The Role of CagA in the Gastric Biology of *Helicobacter pylori*

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See related article by Blaser et al., Cancer Res 1995;55:2111–5.

Helicobacter pylori (H. pylori) was discovered in 1983 by the Australian scientists Warren and Marshall as a gastric pathogen, causing peptic ulcer disease. In 2005, they received the Nobel Prize in Medicine or Physiology for their discoveries, especially because the use of antibiotics to treat ulcers changed the practice of medicine. In the early 1990s, our group and others showed that *H. pylori* is also involved in causing gastric cancer. Between ulcers and cancer, *H. pylori* became firmly established as a major human pathogen.

Yet, colonization by *H. pylori* is extremely common worldwide, affecting about half of the world's population, and most carriers develop neither ulcers nor cancer. As such, we had been investigating whether some strains might be more pathogenic than others. In 1988 to 1989, my laboratory was creating libraries of *H. pylori* genes to identify and clone important antigens. A screen of these libraries using serum from an *H. pylori* carrier (M.J. Blaser) identified a strongly immunoreactive clone, which we ultimately characterized. Concurrently, we were asking whether carriers developed antibody responses to specific bacterial proteins, including the secreted vacuolating cytotoxin (VacA). In 1990, we reported that people carrying VacA$^+$ strains produced antibodies to a band at about 128 kDa that was absent in carriers of strains that did not have toxin activity (1). Both of our approaches identified the very same protein, which we and colleagues in Siena later jointly named as cytotoxin-associated gene A (*cagA* and the resulting protein CagA; refs. 2, 3). Studies reported in 1991 by Jean Crabtree and her colleagues in England confirmed our earlier association of a high molecular weight protein (they measured it at 120 kDa) with peptic ulcer disease (4). That independent confirmation by another team, across an ocean, using parallel but different investigative methods, showing essentially the exact same result, confirmed for us that we were on the track of something important.

In 1995, we reported in *Cancer Research* that carriage of a CagA$^+$ strain was associated with increased risk of gastric cancer (5), that is, the article we are remembering today, but it was one link in a long chain of studies involving multiple scientists around the world. In 1995, we also reported that adjacent to *cagA* on the bacterial chromosome was a homolog to a type IV secretion system (T4SS) protein (*cagE* or *virB4*), and mutating that gene eliminated major CagA effects on epithelial cells (6), but at this stage we did not understand all of the steps in the process. After these early studies, a breathtaking expansion of research on CagA occurred with more than 3,000 citations in PubMed until today. A major question asked by many researchers was: Why are *cagA*$^+$ *H. pylori* strains especially associated with ulcers and cancer?

A major breakthrough in the study of CagA came in 2000 when five groups independently reported that *H. pylori* may encode a functioning membrane-spanning T4SS and that the major substrate that it translocates into host cells is the CagA protein itself (7). All these genes and others are present on a bacterial chromosomal locus that has been called the *cag* island. The island, a locus of about 40 kb carrying up to 32 genes, is typically flanked by 31-bp direct repeats, was acquired horizontally from a yet unknown ancestor and integrated into the *H. pylori* chromosome (6, 7). In later years, we have learned that the *cag* T4SS also translocates other *H. pylori* substrates, including cell wall components (peptidoglycan) driving much of the proinflammatory response. However, *H. pylori* also possesses other T4SSs with different activities.

The CagA protein has been recognized as a marker for the entire *cag* island and has remarkable functions. During infection, CagA localizes to the host plasma membrane and undergoes tyrosine phosphorylation. This was surprising at the time, as the previously published *H. pylori* genome sequences do not encode tyrosine kinases. These data suggested that host tyrosine kinases may target CagA upon delivery (7). After this discovery, we and others showed that phosphorylation occurs at specific Glu-Pro-Ile-Tyr-Ala (EPIYA)-sequence motifs by c-SRC and c-ABL family kinases (8). The action is precise: the bacteria inject CagA molecules that mimic host proteins and thereby act as molecular "Trojan horses" containing a bacterial hidden core message that allows the microbe to substantially influence the host cell (7). In fact, intracellular CagA has since been shown to interact with multiple host cell proteins, either depending on its phosphorylation or not (Fig. 1). A common variant of the *cagA* gene, encoding an EPIYT-motif, has an altered function (9).

