Chapter 4
Roles of the *cag*PAI and CagA on Gastroduodenal Diseases

Steffen Backert, Giuseppe Zanotti, Judith Lind, Carmen Isabell Asche, and Nicole Tegtmeyer

Abstract *Helicobacter pylori* is a highly successful human-specific Gram-negative bacterium. Infections with this pathogen in the stomach can induce pathologies ranging from chronic gastritis, peptic ulcers, to gastric cancer. Highly virulent *H. pylori* isolates harbor the cytotoxin-associated genes (*cag*) pathogenicity island, which encodes a typical type IV secretion system (T4SS). This T4SS constitutes a syringe-like pilus structure for the translocation of virulence factors such as the CagA effector protein into gastric epithelial and immune cells. This is achieved by a number of T4SS proteins such as CagL, CagI, CagY, and CagA, which can interact with the host cell integrin member $\alpha_5\beta_1$ followed by transport of CagA across the host cell membrane. After delivery, CagA undergoes phosphorylation by oncogenic tyrosine kinases and mimics a host factor for the activation or inactivation of multiple intracellular signaling cascades. Here we review the current status in the characterization of phosphorylation-dependent and phosphorylation-independent signaling events by CagA and the CagA-independent T4SS activities in vivo and in vitro, which include the induction of membrane dynamics, actin-cytoskeletal rearrangements, disruption of cell-to-cell junctions, as well as pro-inflammatory, proliferative and anti-apoptotic nuclear responses. The contribution of these signaling pathways to pathogenesis during *H. pylori* infection is discussed.

Keywords *Helicobacter pylori* • Type IV secretion • VirB1–VirB11 • VirD4 • Signaling • Gastric cancer • Infection • Tyrosine kinase • SH2 domain • Inflammasome

S. Backert (✉) • J. Lind • C.I. Asche • N. Tegtmeyer
Division of Microbiology, Department of Biology, Friedrich Alexander University Erlangen-Nuremberg, Staudtstr. 5, D-91058 Erlangen, Germany
e-mail: steffen.backert@fau.de

G. Zanotti
Department of Biomedical Sciences, University of Padua, Viale G. Colombo 3, 35131 Padova, Italy

S. Backert, Y. Yamaoka (eds.), Helicobacter pylori *Research*,
DOI 10.1007/978-4-431-55936-8_4

4.1 Introduction

Helicobacter pylori is one of the best adapted human pathogens that colonizes the surface region in the gastric mucosa of the stomach. Approximately half of the world's population carries this microbe, often causing asymptomatic gastritis in infected people, but also more severe gastric diseases such as mucosa-associated lymphoid tissue (MALT) lymphoma and gastric cancer may arise. Colonization commonly occurs early in childhood and *H. pylori* can persist lifelong, if not treated by antimicrobial therapy. Although *H. pylori* infections are commonly associated with elevated inflammation parameters that are generated by the host innate and adaptive immune systems, the bacteria are not eliminated. Various mechanisms of immune evasion were documented and *H. pylori* became a prime example of chronic bacterial infections. Host-pathogen interactions are highly complex and determine the clinical outcome of infections. The development of gastric diseases is controlled by the bacterial genotype, genetic predisposition of the host, and environmental factors. For example, specific polymorphisms in host genes with important roles in pro-inflammatory and immune-regulatory signal transduction such as interleukin-1β (IL-1β), interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), or Nod-1 (nucleotide oligomerization domain-1) have been linked to a higher risk of developing *H. pylori*-triggered gastric diseases (for more details, see Chaps. 3, 13, and 16 of this book). *H. pylori* strains are highly heterogeneous both in their DNA sequences and virulence properties. Dozens of bacterial genes have been described to control the pathogenesis of *H. pylori*. There are two classical virulence factors expressed by *H. pylori*, the CagA protein encoded in the *cag* (cytotoxin-associated genes) pathogenicity island (*cag*PAI) and the vacuolating cytotoxin A (VacA). VacA interacts with multiple host molecules and can trigger various downstream signaling cascades as discussed in Chap. 5. Here we summarize our current knowledge on the multiple *cag*PAI and CagA functions as well as the multitude of affected host signaling cascades with focus on their importance in *H. pylori* pathogenesis.

4.2 The *cag*PAI Encodes a Type IV Secretion System

In the *H. pylori* research field, worldwide interest is focused on the effector protein CagA. CagA is encoded by highly virulent type I isolates, but is absent in less virulent type II strains. Thus, the protein has been recognized as a molecular marker for the *cag*PAI locus (Hatakeyama 2003; Backert et al. 2015). The *cag*PAI encodes functional components of a type IV secretion system (T4SS). This T4SS represents a pilus-like structure (called the T4SS pilus), induced upon host cell contact and protruding from the bacterial membrane (Fig. 4.1a). T4SS machineries are evolutionary related to DNA conjugation systems (Backert and Meyer 2006). The class

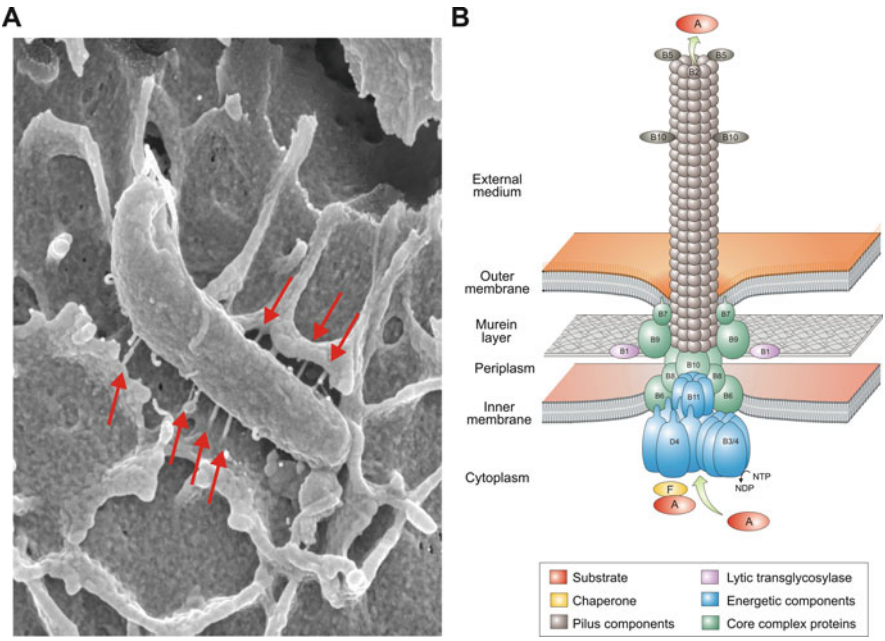

Fig. 4.1 Model for the assembled type IV secretion (T4SS) complex in *Helicobacter pylori* (*Hp*). (**a**) The T4SS encoded by the *cag* pathogenicity island is a multicomponent protein complex spanning the inner and outer membranes. Typical T4SS pili are shown by scanning electron microscopy, connecting the bacterium with the membrane of AGS gastric epithelial cells (*arrows*). (**b**) The *Hp* T4SS exhibits homology to the VirB/VirD4 T4SS machinery of *Agrobacterium tumefaciens*. T4SS assembly and subcellular localization of the proteins are shown in a simplified manner. Pilus components, core complex proteins, energetic components, and others factors are highlighted with different colors as indicated. The reported substrates for the *Hp* T4SS are CagA and peptidoglycan. (For more details, see text. Panel **a** was from Hauck (2007) with kind permission from Nature Publishing and panel **b** was adapted from Tegtmeyer and coworkers (2011) with kind permission from Wiley.)

of T4SSs is highly diverse both with regard to the delivered factors (DNA, proteins, DNA-protein complexes, or peptidoglycan) and recipient organisms. The latter can be either a bacterium of the same or other species or even species from different kingdoms (e.g., mammalian, fungal, or plant cells). In addition to *H. pylori*, pathogenicity-linked T4SSs have also been found in *Agrobacterium*, *Legionella*, *Bordetella*, *Bartonella*, and other species. T4SSs commonly comprise 11 VirB proteins (encoded by the *virB1–virB11* genes) and the NTPase coupling protein (VirD4). The prototypic and by far best characterized T4SS is the T-DNA transfer apparatus of *Agrobacterium tumefaciens* (Waksman and Orlova 2014). The agrobacterial VirB proteins can be classified in three groups: (1) the core or putative channel components (VirB6–VirB10), (2) the pilus-associated proteins (VirB2, and possibly VirB3 and VirB5), and (3) the energetic factors (the NTPases: VirB4 and VirB11). In addition, VirB1 is an enzyme having muraminidase activity, allowing

localized lysis of murein layer in the membrane to achieve T4SS assembly at a specific site of the cell (Backert et al. 2015). A model for the individual steps in assembling of the agrobacterial T4SS has been described in numerous review articles (Backert and Meyer 2006; Waksman and Orlova 2014). When having a look at the *H. pylori* T4SS, all 11 VirB and VirD4 orthologs are encoded in the *cag*PAI as well as some accessory proteins (Fischer 2011), resulting in a T4SS model (Fig. 4.1b). Immunogold labeling and electron microscopy (EM) have shown that the tips of the T4SS pilus are covered with CagL (Kwok et al. 2007). In addition, EM revealed that CagL, CagI, and CagH proteins were involved in T4SS pilus formation and deletion of a conserved C-terminal hexapeptide motif, which is shared among these proteins, abolished CagA delivery (Shaffer et al. 2011). The T4SS pilus is also decorated locally or completely by CagY (VirB10) (Rohde et al. 2003). CagY is a very large protein (about 250-kDa) that contains two transmembrane domains with the mid region (also called the repeat domain) exposed to the extracellular environment (Rohde et al. 2003). It carries an unusual sequence structure owing to an extraordinary number of direct DNA repeats that are predicted to result in in-frame intersections or deletions (Barrozo et al. 2013). Interestingly, CagY rearrangements are driven by the host and are sufficient to result in gain or loss of function in the T4SS. This molecular switch allowed modification of the immune response to benefit persistent infection of *H. pylori* (Barrozo et al. 2013). Major other components of the T4SS structure, also visualized by EM, are the VirB7 and VirB9 proteins (Tanaka et al. 2003). Interestingly, T4SS pilus formation requires a host cell receptor (Kwok et al. 2007). This is achieved by a number of T4SS proteins, including CagI, CagL, CagY, and CagA, interacting with the host cell integrin member $\alpha_5\beta_1$, followed by translocation of CagA into the host (Kwok et al. 2007; Jiménez-Soto et al. 2009). Binding of CagA to phosphatidylserine has also been shown to play a role in the translocation process (Murata-Kamiya et al. 2010).

