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Review Article

Helicobacter pylori infection: An overview of bacterial virulence factors and pathogenesis

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ABSTRACT

Helicobacter pylori pathogenesis and disease outcomes are mediated by a complex interplay between bacterial virulence factors, host, and environmental factors. After *H. pylori* enters the host stomach, four steps are critical for bacteria to establish successful colonization, persistent infection, and disease pathogenesis: (1) Survival in the acidic stomach; (2) movement toward epithelium cells by flagella-mediated motility; (3) attachment to host cells by adhesins/receptors interaction; (4) causing tissue damage by toxin release. Over the past 20 years, the understanding of *H. pylori* pathogenesis has been improved by studies focusing on the host and bacterial factors through epidemiology researches and molecular mechanism investigations. These include studies identifying the roles of novel virulence factors and their association with different disease outcomes, especially the bacterial adhesins, *cag* pathogenicity island, and vacuolating cytotoxin. Recently, the development of large-scale screening methods, including proteomic, and transcriptomic tools, has been used to determine the complex gene regulatory networks in *H. pylori*. In addition, a more available complete genomic database of *H. pylori* strains isolated from patients with different gastrointestinal diseases worldwide is helpful to characterize this bacterium. This review highlights the key findings of *H. pylori* virulence factors reported over the past 20 years.

Helicobacter pylori is a common bacterium, and infects approximately 50% of the world's population. The prevalence of *H. pylori* infection is highly variable across different countries; for example, high prevalence is observed in the Latin American countries (75–83%), in contrast to the low prevalence in Japan (39.6%) and the US (17.1%) [1]. Several gastrointestinal diseases, including gastritis, peptic ulcer, duodenal ulcer, and gastric adenocarcinoma have been proven to be highly associated with *H. pylori* infection. Different disease outcomes are mediated by the complex interplay between bacterial, host, and environmental factors. Clarification of the role of bacterial virulence factors in

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H. pylori pathogenesis will benefit the development of vaccines and alternative therapies. This review highlights recent advances in *H. pylori* virulence factors and pathogenesis.

Overview of *H. pylori* infection and pathogenesis

After entering the host stomach, *H. pylori* utilizes its urease activity to neutralize the hostile acidic condition at the beginning of infection. Flagella-mediated motility is then required for *H. pylori* to move toward host gastric epithelium cells, followed by specific interactions between bacterial adhesins with host cell receptors, which thus leads to successful colonization and persistent infection. Finally, *H. pylori* releases several effector proteins/toxins, including cytotoxin-associated gene A (CagA), and vacuolating cytotoxin A (VacA), causing host tissue damage [Fig. 1]. In addition, the gastric epithelium layer, which forms the major interface between *H. pylori* and the host, secretes chemokines to initiate innate immunity and activate neutrophils, and further lead to the formation of clinical diseases such as gastritis and ulcer. In summary, four steps are critical for *H. pylori* colonization and pathogenesis: (1) Survival under acidic stomach conditions; (2) movement toward epithelium cells through flagella-mediated motility; (3) attaching to host receptors by adhesins; (4) causing tissue damage by toxin release [Fig. 1]. These are discussed below.

Bacterial factors in *H. pylori* pathogenesis

Step 1: Urease and survival under acidic stomach conditions

H. pylori has developed an acid acclimation mechanism that promotes adjustment of periplasmic pH in the harsh acidic environment of the stomach by regulating urease activity. The urease gene cluster is composed of seven genes, including catalytic subunits (*ureA/B*), an acid-gated urea channel (*ureI*), and accessory assembly proteins (*ureE-H*) [2]. The metal cofactor nickel has to be inserted into the apoenzyme for heterodimer urease activity through the action of the four accessory proteins, among which UreE appears to be an important metallochaperone [3]. A recent paper describes how the other metallochaperone, HypA, interacts with UreE, and facilitates nickel transfer from HypA to UreE, and subsequently to downstream partner proteins, possibly UreG [3]. However, the mechanism of nickel transfer and insertion into the apo-urease is currently not well understood and requires further studies.