The first identified binding partner of phospho-CagA was the phosphatase SHP-2, stimulating gastric epithelial (AGS) cell elongation through an ERK signaling pathway (10). CagA can also recruit the tight junction proteins JAM and ZO-1 (11), as well as E-cadherin and PAR1, resulting in altered epithelial cell-to-cell junctions (Fig. 1). Subsequently, the various forms of CagA have been discovered to interact with >25 host signaling factors promoting proinflammatory, proliferative, cell cycle–related, and antiapoptotic gene alterations (Fig. 1). Thus, CagA is a remarkably multifunctional effector protein of *H. pylori*, acting as a signaling "master key" to subvert normal host cell functions, presumably to directions favorable for *H. pylori* survival. Accordingly, alterations in cellular growth and of epithelial integrity by CagA can trigger precancerous alterations in the stomach, which is one explanation for the high frequency of gastric cancer in patients carrying CagA$^+$*H. pylori* (5). On the basis of these findings, the study of CagA biology has evolved in recent years as a paradigm for a group of bacterial effector proteins, which are identified by the host as "self" and are being phosphorylated in the same fashion. Like EPIYA-motif phosphorylation in *H. pylori*, similar tyrosine-phosphorylated effector

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doi: 10.1158/0008-5472.CAN-16-1680