4.3 Crystal Structures of *cag*PAI Proteins

In the last few years, several pieces of information have been gathered about the architecture of bacterial T4SSs, both from EM and single-crystal X-ray diffraction studies. The most complete picture of the apparatus comes from the 18Å–23Å EM structure of the T4SS of *Escherichia coli* conjugative plasmid R388 (Low et al. 2014). This complex includes 8 proteins (from VirB3 to VirB10) that form a supramolecular assembly spanning the entire cell envelope. The complex can be divided in two parts, the core complex, characterized by C_{14} symmetry, and the inner membrane complex, with a lower C_2 symmetry. The two complexes are connected by a small stalk. In the overall, the entire membrane-spanning machinery is about 340Å high and 255Å wide. A more detailed description at the atomic level

comes from the crystal structure of the O-layer (Chandran et al. 2009; Terradot and Waksman 2011) and from EM studies of the outer membrane T4SS core complex from plasmid pKM101 (Fronzes et al. 2009). The O-layer includes 14 copies of VirB10, VirB7, and VirB9, with the VirB10 components forming a tetradecameric channel and the 14 VirB7/VirB9 complexes surrounding and stabilizing it. The T4SS pilus is composed of the major subunit VirB2 and VirB5 (CagL) (Backert et al. 2008). The structure of the latter, which decorates the pilus surface, has been determined (Barden et al. 2013). CagL consists of a four-helix bundle, containing the Arg-Gly-Asp (RGD) motif that represents a recognition site for integrin binding (Kwok et al. 2007). Finally, the structure of VirB11 ATPase revealed a hexameric ring and functions as a gating molecule at the inner membrane, which is proposed to cycle through closed and open forms by ATP binding/hydrolysis (Yeo et al. 2000; Hare et al. 2007).

The crystal structures of some other members of the *cag*PAI, which are important for CagA secretion or IL-8 induction, have also been determined: CagZ, a 23-kDa protein essential for CagA translocation, consists of a single compact L-shaped domain containing seven α-helices (Cendron et al. 2004); CagS is a 23-kDa single-domain protein characterized by an all-α structure and an unusually high methionine content (Cendron et al. 2007); CagD is a covalent dimer in which each monomer folds as a single domain that is composed of five β-strands and three α-helices (Cendron et al. 2009).

The translocated effector protein CagA in strain 26695 consists of 1,186 amino acids and is structurally organized in several domains. Various crystal structures of the large N-terminal portion, residues 1–876 and 261–829 (Hayashi et al. 2012) and residues 1–884 (Kaplan-Türköz et al. 2012), consist of three domains, one of which is made by two sub-domains (or four domains in total, according to a different interpretation). Domain I (24–221) is composed of 10 α-helices. It makes very few contacts with the other domains, such that its orientation is mobile with respect to the rest of the molecule. Domain II (residues 303–644) constitutes the molecular core and contains a large antiparallel β-sheet flanked by an all α-helical subdomain (residues 370–446) and two long α-helices. A long, partially flexible α-helix connects domain II to domain III (residues 645–824), whose structure is that of a classical four-helix bundle. The C-terminal region (residues 825–1186, about 30% of the entire protein) of CagA seems to be intrinsically disordered, a fact that could possibly facilitate the interaction with other proteins inside the host cell. In fact, the structure of microtubule affinity-regulating kinase 2 (MARK2) in complex with 120 amino acids of the C-terminal region (residues 885–1005) has been determined, but only a 14-residue peptide of CagA is ordered in the crystal (Nesić et al. 2010). Recently, the complex between domain I of CagA (residues 25–220) with the apoptosis stimulating protein of p53-2 (ASPP2) has been also solved (Nešić et al. 2014), confirming that different parts of CagA interact with different target proteins to hijack host cell-signaling cascades associated with disease outcome.

4.4 Pathological Function of the *cag*PAI Type IV Secretion System

Clinical, epidemiological, and functional studies have shown that the presence of *cag*PAI and CagA is associated with the development of gastric disease. Various animal infection models have been developed and provided comprehensive evidence for the significance of *cag*PAI and CagA in *H. pylori* pathogenesis (Ogura et al. 2000; Rieder et al. 2005; Franco et al. 2005; Noto et al. 2013). For example, Mongolian gerbils infected with highly virulent *cag*PAI-positive *H. pylori* have been found to develop similar pathology as compared to infected humans. Gerbils developed gastric dysplasia in almost all animals by 4-week infection, which was accompanied by adenocarcinomas in ~25% of gerbils (Franco et al. 2005). After 8 weeks, ~75% of infected animals exhibited gastric adenocarcinomas. Importantly, infection with isogenic mutants indicated that CagA and the T4SS were necessary for gastric cancer development in the gerbil model (Franco et al. 2005; Noto et al. 2013). For more details and other models, please refer to Chap. 10 of this book. In addition to the *H. pylori* infection model systems, a first direct causal link between CagA and oncogenesis in vivo was reported by the production of transgenic C57BL/6J mice expressing CagA (Ohnishi et al. 2008). After 72 weeks, these transgenic mice exhibited gastric epithelial hyperplasia and some mice developed polyps and adenocarcinomas in the stomach and small intestine. Systemic CagA expression in these mice further induced leukocytosis with IL-3/GM-CSF hypersensitivity, and some animals exhibited myeloid leukemias and B-cell lymphomas (Ohnishi et al. 2008). These findings were supported using two other model organisms, zebrafish and Drosophila (Neal et al. 2013; Reid et al. 2012; Muyskens and Guillemin 2011). Transgenic expression of CagA in these systems exhibited significantly increased rates of intestinal epithelial cell proliferation, upregulation of c-Jun N-terminal kinase (JNK) signaling, and Wnt target genes associated with small cell carcinoma and adenocarcinoma (Neal et al. 2013; Wandler and Guillemin 2012). These experiments demonstrate that *H. pylori* can trigger the development of gastric adenocarcinoma in gerbils and other model systems in a manner dependent on a functional T4SS and that sole expression of CagA is sufficient to produce severe malignant lesions in transgenic mice. Thus, CagA and the *cag*PAI T4SS play central roles during *H. pylori* pathogenesis in vivo.

4.5 Phosphorylation-Dependent Host Cell Signaling of Translocated CagA

CagA represents a prime example of tyrosine phosphorylatable effector proteins ($CagA^{PY}$) of bacteria. Site-directed mutagenesis and mass spectrometry revealed numerous phosphorylation sites in CagA known as the Glu-Pro-Ile-Tyr-Ala (EPIYA) motifs A, B, C, and/or D (Hatakeyama 2003; Yamaoka 2010; Backert

et al. 2010). The host tyrosine kinases active on these EPIYA motifs were identified as members of the c-Src and c-Abl families (Wessler and Backert 2008). The resulting phosphotyrosines together with some flanking residues commonly act as recognition motifs for eukaryotic signaling factors. They recruit in particular cellular binding partners that contain SH2 (Src homology 2) domains, but not PTB (phosphotyrosine binding) domains and thereby target and subvert eukaryotic signal transduction pathways in ways that benefit the pathogen (Selbach et al. 2009). This indicates that CagA was specifically designed during evolution to target SH2 domain containing host cell factors (Fig. 4.2a). Altogether, 12 phospho-dependent interaction partners have been identified over the years (Table 4.1). The first reported interaction partner of $CagA^{PY}$ was the tyrosine phosphatase SHP-2 (Higashi et al. 2002). Since then, nine other host cell factors were also found to interact with CagA in a phosphorylation-dependent fashion: the tyrosine phosphatases SHP-1; phosphoinositide-3-kinase (PI3K); the signaling adaptor proteins Crk, Grb2, and Grb7; the tyrosine kinases Csk, c-Src, and c-Abl; as well as the Ras GTPase-activating protein Ras-GAP (Tsutsumi et al. 2003; Suzuki et al. 2005; Tammer et al. 2007; Selbach et al. 2009; Zhang et al. 2015). Thus, $CagA^{PY}$ seems to mimic a tyrosine-phosphorylated host cell protein and