Intrabacterial urease activity is required for acid resistance by *H. pylori*, and this activity is regulated by the proton-gated urea channel UreI, which permits urea entry only under acidic conditions to prevent lethal alkalization during times of relative neutrality. UreI channels present in the inner membrane are closed at pH 7.0 and fully open at pH 5.0, enabling the rapid entry of urea into the bacterium [4]. As a consequence, *H. pylori* produces unusually large amounts of urea-derived ammonium. Surprisingly, UreI may be capable of extruding NH_3 and/or NH_4 across the inner membrane, allowing rapid neutralization of protons entering the periplasm [5]. Moreover, Miller and Maier indicated urea-derived ammonium is possibly assimilated into amino acids, thus connecting acid resistance and nitrogen metabolism [6].

Urease is also found on the *H. pylori* surface due to the lysis of some organisms. Extracellular urease is supposed to break down urea into carbon dioxide and ammonia, and ammonium hydroxide will be produced quickly when ammonia combines with water. Therefore, *H. pylori* can safely pass through the gastric juice when ammonia hydroxide neutralizes the acidic micro-environment close to the bacteria [4,6]. Schwartz and Allen indicated that, in addition to the role of urease in colonization, urease regulates *H. pylori*-macrophage interactions [7]. Although phagocytosis is an element of the innate immune

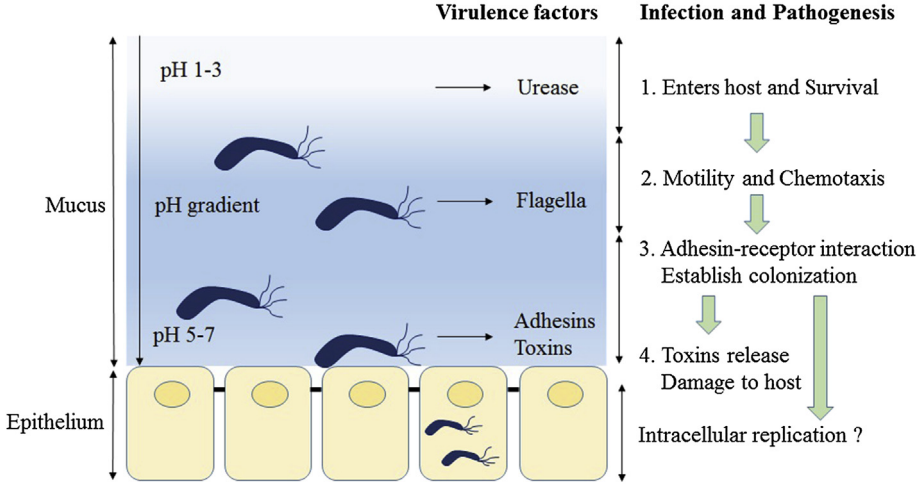

Fig. 1 – Schematic diagram of *Helicobacter pylori* infection and pathogenesis. The urease activity and flagella-mediated motility of *H. pylori* facilitate its survival and movement toward the lower mucus gel above the epithelium, followed by several adhesins, including blood-antigen binding protein A, sialic acid-binding adhesin, and other outer membrane proteins interacting with receptors on the host epithelium cells. After successful colonization, toxins, including cytotoxin-associated gene A, and vacuolating cytotoxin A, are involved in damage of host tissue and intracellular replication.

response, important for killing invading microbes, urease can modulate phagosome pH and megasome formation and as such, is essential for *H. pylori* survival in macrophages [7].

To allow the rapid adjustment of periplasmic pH, the ArsRS two-component system, in an acid-responsive manner, controls the transcription of the urease gene cluster [8]. Recently, a second, cytoplasmically localized acid responsive sensor kinase, FlgS, was identified in *H. pylori*. Although FlgS, with its cognate response regulator, HP0703, is known to regulate flagellar gene transcription, Marcus et al. indicated that a decrease in the cytoplasmic pH, exaggerated in the absence of membrane-located ArsS, may activate cytoplasmic FlgS [9]. However, the regulatory network between the two histidine sensor kinases is still unclear.