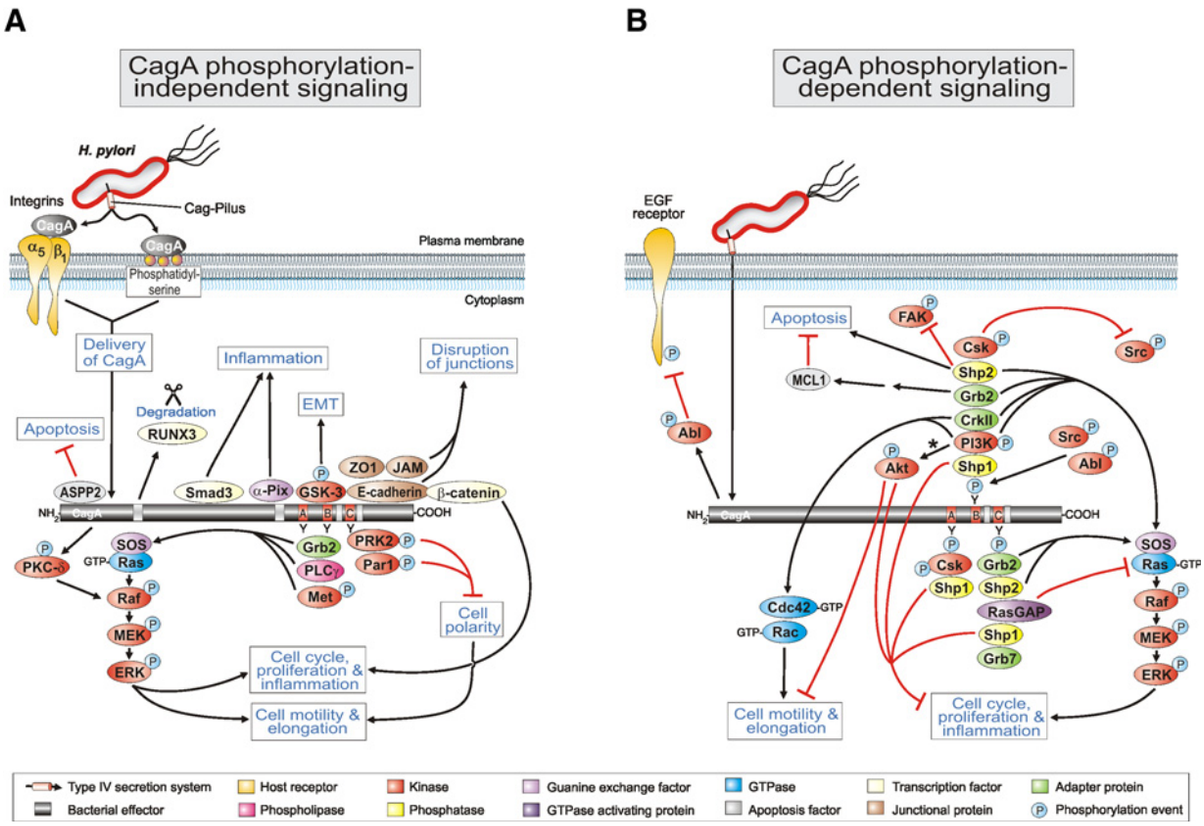

Figure 1.

Model for the translocation of CagA into host cells and role in subverting multiple host signaling cascades. A, CagA phosphorylation-independent and phosphorylation-dependent signal transduction events (B) are displayed. Black arrows, activated signaling events; red lines, inhibitory pathways. Using a type IV secretion system (Cag-pilus), *H. pylori* delivers CagA across both bacterial and host cell membranes into gastric epithelial cells. Translocation requires integrin $\alpha_5\beta_1$ and phosphatidylserine. Upon delivery, members of the oncogenic c-SRC and c-ABL tyrosine kinases can phosphorylate CagA at EPIYA and EPIYT sequences. Intracellular CagA then modulates multiple signaling cascades associated with cell polarity, cell cycle, cell proliferation, disruption of tight and adherent junctions, cytoskeletal rearrangements, cell elongation, proinflammatory responses, and suppression of apoptosis, as indicated. The phospho-EPIYT-motif differs from phospho-EPIYA as it leads to enhanced Akt kinase activation (*) and suppression of IL8 secretion and AGS cell elongation (9). Panels A and B were updated from Backert and colleagues (Helicobacter 2010;15:163–176) with kind permission from Wiley.

proteins have been also identified in other bacteria. In enteropathogenic *Escherichia coli* (EPEC) as well as in *Anaplasma, Bartonella, Chlamydia, Ehrlichia,* and *Haemophilus* species, these effectors also may impact host signaling cascades and disease development.

The *H. pylori* T4SS forms an extracellular, needle-like pilus, and its assembly requires multiple protein–protein interactions and several pilus-associated factors. Upon host cell contact and *H. pylori* adherence, the pilus is induced and then exports CagA across both the bacterial and epithelial membranes into the host cell cytoplasm (12). Studies of the injection mechanism of CagA showed that the T4SS pilus with exposed CagL proteins binds to the epithelial cell integrin member $\alpha_5\beta_1$; this was the first described host cell receptor for a bacterial T4SS (12). Thus, CagA appears to not be randomly delivered across the host cell membrane, but rather in a tightly receptor-controlled fashion. On the basis of the crystal structure of an approximately 100-kDa amino-terminal CagA fragment, a possible mechanism has been proposed for its cell-surface binding (13). Mapping of preserved areas in the CagA crystal structure detected four conserved surface-exposed patches (CSP1-4) that represent putative hotspots for protein–protein interactions. The proximal part of a single-layer β-sheet (covering CSP4) mediates the specific interaction of CagA with β_1 integrin to trigger its own translocation, possibly by an endocytic import pathway (13). Taken together, these data provide comprehensive evidence for a unique receptor-dependent internalization mechanism of CagA into host cells.

Animal models for *H. pylori* infection have been developed in the last 20 years, which highlight the significance of CagA in pathogenesis. For example, in Mongolian gerbils, $CagA^{+}$*H. pylori* may trigger premalignant and malignant pathologies. Four weeks after experimental challenge, nearly every animal developed gastric dysplasia, and by 8 weeks, about two-thirds developed adenocarcinoma (14). A host transcription factor that is aberrantly activated in gastric premalignant lesions is β-catenin, which upregulates cancer-related genes (14). In addition to this infection model, a fundamental experimental connection between CagA and gastric carcinogenesis *in vivo* has been described by the generation of transgenic C57BL/6J mice expressing CagA (13). These transgenic mice developed gastric epithelial hyperplasia

