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Chapter 11

The Role of CagA Protein Signaling in Gastric Carcinogenesis — CagA Signaling in Gastric Carcinogenesis

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1. Introduction

Even though chronic gastritis was well established as a risk factor for the development of several gastric pathologies, such as peptic ulceration, gastric carcinoma, and lymphoma, before the discovery of *Helicobacter pylori* by Warren and Marshall in 1983 (Warren, 1983), the development of chronic gastritis was thought to be due to a plethora of possible causes such as stress, excessive alcohol consumption, ingestion of hot beverages and spicy food, among others (Mobley et al., 2001). With the association of *Helicobacter pylori* as the cause of chronic gastritis, the understanding of bacterial pathogenicity and the host response against the bacterium take enormous importance to understand the pathophysiology of the infection.

It is estimated that almost half of the world's population is infected with *Helicobacter pylori* and although the majority of colonized individuals remain asymptomatic, the infection represents the primary cause of chronic gastritis and it is a risk factor for the development of gastric cancer; it was the first bacterium classified as a type 1 carcinogen by the IARC in 1994 (IARC, 1994). Gastric cancer, despite its declining incidence rate, it remains as the fourth most common cancer and the second cause of cancer-related death worldwide (Ferlay J, 2010) and epidemiological studies have determined that attributable risk for gastric cancer conferred by *H. pylori* infection is approximately 75% (Peek & Blaser, 2002).

The development of gastritis associated with the infection is a multifactorial process, where the bacterium and host factors influence the pathogenesis of the disease. Colonization by *H. pylori* causes chronic gastritis, that can remain asymptomatic, produces gastric epithelial damage (Ricci et al., 2011). In the initial phase of infection, the bacteria penetrate through the mucous layer to multiply in the proximity of epithelial cells. The gastric epithelium responds to the infection by mucin depletion and cellular regenerative changes (Mobley et al., 2001); it also occurs the infiltration of polymorphonuclear cells into surface epithelium and hypochlorhydria and a deficient ascorbic acid secretion are also observed (Sobala et al., 1993). Later, bacterial components penetrate through the damaged epithelium and stimulates the production of IL-8 and IL-1 by macrophages, the activation and degranulation of mast cells and the release of inflammatory mediators that increase vascular permeability and up-regulate the expression of leukocyte adhesion molecules (Mobley et al., 2001). As the immune response fails to eliminate the bacteria, it occurs a gradual accumulation of inflammatory cells, which represents an active chronic phase of gastritis.

2. *Helicobacter pylori* virulence factors in gastritis and gastric cancer

Researchers have studied *H. pylori*'s virulence factors, to elucidate their role in the development of gastritis and gastric cancer, the most studied are the pathogenicity island (*cag*-PAI) and its effector, the cytotoxin associated protein (CagA) and the vacuolating cytotoxin (VacA). However, there are other virulence factors (Figure 1), including the urease, lipopolysaccharide, peptidoglycan, several adhesins, such as outer inflammatory protein (OipA), sialic acid-binding adhesin (SabA), blood group antigen-binding adhesin (BabA/B), that contribute to the damage in the gastric epithelium (Backert et al., 2010).

3. Urease

To colonize the stomach, *H. pylori* has to survive in the presence of acid produced in the stomach. To counteract this acidic environment, *H. pylori* produces an important enzyme, urease, which hydrolyses urea into NH_3 and CO_2. This enzyme has an essential role in the *H. pylori* infection as observed in urease-defective bacteria mutants which cannot colonize the stomach (Montecucco & Rappuoli, 2001). Urease causes damage to the epithelium through the production of ammonia, that in conjunction with neutrophil metabolites (Megraud et al., 1992), form carcinogenic agents that might participate in the development of gastric malignances (Suzuki et al., 1992). Ammonia is capable of cause different cell alterations, including swelling of intracellular acidic compartments, alterations of vesicular membrane transport, repression of protein synthesis and ATP production, and cell-cycle arrest (Montecucco & Rappuoli, 2001). Urease might also help to the recruitment of neutrophils and monocytes in the mucosa and to the production of proinflammatory cytokines (Harris et al., 1996).

