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Role of *Helicobacter pylori* CagL in modulating gastrin expression

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Helicobacter pylori is a Gram-negative bacterium that is highly adapted for persistent colonization of the human stomach. Although most *H. pylori*-infected persons remain asymptomatic, the presence of this organism is a risk factor for gastric adenocarcinoma, peptic ulcer disease, and gastric lymphoma. There is a high level of genetic heterogeneity among *H. pylori* strains, and the risk of gastric disease is determined in part by characteristics of the *H. pylori* strain(s) with which a person is infected¹. One of the most extensively studied genetic features of disease-associated *H. pylori* strains is the *cag* pathogenicity island (PAI). This 40-kb region of chromosomal DNA, comprising about 27 genes, may be present, incomplete, or absent in *H. pylori* strains.

CagA, the first *cag* PAI-encoded protein to be studied in detail, is a highly antigenic protein that is translocated into gastric epithelial cells. Within gastric epithelial cells, CagA undergoes phosphorylation by host cell tyrosine kinases and interacts with multiple host proteins, leading to cytoskeletal rearrangements, disruption of cellular junctions, altered cellular adhesion and polarity, and increased cell proliferation. Experiments in animal models indicate that CagA has an important role in the pathogenesis of gastric cancer, and therefore CagA has been termed a bacterial oncoprotein². CagA is translocated into host cells by a process that requires about 17 genes within the *cag* PAI, several of which are homologous to genes encoding components of type IV secretion systems (T4SS) in other Gram-negative bacterial species³. T4SSs are complex molecular machines utilized by a wide variety of bacteria for delivery of effector proteins or DNA-protein complexes into recipient cells. Thus far, CagA is the only effector protein known to be translocated into host cells by the *H. pylori* *cag* T4SS.

Several recent papers have highlighted functions of a *cag* PAI-encoded protein known as CagL^{4–6}. Early work identified *cagL* as a gene that was required for translocation of CagA

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into host cells, and therefore, CagL is considered to be a component of the *cag* T4SS. More recently, CagL was detected as a component of pilus structures that form at the interface between *H. pylori* and gastric epithelial cells⁴, and was shown to be required for the formation of these *cag* PAI-associated pili⁶. Importantly, CagL has a role in targeting of the *cag* T4SS to the $\alpha 5\beta 1$ integrin receptor on gastric epithelial cells⁴. Several additional Cag proteins, including CagY, CagI, and CagA, can also bind $\beta 1$ integrin⁷. By binding to integrin receptors, CagL can cause a variety of cellular alterations, including the stimulation of cell spreading, focal adhesion formation, and activation of tyrosine kinases such as focal adhesion kinase and the epidermal growth factor receptor (EGFR)^{4,5}. The occurrence of these cellular changes in response to purified recombinant CagL alone indicates that CagL is not simply a component of the T4SS, but is sufficient to trigger cellular signaling events^{4,5}.

A paper published in this issue of Gut analyzes a new function attributed to CagL⁸. Previous work had shown that serum gastrin levels are typically higher in *H. pylori*-infected persons than in uninfected persons, and *H. pylori*-induced hypergastrinemia had also been detected in animal models⁹. Therefore, the authors set out to identify *H. pylori* constituents that could stimulate gastrin gene expression. To do this, they used gastric epithelial cells that were stably transfected with a luciferase reporter regulated by the human gastric gene promoter. Co-culture of these cells with *cag* PAI-positive *H. pylori* strains induced significantly greater gastrin promoter activation than did co-culture with *cag* PAI-negative strains. A *cagL* mutant strain failed to induce gastrin promoter activation, whereas a complemented *cagL* mutant strain had a phenotype similar to the wild-type strain. Since *H. pylori* contact with gastric epithelial cells was necessary for gastrin promoter activation, the observed effect was presumed to be mediated by CagL on the surface of *H. pylori*. Although activation of the gastrin promoter by purified recombinant CagL alone indicated that CagL was sufficient for this effect, stimulation of the gastrin promoter by intact *H. pylori* requires additional *cag* PAI-encoded proteins^{8,9}.

Experiments with anti-integrin blocking antibodies suggested that $\beta 5$ integrin, but not $\beta 1$ integrin, was required for CagL-induced gastrin promoter activation. Consistent with this result, immunoprecipitation analyses indicated that, upon co-culture of *H. pylori* with gastric epithelial cells, both CagL and $\beta 5$ integrin were constituents of a protein complex, and in vitro binding studies showed that CagL bound to $\alpha v\beta 5$ integrin with high affinity. siRNA experiments indicated that integrin linked kinase (a component of focal adhesions) had a role in CagL-induced gastrin promoter activation, and further studies provided evidence that epidermal growth factor receptor (EGFR) and mitogen-activated protein kinase (MAPK) signaling were involved in the process. These results indicate that CagL can target multiple integrins (rather than only $\beta 1$ integrin), and provide a view of the signaling pathways through which CagL can stimulate gastrin expression.

What is the relevance of these findings in vivo? The current study showed that in a Mongolian gerbil model, a wild-type *H. pylori* strain caused inflammation in both the gastric antrum and corpus after 8 weeks of infection, whereas an isogenic *cagL* mutant strain caused inflammation in the antrum but not the corpus⁸. This result indicates that CagL has an important functional role in vivo, but further studies will be required to elucidate the effects of CagL (as well as other products of the *cag* PAI) on gastrin expression and gastric acid production in vivo. *H. pylori* can potentially regulate gastric acid production in vivo not only by altering gastrin expression, but also through direct effects on parietal cells¹⁰ and through the actions of host factors such as IL-1 β . At present, the relative contributions of these different pathways to *H. pylori*-induced alterations in gastric acid production are unclear. Temporal factors also are relevant. For example, *H. pylori* infection in humans is often associated with an initial period of hypochlorhydria and hypergastrinemia, followed by a long-term equilibrium similar to pre-infection conditions, and then a later stage involving

atrophic gastritis, hypochlorhydria and hypergastrinemia. Further studies are warranted to investigate the multiple potential mechanisms by which *H. pylori* can alter gastric acid production, including in vivo analyses of gastrin secretion and gastric pH at multiple timepoints after *H. pylori* colonizes the stomach.

By showing that CagL can activate the gastrin promoter, the current study highlights the ability of *H. pylori* to perturb gastric physiology⁸. Alterations in gastrin expression are of interest because, in addition to its role in stimulating gastric acid secretion, gastrin can regulate the growth and differentiation of gastric epithelial cells; changes in these parameters might be relevant in the pathogenesis of gastric cancer. The findings in the current study add to a large body of evidence indicating that there are striking differences in the interactions of *cag*-positive and *cag*-negative *H. pylori* strains with human hosts. Variation among *H. pylori* strains in the ability to alter gastrin expression may be a factor that helps to determine the risk of *H. pylori*-associated gastric disease.

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Abbreviations

PAI	pathogenicity island
T4SS	type IV secretion system
EGFR	epidermal growth factor receptor
MAPK	mitogen-activated protein kinase

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