even without *H. pylori* infection, and some developed polyps and adenocarcinomas in the stomach (and small intestine) after 72 weeks. In addition, systemic expression of CagA in mice resulted in the development of leukocytosis with IL3/GM-CSF hypersensitivity, and some animals developed B-cell lymphomas and myeloid leukemias (15). In that model, the CagA transgene that could be phosphorylated intracellularly by SRC family kinases was critical to the development of pathology. These observations were further supported by two other transgenic model systems, in *Drosophila* and zebrafish (16). Transgenic expression of CagA in these model organisms revealed significantly elevated rates of JNK activation and Wnt target gene upregulation, resulting in intestinal epithelial cell proliferation as well as the appearance of adenocarcinoma and small cell carcinoma (16). Taken together, these studies establish that *H. pylori* can induce the expansion of gastric adenocarcinoma in gerbils and other model systems. Oncogenic transformation clearly proceeds in a fashion dependent on a functional T4SS during infection, and expression of CagA alone is sufficient to cause severe malignant lesions in transgenic animals. It is reasonable to propose that gastric epithelial cells carrying translocated CagA could acquire cancer stem cell–like properties, making them candidates from which gastric cancer develops; future studies should investigate this hypothesis.

On the basis of the above findings, *cagA* was described as the first bacterial oncogene (15), but as with many other oncogenes in humans, the story became even more complex. The disease-associated factor CagA is also associated with health, and its lack can lead to disease. Although *cagA*$^+$*H. pylori* strains were preferentially associated with both ulcers and gastric cancer, we and others found that these same strains had an inverse (protective) relationship with premalignant and malignant conditions of the esophagus, specifically Barrett esophagus and esophageal adenocarcinoma (17). More recently, numerous studies have shown an inverse association of *H. pylori* with asthma and other manifestations of allergy, including rhinitis and atopy, especially for those conditions that begin in childhood, and especially with *cagA*$^+$ strains (18). Thus, these microbes and their genes do not exist in a vacuum. The context in the stomach, and perhaps elsewhere, is important in terms of the phenotypes conveyed to their hosts. The enhanced host interactions of *cagA*$^+$ strains drive proliferative, inflammatory, and immune phenomena not only *in vitro* but also *in vivo*, which is a double-edged sword. In short, the CagA molecular interactions create a state of chronic inflammation that injures the stomach, which worsens across the decades, leading to atrophy and metaplasia, and in some cases, to cancer. But a similar (if not identical) process leads to the recruitment of immunocytes to the stomach wall. Some of these, for example, FOXP3$^+$-regulatory T cells, may have favorable actions to prevent childhood-onset asthma, as shown in experimental asthma models in mice (19).

In aggregate, new data suggest that carrying a CagA$^+$*H. pylori* strain may not lead to enhanced mortality, compared with a CagA$^-$ strain, or to no strain at all (20). It may just be that the causes of death vary for all three groups, with *H. pylori* inducing gastric cancer cost, but possibly vascular disease benefits. This is in line with the longstanding coevolution of humans with *H. pylori*. Although progress is being made, especially in the experimental models of asthma, the key mechanisms and its switches are not identified to the degree needed to intervene, so as to prevent or treat disease. One future for CagA is to understand these interactions at the cellular and tissue levels to optimize health. Another future is to harness CagA and its parts, as drugs to force cells to respond according to our medical goals, not the organism's biological needs. By comparison, as *Clostridium botulinum* has yielded "botox" for therapeutic (and other) purposes, an ancient molecule so well evolved for interacting with host cells as CagA likely will have future utility. Perhaps, it might be an antioncogene to reduce cell growth, unbridled proliferation, or to trigger apoptosis via particular pathways. At this interface of host and coevolved microbe, with continued investigation nearly 30 years after its discovery, we predict a bright future. It appears that *H. pylori* and CagA will continue as rewarding research topics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was supported by grants from the German Science Foundation (project B10 in CRC-796 and project A4 in CRC-1181 to S. Backert), R01DK090989 and R01GM63270 from the NIH, and the C&D fund (M.J. Blaser).

Received June 17, 2016; accepted June 17, 2016; published online July 15, 2016.

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