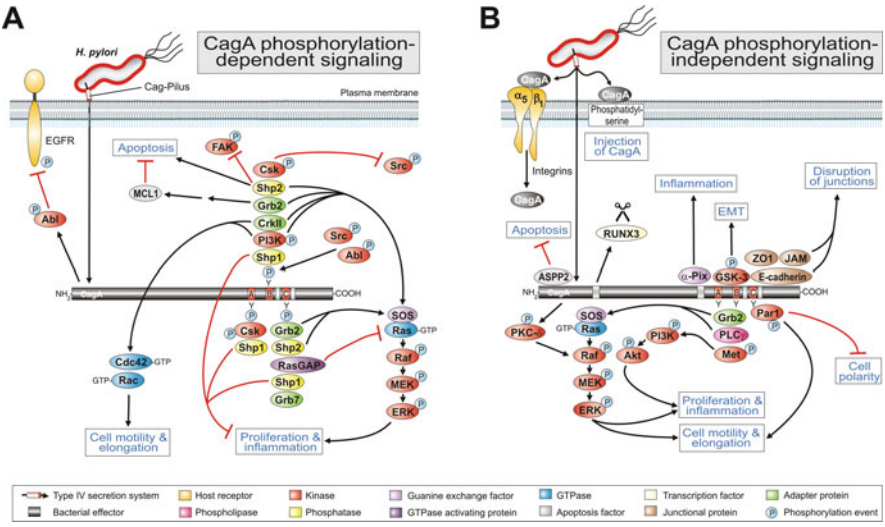

Fig. 4.2 Model for the role of *H. pylori* CagA in host cell-signaling processes which may affect pathogenesis. CagA phosphorylation-dependent (**a**) and phosphorylation-independent (**b**) signal transduction events are shown. CagA is translocated across the host cell membrane of infected gastric epithelial cells which requires integrin β_1 and phosphatidylserine. The tyrosine kinases c-Src and c-Abl phosphorylate delivered CagA. CagA can then modulate various signaling cascades associated with cell polarity, cell proliferation, actin-cytoskeletal rearrangements, cell elongation, disruption of tight and adherens junctions, pro-inflammatory responses, and suppression of apoptosis, as depicted. *Black arrows* indicate activated signaling pathways and *red arrows* correspond to inactivated cascades. (For more details, see text. Panels **a** and **b** were updated from Backert and coworkers (2010) with kind permission from Wiley.)

Table 4.1 Host cell proteins described to interact with phosphorylated CagA and proposed roles in *H. pylori* infections

Interaction partner	Proposed function	Experimental evidence	Applied methods[a]	References
Abl	Phosphorylation of CagA and CrkII adapter proteins	Infection, transfection of CagA	coIP, IF	Tammer et al. (2007)
				Poppe et al. (2007)
				Brandt et al. (2007)
CrkI, CrkII, CrkL[b]	Cell scattering, loss of AJs, MAPK signaling	Infection, transfection of CagA	coIP, IF, PD, immunoblot	Suzuki et al. (2005)
				Tammer et al. (2007)
				Brandt et al. (2007)
Csk	c-Src inactivation, loss of CagA-Shp-2 interaction	Transfection of CagA	coIP	Tsutsumi et al. (2003)
	Dephosphorylation of cortactin, ezrin, and vinculin	Proteomics	SILAC/MS	Selbach et al. (2009)
Grb2[b]	Cell scattering, activation of Ras-MAPK signaling	Proteomics	SILAC/MS	Selbach et al. (2009)
Grb7	Yet unknown	Proteomics, infection	SILAC/MS, coIP	Selbach et al. (2009)
PI3-kinase[b]	ATK signaling	Proteomics, infection	SILAC/MS, coIP	Selbach et al. (2009)
				Zhang et al. (2015)
Ras-GAP	Yet unknown	Proteomics	SILAC/MS	Selbach et al. (2009)
Shp-1	Yet unknown	Proteomics, infection	SILAC/MS, coIP	Selbach et al. (2009)
Shp-2	Cell scattering, activation of ERK	Transfection of CagA	coIP	Higashi et al. (2002)
	Tyrosine dephosphorylation of FAK	Transfection of CagA	coIP	Higashi et al. (2004)
	Downregulation of hBD3	Infection	IF, RT-PCR, immunoblot	Tsutsumi et al. (2006)
c-Src[b]	Phosphorylation of CagA	Infection	coIP	Selbach et al. (2003)

[a]Abbreviations: AJs adherens junctions, *IF* co-localization by immunofluorescence, *coIP* co-immunoprecipitation, *PD* pull-down experiments, *SILAC/MS* stable isotope labeling with amino acids in cell culture combined with mass spectrometry of bound proteins

[b]Using the coexpression system in COS-7 cells, Tsutsumi et al. (2003) overexpressed CagA together with the p85 subunit of PI3-kinase, c-Src, Grb2, or Crk-II, but none of the above reported interactions during infection between CagA and these proteins were found during these transfection studies

therefore appears to function as a master key or picklock to hijack specific cascades of the host. The various CagA^{PY}-SH2 domain interactions appear to have complex roles in *H. pylori*-triggered cytoskeletal rearrangements, cell scattering, and elongation (summarized in Fig. 4.2a).

Infection of AGS gastric epithelial cells with *H. pylori* in vitro results in migration of the cells and an elongated morphology, known as the "hummingbird phenotype" (Segal et al. 1999). This phenotype requires host cell motility by a yet unknown T4SS factor (Churin et al. 2003), while cell elongation is clearly induced by CagA^{PY} (Backert et al. 2001). Transfection experiments have shown that CagA^{PY}-SHP-2 interaction activates the phosphatase activity of SHP-2, which contributes to AGS cell elongation by stimulating the Rap1 → B-Raf → Erk signaling pathway (Higashi et al. 2004). It was also shown that the CagA^{PY}-SHP-2 complex suppresses the activation of epidermal growth factor receptor (EGFR) and downstream signaling, which is associated with enhanced *H. pylori* survival (Bauer et al. 2012). The authors found that an antimicrobial peptide, human β-defensin 3 (hBD3), which is highly active against *H. pylori*, is downregulated by EGFR suppression. These findings revealed a novel mechanism how T4SS-positive strains make use of CagA to evade a key innate mucosal defense pathway to support persistent *H. pylori* infection (Bauer et al. 2012).

Additional reports demonstrated that the elongation phenotype also requires tyrosine dephosphorylation of three well-known actin-binding proteins—vinculin, ezrin, and cortactin (Selbach et al. 2003, 2004; Moese et al. 2007). The phosphatase involved in this scenario is not SHP-2 and still remains to be identified. Instead it was shown that CagA^{PY} can hamper c-Src activity in two ways, by direct binding of both proteins to each other and by interaction of CagA^{PY} with Csk, a negative regulator of c-Src (Tsutsumi et al. 2003; Selbach et al. 2003). Since c-Src is the first kinase phosphorylating CagA, inactivation of c-Src by CagA^{PY} forms a typical negative feedback-loop mechanism, controlling the amount of intracellular CagA^{PY}. Interestingly, vinculin, ezrin, and cortactin are also targets of c-Src, and c-Src inactivation by CagA^{PY} causes the tyrosine dephosphorylation of these factors (Tegtmeyer and Backert 2011). Moreover, binding of CagA^{PY} to PI3K and/or CrkII is involved in activating the small Rho family GTPase members Rac1 and Cdc42 (Suzuki et al. 2005; Selbach et al. 2009), while binding of CagA^{PY} to SHP-2 or Grb2 can stimulate pro-inflammatory and proliferative responses through the mitogen-activated protein (MAP) kinase cascades (Fig. 4.2a). Finally, CagA^{PY} can also interact with SHP-1, Grb7, and Ras-GAP with yet unknown consequences for the host cell (Fig. 4.2a). Taken together, CagA^{PY} can bind to a remarkably high number of host cell factors to activate signaling mediating cell scattering, elongation, and probably other phenotypes.

Translocation and phosphorylation of CagA appear also important for the interplay of *H. pylori* with macrophages. Heme oxygenase (HO-1), an anti-inflammatory enzyme, is released by macrophages in response to CagA phosphorylation in *H. pylori*-infected human patients and mice, while blocking of phagocytosis prevented CagA^{PY} and HO-1 induction (Gobert et al. 2014). Genetic ablation

of the *hmox-1* gene in mice resulted in increased gastritis, which was associated with enhanced M1/Th1/Th17 responses, reduced regulatory macrophage response, as well as lower *H. pylori* colonization. These findings provide a mechanism by which *H. pylori* manipulate immune responses, supporting its own survival by induction of macrophage HO-1 (Gobert et al. 2014).