Treatment of *H. pylori* infection is becoming less effective as a result of increasing antibiotic resistance worldwide, suggesting that an alternatively targeted approach to eradicate *H. pylori* would be beneficial. Previous studies showed that a urease-negative mutant is unable to colonize gastric epithelium cells for persistent infection in gnotobiotic piglets [10,11]. Interestingly, the addition of a nickel-free diet to standard triple therapy significantly increases the *H. pylori* eradication rate, supposedly due to the reduction of *H. pylori* urease activity [12]. As a result, the inhibition of urease activity would compromise the ability to colonize the stomach and therefore provide a target for the prevention or eradication of *H. pylori* infection. Recently, the UreI channel structure has been resolved, and it may guide the discovery of small-molecule inhibitors, providing the possibility of monotherapy without the use of conventional antibiotics [13].

Step 2: Flagella and movement toward epithelium cells

H. pylori moves through the gastric mucosa epithelium layer to the basal layer where the pH value is close to 7.0 by the action of 4–7 polar sheathed flagella. Previous studies showed that flagella-mediated motility is essential for the *H. pylori* colonization of the gnotobiotic piglet and mouse gastric mucosa [14,15]. Mutagenesis of just about any gene of the motility and chemotaxis systems abolishes the ability of *H. pylori* to infect the stomach and establish colonization [14–16]. Kao et al. showed that patients infected with higher motility *H. pylori* may show enhanced bacterial density, triggering a higher inflammatory response in the upper stomach, and thus being associated with severe pathological outcomes [17]. In these respects, flagella can be considered as an early stage colonization/virulence factor. Moreover, mice immunized with a vaccine enriched for *H. pylori* flagella sheath proteins exhibited significantly reduced colonization, equivalent to that observed in mice immunized with whole-cell lysate. Due to the high antigenicity of flagella related proteins, flagella can be considered a suitable diagnostic and vaccine target [18,19].

H. pylori flagella are mainly composed of the basal body, hook, and flagellar filament [20]. The flagellar filament is consisted of two flagellins (FlaA and FlaB) encoded by *flaA* and *flaB* [20]. The hook is composed of FlgE, and it links the basal body and flagellar filament [20]. The basal body is composed of several protein structures, and it plays a role in providing the energy source for motility. In a previous study, *flaA* and *flaB* have been indicated as necessary genes for the complete motility of *H. pylori* [21]. Moreover, the serological response to FlaA can be used as a marker to show the presence of *H. pylori* infection. The titer of anti-FlaA antibody is increased with the increment of colonization density of *H. pylori*, and serves as a noninvasive biomarker for early detection of gastric cancer [22]. However, a single predicted biomarker for screening gastric cancer always results in a relatively lower positive predictive value. Therefore, serum FlaA antibody should be used in combination with other markers to screen for gastric cancer.

More than 40 proteins are involved in the biosynthesis and operation of flagella, making motility one of the most complex processes in the bacterial cell [20]. Flagellar related genes are divided into three classes, governed by the housekeeping sigma factor σ^{80} (RpoD, regulating class 1 genes), the alternative sigma factors σ^{54} (RpoN, regulating class 2 genes), and σ^{28} (FliA, regulating class 3 genes) [Fig. 2] [23]. Class 1 flagellar genes comprise the major regulatory genes (*rpoN*, *flgR*, *flgS*, and *flhA*) and structural genes (*motA* and *motB*) of the flagellar system [23]. Transcription of the class 2 middle flagellar genes (*flaB*, *flgE*, *flgK*, *flgM*, and *flgL*) and the sigma factor σ^{28} are governed by RpoN, assisted by the histidine kinase FlgS and the response regulator FlgR [23]. Late structural genes, including *flaA*, belong to the class 3 flagellar genes, regulated by σ^{28} [23]. In addition, FlhA is necessary for full transcription of flagellar class 2 and 3 genes [23]. Surprisingly, no flagellar master regulator similar to FlhDC in the *Enterobacteriaceae* has been found in the *H. pylori* genome. Recently, Kao et al. showed CsrA, a RNA binding protein, controls *H. pylori* J99 motility by regulating RpoN expression and flagella formation [24]. However, despite intensive research about the roles of motility in *H. pylori* pathogenesis, the complex transcriptional network that controls the expression of flagellar genes in *H. pylori* is still incompletely understood.

