Trends in Microbiology

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Opinion

Classification of *Helicobacter pylori* Virulence Factors: Is CagA a Toxin or Not?

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Since its discovery, *Helicobacter pylori* has been identified as the causative agent of various gastric diseases. *H. pylori* produces myriads of disease-associated virulence factors. These bacterial determinants can be distinguished as cell-binding factors, immunoregulatory components, survival factors, toxins, and effector proteins. For most of these factors there is consensus about their classification. However, there is a strong dispute in the literature as to whether one of the best-studied factors, CagA, represents a toxin or not. CagA displays unique functions that are clearly different from conventional toxins, and CagA counteracts the activities of an established *H. pylori* toxin, VacA. Canonical toxins commonly have specific (and narrow) targets, can act even in the absence of the bacterial cell, and elicit acute damage to host cells. However, there is still no agreement on the classification of CagA. Here we discuss whether CagA acts as a toxin, and propose a classification consensus for CagA.

Highlights

H. pylori represents an emerging paradigm for chronic bacterial infections and pathogenesis in humans.

The *cag* type IV secretion system and CagA of *H. pylori* represent major virulence factors involved in establishing bacterial persistence and gastric cancer development.

Intracellular CagA is phosphorylated and activated by host tyrosine kinases of the Src and Abl families, but exhibits no typical toxic activities as known from canonical toxins, and should not be categorized as a toxin.

Translocated CagA mimics one or more host-cell proteins and can interact with at least 25 signaling factors to hijack intracellular signal transduction pathways, and therefore can be categorized as an effector protein.

More work needs to be done to clarify how CagA is translocated and decipher the evolutionary advantage of the entire type IV secretion system.

Helicobacter pylori Virulence

H. pylori colonizes the gastric epithelium of about half of the world's population and has accompanied humans for at least 100 000 years [1]. The bacterium has been categorized as a class-I carcinogen and represents the strongest known risk factor for severe gastric diseases such as chronic gastritis, peptic ulceration, and gastric adenocarcinoma [2]. Gastric cancer formation is a long-term, multistep, and multifactorial process, which is influenced by nutritional aspects, host genetic susceptibility, and *H. pylori* virulence potential [3]. Bacterial factors comprise cell-binding molecules (e.g., BabA/B, SabA/B, OipA, HopQ, HopZ, CagL, CagY), immunoresponsive elements (e.g., NapA, GGT, peptidogylcan, ADP-Heptose), and survival proteins (e.g., urease, flagellin, arginase, TlpB). In addition, the two major virulence determinants of *H. pylori* are the vacuolating toxin A (VacA; Box 1) and the *cag* pathogenicity island (*cag*PAI; Box 2) encoded type IV secretion system (T4SS) with its substrate protein CagA [2–5]. Historically, the vacuolating cytotoxin was discovered first [6], and the second factor, CagA, was identified later and named in relation to VacA as cytotoxin-associated gene A, but was devoid of cytotoxic activity itself [7]. Today, we know that translocated CagA can interact with about two dozen known cellular factors to manipulate intracellular signaling [2,8]. Many clinical studies, transgenic models, and *H. pylori* infection of Mongolian gerbils indicated that the expression of CagA correlated with gastric disease development [9–12]. However, after more than two decades of research, there is still no consensus on the classification of CagA. Some groups defined CagA as a toxin [13–19], while others refer to it as an effector protein [2,8,20–25]. Here we debate the pros and cons of these CagA activities, and suggest a classification consent.

What Makes a Canonical Bacterial Protein Toxin: The Definition

Generally, a toxin can be defined as a protein, peptide, or other substance which is produced by plants, animals, or pathogenic bacteria, and is highly poisonous for living cells in other organisms[i,ii]. Bacterial protein toxins are secreted into the extracellular environment and act

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Box 1. Vacuolating Cytotoxin A