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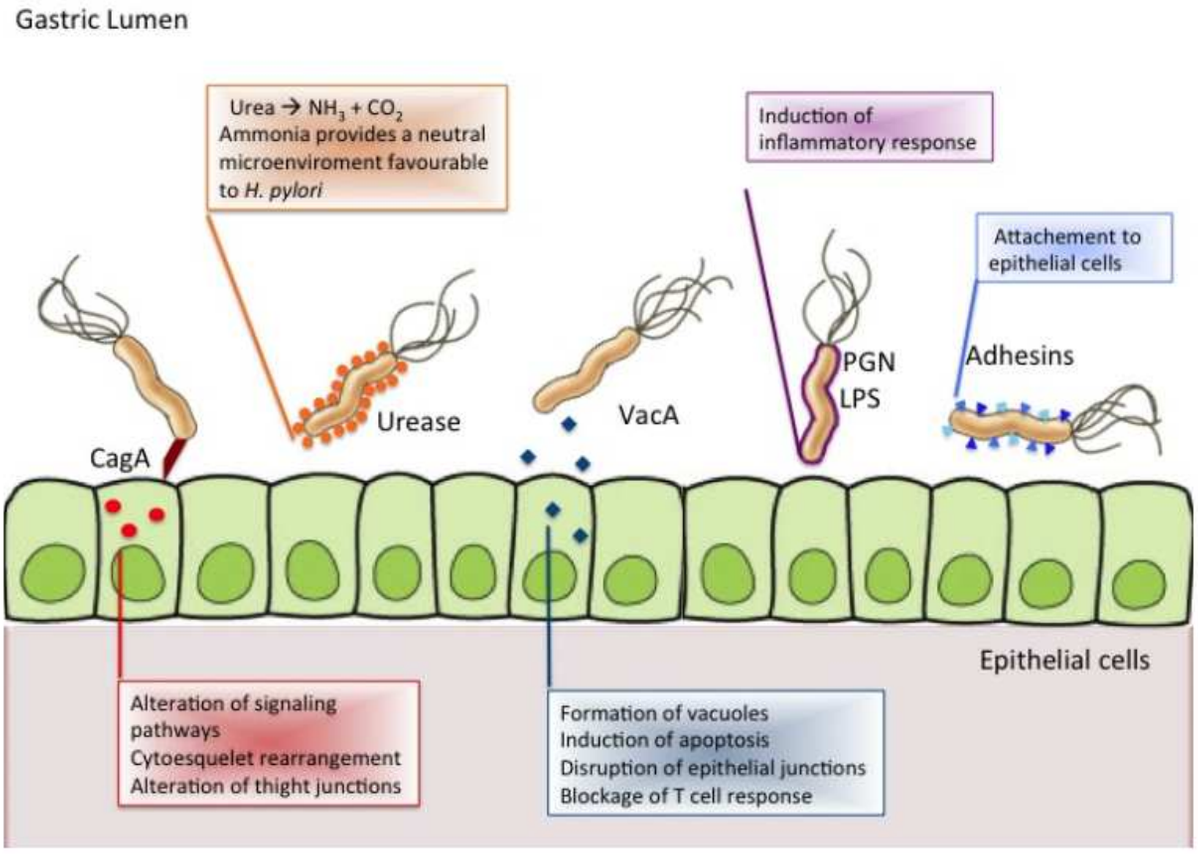

Figure 1. *Helicobacter pylori* virulence factors activities. Although CagA and VacA are the most studied virulence factors, there are more bacterial proteins that promote damage to the host cells.

4. Lipopolysaccharides (LPS) and peptidoglycan

H. pylori LPS are essential components of the bacterial outer membrane that induces a low immunological response in contrary to other bacteria (Moran, 1995). However, despite the low immunological activity of LPS, *H. pylori* colonization is associated with an inflammatory response, because *H. pylori* has the ability of activate mononuclear cells by LPS-independent mechanisms as well as other bacterial surface molecules (Mai et al., 1991, Moran, 1998). Another important component of the cell wall of *H. pylori*, peptidoglycan, interacts with the intracellular pattern recognition receptor Nod1 (an intracellular sensor for peptidoglycan from Gram-negative bacteria), this interaction leads to activation of NF-κB signaling, whit the subsequent secretion of the inflammatory molecules IL-8 (Viala et al., 2004) and β-defensin-2 (Boughan et al., 2006). On the other hand, peptidoglycan translocation of *H. pylori*, leads to enhanced PI3K-AKT signaling, which mediates protection from apoptosis and cell migration, both phenotypes related to carcinogenesis (Nagy et al., 2009).

5. Adhesins

Approximately 4% of the *H. pylori* genome is predicted to encode outer membrane proteins (OMPs) many of which serve as adhesins (Wroblewski et al., 2010), epithelial adhesion provides to *H. pylori* better access to epithelial surface and in this way contributes to deliver bacterial toxins such as CagA (Yamaoka, 2010). Fucosylated ABO blood group antigens and sialyl-Lewis^x and sialyl-Lewis^a antigens (sLe^x and sLe^a) have been identified as functional receptors for *H. pylori* (Boren et al., 1993).

The outer membrane inflammatory protein (OipA), identified in 2000 (Yamaoka et al., 2000), is involved in the attachment of *H. pylori* to gastric epithelial cells *in vitro* (Yamaoka, 2010). OipA is also involved in up-regulation of matrix metalloproteinase 1 (MMP-1), in inhibition of glycogen synthase kinase 3β (GSK-3β) and in β-catenin translocation to the nucleus, this protein also induces an inflammatory response and actin rearrangement through phosphorylation signaling pathways (Wroblewski et al., 2010), although, most of these are also involved in *cag*-PAI signaling alterations, suggesting that there might be some interaction between OipA and *cag*-PAI, it is important to note that it has been found a correlation coefficient of 0.82 between the *oipA* and *cagA* positivity in some virulent strains (Yamaoka et al., 2002).