4.6 Phosphorylation-Independent Signaling of CagA

Early microarray studies of *H. pylori*-infected T84 cells showed that expression of 670 host genes changed and 479 of these genes occurred independent of the phosphorylation state of the CagA protein (El-Etr et al. 2004). Thus, not all the interactions of intracellular CagA require its tyrosine phosphorylation. Subsequently, 12 cellular binding partners of non-phosphorylated CagA have been reported (Table 4.2). Non-phospho CagA interactions have been found to induce loss of cell polarity, mitogenic responses, and pro-inflammatory signaling (Fig. 4.2b). The first interaction partner of non-phosphorylated CagA described has been the adapter protein Grb2 (Mimuro et al. 2002). Interestingly, Grb2 is the only binding factor that has been described to interact with both non-phosphorylated and phosphorylated CagA (Mimuro et al. 2002; Selbach et al. 2009). In particular, non-phosphorylated CagA was shown to utilize Grb2 for recruiting Grb2-associated Sos (son of sevenless), a guanine-exchange factor (GEF) of the small GTPase Ras, to the plasma membrane (Fig. 4.2b). This CagA/Grb2/Sos complex triggers Ras-GTP production, which in turn activates the Raf → MEK → ERK signaling pathway contributing to cell scattering (Mimuro et al. 2002) and stimulation of nuclear responses mediating cell proliferation and transcription of the anti-apoptotic myeloid cell leukemia sequence-1 (MCL-1) protein (Mimuro et al. 2007). CagA was also described to function as a mimetic of the eukaryotic Grb2-associated binder (Gab) adaptor protein in transgenic Drosophila which feeds into the same MAP kinase signaling pathway (Hatakeyama 2003; Botham et al. 2008). CagA-triggered MAP kinase activation can stimulate the transcription factor NF-κB mediating the onset of multiple target genes such as IL-8 (Brandt et al. 2005). In addition, it was shown that CagA targets various tumor suppressors such as p53 and RUNX3. CagA-positive *H. pylori* strains more strongly suppressed p53 as compared with low-risk strains during infection in vivo and in vitro. Degradation of p53 protein was shown to be induced by CagA-mediated signaling via host-specific E3 ubiquitin ligases, thus contributing in tumorigenicity (Wei et al. 2015). In addition, non-phosphorylated CagA can bind to RUNX3, which is often inactivated in gastric cancer. Interaction with RUNX3 proceeds by a WW domain located at the amino-terminus of CagA (Tsang et al. 2010). Importantly, CagA induces the ubiquitination and subsequent degradation of RUNX3, in this way turning off the transcriptional activity of RUNX3 (Fig. 4.2b). These data

Table 4.2 Host cell proteins described to interact with non-phosphorylated CagA and proposed roles in *H. pylori* infections

Interaction partner	Proposed function	Experimental evidence	Applied methods[a]	References
α-Pix [b]	Inflammation	Infection	coIP, MS	Baek et al. (2007)
ASPP2	Activation of ASPP2 and p53 degradation, apoptosis inhibition	Proteomics	LC-MS/MS, coIP, IF, Y2H	Buti et al. (2011), Nešić et al. (2014)
β1-integrin	Internalization of CagA	Infection	Y2H, coIP, Biacore studies	Jiménez-Soto et al. (2009)
CagA	Multimerization	Transfection of CagA	coIP, mutagenesis	Ren et al. (2006)
Calcineurin	Dephosphorylation of NFAT, nuclear translocation	Transfection of CagA	Coexpression, IF	Yokoyama et al. (2005)
c-Met [c]	Cell scattering, activation of PI3-kinase, Erk, β-catenin, and NF-κB [d]	Infection, transfection of CagA	coIP, IF	Churin et al. (2003), Suzuki et al. (2009)
E-cadherin	Destabilization of AJs, β-catenin signaling	Transfection of CagA	coIP	Murata-Kamiya et al. (2007)
GSK-3	Snail-mediated EMT trough GSK-3 depletion	Infection, transfection of cagA	IF, coIP, immunoblot	Lee et al. (2014)
p120-catenin	E-cadherin/c-Met/ p120 complex, cell scattering, invasion	Infection	IF, coIP, cell invasion assay, immunoblot	Oliveira et al. (2006, 2009)
Grb2 [e]	Cell scattering, activation of Ras-MAPK signaling	Transfection of CagA	PD, coIP, IF	Mimuro et al. (2002)
Par1b/ MARK2	Inhibition of Par1 activity, disruption of apical junctions	Transfection of CagA	coIP, IF, MF	Saadat et al. (2007), Zeaiter et al. (2008)
Par1b	Inhibition of mitosis	Transfection of CagA	IF, FACS	Umeda et al. (2009)
Par1a, Par1c, Par1d	Enhancement of cell elongation	Transfection of CagA	coIP, IF	Lu et al. (2009)
Par1b	Inhibition of Par1	3D structure	Crystallization, binding, and kinase assays	Nesić et al. (2010)
PLC-γ [f]	Cell scattering	Infection	coIP	Churin et al. (2003)

(continued)

Table 4.2 (continued)

Interaction partner	Proposed function	Experimental evidence	Applied methods[a]	References
RUNX3	Oncogene by blocking tumor suppressor RUNX3	Infection, transfection of CagA	Mutagenesis, coIP	Tsang et al. (2010), Liu et al. (2012)
TAK1[g]	Activation of NF-κB	Transfection of CagA, infection	EMSA, qPCR, coIP, PD, IF	Lamb et al. (2009)
ZO-1, JAM	Disruption of lateral junctions and cell motility	Infection, transfection of CagA	IF, MF	Amieva et al. (2003), Bagnoli et al. (2005)

[a]Abbreviations: *AJs* adherens junctions, *EMSA* electrophoretic mobility-shift assay, *FACS* fluorescence-activated cell sorting, *IF* co-localization by immunofluorescence, *coIP* co-immunoprecipitation, *EMT* epithelial-mesenchymal transition, *MF* membrane fractionation by Iodixanol Gradients, Opti-Prep™, *MS* mass spectrometry of bound proteins, *PD* pull-down experiments, *qPCR* quantitative real-time polymerase chain reaction, *Y2H* yeast two-hybrid screen
[b]Dependency of $CagA^{PY}$ was not investigated but α-PIX does not contain an SH2 domain and is therefore unlikely to interact with CagA in a phospho-specific manner
[c]Activation of c-Met was not observed in another study and attributed to cross-reactivity of the phospho-c-Met antibody with $CagA^{PY}$ (Snider and Cardelli 2009)
[d]Activation of CagA-induced NF-κB activation and IL-8 secretion was not observed when CagA was co-transfected with dominant-negative c-Met in AGS cells (Brandt et al. 2005)
[e]An interaction of Grb2 with non-phosphorylated CagA (Mimuro et al. 2002) or $CagA^{PY}$ (Selbach et al. 2009) was reported during *Hp* infection; but none of the latter interactions were found in studies using transfected CagA (Tsutsumi et al. 2003; Churin et al. 2003)
[f]PLC-γ coIP revealed a strong background signal for non-injected CagA in a translocation-defective Δ*virb11* mutant
[g]An interaction of CagA with TAK1 was excluded in another study showing that anti-TAK1 antibodies artificially precipitate CagA from a T4SS-defective strain (Sokolova et al. 2014)

together suggest the presence of distinct EPIYA-independent domains within CagA, which have crucial functions in protein targeting and modification of host cell transcription.

Another remarkable outcome of phosphorylation-independent CagA activities in polarized epithelial cells is the disturbance of the cell-to-cell integrity (Fig. 4.2b). In particular, the proper architecture of the gastric epithelium is controlled by highly organized tight and adherent junctions (Wessler and Backert 2008). Infection and transfection experiments showed that CagA interferes with these intercellular junctions in multiple ways. For example, CagA associates with ZO-1 (zona occludens-1), a tight-junction scaffolding protein and the transmembrane protein JAM (junctional adhesion molecule), causing an ectopic assembly of tight-junction components at sites of bacterial attachment (Amieva et al. 2003). The non-

phosphorylated form of CagA has also been reported to bind to the cell-cell junctional transmembrane protein E-cadherin (Murata-Kamiya et al. 2007). Later on, immunoprecipitation studies showed that CagA forms a complex with c-Met recruiting E-cadherin and the armadillo-domain protein p120 catenin, suggesting that binding of CagA to E-cadherin is probably not direct (Oliveira et al. 2009). However, there is much debate going on whether or not the 135-kDa c-Met receptor is phosphorylated and activated upon infection with *H. pylori* (Snider and Cardelli 2009). Thus, the function of c-Met signaling during *H. pylori* infection is not fully clarified and should be investigated more thoroughly in future. Some controversy also exists whether CagA can disrupt the E-cadherin complex associated with the release of β-catenin, which has been proposed for transfected CagA or *H. pylori*-infected AGS cells (Franco et al. 2005; Murata-Kamiya et al. 2007). It has been noted by some authors that AGS cells do not express E-cadherin and exhibit abnormal β-catenin allocation, making them not suitable for the study of related signaling (Oliveira et al. 2009). Considering this fact, it was shown, using MDCK cells expressing wild-type E-cadherin and β-catenin without any mutations, that *H. pylori*-induced β-catenin signal transduction proceeds independently of CagA during infection (Sokolova et al. 2008). Similarly, some controversy also exists with regard to the proposed interaction of CagA with transforming growth factor beta-activated kinase 1 (TAK1) (Lamb et al. 2009; Sokolova et al. 2014) (Table 4.2).

However, the importance of CagA in inducing the loss of cell polarity is much clearer. The host kinase Par1b (partitioning-defective 1), also called MARK2 (microtubule affinity-regulating kinase), is a central regulator of cell polarity and was reported to have a crucial impact on *H. pylori*-induced signal transduction. Non-phosphorylated CagA can directly bind Par1b that results in the inhibition of its kinase activity, triggering the loss of cell polarity (Saadat et al. 2007; Nesić et al. 2010). Furthermore, more recent studies showed that CagA not only binds to Par1b but also to other members of this kinase family (Par1a, Par1c, and Par1d) and that these interactions contribute to the *H. pylori*-triggered AGS cell elongation phenotype (Lu et al. 2009). Recent data also suggest that CagA can also interact with glycogen synthase kinase 3 (GSK-3) and acts as a pathogenic scaffold protein that induces a Snail-mediated epithelial-mesenchymal transition via the depletion of GSK-3 activity (Lee et al. 2014). Taken together, these results suggest that transfected CagA can interfere with Par1 members, c-Met, GSK-3, and E-cadherin signaling and may also activate NF-κB, thereby contributing to *H. pylori*-induced pro-inflammatory responses. Finally, there are two more reported binding partners of CagA, α-Pix (Baek et al. 2007), and integrin β_1 (Jiménez-Soto et al. 2009; Kaplan-Türköz et al. 2012). While the interaction with α-Pix has a proposed role in inflammation, CagA-integrin β_1 interaction may be involved in delivery of CagA into the host cell (Fig. 4.2b). Importantly, the downstream pathways of CagA emerged to be highly diverse and possible cross talk among them and other bacterial factors need to be dissected in more detail.