Virtually all *H. pylori* isolates harbor the *vacA* gene encoding the vacuolating cytotoxin VacA. VacA represents an autotransporter and pore-forming toxin, which is inserted as oligomers into the host cell membrane, forms anion-conducting channels, and exhibits the capability to induce vacuoles in epithelial cells *in vitro* [4,26,29]. The secreted VacA toxin is about 88 kDa in size and comprises the N-terminal p33 and the C-terminal p55 domains. Various VacA functions have been described, including autophagy and apoptosis by cytochrome c release from mitochondria in epithelial cells, the promotion of immune tolerance, and bacterial persistence through inhibitory activities on T cells and antigen-presenting cells [4,26,29]. Clinical and epidemiological analyses have shown a correlation between specific *vacA* gene polymorphisms and the severity of *H. pylori*-associated gastric diseases. Genetic polymorphisms are well characterized for the signal (s) sequence region, which comprises part of the N terminus, and were typed as s1 or s2, respectively. This is followed by the so-called middle (m) region of *vacA*, which has been called m1 or m2. A higher risk for development of peptic ulcer disease and gastric cancer has been described for the *vacA* s1/m1 allelic combination, as compared with the less-virulent s2/m2 allelic pair. Additionally, the intermediate (i) and the deletion (d) sections have been discovered in *vacA,* but these sequences are less well characterized.

independently of the bacterial cell by exhibiting specific molecular toxic activities on host target cells. According to their site of action, they are classified into two main categories: (i) toxins active on the surface of target cells, and (ii) toxins active intracellularly [26]. On the cell surface, bacterial toxins can: (i) subvert cell signaling by mimicking a natural ligand (e.g., the heat-stable STa toxin from enterotoxigenic *Escherichia coli* mimics the intestinal hormone guanylin), (ii) exert an enzymatic activity on specific surface-exposed molecules (e.g., fragilysin from *Bacteroides fragilis*, which is a metalloprotease active on the extracellular domain of **E-cadherin**) (see Glossary), (iii) act as superantigens (by cross-linking MHC class II molecules on the surface of antigen-presenting cells with T cell receptors they cause a massive nonspecific activation of T lymphocytes, resulting in toxic shock syndromes), (iv) form pores in the plasma membrane of host cells (e.g., streptolysin O from *Streptococcus pyogenes*), or (v) damage the cell membrane, through either a detergent-like or an enzymatic activity (e.g., phospholipase such as the alpha toxin of *Clostridium perfringens*) [26,27]. Canonical bacterial toxins active inside target cells are formed by two different domains or subunits, named A and B, respectively, and thus are also known as 'A–B toxins'. Domain A represents the active part of the toxin, while domain B is devoted to enabling subunit A to reach its intracellular target (located either in the cytoplasm or in the nucleus) governing the process of cell binding and membrane translocation of the toxin. Interestingly, the A and B moieties can be represented not only by different parts of the same protein but also by separate gene products and proteins (thus forming the so-called 'binary toxins', as in the case of *Bacillus anthracis* toxins). A–B toxins are extremely powerful because the subunit A generally exerts an enzymatic activity on its molecular target, and thus few toxin molecules can rapidly modify a large number of substrate molecules. For instance, one single molecule of diphtheria toxin subunit A reaching the cytosol is sufficient to kill a target cell [28]. An exception

Box 2. *cag* Pathogenicity Island

Highly virulent *H. pylori* strains contain the cytotoxin-associated genes pathogenicity island (*cag* PAI). This represents a ~40 kb chromosomal stretch of DNA carrying up to 31 genes, which was acquired by a horizontal DNA transfer event from a yet unknown donor. The *cag*PAI encodes proteins forming a functional type IV secretion system (T4SS) with homology to the prototypic T4SS of *Agrobacterium tumefaciens* [5]. This T4SS represents a syringe-like multiprotein complex, which assembles as a core complex in the bacterial inner and outer membranes. This core structure is connected to an extracellular pilus, called the T4SS pilus, which is induced during host cell infection. This T4SS presumably acts as a syringe-like delivery device for virulence factors, including the major effector protein CagA, into host target cells. Following translocation, CagA is then sequentially phosphorylated at the C-terminal EPIYA (Glu-Pro-Ile-Tyr-Ala) sequence motifs by host tyrosine kinases of the c-Src and c-Abl family [58]. These EPIYA motifs are closely associated with the so-called CM (CagA multimerization) sequences at the C terminus, also called CRPIA (conserved repeats responsible for phosphorylation-independent activity) sites [2,8]. Delivered CagA can bind to about 25 known host cell signaling factors, and in this way, CagA can hijack various signal transduction cascades within gastric epithelial cells to induce inflammation, proliferation, and genetic instability, while it inhibits cell polarity and apoptosis. According to this cancer-inducing potential, CagA has been designated as the first oncoprotein in the bacterial world [2,8].