Recently, *H. pylori* flagellin was found to be heavily glycosylated with the novel sialic acid-like nonulosonate, pseudaminic acid (Pse). The glycosylation process is essential for assembly of functional flagellar filaments and consequent bacterial motility [25]. Therefore, the Pse biosynthetic pathway offers considerable potential as an antivirulence drug target, especially since motility is required for *H. pylori* colonization and persistence in the host. Small-molecule inhibitors of the Pse biosynthetic pathway that penetrate the *H. pylori* cell membrane and prevent the formation of flagella were identified, however, the binding modes and *in vivo* inhibition activity are still unclear [26]. Moreover, the role of flagella posttranslational modification in immune recognition clearly needs further investigation.

In addition to motility, the role of flagella in bacterial adherence to mammalian hosts has been demonstrated for various bacterial species in a number of hosts. For example, polar flagella glycosylation is extremely important for *Aeromonas hydrophila* adhesion to Hep-2 cells, biofilm formation, and immune stimulation of interleukin-8 (IL-8) production via toll-like receptor 5 (TLR5) [27]. In animal cell models, *Pseudomonas aeruginosa* flagella can recognize lung epithelial cells through heparan sulfate, a highly sulfated proteoglycan [28]. However, there is no evidence of specific attachment of *H. pylori* flagella to epithelial cells, and the role of flagella in cell adhesion is controversial. Clyne et al. have studied whether *H. pylori* flagella are directly involved in adhesion by constructing

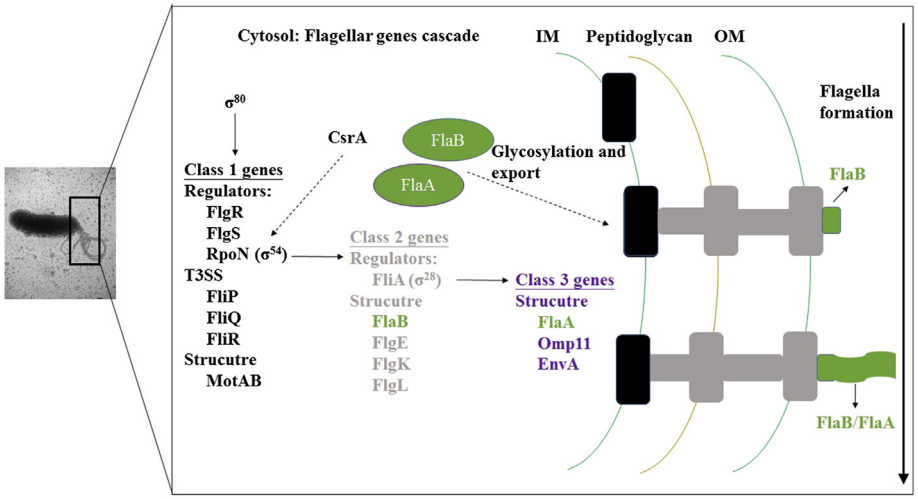

Fig. 2 – Current model of the flagellar transcriptional regulatory cascade for *Helicobacter pylori* flagellar biosynthesis. These genes are color-coded on the basis of their classification in the transcriptional regulatory cascade: black (class 1 genes), gray (class 2 genes), and purple (class 3 genes). Class 1 flagellar genes comprise most of the major regulatory genes of the flagellar system, including σ^{54} (RpoN), and FlgR. Formation of the flagellar T3SS has been proposed to create a signal detected by the FlgS sensor kinase, resulting in autophosphorylation of the protein. Phosphotransfer to the FlgR response regulator activates the protein, allowing for interactions with and stimulation of σ^{54}. The expression of σ^{54} is positively regulated by CsrA through unclear mechanism. Alternative sigma factor σ^{28} (FliA) and structural proteins, including the minor flagellin FlaB, belong to Class 2 flagellar genes and are under the control of σ^{54}. The T3SS facilitates the ordered secretion of the class 2 rod, ring, and hook proteins. The class 3 genes include *flaA*, which encodes the major flagellin, and those for other minor filament proteins, under the control of σ^{28}.

flagellin (*flaA* and/or *flaB*) mutants and a flagellar regulator (*flbA*) mutant, and it appeared that all mutants adhered to gastric cells, indicating that flagella do not play a direct role in adhesion of *H. pylori* [29]. Although a lower adhesion rate in a *flbA* mutant was observed, the authors suggested that in addition to regulating flagella, FlbA may regulate some *H. pylori* adhesins [29]. Recently, Kao et al. revealed flagella associated regulator (*csrA* or *rpoN*) mutants showed decreased bacterial adhesion to AGS cells [24]. Taken together, although flagella may not directly participate in cell adhesion, regulators controlling flagellar-related genes are thought to affect adhesin expression. Therefore, the complex flagella formation process and its role in *H. pylori* pathogenesis needs further investigation.