4.7 T4SS-Dependent but CagA-Independent Cellular Signaling Induced by *H. pylori*

In this section, various T4SS-dependent but CagA-independent signaling events induced by *H. pylori* will be discussed (Fig. 4.3). Early studies indicated that *H. pylori* can actively inhibit its own uptake and killing by professional phagocytes (Ramarao et al. 2000). This anti-phagocytic effect was dependent on vital bacteria expressing the T4SS, because various isogenic *virB* mutants blocked this phenotype (Ramarao et al. 2000). Interestingly, the actual factor involved was not CagA, because isogenic Δ*cag*A mutants also abrogated phagocytosis. These experiments indicated that *H. pylori* express a yet unknown T4SS effector with anti-phagocytic capability that may play a crucial function in the immune escape of this persistent pathogen (Fig. 4.3). Infection of bone-marrow-derived macrophages by *H. pylori*-induced pathology via microRNAs, such as miR-155, as important regulators of inflammatory and innate immune responses. Increase of miR155 expression was T4SS-dependent but CagA-independent and resulted in reduced macrophage apoptosis (Koch et al. 2012). However, most of the studies were performed to

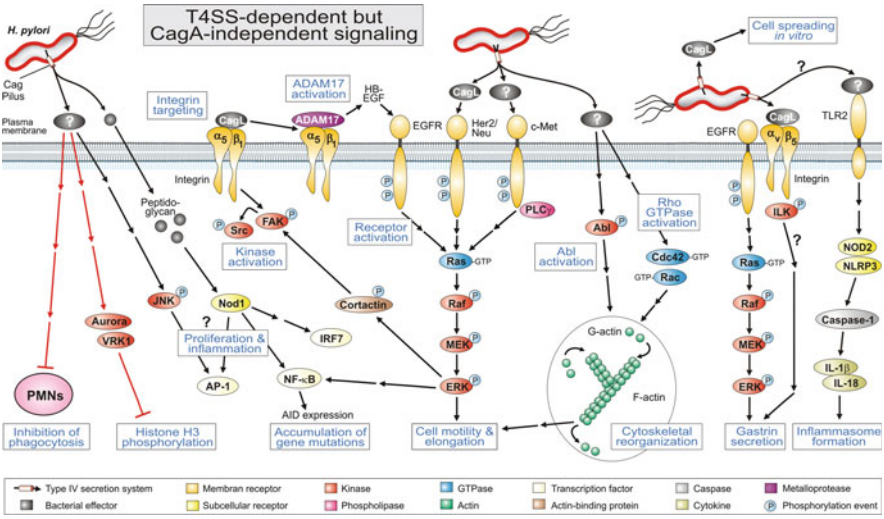

Fig. 4.3 Model for the role of *H. pylori* T4SS-dependent but CagA-independent host cell-signaling processes which may affect pathogenesis. The multitude of known T4SS-dependent but CagA-independent pathways involve in the activation of receptor and non-receptor tyrosine kinases, pro-inflammatory signaling, Rho GTPase activation, scattering and motility of gastric epithelial cells, as well as suppression of histone phosphorylation and *H. pylori* phagocytosis by immune cells. Two particular T4SS factors have been reported to be involved in some but not all of these responses. The known signaling functions for injected peptidoglycan as well as pilus-exposed or recombinant CagL are shown. For numerous other pathways, the actual T4SS factor is yet unknown as also indicated. (For more details see text. This figure was updated from Backert and coworkers (2010) with kind permission from Wiley.)

investigate the interaction of *H. pylori* with gastric epithelial cells. Phosphoproteomics of epithelial cells infected with *H. pylori* revealed the induction of multiple tyrosine-phosphorylated proteins. The majority of enriched phosphopeptides were from kinases of the MAPK family and the use of isogenic mutants showed that both CagA and the T4SS are key regulators of tyrosine phosphorylation events (Glowinski et al. 2014). In addition, histone H3 phosphorylation was found to be altered by a T4SS-dependent but CagA-independent pathway (Fig. 4.3). Infection with *cag*PAI-positive *H. pylori* strains decreased H3 phosphorylation levels at two phosphorylation sites, serine residue 10 and threonine residue 3 (Fehri et al. 2009; Ding et al. 2010). It appeared that mitotic histone H3 kinases such as Aurora B and vaccinia-related kinase 1 (VRK1) were not fully activated in *H. pylori*-infected cells, leading to a transient pre-mitotic cell cycle arrest (Fehri et al. 2009). Together, these data demonstrate that *H. pylori* subvert cellular key processes, including cell cycle progression, by a not yet identified T4SS effector. Furthermore, the results of numerous reports revealed that other components of the T4SS, but not CagA itself, were necessary for the induction of pro-inflammatory signaling, including the activation of transcription factors NF-κB and AP-1 (Fig. 4.3). This implicated that the T4SS might deliver effectors in addition to CagA or that the T4SS itself triggers the effect. Despite systematic mutagenesis of all *cag*PAI genes and other efforts, the hypothetical additional effector remained unknown for many years. One proposed candidate was bacterial peptidoglycan, because it can be identified by Nod1, an intracellular pathogen-recognition molecule (Viala et al. 2004). These results implicated that T4SS-dependent delivery of peptidoglycan is responsible for activation of Nod1 → NF-κB-dependent pro-inflammatory responses such as secretion of IL-8 (Viala et al. 2004). However, the actual bacterial T4SS factor(s) and pathways that activate both transcription factors, NF-κB and AP-1, are highly controversial in the literature and still not fully defined (Backert and Naumann 2010). Remarkably, T4SS-positive *H. pylori* can activate the NF-κB-dependent induction of a DNA-editing enzyme (AID) in gastric epithelial cells, that leads to the accumulation of mutations in tumor suppressor p53 (Matsumoto et al. 2007). Thus, induction of AID by *H. pylori* infection might be a mechanism whereby gastric carcinogenesis-related gene mutations accumulate.

Infection of gastric epithelial cells with *H. pylori* was also reported to profoundly activate various receptor tyrosine kinases (RTKs) in a T4SS-dependent manner including EGFR (Keates et al. 2001; Churin et al. 2003), hepatocyte growth factor receptor c-Met, and Her2/Neu (Churin et al. 2003). Studies on the downstream signaling indicated that each of these RTKs can activate the MAP kinase members MEK and ERK1/2 (Fig. 4.3). However, while induction of EGFR has been shown to induce pro-inflammatory responses leading to the secretion of IL-8 (Keates et al. 2001), activation of c-Met (but not EGFR or Her2/Neu) was involved in cell scattering and motogenic responses of infected gastric epithelial cells (Churin et al. 2003). At later time points of infection, EGFR can be inactivated by CagA as mentioned above (Bauer et al. 2012). Interestingly, the small Rho GTPases Rac1

and Cdc42 and the non-receptor tyrosine kinase c-Abl are also activated by a T4SS-dependent but CagA-independent process and play a role in stimulating scattering and motility of infected gastric epithelial cells (Fig. 4.3). However, the actual T4SS factor involved for many of the above events is still unknown.

In vitro studies showed a profound role of recombinant CagL in activating the host tyrosine kinases EGFR, ErbB3/Her3, FAK, and c-Src (Tegtmeyer et al. 2010). Investigation on the molecular mechanism of EGFR activation by CagL has demonstrated the involvement of ADAM17, a metalloprotease implicated in catalyzing ectodomain shedding of receptor tyrosine kinase ligands. In non-stimulated cells, the inactive form of ADAM17 forms a complex with integrin $\alpha_5\beta_1$ (Fig. 4.3). During acute *H. pylori* infection, however, it was demonstrated that CagL binding to integrin $\alpha_5\beta_1$ activates ADAM17 by dissociating ADAM17 from the complex (Saha et al. 2010). In addition, CagL immobilized on petri dishes binds host cells and, thus, mimics human fibronectin (Tegtmeyer et al. 2010). Fibronectin is a 250-kDa protein containing an RGD motif that plays crucial roles in promoting cell adhesion, migration, and intracellular signaling. It was shown that purified CagL alone can directly trigger intracellular signaling pathways upon contact with mammalian cells and can even complement the spreading defect of fibronectin$^{-/-}$ knockout cells in vitro (Tegtmeyer et al. 2010). Treatment of AGS cells with purified CagL was also demonstrated to be sufficient to result in IL-8 induction, which required the RGD motif in CagL and activation of integrin $\alpha_5\beta_1$ (Gorrell et al. 2012). Using different wild-type or Δ*cagL* mutant strains showed that IL-8 induction occurred independently of CagA translocation. Further studies revealed another surface-exposed Phe-Glu-Ala-Asn-Glu (FEANE) interaction motif located close to the RGD site. This site enhanced the interaction of CagL with integrin $\alpha_5\beta_1$ supporting CagA translocation and was referred to as RGD helper sequence, RHS (Conradi et al. 2012). CagL was also shown to be related to increased gastrin expression resulting in hypergastrinemia, a major risk factor for gastric adenocarcinoma formation. Gastric epithelial cells stably transfected with a human gastrin promoter luciferase construct increased the promoter activation of gastrin via integrin-linked kinase (ILK) and integrin $\alpha_V\beta_5$ (Wiedemann et al. 2012). Another role of CagL was seen in bone-marrow derived DCs, where it resulted in induction of pro-IL-1β and formation of mature IL-1β and NLRP3 (NOD-like receptor pyrin domain-containing 3) induction in response to *H. pylori* infection. This activity was mediated via different host innate immune receptors including Toll-like receptor 2 (TLR2) and nucleotide-binding oligomerization domain 2 (NOD2) (Kim et al. 2013). CagL and the activity of MAP kinases were also important for *H. pylori*-mediated regulation of eosinophil migration (Nagy et al. 2011). The results indicate that *H. pylori* increases production of the chemokines CCL2, CCL5, and granulocyte-macrophage colony-stimulating factor (GMC-SF) by gastric epithelial cells and that these molecules induce eosinophil migration. Interestingly, CagL sequence analyses revealed that isolates from the gastric cancer patients had a higher rate of amino acid sequence polymorphisms—Y58 and

