Helicobacter pylori Makes a Molecular Incision to Gain Epithelial Entry

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Helicobacter pylori type IV secretion system injects the oncoprotein CagA into epithelial cells to drive carcinogenesis. In this issue of *Cell Host & Microbe*, Tegtmeyer et al. (2017) show that a secreted bacterial protease disrupts apical-junctional complexes, paving the way for *H. pylori* to access the basolateral compartment and trigger pathogenesis.

Gastric adenocarcinoma is the third leading cause of cancer-related death worldwide, accounting for more than 720,000 deaths annually. *Helicobacter pylori*, the most common bacterial infection of humans, is the strongest known risk factor for this disease. Pathologic outcomes of *H. pylori* infection are mediated by complex interactions among bacterial virulence determinants, host constituents, and environmental factors. However, a major driver of susceptibility to gastric carcinogenesis is the *H. pylori* Cag type IV secretion system (T4SS), which delivers the oncoprotein CagA into gastric epithelial cells. The Cag T4SS is assembled only after host cell contact and has been shown to directly interact with the host cell receptor integrin-α5β1 (Kwok et al., 2007). Interestingly, this receptor is typically localized to the basolateral cell surface, a niche that is protected by apical-junctional complexes.

Integrity of the gastric epithelium is tightly regulated by cell polarity and functional apical-junctional complexes, comprised of tight junctions and adherens junctions. Tight junctions regulate cell polarity, epithelial barrier function, and paracellular permeability, and are composed of transmembrane proteins, including occludin, claudins, and junctional adhesion molecules (JAMs), as well as a complex network of scaffolding proteins, such as zonula occludens (ZO) (Figure 1). Adherens junctions are positioned directly below tight junctions and primarily function to regulate intracellular adhesion and cell signaling events. The integrity of adherens junctions is maintained through precise interactions between E-cadherin and members of the catenin family, such as β-catenin and p120-catenin, which serves to provide structural stability (Figure 1).

Following *H. pylori* colonization, normal gastric epithelial integrity and host cell signaling pathways are disrupted, which can lead to a variety of pathologic outcomes ranging from gastritis to premalignant lesions, such as atrophic gastritis, intestinal metaplasia, dysplasia, and gastric adenocarcinoma. The presence of the Cag T4SS significantly increases the risk for development of gastric cancer, which is likely dependent on the ability of the T4SS to translocate CagA into host cells. Once CagA is delivered into host cells, it can exert numerous effects, many of which are linked with carcinogenesis. Transgenic mice that overexpress CagA develop gastric epithelial cell hyperproliferation and gastric adenocarcinomas, further implicating this molecule as a bacterial oncoprotein (Ohnishi et al., 2008). Following its injection into epithelial cells, CagA undergoes tyrosine phosphorylation by Src/Abl kinases and, in turn, activates a eukaryotic phosphatase (SHP-2) and extracellular signal-regulated kinase 1 and 2 (Erk1/2), leading to cell scattering, cytoskeletal changes, and other morphologic changes reminiscent of unrestrained stimulation by growth factors (Segal et al., 1999; Odenbreit et al., 2000; Backert et al., 2000) (Figure 1). Non-phosphorylated CagA also exerts detrimental effects within gastric epithelial cells that contribute to pathogenesis. CagA, in its non-phosphorylated form, leads to disruption of apical-junctional complexes, and directly associates with the epithelial tight-junction scaffolding protein zona occludens 1 (ZO-1) and the transmembrane protein junctional adhesion molecule A (JAM-A). These interactions result in nascent but incomplete assembly of tight junctions at ectopic sites of bacterial attachment (Amieva et al., 2003) (Figure 1). In addition, unmodified CagA disrupts adherens junctions through an interaction with E-cadherin, leading to aberrant activation of β-catenin and an overall loss of barrier function and cellular polarity (Franco et al., 2005; Murata-Kamiya et al., 2007) (Figure 1).