Step 3: Adhesins and attachment to cellular surface receptors

Adhesins

When *H. pylori* colonizes on the mucosal layer lining the gastric epithelium, the interaction of bacterial adhesins with cellular receptors protects the bacteria from displacement from the stomach by forces such as those generated by peristalsis and gastric emptying, and then bacteria get metabolic substrates and nutrients to improve growth through releasing toxins to damage the host cells. Although blood-antigen binding protein A (BabA) and sialic acid-binding adhesin (SabA) are the well-characterized adhesins studied so far, not all *H. pylori* strains express these adhesins [30,31]. There are several other known adhesins in *H. pylori* for adapting to different hosts/tissues, including neutrophil-activating protein (NAP) [32], heat shock protein 60 (Hsp60) [33], adherence-associated proteins (AlpA and AlpB) [34], *H. pylori* outer membrane protein (HopZ) [35], and lacdiNAc-binding adhesin (LabA) [36].

Neutrophil activating protein A

H. pylori-NAP belongs to the DNA-protecting proteins under starved conditions (Dps) family, which has significant structural similarities to the dodecameric ferritin family. NAP was first identified to stimulate high production of oxygen radicals from neutrophils, leading to damage of local tissues, and promote neutrophil adhesion to endothelial cells during *H. pylori* infection [37]. This NAP-induced adhesion depends on the acquisition of a high-affinity state of β2 integrin on the neutrophil surface membrane [38]. In addition to the stimulation of reactive oxygen species production, NAP induces the expression and release of IL-8, macrophage inflammatory protein (MIP)-1α, and MIP-1β by neutrophils [38]. As a result, NAP is highly associated with the hallmark of chronic gastritis, and infiltration of neutrophils and mononuclear cells into the gastric mucosa, caused by *H. pylori* infection.

The glycosphingolipids expressed on the neutrophil surface serve as a major receptor to interact with the NAP expressed on bacterial surface [38]. Moreover, NAP is supposed to facilitate SabA-mediated binding of sialylated antigens on the host cell surface [39]. In an animal study to investigate *H. pylori* colonization in mice infected with both the wild-type and *napA* mutant strains, the degree of survival of the *napA* mutant was found to be much lower than that of the wild-type strain [40]. Several studies showed that NAP can protect *H.*

pylori DNA from damage, either due to its ability to bind DNA and thus to prevent DNA from attack by free radicals or through its iron-sequestering ability to reduce the oxidative stress produced in ferrous ion-mediated Fenton reactions [40,41]. However, for gastroduodenal diseases, only one study showed that the level of NAP-specific antibodies in sera from *H. pylori*-infected patients with gastric cancer was significantly higher than that from patients with chronic gastritis [42]. No report has shown the direct association of NAP with *H. pylori*-induced gastric inflammation in patients so far.

Interestingly, NAP can also stimulate either neutrophils or monocytes to increase the expression of IL-12, and induces T helper cells to differentiate toward the T helper 1 phenotype [43]. Thus, NAP has been suggested as an immunotherapeutic anticancer agent and adjuvant for vaccination in clinical applications. Further study showed that NAP-activated DCs had a Th1 cytokine secretion profile, with high IL-12 and relatively low IL-10 secretion. Therapeutic effects of NAP can be mediated by the maturation of DCs and subsequent activation of Ag-specific T-cells, in addition to provoking innate immunity [44]. However, further studies are required to confirm that activation of DC by NAP can be an adjuvant for DC-based cancer vaccines and cancer immunotherapy.