Blood group antigen binding adhesin (BabA), has been thought to be the primary protein involved in adherence to the gastric mucosa (Boren et al., 1993). It is encoded by the *babA2* gene (the *babA1* although is present, is silent because of lack of an initiation codon (Mobley et al., 2001) and binds to fucosylated Lewis^b antigen. Some studies report that the presence of *babA2* is associated with duodenal ulcer and gastric cancer and found in conjunction with *cagA* and *vacA* s1 allele, provides a greater risk of develop a severe gastric disease (Gerhard et al., 1999).

Sialic acid-binding adhesin (SabA) is responsible of *H. pylori* binding to syalylated Le^x and Le^a in epithelial cells. Infiltration of neutrophils into the gastric mucosa is a characteristic feature of chronic gastritis caused by *H. pylori* and SabA binds to neutrophils through this sialylated carbohydrates and induces oxidative burst in these cells, which has a consequence of produce oxidative damage in gastric epithelium. It has been proposed that SabA-positive status is associated with gastric cancer, intestinal metaplasia, and corpus atrophy and negatively associated with duodenal ulcer and neutrophil infiltration (Yamaoka, 2008).

6. Vacuolating cytotoxin (VacA)

VacA is a pore-forming cytotoxin identified in supernatants of *H. pylori* broth cultures that cause aberrant vacuolation of cultured cells (Leunk et al., 1988). This cytotoxin is secreted from the bacteria as a large 140-kilodalton polypeptide and latter trimmed at both ends to finally deliver it in an active form to host cells, where it exerts its activity.

VacA induces multiple cellular activities, the best studied is the alteration on endosomal maturation which consequently leads to vacuolation of epithelial cells, VacA is also capable

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of induce membrane-channel formation, cytochrome c release from mitochondria, and binding to cell-membrane receptors activating a proinflammatory response (Amieva & El-Omar, 2008). VacA has the ability to cause leakage of ions and small molecules, by disrupting the barrier function of tight junctions (Papini et al., 1998) and also inhibit T-cell activation and proliferation (Gebert et al., 2003).

All strains of *H. pylori* contain the *vacA* gene, although, there is considerable genetic diversity among them, and consequently the cytotoxicity activity of the toxin varies between strains (Amieva & El-Omar, 2008). The diversity observed is attributed to variations in *vacA* gene structure within the signal (s) region, the middle (m) region, and the intermediate (i) region (Wroblewski et al., 2010).

The amino terminus contains the signal sequence that shows allelic variability and has been classified into different types. Strains with s1 allele secrete an active toxin and are also highly associated with ulcers and gastric cancer (Atherton et al., 1995), however, s1/s2 combination or s2 genotypes are found in patients with gastric cancer (Lopez-Vidal et al., 2008). The middle region of the gene also shows allelic variation, with m1 subtype having stronger vacuolating activity and it is associated with an increased risk for development of gastric epithelial injury and gastric cancer (Yamaoka, 2010).

The clinical isolates of *H. pylori* have been grouped into two broad families defined as type I and type II on the basis of whether they have a functional pathogenicity island (*cag*-PAI), and secrete an active vacuolating cytotoxin (VacA). Type I strains are positive for all these characteristics. In contrast, type II strains do not have a functional *cag*-PAI and possess the *vacA* gene that encodes for non-toxic protein (Censini et al., 1996, Tomb et al., 1997).

7. Cytotoxin associated gen-Pathogenicity Island (*cag*-PAI)

Colonization of the human stomach by *H. pylori* virulent strains is associated with a significantly increased risk for the development of several gastric diseases, such as gastric cancer. These highly virulent *H. pylori* strains harbor a (cytotoxin-associated genes) pathogenicity island (*cag*-PAI) that encode proteins that are components of a type IV secretion system (T4SS) apparatus and the CagA effector protein into host target cells (Castillo-Rojas et al., 2004). T4SS translocates the bacterial oncoprotein CagA and peptidoglycan that induces proinflammatory chemokine and cytokine secretion, including interleukin-8 (IL-8) in gastric epithelial cells (Figueiredo et al., 2005). The hallmark of *H. pylori* virulent strains is the presence of *cag*-PAI that induce of gastric mucosal inflammation, which is a risk factor for developing severe gastric pathologies (Antonio-Rincon et al., 2011).

The presence of flanking direct repeated sequences 605 (IS605) in the *cag*-PAI have been reported to cause rearrangements and partial or total deletions of it. This has generated *H. pylori* strains with varying virulence, and consequently, the clinical outcome of the infection (Censini et al., 1996).

T4SSs are a large group of highly versatile secretion machineries in many Gram-negative pathogenic bacteria that are evolutionarily related to bacterial conjugative systems (Fronzes et al., 2009). Although the *H. pylori cag*-PAI has been evolutionarily related to others T4SSs based on their common features like structural and sequence similarities, only a few *cag* PAI proteins (also termed as HP o VirB/D) show clear sequence similarities to the prototypical system of *A. tumefaciens* and other bacterial species.