Most prior studies investigating *H. pylori* T4SS and CagA function have used non-polarized gastric epithelial cell models, in which apical-junctional complexes are incompletely formed; therefore, integrins and other receptors, typically located on the basolateral surface, are easily accessible to pathogens. Using *in vivo* experiments and polarized cell models, Tegtmeyer et al. have now elucidated a unique mechanism by which *H. pylori* gains access to the basolateral cell surface, thereby facilitating Cag T4SS interactions with the previously identified integrin-α5β1 host cell receptor (Kwok et al., 2007) and targeted injection of CagA (Tegtmeyer et al., 2017) (Figure 1). Prior work had demonstrated that a secreted *H. pylori* serine protease, HtrA, cleaves E-cadherin to disrupt adherens junctional complexes (Hoy et al., 2010). Tegtmeyer et al. now demonstrate that HtrA is secreted *in vivo* in *H. pylori*-infected patients and functions to cleave gastric epithelial adherens junctions and disrupt mucosal barrier function (Tegtmeyer et al., 2017) (Figure 1). In addition to cleavage of E-cadherin, HtrA also facilitates cleavage of other tight junction proteins, namely occludin and claudin-8,

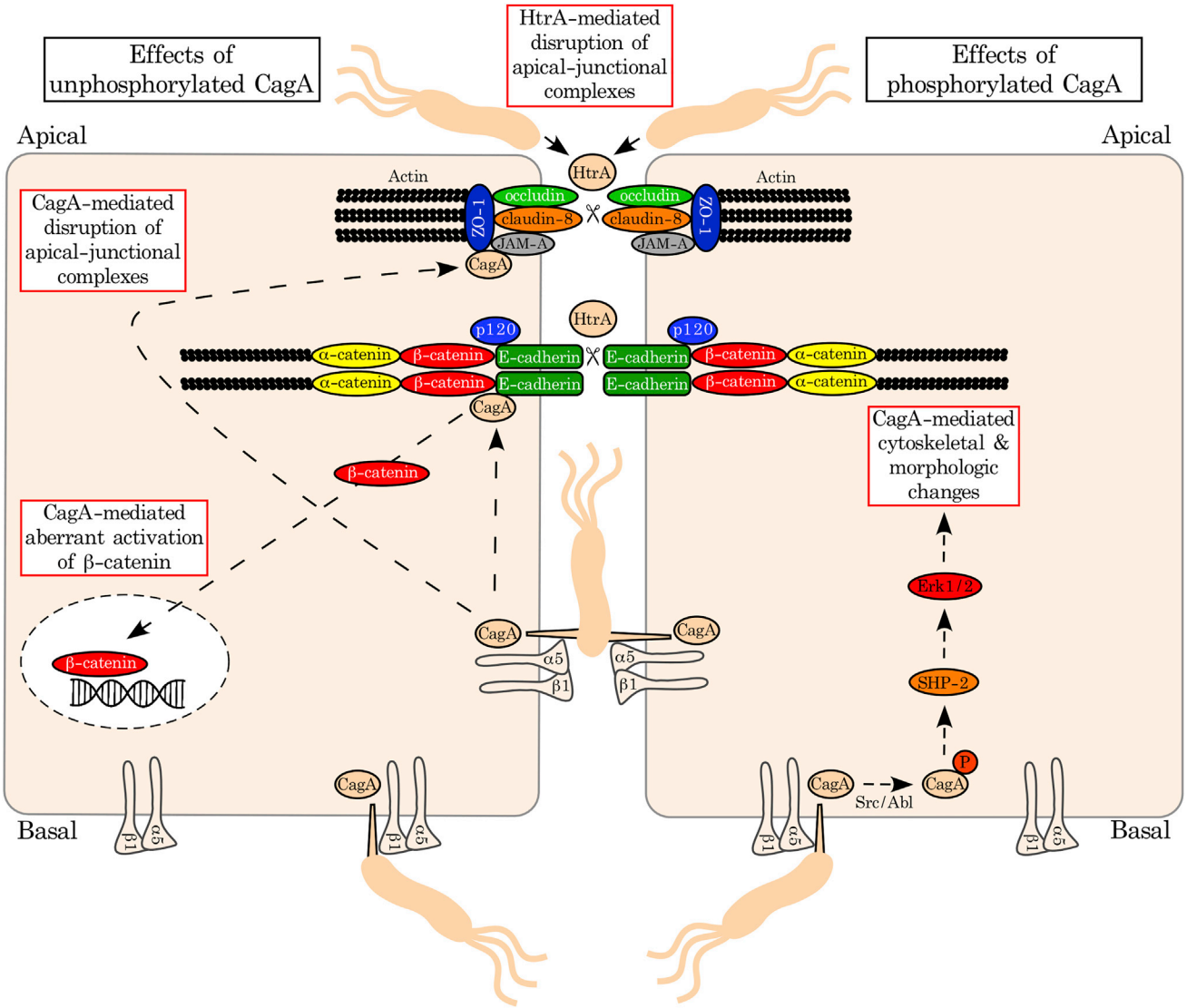

Figure 1. HtrA Disrupts Apical-Junctional Complexes, Allowing *H. pylori* Access to the Basolateral Compartment for Deployment of the Cag T4SS

H. pylori secretes HtrA to cleave proteins within the tight junction (occludin and claudin-8) and adherens junction (E-cadherin). Disruption of the apical-junctional complex permits *H. pylori* transmigration to the basolateral compartment, where *H. pylori* assembles and deploys the Cag T4SS via interaction with its cognate integrin-α5β1 receptor. T4SS-mediated translocation of CagA exerts numerous effects on host cells. Phosphorylated CagA induces cytoskeletal and morphological changes, while unphosphorylated CagA disrupts apical-junctional complexes and leads to aberrant activation of β-catenin.