Heat shock protein 60

Heat shock proteins, a highly conserved protein family detected not only in prokaryotes but also in eukaryotes, are induced by a variety of environmental stresses such as temperature, pH change, ischemia, and microbial infection. *H. pylori* produces mainly two Hsps, GroES-like HspA (Hsp10), and GroEL-like HspB (Hsp60). The high expression of Hsp60 at low pH, which interacts with the receptor-like sulfatide (sulfoglycolipid), indicates the stress of acid may change the specificity of *H. pylori* to receptors [33,45].

Heat shock protein has been identified as one of the potential immunogens of the bacterium that induces IL-6, IL-8, tumor necrosis factor alpha (TNF-α), and GRO production from monocytes or gastric epithelial cells [46]. Hsp60 induces activation of NF-κB via TLR2 and the mitogen-activated protein kinase pathway, and thereby induces human monocytes to secrete IL-8 [47]. Moreover, anti-Hsp60 antibodies are consistently detected in *H. pylori*-infected patients, and the titers are associated with the progression of gastritis or gastric cancer [48,49]. Further study showed that mAbs against *H. pylori* Hsp60 could modulate bacterial pathogenesis by increasing IL-8 and TNF-α production [50]. The pathogen-specific antibodies are supposed to execute potential immune functions rather than recognize or neutralize microbes. However, further studies are required to provide important insights into the role of anti-Hsp60 antibodies in *H. pylori*-associated gastric diseases.

Blood group antigen binding adhesin (BabA and BabB)

Three *bab* allelic types have been identified, including *babA*$_1$, *babA*$_2$, and *babB*. The molecular mass of the BabA protein is nearly 78 kDa, encoded by *babA*$_2$. The *babA*$_1$ and *babA*$_2$ coding sequences are highly similar, but the translational start codon is lacking in *babA*$_1$. *H. pylori* employs BabA to bind to fucosylated Lewis B blood-group antigen (Lewis b [Le^b]) expressed on host gastric epithelium cells, when *H. pylori* initially infects the human stomach [30]. The structure of the BabA receptor is similar to the O type blood antigen, and the statistics of epidemiology reveal the correlation between type O blood and gastric related diseases [51].

Blood-antigen binding protein A and BabB are nearly identical in their 5′ and 3′ regions, with most of their sequence divergence being in their mid-regions. Importantly, the middle region of the BabA sequence determines the adhesion ability of BabA. In the western countries, the expression of BabA contributes to increased risk of peptic ulcer disease and gastric cancer [52,53]. However, the existence of BabA is not correlated to gastric related diseases in Asians [52,54]. The function of BabB is still unclear, but the expression of BabB was associated with increased gastric histologic lesions in patients [55].

Some strains do not carry *babA*$_2$ in their genome, or are deficient in BabA resulting from mutation, yet the bacteria still express a chimeric BabB/A (which has the ability to bind to the Le^b antigen) by genetic recombination of *babA*$_1$ and *babB* in certain conditions [56,57]. To study the dynamics of Le^b adherence during human infection, a study analyzed paired *H. pylori* isolates obtained sequentially from chronically infected individuals. The results showed that a complete loss or significant reduction of Le^b binding was observed in strains from 5 out of 23 individuals, indicating that the BabA-Le^b binding phenotype is quite stable during chronic human infection. Sequence comparisons revealed that most amino acid changes were found in the putative N-terminal extracellular adhesion domain [58]. In conclusion, recombination mediates dynamic changes in adherence properties, which suggests that it contributes to the persistence and adaption of *H. pylori* in ever-changing gastric environments [56,58].

Sialic acid-binding adhesin

At sites of vigorous local inflammatory response due to *H. pylori* infection, the expression of sialyl-Lewis x glycosphingolipid (sLe^x) antigen is increased on the cellular surface. This suggests that SabA adhesin plays a critical role to assist *H. pylori* to adhere to and colonize the gastric epithelium cells of a patient with gastritis [31,59]. Especially when lacking gastric Le^b expression, Le^x and Le^a were closely related to *H. pylori* colonization [60]. The *sabB* gene is homologous to *sabA*, but appears not to be involved in sLe^x binding [31]. Therefore, the function of *sabB* in bacterial adhesion and pathogenesis is worth investigating.