Glossary

AKT-mTOR pathway: a signal transduction cascade downstream of **phosphoinositide 3-kinase** involved in cell proliferation and apoptosis. Activation phosphorylates and activates AKT; in many cancers this pathway is overactive, allowing strong cell proliferation, while inhibiting apoptosis.
Autophagy: the orderly degradation and recycling of unnecessary or dysfunctional cellular molecules or organelles through autophagosomes.
B cell lymphoma 2 (Bcl-2): this represents a family of regulator proteins, consisting of a number of evolutionarily conserved proteins that share Bcl-2 homology (BH) domains, displaying either pro- or antiapoptotic effects. BCL2L11 (BIM) shows proapoptotic effects, whereas Bcl-2 and Bcl-X_L exhibit antiapoptotic properties.
CD44 variant 9 (CD44v9): a splicing variant of the CD44 hyaluronan cell-surface receptor, which has been shown to correlate with a resistance to cancer chemotherapy.
c-Met: this transmembrane tyrosine kinase (also called the HGF receptor) triggers various cellular processes, including proliferation, survival, migration, and invasion.
E-cadherin: a transmembrane protein in the adherens junctions, which links adjacent cells and plays an important role in stabilizing the polarized epithelium and signal transduction.
Focal adhesion kinase (FAK): this tyrosine kinase is a major regulator in the focal adhesions, which connect mammalian cells to extracellular matrix. FAK signaling plays an important role in multiple cellular responses, including motility and apoptosis.
Glycogen synthase kinase-3 beta (GSK3β): this serine/threonine kinase has been described as a major regulator for various signal transduction cascades such as Wnt and insulin signaling, glycogen synthesis, and microtubule dynamics.
Leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5): a potentiator for the canonical Wnt signaling pathway; it represents a biomarker of adult stem cells.
Myeloid cell leukemia 1 (MCL1): this Bcl-2 family protein is involved in the regulation of apoptosis versus cell survival, and in the maintenance of viability, but not of proliferation.

might be represented by the *H. pylori* toxin VacA, which has been suggested to be a new type of intracellularly active A–B toxin in which the subunit A is endowed with pore-forming instead of enzymatic activity, thus behaving as a 'cell-invasive' chloride channel [26,29].

CagA Exhibits No Classical Toxic Activities

Crystallographic analyses of a large ~100 kDa N-terminal CagA fragment revealed the presence of three discrete subdomains [30,31]. While domain-I represents a mobile N terminus of CagA, domain-II is responsible for CagA tethering to the plasma membrane and domain-III interacts intramolecularly with the intrinsically disordered C-terminal section containing various EPIYA phosphorylation sites (Figure 1A). However, no structural or sequence similarities to any known bacterial protein toxins were noted [30,31]. In addition, CagA does not exhibit any of the aforementioned toxic activities exerted by canonical bacterial toxins [2,8]. It is widely accepted that CagA acts inside the target cell where, however, it does not exhibit any enzymatic or pore-forming activity. Moreover, in the absence of the bacterium, CagA is apparently unable to autonomously translocate across the host membrane [30], a property strictly required for defining an intracellularly active bacterial virulence factor as a toxin. After entry into the cytosol through the bacterial T4SS, CagA acts as a 'Trojan horse', allowing *H. pylori* to subvert many host cell functions by acting as a nonphysiological scaffold/hub protein [2,8], as a result a few CagA molecules can have enormous impact on a variety of signaling cascades in the host cell, even without utilizing any enzymatic activity (Figure 1A,B).

Delivery of CagA is achieved by specific binding to phosphatidylserine [31] and/or integrin-β1 [30]. This binding step might be followed by membrane translocation of CagA through a yet uncharacterized endocytic process. Furthermore, most of the intracellular activities of CagA are accounted for the ~35-kDa C-terminal part through the phosphorylatable EPIYA-motifs and phosphorylation-independent CM/CRPIA sites, whose deletion does not affect CagA translocation [2,5,32]. Taken together, these findings might raise the speculation that CagA may be envisaged as the prototype of a novel class of A–B toxins that require bacteria–host cell contact for their productive delivery to target cells. The C-terminal EPIYA region of CagA would represent its atypical (i.e., nonenzymatic) domain A, while the N-terminal portion of the molecule would serve as a domain B, enabling the EPIYA region to reach the cytosol. However, the N-terminal portion of CagA also seems to play a relevant role in its intracellular activity [2,8], and mounting evidence suggests that CagA delivery is a highly sophisticated process widely exceeding a simple transmembrane penetration by a bacterial pilus or by an A–B toxin mechanism.

