin-associated gene A (CagA) from the bacteria into the host cell cytosol results in CagA phosphorylation dependent and CagA phosphorylation independent events occurring which subvert epithelial cell function.

Polymorphisms in genes involved in the innate and adaptive immune response play a role in host susceptibility to infection and disease progression. IL-2 plays an important role in mediating the T lymphocyte response including the Th1 phenotype which is known to dominate the immune response to *H. pylori* infection. An increase in IL-2 production as a result of a gene polymorphism, T330G, has been shown to be negatively associated with *H. pylori* infection in adults[130]. Autophagy plays a critical role in the modulation of host immunity/inflammatory responses and is thought to serve as an innate defence mechanism against infection[131,132]. A single nucleotide polymorphism in the autophagy gene, *ATg16L1* (T300A), has been identified as a causal risk variant for Crohn's disease. The functional relevance of this polymorphism is not known but it is thought to result in production of an unstable ATg16L1 protein leading to impaired cytokine responses and antimicrobial autophagy. Induction of autophagy in response to the *H. pylori* VacA cytotoxin is significantly reduced in cells harbouring the T300A polymorphism and a survey of Scottish and German individuals showed that the T300A polymorphism is associated with a higher odds ratio of *H. pylori* infection[133], suggesting that the T300A polymorphism in *ATg16L1* confers a modest but significant host genetic risk of *H. pylori* infection.

There is an association between polymorphisms in the gene encoding the membrane-associated mucin MUC1 which result in shorter MUC1 alleles and *H. pylori* related gastritis[134]. Short *MUC1* alleles have also been linked to the development of gastric adenocarcinoma[135] and intestinal metaplasia in patients with chronic gastritis[136]. In support of the hypothesis that a link exists between short *MUC1* alleles and *H. pylori* related pathologies it has been shown that *Muc1*$^{-/-}$ mice display increased colonization by *H. pylori*, compared to wildtype controls, and also display increased severity of *H. pylori* induced gastritis. Shorter *MUC1* alleles encode proteins with a much smaller extracellular domain, thereby reducing the ability of MUC1 on the surface of cells to maintain a distance between the microbe and the cell surface and thus protect the cell from microbial adhesion and the subsequent development of disease[67].

H. pylori is rarely found in the mucus covering the gastric gland[59] where mucins are capped with a terminal α-1,4-linked *N*-acetylglucosamine[137]. Glycans possessing this residue suppress *H. pylori* growth *in vitro*[61]. The transfer of α-1,4-linked *N*-acetylglucosamine is mediated by a transferase encoded by the gene *a4GnT*[137]. Polymorphisms in this gene may lead to altered expression or function of the encoded transferase. An analysis of single nucleotide polymorphisms in *a4GnT* found that changes in *H. pylori* seropositivity were associated with changes in *a4GnT* and may be related to an increased risk of *H. pylori* infection[138].

The secretor status of an individual is also thought to influence their susceptibility to infection. A Fucα1,2-glycan is common to all three ABH blood group antigens. These carbohydrate structures are expressed along the gastro-intestinal tract of individuals with a positive secretor status[139,140]. Secretor individuals express the secretor-(fucosyl)transferase, an enzyme involved in the synthesis of Fucα1,2-glycan. Non-secretors lack expression of this fucosyl-transferase[141] while weak secretors express a mutated secretor-(fucosyl)transferase with reduced enzymatic activity. Studies involving infected secretor and non-secretor mice have revealed that the non-secretor mice display reduced adhesion of *H. pylori* to gastric tissue[142]. In addition experimental infection of Rhesus monkeys has revealed that the weak secretor phenotype displays reduced *H. pylori* density and reduced inflammation[143]. Non-secretor phenotypes have been shown to express higher inflammatory reactivity and expression of sialylated antigens in response to *H. pylori* infection, and this may explain the higher incidence of peptic ulcer disease previously reported in these individuals[144]. Bacterial factors alone cannot explain why some individuals develop disease upon *H. pylori* infection whereas others remain asymptomatic. The discovery of host factors such as the polymorphisms described above go some way to explaining the different outcomes of infection in different individuals.

CONCLUSION

H. pylori has evolved with humans over thousands of years and is a paradigm of chronic infection. Both bacterial and host factors play a role in mediating bacterial virulence and susceptibility to infection and can explain somewhat the outcomes of infection in different individuals. However, we suggest that *H. pylori* colonization of gastric mucus is key to the ability of the organism to cause chronic infection in humans. The establishment of a reservoir of bacteria in gastric mucus close to the epithelial surface would allow *H. pylori* to avoid removal by mucus flow and killing by gastric acid. The interaction of *H. pylori* with gastric mucus could limit the number of bacteria that can interact with gastric epithelial cells and thus also limit the inflammatory response and promote chronic infection. Modulation of the interaction of *H. pylori* with gastric mucus may be a viable alternative or even adjuvant therapy to antimicrobials for prevention of colonization and the eradication of the organism. Given that the majority of infections in humans and animals occur through mucosal surfaces combined with the results of recent studies which show that alterations in the composition of the gut microflora, sometimes referred to as dysbiosis, are associated with a number of chronic diseases including obesity[145], diabetes[146] and inflammatory disease[147] there is now intense interest in how bacteria living in mucus cause disease. Studies on *H. pylori* are likely to serve as a valuable reference system for how other organisms colonise and infect mucosal surfaces.

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