to further disrupt barrier function and increase paracellular permeability (Tegtmeyer et al., 2017) (Figure 1). HtrA-mediated cleavage of E-cadherin, occludin, and claudin-8 subsequently permits transmigration of *H. pylori* from the apical surface to the basolateral cell membrane (Tegtmeyer et al., 2017) (Figure 1). Following localization to the basolateral cell surface, *H. pylori* actively assembles and deploys the Cag T4SS, which then directly interacts with its cognate integrin-α5β1 receptor, allowing for translocation of CagA into host cells (Tegtmeyer et al., 2017) (Figure 1). These results suggest that formation and function of the Cag T4SS occur only after *H. pylori* surreptitiously gains access to a tightly protected epithelial site, the basolateral surface, which is dependent on the function of bacterial HtrA (Tegtmeyer et al., 2017).

Many roles for the Cag T4SS in the pathogenesis of *H. pylori* have been uncovered over the years through investigating CagA-dependent host cell effects. Within this study, Tegtmeyer et al. have now built a novel model that incorporates many of these findings (Tegtmeyer et al., 2017) (Figure 1). However, there are questions that remain and future research opportunities that emanate from these findings. For example, does HtrA function as a molecular scalpel in strains that lack the Cag T4SS? If so, this would suggest that additional downstream

consequences may occur after *H. pylori* disrupts the junctions to invade the paracellular space since *cag*− strains would not engage the integrin-α5β1 receptor. Do *H. pylori* strains that access the paracellular space remain at this site or is there free exchange of bacteria between the apical and basolateral niches? What is the purpose of CagA injection at the basolateral site? Prior studies have indicated that CagA induces an aberrant transcytosis of basolateral transferrin receptors to the apical membrane as a means of acquiring iron from bound transferrin and promoting apical colonization. Is it also possible that *H. pylori* is able to induce mislocalization of the integrin-α5β1 receptor as well? Only future research will provide answers to these interesting questions.

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