CagA Even Counteracts VacA Toxin Activity

An emerging concept in microbial pathogenesis is that, in order to achieve the best fitness in their hosts, bacteria may produce different virulence factors able to work either together as a team or counteracting each other with a sort of 'friendly fire' [33,34]. For fine-tuning its interaction with the human stomach, *H. pylori* seems to exploit a functional relationship between CagA and VacA [29,34]. Acting in concert, CagA and VacA would provide bacteria attached to the luminal pole of gastric epithelium with specific nutrients, such as iron, by increasing nutrient transepithelial delivery [3]. However, CagA and VacA are mainly counteracting each other. In this respect, many pathogens (e.g., enteropathogenic *E. coli*, *Shigella flexneri*, and *Chlamydia trachomatis*) exploit such a mechanism of virulence downregulation (to moderate cell damage and thus partly preserving host cells) as a key part of their overall pathogenic strategy [33]. CagA not only reduces the namesake cellular effect of VacA (i.e., cytoplasmic vacuole formation) [35], but also counteracts VacA-induced apoptosis by affecting the endocytosis and intracellular trafficking of the toxin and increasing the expression of antiapoptotic factors [20–22]. CagA activates, while VacA inhibits, the transcription factor **nuclear factor of activated T cells (NFAT),** which exerts pleiotropic actions on cell proliferation and differentiation, and whose overall

Depending on the isoform, the protein can either inhibit or promote apoptosis.

Nuclear factor of activated T cells (NFATs): a family of transcription factors known to play a role in the immune response as well as in the development of several diseases and cancers, through their influence on proliferation.

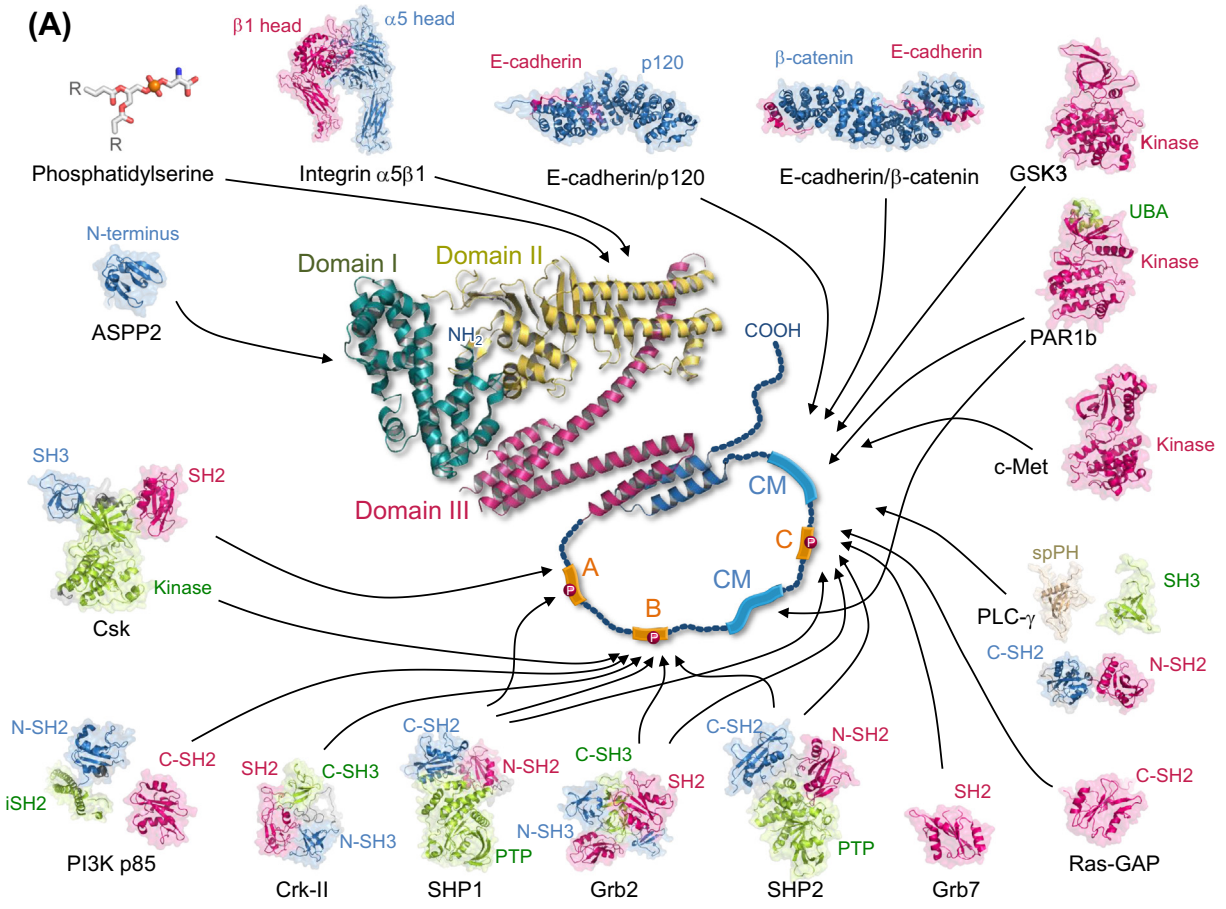
Phosphoinositide 3-kinases (PI3Ks): represent a family of kinases involved in many cell functions such as cell growth, proliferation, survival, differentiation, motility, and intracellular trafficking.