A systematic mutagenesis study with isogenic mutants in each of the *cag*-PAI genes, reported that 14 genes out of 27 of *cag*-PAI are essential for CagA translocation and induction of IL-8 secretion in gastric epithelial cells (Aguilar et al., 2001).

Twelve genes out of 27 of *cag*-PAI encoded proteins share sequence similarities with components of the prototypical T4SS system VirB/D4 of *Agrobacterium tumefaciens*, which contains 11 essential proteins of the secretion apparatus (VirB1–VirB11) encoded by the operon *virB* and a coupling protein (VirD4) that mediates substrate recognition (Fronzes et al., 2009). The majority of these proteins are conserved among the known T4SSs of other bacteria (Alvarez-Martinez & Christie, 2009).

The prototypical (macromolecular complex) T4SS of *A. tumefaciens*, is among the best-studied T4SS and is composed of two major structural complexes: the T-pilus and a membrane-associated complex responsible for the translocation of substrates acrosss the inner and the outer membrane (Fronzes et al., 2009, Alvarez-Martinez & Christie, 2009). The membrane-associated complex includes the energetic components VirB4, VirB11 and VirD4, which are conserved among the known T4SSs of other bacteria. These proteins contain Walker nucleotide binding, and hydrolysis motifs and are known to energize T4SS for its function. The membrane-associated complex also includes the proteins VirB1, VirB3, VirB6, VirB7, VirB8, VirB9 and VirB10 that form the substrate translocation channel that spans both bacterial membranes. VirB6, VirB8, and VirB10 are part of the subcomplex of proteins attached to the inner membrane with domains spanning the periplasm, while VirB7 and VirB9 are part of the outer membrane subcomplex. The T4SS-determined T-pilus is an extracellular appendage that is thought to initiate cell–cell contact with plant target cells before the initiation of T-DNA transfer. This structure includes the proteins VirB2 and VirB5 as major and minor components, respectively (Backert et al., 2008).

H. pylori and *A. tumefaciens* share similarity in several components of their T4SSs and probably function in a similar manner (Backert & Meyer, 2006). Nevertheless, the molecular mechanisms responsible for assembly, activity and specific recognition of host target cells of these macromolecular complexes remains unclear. It has been suggested that after the induction by cell contact, *H. pylori* forms T4SS pili located at one bacterial pole for the injection of virulence factors into target cells such as the oncoprotein CagA.

8. Cytotoxin-associated protein (CagA)

CagA protein is the only known effector protein translocated to the host cell by the T4SS (Backert et al., 2010). It was firstly described as a very immunogenic protein in humans infected

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with cytotoxin-producing strains (Covacci et al., 1993). After that, its association with gastric cancer and peptic ulcer was well established.

Recent reports suggest that CagA is able to down regulate the vacuolation effects of VacA on host cells, and conversely, VacA is able to down regulates CagA activity (Oldani et al., 2009, Tegtmeyer et al., 2009). Clinical isolates that contain the *cag*-PAI generally are *babA, oipA* and *vacA* s1 positive, Yamaoka proposes that these virulence factors may interact with each other with a certain biological significance; therefore, these factors interact synergistically with each other to induce gastric diseases (Yamaoka, 2010).

CagA is encoded by the *cag*A gene, located at one end of the *cag*-PAI (Censini et al., 1996). One copy per genome is present in most of the strains whose genome has been sequenced but strains with two gene copies might exist, as revealed by the genome sequence of the Amerindian strains *H. pylori* Shi470 and V225d, although functionality of both copies in these strains is unknown (Kersulyte et al., Mane et al., 2010). It has been estimated that 60-95% of strains worldwide carry the *cag*A gene, which is different between geographic regions (Hatakeyama, 2004). However, the complete *cag*-PAI is important for the translocation of this protein and the completeness of the island may impact on the association of CagA presence and the outcome of the disease.

Other main feature of CagA protein is its high variable size, which range from 120-145 kDa (Covacci et al., 1993, Tummuru et al., 1993). This variation is due to the presence of polymorphism at the carboxy-terminal region given by the presence of repeat sequences called EPIYA motifs, which are present in a copy number varying from one to seven (Xia et al., 2009). Four major types of EPIYA motifs (A, B, C, and D) have been described based on the specific amino acid sequence that flanks the Glu-Pro-Ile-Tyr-Ala motif at both sides (Hatakeyama, 2004).

The EPIYA-repeat region of CagA is made of tandem alignment of the four distinct EPIYA segments (A, B, C, D) in various combinations (Hatakeyama, 2011). CagA molecules containing the EPIYA-A, EPIYA-B and EPIYA-C segments in tandem are denoted as ''western type'', and are found in many *H. pylori* isolates from Europe, North America, and Australia. On the other hand, CagA carrying EPIYA-A, EPIYA-B and EPIYA-D segments are called "East Asian type" and have been isolated in East Asian countries such as Japan, Korea and China (Furuta et al., 2011).