E59—than those of the non-gastric cancer patients (Yeh et al. 2011). The CagL Y58E59 polymorphism increased risk of gastric cancer up to 4.6-fold and infected patients had higher integrin $\alpha_5\beta_1$ expression than noninfected patients. Furthermore, CagL-Y58E59 *H. pylori* infection predisposed an upward shift in integrin $\alpha_5\beta_1$ in the corpus, leading to more severe corpus chronic inflammation (Yeh et al. 2011). However, expression of isogenic CagL Y58/E59 variants in *H. pylori* strain 26695 significantly blocked translocation and phosphorylation of CagA as compared to complemented wild-type CagL (Tegtmeyer et al. 2014). The involved signaling should be studied in detail in future studies.

4.8 Conclusions

H. pylori represents a highly successful human pathogen, which can trigger severe clinical symptoms in a small subset of patients. The investigation of bacteria-host interactions and virulence factors such as CagA and the T4SS has provided us with crucial insights in mechanisms leading to *H. pylori* pathogenesis. A list of more than 20 known cellular interaction partners of CagA is quite amazing for a bacterial effector protein. The current model suggests that CagA mimics a eukaryotic signaling factor either located in a large multiprotein complex or simultaneously in various subcellular areas of infected host cells. The large variety of binding partners also reflects the integrated network of complex signal transduction pathways in target cells, which may have important impact on the multi-step pathogenesis of *H. pylori*. In the future, it will be important to search for additional translocated effector molecules of the *cag*PAI T4SS and various other T4SSs present in the *H. pylori* chromosome (Backert et al. 2015). Finally, the importance of CagA for *H. pylori* itself is also not yet clear. Using a polarized epithelium model system, Δ*cagA* mutants were shown to be defective in cell surface colonization, but exogenous addition of iron to the apical medium partially rescues this defect, suggesting that one of CagA's effects on host cells is to facilitate iron acquisition from the host via a mechanism involving the transferrin receptor (Tan et al. 2011). To test whether CagA is important in promoting iron acquisition in vivo, the colonization of *H. pylori* in iron-replete *vs.* iron-deficient Mongolian gerbils was carried out. While wild-type *H. pylori* and Δ*cagA* mutants colonized iron-replete gerbils at similar levels, Δ*cagA* mutants are markedly impaired in colonizing iron-deficient gerbils (Tan et al. 2011). Iron depletion accelerated the development of *H. pylori*-induced premalignant and malignant lesions in a CagA-dependent manner (Noto et al. 2013). *H. pylori* strains harvested from iron-depleted gerbils or grown under iron-limiting conditions exhibited enhanced virulence and induction of inflammatory factors. Future studies should investigate the molecular basis of this important disease-associated phenomenon.

References

Amieva MR, Vogelmann R, Covacci A, Tompkins LS, Nelson WJ, Falkow S (2003) Disruption of the epithelial apical-junctional complex by *Helicobacter pylori* CagA. Science 300:1430–1434

Backert S, Meyer TF (2006) Type IV secretion systems and their effectors in bacterial pathogenesis. Curr Opin Microbiol 9:207–217

Backert S, Naumann M (2010) What a disorder: proinflammatory signaling pathways induced by *Helicobacter pylori*. Trends Microbiol 18:479–486

Backert S, Moese S, Selbach M, Brinkmann V, Meyer TF (2001) Phosphorylation of tyrosine 972 of the *Helicobacter pylori* CagA protein is essential for induction of a scattering phenotype in gastric epithelial cells. Mol Microbiol 42:631–644

Backert S, Fronzes R, Waksman G (2008) VirB2 and VirB5 proteins: specialized adhesins in bacterial type-IV secretion? Trends Microbiol 16:409–413

Backert S, Tegtmeyer N, Selbach M (2010) The versatility of *Helicobacter pylori* CagA effector protein functions: The master key hypothesis. Helicobacter 15:163–176

Backert S, Tegtmeyer N, Fischer W (2015) Composition, structure and function of the *Helicobacter pylori cag* pathogenicity island encoded type IV secretion system. Future Microbiol 10:955–965

Baek HY, Lim JW, Kim H (2007) Interaction between the *H. pylori* CagA and alpha-Pix in gastric epithelial AGS cells. Ann N Y Acad Sci 1096:18–23

Bagnoli F, Buti L, Tompkins L, Covacci A, Amieva MR (2005) *Helicobacter pylori* CagA induces a transition from polarized to invasive phenotypes in MDCK cells. Proc Natl Acad Sci U S A 102:16339–16344

Barden S, Lange S, Tegtmeyer N, Conradi J, Sewald N, Backert S, Niemann HH (2013) A helical RGD motif promoting cell adhesion: crystal structures of the *Helicobacter pylori* type IV secretion system pilus protein CagL. Structure 21:1931–1941

Barrozo RM, Cooke CL, Hansen LM, Gaddy JA, Johnson EM, Cariaga TA, Suarez G, Peek RM Jr, Cover TL, Solnick JV (2013) Functional plasticity in the type IV secretion system of *H. pylori*. PLoS Pathog 9:e1003189

Bauer B, Pang E, Holland C, Kessler M, Bartfeld S, Meyer TF (2012) The *H. pylori* virulence effector CagA abrogates human β-defensin 3 expression via inactivation of EGFR signaling. Cell Host Microbe 11:576–586

Botham CM, Wandler AM, Guillemin K (2008) A transgenic Drosophila model demonstrates that the *Helicobacter pylori* CagA protein functions as a eukaryotic Gab adaptor. PLoS Pathog 4: e1000064

Brandt S, Kwok T, Hartig R, König W, Backert S (2005) NF-kappaB activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. Proc Natl Acad Sci U S A 102:9300–9305

Brandt S, Shafikhani S, Balachandran P, Jin S, Hartig R, König W, Engel J, Backert S (2007) Use of a novel coinfection system reveals a role for Rac1, H-Ras, and CrkII phosphorylation in *H. pylori*-induced host cell actin cytoskeletal rearrangements. FEMS Immunol Med Microbiol 50:190–205

Buti L, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL (2011) *Helicobacter pylori* cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. Proc Natl Acad Sci U S A 108:9238–9243

Cendron L, Seydel A, Angelini A, Battistutta R, Zanotti G (2004) Crystal structure of CagZ, a protein from the *H. pylori* pathogenicity island that encodes for a Type IV Secretion System. J Mol Biol 340:881–889

Cendron L, Tasca E, Seraglio T, Seydel A, Angelini A, Battistutta R, Montecucco C, Zanotti G (2007) The crystal structure of CagS from the *Helicobacter pylori* pathogenicity island. Proteins 69:440–443

Cendron L, Couturier M, Angelini A, Barison N, Stein M, Zanotti G (2009) The *H. pylori* CagD (HP0545, Cag24) protein is essential for CagA translo-cation and maximal induction of IL-8 secretion. J Mol Biol 386:204–217

Chandran V, Fronzes R, Duquerroy S, Cronin N, Navaza J, Waksman G (2009) Structure of the outer membrane complex of a type IV secretion system. Nature 462:1011–1016

Churin Y, Al-Ghoul L, Kepp O, Meyer TF, Birchmeier W, Naumann M (2003) *Helicobacter pylori* CagA protein targets the c-Met receptor and enhances the motogenic response. J Cell Biol 161:249–255

Conradi J, Tegtmeyer N, Woźna M, Wissbrock M, Michalek C, Gagell C, Cover TL, Frank R, Sewald N, Backert S (2012) An RGD helper sequence in CagL of *Helicobacter pylori* assists in interactions with integrins and injection of CagA. Front Cell Infect Microbiol 2:70

Ding SZ, Fischer W, Kaparakis-Liaskos M, Liechti G, Merrell DS, Ferrero RL, Crowe SE, Haas R, Hatakeyama M, Goldberg JB (2010) *Helicobacter pylori*-induced histone modification, associated gene expression in gastric epithelial cells, and its implication in pathogenesis. PLoS One 5:e9875

El-Etr SH, Mueller A, Tompkins LS, Falkow S, Merrell DS (2004) Phosphorylation-independent effects of CagA during interaction between *Helicobacter pylori* and T84 polarized monolayers. J Infect Dis 190:1516–1523