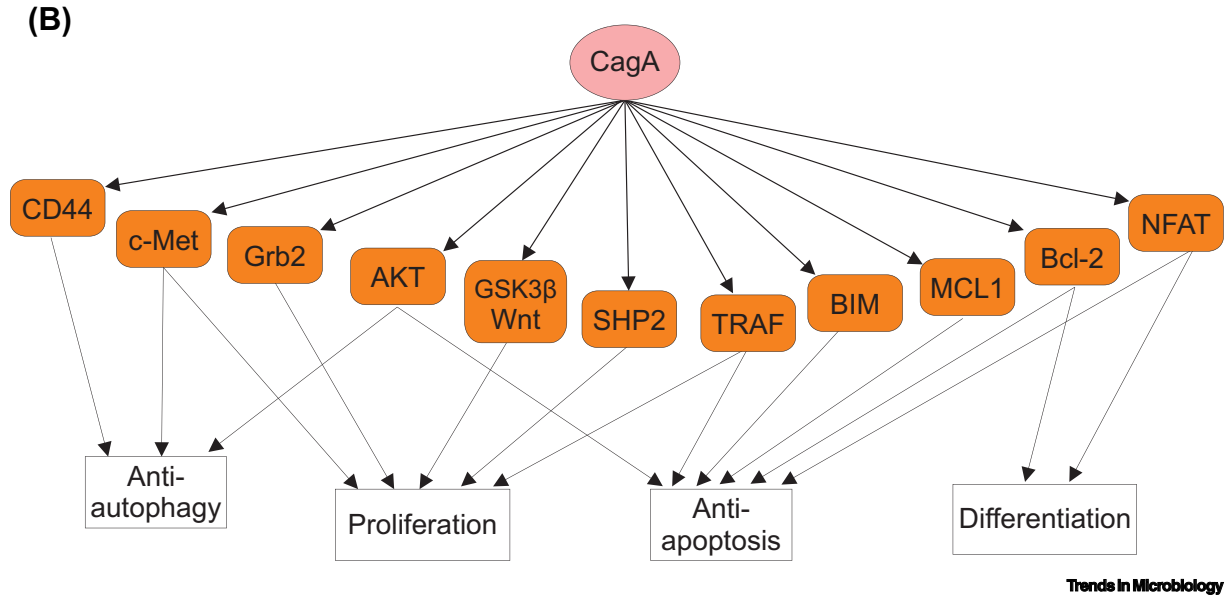
Ras-extracellular signal-regulated kinases (ERKs): members of the mitogen-activated protein kinases (MAPKs) pathways, regulating differentiation, proliferation, and apoptosis.

Src homology 2 (SH2) domain-containing protein tyrosine phosphatase-2 (SHP-2): a protein tyrosine phosphatase carrying two SH2 domains, which controls the signaling cascades of numerous cytokines and growth factors.