The EPIYA-C segment in "Western type" isolates can be present in up to five copies which might be due to recombination mediated by a 16 amino acid sequence designated CagA multimerization sequence that is present at both sides of EPIYA-C motif. The CagA multimerization sequence is also present in EPIYA-D but only at one end, therefore cannot duplicate by this mechanism (Furuta et al., 2011). Other implications of CagA multimerization sequence in the bacterial pathogenicity will be discussed later in the text.

It has been proposed that EPIYA motifs of CagA act as ''master keys'' that can potentially be tyrosine-phosphorylated by host kinases and are capable to interact with a wide range of different host cell proteins (Backert et al., 2010). In the same way, the observation that the EPIYA-region is unstructured and can adopt multiple conformations mimicking host sub-

strates of kinase families, thus manipulating eukaryotic cellular biochemistry during infection, provides further support for this "master key" hypothesis (Nesic et al., 2010).

In contrast to the polymorphic C-terminal EPIYA region of CagA, N-terminal region is well conserved among CagA proteins (Hatakeyama, 2011). The N-terminal CagA region is required for the membrane association of CagA in polarized epithelial cells (Bagnoli et al., 2005) and is important for its translocation into host cells (Murata-Kamiya et al., 2010), the first 200 amino acids at the N-terminus of CagA form a membrane-binding domain in *H. pylori*, mediating proper translocation of the CagA protein via the T4SS (Steininger et al., 2011).

9. CagA tridimensional structure and membrane localization

It has been shown that CagA targeting to the epithelial cell membrane is important for host signaling (Higashi et al., 2002, Bagnoli et al., 2005, Higashi et al., 2005) and there is evidence that membrane binding of CagA is important for its translocation into host cells (Murata-Kamiya et al., 2010, Steininger et al., 2011).

Recently, it has been proposed that interaction of CagA with host cell proteins depends on the localization of CagA into distinct compartments in host cells (Pelz et al., 2011). The model proposed states that membrane localization is mediated by two membrane domains, one composed by residues 1-200 (N-terminal domain) and the other from 200-1216 (C-terminal domain), detailed description of the function of these domains are described in the Figure 2. In addition, C-terminal domain is comprised of two regions that interact with each other and are located in the membrane together when linked to N terminal domain, the first region is from amino acids 200-800 containing phosphatidyl serine binding domain and the second from 800-1216 containing EPIYA and CagA multimerization motifs. The region from 800-1216 when expressed alone localizes in cytosol and produces a strong alteration in cell morphology (Steininger et al., 2011).

According with the above-mentioned model, CagA spatial conformation may play an important role for the proper translocation and compartmentalization, so a tridimentional structure prediction of CagA was made using I-TASSER, which has been ranked as the best method for the automated protein structure prediction in the last two community-wide critical assessment of protein structure prediction (CASP) experiments (Roy et al., 2010). The best predicted model for both East-Asia and Western type of CagA consists of a sinuous super helical structure in which N-terminal region protrudes at one side and C-terminal region forms a loop (Figure 3). These predicted structural models agree with the model proposed of Steininger et al., in which amino acids 200-1216 forms two domains that interact with each other (Steininger et al., 2011). According to the results obtained with I-TASSER the most similar structure available in the PDB database (www.rcsb.org/pdb/home/home.do) is from Cand1 protein, however the only function similarity shared with CagA is the capacity of protein binding.

Interestingly, it is also proposed that N-terminal membrane-binding domain functions as an inhibitory domain of the C-terminal region effects, and that region from 800-1216 when ex-

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pressed alone induces the highest percentage of epithelial cell elongation (Pelz et al., 2011). Also it has been proposed that CagA is cleaved by proteases in human cells yielding two fragments of about 100-105 kDa and 35-40 kDa (Moese et al., 2001), the last would correspond to the region from amino acid 800-1216. This leads to the question if some structural variants of CagA are better cleaved inside the host cell so that can not be totally inhibited by the N-terminal membrane-binding domain, but this requires investigation.