Fehri LF, Rechner C, Janssen S, Mak TN, Holland C, Bartfeld S, Brüggemann H, Meyer TF (2009) *Helicobacter pylori*-induced modification of the histone H3 phosphorylation status in gastric epithelial cells reflects its impact on cell cycle regulation. Epigenetics 4:577–586

Fischer W (2011) Assembly and molecular mode of action of the *Helicobacter pylori* cag type IV secretion apparatus. FEBS J 278:1203–1212

Franco AT, Israel DA, Washington MK, Krishna U, Collier-Hyams L, Perez-Perez GI, Hatakeyama M, Whitehead R, Gaus K, O'Brien DP, Romero-Gallo J, Peek RM Jr (2005) Activation of beta-catenin by carcinogenic *Helicobacter pylori*. Proc Natl Acad Sci U S A 102:10646–10651

Fronzes R, Schäfer E, Wang L, Saibil HR, Orlova E, Waksman G (2009) Structure of a type IV secretion system core complex. Science 323:266–268

Glowinski F, Holland C, Thiede B, Jungblut PR, Meyer TF (2014) Analysis of T4SS-induced signaling by *H. pylori* using quantitative phosphoproteomics. Front Microbiol 5:356

Gobert AP, Verriere T, Asim M, Barry DP, Piazuelo MB, de Sablet T, Delgado AG, Bravo LE, Correa P, Peek RM Jr, Chaturvedi R, Wilson KT (2014) Heme Oxygenase-1 Dysregulates Macrophage Polarization and the Immune Response to *Helicobacter pylori*. J Immunol 193:3013–3022

Gorrell RJ, Guan J, Xin Y, Hutton ML, McGuckin MA, Ferrero RL, Kwok T (2012) A novel NOD1- and CagA-independent pathway of IL-8 induction mediated by the H.pylori type IV system. Cell Microbiol 15:554–570

Hare S, Fischer W, Williams R, Terradot L, Bayliss R, Haas R, Waksman G (2007) Identification, structure and mode of action of a new regulator of the *Helicobacter pylori* HP0525 ATPase. EMBO J 26:4926–4934

Hatakeyama M (2003) *Helicobacter pylori* CagA-a potential bacterial oncoprotein that functionally mimics the mammalian Gab family of adaptor proteins. Microbes Infect 5:143–150

Hauck CR (2007) Microbiology: preparing the shot. Nature 449:798–799

Hayashi T, Senda M, Morohashi H, Higashi H, Horio M, Kashiba Y, Nagase L, Sasaya D, Shimizu T, Venugopalan N, Kumeta H, Noda NN, Inagaki F, Senda T, Hatakeyama M (2012) Tertiary structure-function analysis reveals the pathogenic signaling potentiation mechanism of *Helicobacter pylori* oncogenic effector CagA. Cell Host Microbe 12:20–33

Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M (2002) SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 295:683–686

Higashi H, Nakaya A, Tsutsumi R, Yokoyama K, Fujii Y, Ishikawa S, Higuchi M, Takahashi A, Tanaka S, Azuma T, Hatakeyama M (2004) *Helicobacter pylori* CagA induces Ras-independent morphogenetic response through SHP-2 recruitment and activation. J Biol Chem 279:17205–17216

Jiménez-Soto LF, Kutter S, Sewald X, Ertl C, Weiss E, Kapp U, Rohde M, Pirch T, Jung K, Retta SF, Terradot L, Fischer W, Haas R (2009) *Helicobacter pylori* type IV secretion apparatus exploits beta1 integrin in a novel RGD-independent manner. PLoS Pathog 5:e1000684

Kaplan-Türköz B, Jiménez-Soto LF, Dian C, Ertl C, Remaut H, Louche A, Tosi T, Haas R, Terradot L (2012) Structural insights into *H. pylori* oncoprotein CagA interaction with β1 integrin. Proc Natl Acad Sci 109:14640–14645

Keates S, Sougioultzis S, Keates AC, Zhao D, Peek RM Jr, Shaw LM, Kelly CP (2001) cag+ *H. pylori* induce transactivation of the epidermal growth factor receptor in AGS gastric epithelial cells. J Biol Chem 276:48127–48134

Kim DJ, Park JH, Franchi L, Backert S, Núñez G (2013) The Cag pathogenicity island and interaction between TLR2/NOD2 and NLRP3 regulate IL-1β production in *Helicobacter pylori* infected dendritic cells. Eur J Immunol 43:2650–2658

Koch M, Mollenkopf HJ, Klemm U, Meyer TF (2012) Induction of microRNA-155 is TLR- and T4SS-dependent in macrophages and inhibits DNA-damage induced apoptosis. Proc Natl Acad Sci U S A 109:E1153–E1162

Kwok T, Zabler D, Urman S, Rohde M, Hartig R, Wessler S, Misselwitz R, Berger J, Sewald N, König W, Backert S (2007) *Helicobacter* exploits integrin for type IV secretion and kinase activation. Nature 449:862–866

Lamb A, Yang XD, Tsang YH, Li JD, Higashi H, Peek RM, Blanke SR, Chen LF (2009) *Helicobacter pylori* CagA activates NF-κB by targeting TAK1 for TRAF6-mediated Lys 63 ubiquitination. EMBO Rep 10:1242–1249

Lee DG, Kim HS, Lee YS, Kim S, Cha SY, Ota I, Kim NH, Cha YH, Yang DH, Lee Y, Park GJ, Yook JI, Lee YC (2014) *Helicobacter pylori* CagA promotes snail-mediated epithelial-mesenchymal transition by reducing GSK-3 activity. Nat Commun 5:4423

Liu Z, Xu X, Chen L, Li W, Sun Y, Zeng J, Yu H, Chen C, Jia J (2012) *H. pylori* CagA inhibits the expression of Runx3 via Src/MEK/ERK and p38 MAPK pathways in gastric epithelial cell. J Cell Biochem 113:1080–1086

Low HH, Gubellini F, Rivera-Calzada A, Braun N, Connery S, Dujeancourt A, Lu F, Redzej A, Fronzes R, Orlova EV, Waksman G (2014) Structure of a type IV secretion system. Nature 508:550–553

Lu H, Murata-Kamiya N, Saito Y, Hatakeyama M (2009) Role of partitioning-defective 1/microtubule affinity-regulating kinases in the morphogenetic activity of *Helicobacter pylori* CagA. J Biol Chem 284:23024–23036

Matsumoto Y, Marusawa H, Kinoshita K, Endo Y, Kou T, Morisawa T, Azuma T, Okazaki IM, Honjo T, Chiba T (2007) *Helicobacter pylori* infection triggers aberrant expression of activation-induced cytidine deaminase in gastric epithelium. Nat Med 13:470–476

Mimuro H, Suzuki T, Tanaka J, Asahi M, Haas R, Sasakawa C (2002) Grb2 is a key mediator of *H. pylori* CagA protein activities. Mol Cell 10:745–755

Mimuro H, Suzuki T, Nagai S, Rieder G, Suzuki M, Nagai T, Fujita Y, Koyasu S, Haas R, Sasakawa C (2007) *Helicobacter pylori* dampens gut epithelial self-renewal by inhibiting apoptosis, a bacterial strategy to enhance colonization of the stomach. Cell Host Microbe 2:250–263

Moese S, Selbach M, Brinkmann V, Karlas A, Haimovich B, Backert S, Meyer TF (2007) The *Helicobacter pylori* CagA protein disrupts matrix adhesion of gastric epithelial cells by dephosphorylation of vinculin. Cell Microbiol 9:1148–1161

Murata-Kamiya N, Kurashima Y, Teishikata Y, Yamahashi Y, Saito Y, Higashi H, Aburatani H, Akiyama T, Peek RM Jr, Azuma T, Hatakeyama M (2007) H.pylori CagA interacts with E-cadherin and deregulates catenin that promotes intestinal transdifferentiation in cells. Oncogene 26:4617–4626

Murata-Kamiya N, Kikuchi K, Hayashi T, Higashi H, Hatakeyama M (2010) *H. pylori* exploits host membrane phosphatidylserine for delivery, localization, and pathophysiological action of CagA. Cell Host Microbe 7:399–411

Muyskens JB, Guillemin K (2011) *Helicobacter pylori* CagA disrupts epithelial patterning by activating myosin light chain. PLoS One 6:e17856

Nagy TA, Allen SS, Wroblewski LE, Flaherty DK, Slaughter JC, Perez-Perez G, Israel DA, Peek RM Jr (2011) *Helicobacter pylori* induction of eosinophil migration is mediated by the cag pathogenicity island via microbial-epithelial interactions. Am J Pathol 178:1448–1452

Neal JT, Peterson TS, Kent ML, Guillemin K (2013) *H. pylori* virulence factor CagA increases intestinal cell proliferation by Wnt pathway activation in a transgenic zebrafish model. Dis Model Mech 6:802–810

Nesić D, Miller MC, Quinkert ZT, Stein M, Chait BT, Stebbins CE (2010) *Helicobacter pylori* CagA inhibits PAR1-MARK family kinases by mimicking host substrates. Nat Struct Mol Biol 17:130–132

Nešić D, Buti L, Lu X, Stebbins CE (2014) Structure of the *Helicobacter pylori* CagA oncoprotein bound to the human tumor suppressor ASPP2. Proc Natl Acad Sci U S A 111:1562–1567