TNF receptor-associated factor 1 and 4-1BB (TRAF1 and TRAF4-1BB): TNF receptor-associated factor 1 (TRAF1) is a scaffold protein that participates in TNFR2 signaling, and TNF receptor family member 4-1BB (CD137) is a TNF receptor molecule encoded by TNFRSF9. Both molecules are involved in the regulation of inflammation and antiviral responses.

Wnt-β-catenin pathway: Wnt molecules activate, as ligands, the frizzled receptors and thereby the transcription factor β-catenin. This pathway regulates many vital cellular functions, such as cell determination and proliferation. Deregulation in this pathway has been shown to lead to a wide array of human diseases, including cancer.





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deregulation may play a significant role in the pathogenic action of *H. pylori* [36]. In addition, a short half-life of CagA in host cells was reported, which depends on autophagic degradation induced by VacA [37]. CagA can escape degradation in gastric cells where VacA-induced **autophagy** is suppressed, such as in cells expressing **CD44 variant 9 (CD44v9)**, a cell-surface marker associated with cancer stem cells [37]. However, a recent report provides discrepant results, suggesting a different scenario in which CagA undergoes both autophagic and proteasomal degradation in the absence of VacA [38]. VacA would cause accumulation (and not degradation) of CagA in gastric epithelial cells because the toxin disrupts a late step of the autophagic pathway leading to accumulation of dysfunctional autophagosomes. To explain the functional antagonism between CagA and VacA it was speculated that CagA may accumulate in dysfunctional autophagosomes induced by VacA. CagA would be thus functionally sequestered and its downstream signaling greatly limited [38]. On the other hand, the proinflammatory and carcinogenic action of CagA would be contributed by its direct inhibitory action on autophagy via the **c-Met**-PI3K/**AKT-mTOR** signaling pathway [39]. Indeed, in nontransformed cells, autophagy exerts a homeostatic activity counteracting malignant transformation, and many oncoproteins inhibit it [34]. This seems also the case for CagA. The inhibitory action on autophagy would thus represent another mechanism through which CagA counteracts VacA action.

CagA Acts as an Antikilling Factor

Since CagA has been identified to play a crucial role in the development of gastric cancer in *H. pylori*-infected individuals, it is not surprising that the protein exhibits antiapoptotic effects. For example, infection of Mongolian gerbils by wild-type *H. pylori* revealed a reduced amount of apoptotic cells in gastric pits after treatment with the apoptosis-inducing drug etoposide [20]. On the other hand, *cagA*- or T4SS-defective mutants showed levels of apoptosis comparable with uninfected cells. This decreased rate of apoptotic cells infected with the *cagA*-positive strains coincided with the increased expression of prosurvival factor phospho-ERK and antiapoptotic protein **myeloid cell leukemia 1 (MCL1)** *in vitro* [20]. Infection with *cagA*-positive strains have also revealed the phosphorylation of AKT, which coincided with the phosphorylation-dependent inactivation of the proapoptotic protein BIM of the **B cell lymphoma 2 (Bcl-2)** family [40]. Conversely, two other proteins of the Bcl-2 family (Bcl-2 and Bcl-X_L), which exhibit antiapoptotic effects, revealed increased activation in B lymphoid cells infected with wild-type *H. pylori* [41]. Human GES-1 cells transfected with CagA also displayed a marked resistance to apoptosis coupled with increased expression of **TNF receptor-associated factor 1 and 4-1BB (TRAF1)** and TRAF4-1BB, both important proteins in the antiapoptotic NF-κB signaling pathway [42]. Furthermore, it was reported that gastric cancer cells, after stable transfection with the *cagA* gene, showed an increased resistance against etoposide and other apoptosis-inducing chemicals [43]. In contrast to CagA, only a few known toxins were reported to promote antiapoptotic signals, for example the *Pasteurella multocida* toxin [44], whereas the induction of apoptosis by toxins appears to be a more widespread effect [45].