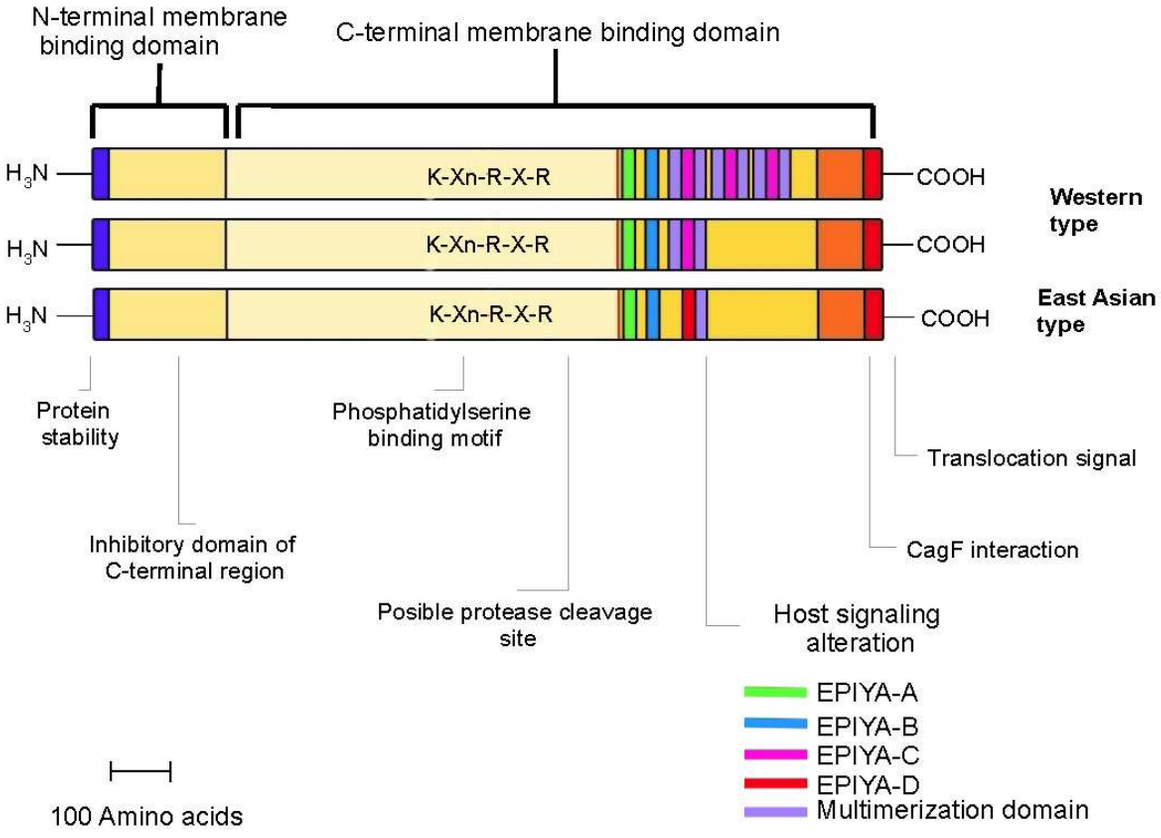

Figure 2. CagA structure in schematic representation. The most common variants of western and East Asian type are depicted. CagA possess at least two membrane-binding domains (Steininger et al., 2011), which are marked with quotation. Main known functions of certain regions are mentioned.

10. Phosphorylation-dependent cell signaling of CagA

Phosphorylation of CagA deregulates several signaling pathways leading to modification in host cell shape and adhesion, contributing to the transformation of cells (Hatakeyama, 2011, Jones et al., 2010). All of the EPIYA motifs in the four distinct EPIYA segments serve as tyrosine phosphorylation sites of CagA in host cells (Hatakeyama, 2011). Enzymes that can phosphorylate CagA *in vitro* and during infection *in vivo* are members of the Src and Abl families, which have been, recognized as oncogenic tyrosine kinases (Selbach et al., 2002, Stein et al., 2002,

Poppe et al., 2007, Tammer et al., 2007). The phosphorylation is a hierarchic regulated process in which c-Src phosphorylates EPIYA-C or EPIYA-D early in infection, while c-Abl phosphorylates either EPIYA-A, EPIYA-B, EPIYA-C, or EPIYA-D later in infection but only 2 EPIYA sites can be simultaneously phosphorylated per CagA molecule (Mueller et al., 2012). Furthermore, simultaneous phosphorylation of EPIYA-AC or EPIYA-BD confers strongest elongation phenotype in an AGS cellular model, even in a single CagA protein with both of the phosphorylatable EPIYA motifs (preferably EPIYA-AC), or in 2 independent CagA proteins, each with a single phosphorylatable EPIYA motif, which may form dimers to trigger this response (Mueller et al., 2012).