The prevalence of *sabA* in clinical strains is nearly 80%, and the sequence results revealed two types of *sabA* genotypes [59]. The expression of type I *sabA* is regulated by a CT repeat sequence in the 5′ ORF of *sabA*, and this repeat can be regulated by slipped strand mispairing (SSM) [59,61]. The functional protein is only expressed in those strains in which the numbers of the CT dinucleotide repeats are 4, 7, and 10, and allow an ORF encoding the full length SabA. This expression

status is defined as "On". When the CT repeat number is varied to 3, 5, 6, 8, 9, and 11, there will be early stop codons in the ORF resulting from frame-shift. In this status, the gene expression is defined as "Off", and the truncated SabA is expressed. The type II *sabA* has a unique deletion of the CT repeats and a distinctive sequence in this region. Moreover, not only *sabA*, but also adhesins *oipA* and *hopZ* genes, are characterized by CT dinucleotide repeats in their 5′-coding regions [62].

The expression of SabA detected by western blotting is dramatically different from the sequence-based prediction [59]. In type I strains, which are predicted to be "On", the expression of SabA is only 43% by western blotting. Kao et al. showed the length of a polyT tract close to the *sabA* promoter region is variable, with the variation also arising through SSM [61]. In addition, the length of the polyT tract is supposed to modulate *sabA* promoter activity, providing an alternative mechanism for transcriptional regulation in *H. pylori*, which possesses a limited repertoire of classical trans-acting regulatory factors [61,63,64]. A mixed-genotype population is developed by SSM during infection by *H. pylori* which may benefit bacterial immune evasion and adaptation to different hosts.

The analysis of clinical statistics indicates that in patients infected with SabA-positive strains, the density of *H. pylori* in the body is dramatically higher than in patients infected by SabA-negative strains [59]. This finding indicates that SabA interacting with the sLe^x antigen can enhance *H. pylori* colonization in those patients with weak or no Le^b expression [59]. Although the intensity of Le^b is the key host factor regulating *H. pylori* density in patients with a *babA*$_2$-positive *H. pylori* infection, the weakness of the sLe^x-mediated adherence, and its metastable On/Off switching resulting from phase variation, may benefit *H. pylori* by allowing escape from sites where bactericidal host defense responses are most vigorous.

Step 4: Toxin and host tissue damage

Cytotoxin-associated gene A

The epidemiological prevalence of CagA-positive *H. pylori* infection in western countries is nearly 60% [65,66], and the prevalence is about 90% in Asian countries [67,68]. Several studies indicated that the CagA-positive strains are directly associated with acute gastritis, gastric ulcer, and gastric cancer development [69–71]. As a result, the virulence of individual *H. pylori* isolates has been measured by their ability to produce CagA.

Cytotoxin-associated gene A protein can be further divided into the Western-type CagA and East Asian-type CagA, by the repeat sequence Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs at the N-terminus of CagA [72,73]. The affinity of the East Asian-type CagA to SHP-2 is significantly higher than that of the Western-type CagA [72,73]. As a result, East Asian-type CagA induces more cytoskeleton changes, and is more likely to be associated with gastric cancer [73].

The *cag* pathogenicity island (*cag*PAI) is located on the chromosome in *H. pylori*, is 35–40 kb, and contains more than 30 genes [62]. Among them, *cag*PAI carries at least 6 genes with homology to type IV secretion systems, and thus translocate the bacterial protein CagA into the host gastric cell cytoplasm upon contact with epithelium cells [74]. Moreover, several proteins, including CagL, and CagY, that are present in the T4SS use β1-integrin as a receptor to deliver CagA into the host cell [75,76].

The translocated CagA protein localizes to the inner surface of the plasma membrane via interactions with phosphatidylserine and subsequently undergoes tyrosine phosphorylation by the Src family protein tyrosine kinase. However, once injected into the cytoplasm via the T4SS, CagA can alter host cell signaling in both a phosphorylation-dependent and phosphorylation-independent manner. The phosphorylated CagA binds to the phosphatase SHP-2 and affects the adhesion, spreading, and migration of the cell [72,77]. Moreover, CagA can also affect the host cell in several aspects, such as the formation of gastric epithelium cell pedestals, the change of the cytoskeleton, affecting the proliferation of cells, and stimulating the gastric epithelium cells to secrete IL-8 [78–80].