CagA Induces Host Cell Proliferation

Typically, many bacterial protein toxins can blunt cell growth and proliferation by disintegrating fundamental cell structures and/or functions, occasionally leading to cell death [26]. In contrast,

Figure 1. Nontoxic Signaling Events Induced by *Helicobacter pylori* CagA. (A) 3D structure of the N terminus of CagA (domains I, II, and III) with modeled C-terminal tail containing the EPIYA phosphorylation sites (A, B, and C) and CM (CagA multimerization) sequences. Selected host cell binding partners are shown with proposed or confirmed interaction sites as marked with arrows. (B) Using a subset of the above signaling factors, injected CagA hijacks multiple cellular signal transduction cascades and thereby induces different responses, including varied antitoxic responses. The clearest antitoxic effects by CagA are the increase in cell proliferation as well as an increased resistance to apoptosis, both of which can be induced by multiple pathways. Additionally, other nontoxic effects, such as increased resistance to autophagy and increased differentiation of the infected cell, are also induced by some of these interaction partners. Additional abbreviations used: SH2, Src homology domain 2; SH3, Src homology domain 3; N, N-terminal; I, internal; C, C-terminal; PTP, protein tyrosine phosphatase; UBA, ubiquitin-associated domain.

very early studies indicated that CagA has a positive effect on the rate of proliferation of infected cells, as observed in early clinical studies. Patients infected with *cagA*-positive *H. pylori* strains exhibited a significant increase in epithelial cell proliferation compared with uninfected patients or individuals infected with *cagA*-negative strains [46]. CagA has then been shown to stimulate two major mitogenic signaling pathways in delivered cells, the **Ras**-ERK MAP kinase and **Wnt**-β-catenin pathways, each of which potently stimulates cell proliferation [2]. CagA promotes the Ras-ERK pathway by forming a physical complex with the corresponding activators, tyrosine-phosphatase **Src homology 2 (SH2) domain-containing protein tyrosine phosphatase-2** (SHP2) or the adapter protein Grb2 [47–49]. Likewise, CagA stimulates the Wnt-β-catenin signaling cascade by interacting intracellularly with E-cadherin, hepatocyte growth factor (HGF)-receptor c-Met, and/or **glycogen synthase kinase-3 beta (GSK3β)** [2,9,50,51]. Growth-stimulating activity of CagA has also been demonstrated in transgenic studies of the *cagA* gene in animals. Systemic expression of CagA in mice induced gastric epithelial hyperplasia and granulocytosis without signs of cell death [10]. Likewise, transgenic expression of *cagA* in zebrafish or *Drosophila* gave rise to hyperproliferation of intestinal epithelial cells [11,12]. Mouse infection experiments also revealed that *H. pylori* colonized the progenitor and **leucine-rich repeat-containing G-protein-coupled receptor 5** (**Lgr5$^+$**) stem cell compartments of the gastric antral glands and induced hyperplastic responses that expanded Lgr5$^+$ stem cell populations in a T4SS-dependent manner, suggesting that CagA could be involved [52]. Additionally, *in vitro* infection with *H. pylori cagA*-positive strains induced epithelial cell proliferation in human and mouse gastric organoids [23,53,54]. As an example, Figure 2A shows abnormal proliferation of epithelial cells following expression of CagA in polarized epithelial monolayers [49].