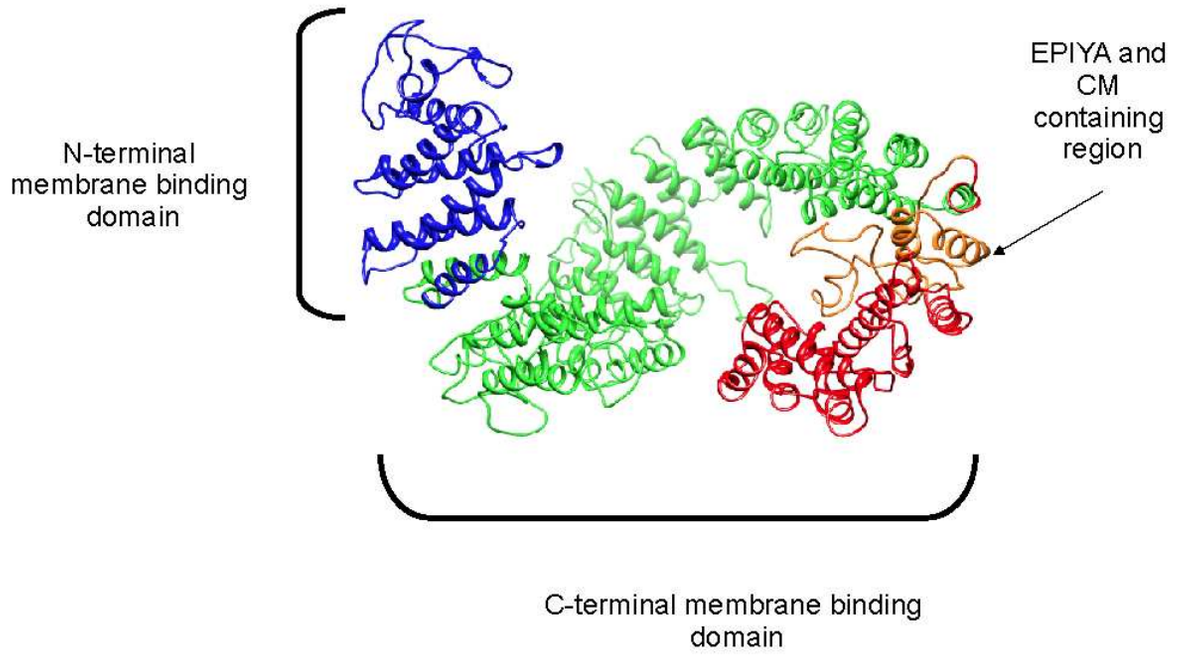

Figure 3. Tridimensional structure of CagA. The model was predicted using I-TASSER, image was obtained using UCSF CHIMERA software (Pettersen et al., 2004).

Once tyrosine phosphorylated, EPIYA-C or -D can bind to SHP-2 phosphatase (Hatakeyama, 2011) and activates the phosphatase activity of Shp2 producing an extremely elongated cell shape known as the hummingbird phenotype and altered cell motility (Backert et al., 2010, Higashi et al., 2004, Tsutsumi et al., 2006). SHP2 is required for full activation of the MAPK/ERK pathway, which conveys a potent mitogenic signal (Matozaki et al., 2009) and for direct dephosphorylation and inactivation of focal adhesion kinase, FAK, that deregulates integrin signaling (Tsutsumi et al., 2006).

Carrying the type EPIYA-D or multiple EPIYA-C repeats is associated with increased SHP-2 phosphatase activity induced by CagA, increasing the risk of precancerous and cancer lesions. This is because East-specific D segment shows higher binding affinity to SHP2 than the West-specific C segment but the C-type motif is variable in copy number and sequence, which can provide more sites for SHP2 binding.

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On the other hand, phosphorylated EPIYA-A and EPIYA-B have been shown ability to bind to Csk (Tsutsumi et al., 2003). Interaction of CagA with Csk stimulates the kinase activity of Csk, which in turn inhibits Src in a negative feedback loop that constrains the phosphorylation-dependent CagA activity below a certain threshold to ensure long-term colonization of *H. pylori* in the stomach without causing fatal damages (Hatakeyama, 2011). In addition, phosphorylated EPIYA-B can interact with phosphatidylinositol 3-kinase (PI3K) that regulates diverse cellular functions including proliferation and survival via AKT pathway (Nagy et al., 2009).

11. Phosphorylation-independent cell signaling of CagA

Recent studies have shown that not all cellular interactions exerted by CagA depend on its tyrosine phosphorylation so far more than 13 cellular interaction partners of non-phosphorylated CagA have been identified (Backert et al., 2010, Tegtmeyer et al., 2009, Mimuro et al., 2002). It has been shown that these interactions exert effects such as pro-inflammatory and mitogenic responses, disruption of cell-to-cell junctions or loss of cell polarity.

The first cellular interaction partner described of non-phosphorylated CagA was the adapter protein Gbr2 (Mimuro et al., 2002). Recently, it was reported that Gbr2 is the only host factor that interacts with both phosphorylated and non-phosphorylated EPIYA motifs (Selbach et al., 2009). Non-phosphorylated CagA was shown to interact with Gbr2 *both in vitro* and *in vivo*, which provides a mechanism by which Gbr2-associated SOS (son of sevenless) is recruited to the plasma membrane, this complex (CagA-Gbr2-SOS) can promote Ras-GTP formation, leading to cell scattering by stimulation of the MAPK/ERK signaling pathway as well as activation of nuclear transcription factors involved in cell proliferation and expression of the anti-apoptotic myeloid cell leukemia sequence-1 protein (Mimuro et al., 2007).

On the other hand, Chang YJ, et al., 2006, reported that *cagA*-positive *H. pylori* strains affect the cell cycle during infection by stimulating cyclin D1 expression, G1-S phase progression and host cell survival, suggesting the presence of distinct functional domains within CagA that play essential roles in protein targeting and alteration of host-cell transcription signaling pathways (Chang et al., 2006).