Cytotoxin-associated gene A has phosphorylation-independent effects, many of which remain unclear. A conserved motif in the C-terminus of the nonphosphorylated CagA 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 signaling pathway, which activates NF-κB and β-catenin [81].

Vacuolating cytotoxin A

Vacuolating cytotoxin A is predicted to encode a protoxin with a mass of about 140 kDa, but the secreted VacA toxin is composed of the p33 and p55 domains that form an oligomeric structure. This complex can embed into the host cell membrane, and also has the characteristic of an anion-selection channel. This channel can release bicarbonate and organic anions in the host cytoplasm [82]. In this way, the channel might help *H. pylori* colonization by allowing the efflux of potential metabolic substrates for bacterial growth. This complex can also get into the endosome via endocytosis. The endocytosed VacA channel will allow anions to permeate into late endosomes, which leads to accumulation of weak bases and thence to large vacuole formation by water influx [83,84]. Previous studies also indicate that VacA applied extracellularly apparently targets mitochondria, since it induces the release of cytochrome C, ER stress, and apoptosis [85]. In addition, VacA disrupts the balance of cell proliferation and death by affecting genes that regulate the cell cycle. It also can induce acute inflammatory responses through inducing host cell release of IL-8 [86].

All *H. pylori* strains carry the *vacA* gene encoding protein production, with various degrees determined by the different genopatterns of the signal sequence (s1a, s1b, s1c, and s2), mid-region (m1, m1T, and m2), and the intermediate region (i1, i2, and i3) [87,88]. The genotype can be divided into different subtypes, according to the combinations of the diversity of these three regions [87,88]. For the genotype s1/m1, the expression of VacA is highly active and can damage cells in a more acute manner [87]. It has been shown that *H. pylori*

vacA s1 and m1 strains are associated with high levels of inflammation in the gastric mucosa and increased risk for gastric atrophy and carcinoma, compared with the less virulent *vacA* s2 and m2 strains [89]. Furthermore, the *vacA* i1 genotype is strongly associated with *vacA* s1, *vacA* m1, and *cagA*-positive genotypes, while the *vacA* i2 genotype is closely associated with *vacA* s2, *vacA* m2, and *cagA*-negative genotypes [88]. A previous study showed that, in patients with gastric cancer, the *vacA* s1a and s1c subtypes are less common, and m1T is more prevalent in patients with peptic ulcer and chronic gastritis [90]. However, the association of *vacA* subtypes with disease is not consistent in different countries.

Although *H. pylori* is generally viewed as an extracellular microorganism, Chu et al. showed that in a gentamicin protection assay on AGS or MKN45 cells, *H. pylori* could invade the epithelial cells and multiply within double-layer vesicles either on the plasma membrane or in the cytoplasm. The autophagic vesicles induced by *H. pylori* are supposed to be the location of replication, and also of the degradation of the replicating bacteria after fusion with lysosomes [91]. The multiplication of *H. pylori* within cells provides a niche for its resistance to antibacterial therapy and has a significant impact on its biological life cycle. In addition, the VacA or CagA mutants of *H. pylori* have lower levels of multiplication in macrophages [92].

H. pylori infection is supposed to induce autophagosome formation, and these autophagic vesicles are adapted for the multiplication of *H. pylori* in the host. The use of different host cell lines and bacterial strains has produced inconsistent results, indicating that *H. pylori* may affect autophagy in a host cell/bacterial strain dependent manner [93]. However, it is still uncertain whether autophagy serves as an effective front line of host defense against intracellular *H. pylori*, and the precise mechanisms by which *H. pylori* exploits host cell machineries for intracellular survival are poorly understood. As a result, the investigation of the roles of *vacA*, *cagA*, and other virulence factors in *H. pylori* intracellular multiplication is necessary.

Conclusions

In summary, over the past year, the knowledge of *H. pylori* pathogenesis and disease development has been improved by the studies focusing on investigation of bacterial factors. Application of large-scale screening methods should have broad relevance to understanding *H. pylori* infection-mediated carcinogenesis. Moreover, continually clarifying and refining the roles of bacterial virulence factors in *H. pylori* pathogenesis will highly benefit vaccine and alternative therapy development.

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Nil.

Conflict of interest

None declared.

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