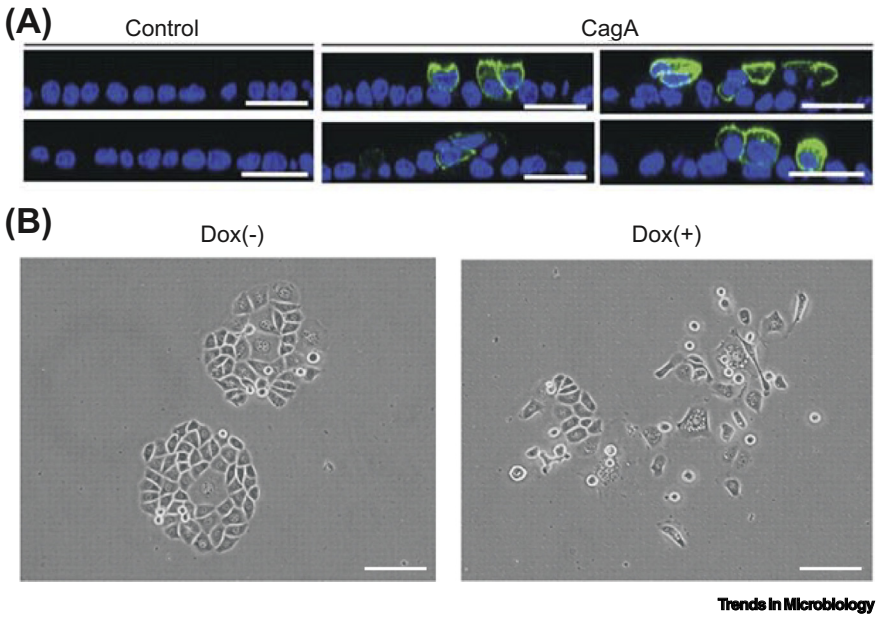

Figure 2. Intracellular Expression of *Helicobacter pylori* CagA Results in Cell Proliferation and Scattering. (A) Transfection of CagA in a MDCK (Madin-Darby Canine Kidney) polarized epithelial monolayer induces extrusion of CagA-expressing cells (shown in green) from the monolayer, which is followed by multiple rounds of cell division. The images are X–Z confocal views. Nuclei are stained with DAPI (4′,6-diamidino-2-phenylindole) (blue). The figure was modified with permission from Saito *et al*. [49]. (B) Foci of 1804 cells with (right) or without (left) CagA induction by the Tet-on system using doxycycline (Dox). Following CagA induction (right), the foci were diffusely scattered because of elevated cell motility. A fraction of CagA-expressing cells also showed the elongated cell shape known as the 'hummingbird phenotype'. The figure was modified with permission from Higashi *et al*. [56]. The scale bars are 10 μm.

CagA Promotes Cell Motility

Morphogenetic/motogenetic activity of CagA has been well documented by a number of studies. Infection by *cagA*-positive *H. pylori* or ectopic expression of CagA in various cell types causes an elongated cell shape (known as the 'hummingbird phenotype') [55], which is concomitantly associated with cell scattering (Figure 2B) [56]. This phenotype closely resembles that of MDCK cells treated with HGF leading to c-Met receptor-mediated cell–cell dissociation and motility [57]. This phenotype requires CagA tyrosine phosphorylation, which is mediated sequentially by **Src** and Abl kinases [58] and involves the CagA-deregulated SHP2-**FAK (focal adhesion kinase)** signaling axis [2] as well as dephosphorylation of the actin-binding proteins cortactin, ezrin, and vinculin [59–62]. Since the hummingbird phenotype is associated with dramatic cytoskeletal rearrangements, CagA may induce the cell-elongation through small GTPases Cdc42 and Rac1, which also promote cell scattering. Abl-mediated CagA–Crk interaction might additionally promote these morphological changes through Rac1 [63].

Concluding Remarks

Bacterial pathogens produce toxins, effector proteins, and other molecules to influence host–pathogen interactions. Here, we discussed the molecular structure and activities of *H. pylori* CagA, an important virulence factor and gastric cancer-triggering protein. Antonello Covacci and coworkers [7] noted in their first CagA report '…We have found that the 128-kDa molecule (CagA) purified by gel chromatography is devoid of cytotoxic activity and that cytotoxicity is associated with fractions containing an 87-kDa protein (VacA) not crossreacting with the CagA antigen. These data indicate that the 128-kDa molecule is not the cytotoxin, but is somehow associated with it.'. We think that these very early findings are still valid, and the function of CagA has been corroborated by many following studies during the past 26 years [2,8,20–24,47,56]. Thus, as a consensus, we recommend naming CagA as an effector protein, and not a toxin.

Outstanding Questions

Can any classical toxin activity be defined for CagA in future studies?

How is CagA transported through the T4SS apparatus and across the host cell membrane?

What is the evolutionary benefit of certain CagA-dependent signaling cascades for *H. pylori*?

Is there any direct function of CagA inside the bacterium itself?

Will it be possible in the near future to generate a crystal structure of the CagA C terminus?

How is the recruitment of approximately 25 interaction partners of CagA and downstream signaling organized?

Is there a specific strategy by CagA for sequential targeting of multiple host cell interaction partners?

How many interaction partners can be recruited by a single CagA molecule?

What is/are the most important CagA signaling pathway(s) contributing to gastric cancer development?

What is the fate of CagA in the host cell *in vivo*?

Acknowledgments

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Resources

[i]https://medical-dictionary.thefreedictionary.com/toxin

[ii]www.merriam-webster.com/dictionary/toxin

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