Yokoyama et al., 2005, found that CagA can stimulate the calcium-dependent serine/threonine phosphatase calcineurin in gastric epithelial cells, inducing translocation of the nuclear factor of activated T cells (NFAT) from the cytoplasm to the nucleus, where activates NFAT-dependent genes (Yokoyama et al., 2005). It is possible that the reported CagA-PLC interaction triggers Ca^{2+} mobilization and subsequent activation of calcineurin.

One of the NFAT-dependent genes activated by CagA in gastric epithelial cells is p21Cip1, a cyclin-dependent kinase inhibitor. Accordingly, although CagA activates a growth-promoting signal via the SHP-2-MAP kinase pathway or the Grb2-Ras-MAP kinase pathway, it simultaneously inhibits cell proliferation through NFAT-dependent p21Cip1 induction. Intriguingly, the *H. pylori*-vacuolating toxin *vac*A counteracts the activity of CagA to stimulate NFAT. Thus,

VacA has a role in determining the magnitude of NFAT deregulation in gastric epithelial cells expressing CagA. Such a functional interplay between CagA and VacA has already been suggested from the observation that secretion of VacA protein is associated with the presence of CagA despite the presence of vacA gene in all *H. pylori* strains (Yokoyama et al., 2005).

The CagA multimerization sequence mediates a stable binding with SHP-2 protein potentiating CagA virulence and is also responsible of the phosphorylation-independent activity of CagA (Ren et al., 2006, Nagase et al., 2011). Furthermore, it has been shown that CagA binds and inhibits the polarity-regulating kinase partitioning-defective 1 (PAR1)/ microtubule affinity-regulating kinase (MARK) through CagA multimerization sequence (Nagase et al., 2011). Kurashima et al., showed that CagA multimerization sequence destabilizes the E-cadherin/beta-catenin complex to trigger aberrant activation of the beta-catenin signal that underlies intestinal metaplasia (Kurashima et al., 2008). Recently, it was shown that the diversity in the structure and number of CagA multimerization sequences influence the strength of pathological activities of individual CagA with its cellular targets (PAR1b and SHP-2), emphasising its importance as a determinant of the degree of virulence of CagA (Lu et al., 2008) for development of gastric cancer.

A recent study shows that CagA can also interact with runt-related transcription factor gene 3 (RUNX3, a tumor suppressor that is frequently inactivated in gastric cancer) by a novel identified WW domain in the amino-terminal region of CagA (Tsang et al., 2010). In particular, CagA induces the ubiquitination and degradation of RUNX3, thereby extinguishing its ability to inhibit the transcriptional activation of RUNX3; currently, functional inactivation of RUNX3 can be caused by mutation, epigenetic gene silencing (promoter methylation) or cytoplasmic protein mislocalization (Ito, 2011). Recent evidence shows a strong association between methylation of the RUNX3 promoter and gastric cancer (Fan et al., 2011, Li et al., 2011). Recently, Lin et al., found that loss of RUNX3 in gastric cancer prevents G1 phase arrest and promotes tumorigenesis through Akt1/ß-catenin/cyclin D1 signaling pathway (Lin et al., 2012).

A consequence of non-phosphorylated CagA interactions in polarized epithelial cells is the disruption of cell junctions. In particular, tight and adherence junctions are essential for the integrity of the gastric epithelium (Wessler & Backert, 2008). CagA interferes with these intercellular junctions via several pathways. 1) Injected CagA associates with the epithelial tight-junction scaffolding protein, zone occludens-1 (ZO-1), and the transmembrane protein, junction adhesion molecule (JAM2), causing an ectopic assembly of tight-junction components at sites of bacterial attachment (Amieva & El-Omar, 2008), leading to alterations of cell polarity. 2) Non-phosphorylated CagA can interact with the transmembrane cell-cell junction protein E-cadherin (Hatakeyama, 2008). Subsequently, it was found that CagA forms a complex with c-Met recruiting E-cadherin and the Armadillo-domain protein, catenin delta, indicating that the interaction between CagA and E-cadherin is not direct (Oliveira et al., 2009). Baek et al., in 2007, reported that the interaction of alpha-Pix (PAK-interactive exchange factor) with CagA activates PAK1, ERK and NF-κB, which induces IL-8 expression (Baek et al., 2007, Lim et al., 2009); beta 1 integrin (integrin ß1) is another CagA-independent signaling that promotes cell

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motility (Snider et al., 2008), although the functional importance of this interaction needs to be investigated.

12. Concluding remarks

Studies host interactions with *H. pylori* virulence factors, such as CagA and the T4SS, have provided us many fundamental insights into the processes leading to *H. pylori* pathogenesis. The current hypothesis implies a model with translocated CagA as an "eukaryotic" signaling mimetic molecule either present in a large multiprotein complex or simultaneously in separated locations whitin infected target cells, which may have an important impact on the multistep pathogenesis of *H. pylori*. In the future, it will be important to search for additional injected proteins because it is rather unlikely that *cag*-PAI acquisition during evolution was intended to inject few effectors like CagA and peptidoglycan. Future studies are required to uncover the complex mechanisms that explain how *H. pylori* interacts whit host cells at the molecular level to accomplish its persistence through host life.

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