Noto JM, Gaddy JA, Lee JY, Piazuelo MB, Friedman DB, Colvin DC, Romero-Gallo J, Tan S, Morgan DR, Wilson KT, Bravo LE, Correa P, Cover TL, Amieva MR, Peek RM Jr (2013) Iron deficiency accelerates *H. pylori*-induced carcinogenesis in rodents and humans. J Clin Invest 123:479–492

Ogura K, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M (2000) Virulence factors of *H. pylori* responsible for gastric diseases in Mongolian gerbil. J Exp Med 192:1601–1610

Ohnishi N, Yuasa H, Tanaka S et al (2008) Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci U S A 105:1003–1008

Oliveira MJ, Costa AC, Costa AM, Henriques L, Suriano G, Atherton JC, Machado JC, Carneiro F, Seruca R, Mareel M, Leroy A, Figueiredo C (2006) *H. pylori* induces gastric epithelial cell invasion in a c-Met and type IV secretion system-dependent manner. J Biol Chem 281:34888–34896

Oliveira MJ, Costa AM, Costa AC, Ferreira RM, Sampaio P, Machado JC, Seruca R, Mareel M, Figueiredo C (2009) CagA associates with c-Met, E-cadherin, and p120-catenin in a multiproteic complex that suppresses *H. pylori*-induced cell-invasive phenotype. J Infect Dis 200:745–755

Poppe M, Feller SM, Römer G, Wessler S (2007) Phosphorylation of *Helicobacter pylori* CagA by c-Abl leads to cell motility. Oncogene 26:3462–3472

Ramarao N, Gray-Owen SD, Backert S, Meyer TF (2000) *Helicobacter pylori* inhibits phagocytosis by professional phagocytes involving type IV secretion components. Mol Microbiol 37:1389–1404

Reid DW, Muyskens JB, Neal JT, Gaddini GW, Wandler AM, Botham CM, Guillemin K (2012) Identification of genetic modifiers of CagA-induced epithelial disruption in Drosophila. Front Cell Infect Microbiol 2:24

Ren S, Higashi H, Lu H, Azuma T, Hatakeyama M (2006) Structural basis and functional consequence of *Helicobacter pylori* CagA multimerization in cells. J Biol Chem 281:32344–32352

Rieder G, Merchant JL, Haas R (2005) *Helicobacter pylori* cag-type IV secretion system facilitates corpus colonization to induce precancerous conditions in Mongolian gerbils. Gastroenterology 128:1229–1242

Rohde M, Püls J, Buhrdorf R, Fischer W, Haas R (2003) A novel sheathed surface organelle of the *Helicobacter pylori cag* type IV secretion system. Mol Microbiol 49:219–234

Saadat I, Higashi H, Obuse C, Umeda M, Murata-Kamiya N, Saito Y, Lu H, Ohnishi N, Azuma T, Suzuki A, Ohno S, Hatakeyama M (2007) *Helicobacter pylori* CagA targets PAR1/MARK kinase to disrupt epithelial cell polarity. Nature 447:330–333

Saha A, Backert S, Hammond CE, Gooz M, Smolka AJ (2010) *Helicobacter pylori* CagL activates ADAM17 to induce repression of the gastric H, K-ATPase alpha subunit. Gastroenterology 139:239–248

Segal ED, Cha J, Lo J, Falkow S, Tompkins LS (1999) Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by *H. pylori*. Proc Natl Acad Sci U S A 96:14559–14564

Selbach M, Moese S, Hurwitz R, Hauck CR, Meyer TF, Backert S (2003) The *Helicobacter pylori* CagA protein induces cortactin dephosphorylation and actin rearrangement by c-Src inactivation. EMBO J 22:515–528

Selbach M, Moese S, Backert S, Jungblut PR, Meyer TF (2004) The *Helicobacter pylori* CagA protein induces tyrosine dephosphorylation of ezrin. Proteomics 4:2961–2968

Selbach M, Paul FE, Brandt S, Guye P, Daumke O, Backert S, Dehio C, Mann M (2009) Host cell interactome of tyrosine-phosphorylated bacterial proteins. Cell Host Microbe 5:397–403

Shaffer CL, Gaddy JA, Loh JT, Johnson EM, Hill S, Hennig EE, McClain MS, McDonald WH, Cover TL (2011) *Helicobacter pylori* exploits a unique repertoire of type IV secretion system components for pilus assembly at the bacteria-host cell interface. PLoS Pathog 7:e1002237

Snider JL, Cardelli JA (2009) *Helicobacter pylori* induces cancer cell motility independent of the c-Met receptor. J Carcinog 8:7

Sokolova O, Bozko PM, Naumann M (2008) *Helicobacter pylori* suppresses glycogen synthase kinase 3beta to promote beta-catenin activity. J Biol Chem 283:29367–29374

Sokolova O, Maubach G, Naumann M (2014) MEKK3 and TAK1 synergize to activate IKK complex in *H. pylori* infection. Biochim Biophys Acta 1843:715–724

Suzuki M, Mimuro H, Suzuki T, Park M, Yamamoto T, Sasakawa C (2005) Interaction of CagA with Crk plays an important role in *H. pylori*-induced loss of gastric epithelial cell adhesion. J Exp Med 202:1235–1247

Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, Morikawa H, Nagai S, Koyasu S, Gilman RH, Kersulyte D, Berg DE, Sasakawa C (2009) *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. Cell Host Microbe 5:23–34

Tammer I, Brandt S, Hartig R, König W, Backert S (2007) Activation of Abl by *Helicobacter pylori*: a novel kinase for CagA and crucial mediator of host cell scattering. Gastroenterology 132:1309–1319

Tan S, Noto JM, Romero-Gallo J, Peek RM Jr, Amieva MR (2011) *H. pylori* perturbs iron trafficking in the epithelium to grow on the cell surface. PLoS Pathog 7:e1002050

Tanaka J, Suzuki T, Mimuro H, Sasakawa C (2003) Structural definition on the surface of *H. pylori* type IV secretion apparatus. Cell Microbiol 5:395–404

Tegtmeyer N, Backert S (2011) Role of Abl and Src family kinases in actin-cytoskeletal rearrangements induced by the *Helicobacter pylori* CagA protein. Eur J Cell Biol 90:880–890

Tegtmeyer N, Hartig R, Delahay RM, Rohde M, Brandt S, Conradi J, Takahashi S, Smolka AJ, Sewald N, Backert S (2010) A small fibronectin-mimicking protein from bacteria induces cell spreading and focal adhesion formation. J Biol Chem 285:23515–23526

Tegtmeyer N, Wessler S, Backert S (2011) Role of the cag-pathogenicity island encoded type IV secretion system in *Helicobacter pylori* pathogenesis. FEBS J 278(8):1190–1202

Tegtmeyer N, Lind J, Schmid B, Backert S (2014) *Helicobacter pylori* CagL Y58/E59 mutation turns-off type IV secretion-dependent delivery of CagA into host cells. PLoS One 9:e97782

Terradot L, Waksman G (2011) Architecture of the *Helicobacter pylori* Cag-type IV secretion system. FEBS J 278:1213–1222

Tsang YH, Lamb A, Romero-Gallo J, Huang B, Ito K, Peek RM Jr, Ito Y, Chen LF (2010) *Helicobacter pylori* CagA targets gastric tumor suppressor RUNX3 for proteasome-mediated degradation. Oncogene 29:5643–5650

Tsutsumi R, Higashi H, Higuchi M, Okada M, Hatakeyama M (2003) Attenuation of *Helicobacter pylori* CagA x SHP-2 signaling by interaction between CagA and C-terminal Src kinase. J Biol Chem 278:3664–3670

Tsutsumi R, Takahashi A, Azuma T, Higashi H, Hatakeyama M (2006) Focal adhesion kinase is a substrate and downstream effector of SHP-2 complexed with *Helicobacter pylori* CagA. Mol Cell Biol 26:261–276

Umeda M, Murata-Kamiya N, Saito Y, Ohba Y, Takahashi M, Hatakeyama M (2009) *Helicobacter pylori* CagA causes mitotic impairment and induces chromosomal instability. J Biol Chem 284:22166–22172

Viala J, Chaput C, Boneca IG, Cardona A, Girardin SE, Moran AP, Athman R, Mémet S, Huerre MR, DiStefano PS, Sansonetti PJ, Labigne A, Bertin J, Philpott DJ, Ferrero RL (2004) Nod1 responds to peptidoglycan delivered by the *H. pylori cag* pathogenicity island. Nat Immunol 5:1166–1174

Waksman G, Orlova EV (2014) Structural organisation of the type IV secretion systems. Curr Opin Microbiol 17:24–31

Wandler AM, Guillemin K (2012) Transgenic expression of the *Helicobacter pylori* virulence factor CagA promotes apoptosis or tumorigenesis through JNK activation in Drosophila. PLoS Pathog 8:e1002939

Wei J, Noto JM, Zaika E, Romero-Gallo J, Piazuelo MB, Schneider B, El-Rifai W, Correa P, Peek RM, Zaika AI (2015) Bacterial CagA protein induces degradation of p53 protein in a p14ARF-dependent manner. Gut 64:1040–1108

Wessler S, Backert S (2008) Molecular mechanisms of epithelial-barrier disruption by *Helicobacter pylori*. Trends Microbiol 16:397–405

Wiedemann T, Hofbaur S, Tegtmeyer N, Huber S, Sewald N, Wessler S, Backert S, Rieder G (2012) *Helicobacter pylori* CagL dependent induction of gastrin expression via a novel αvβ5-integrin linked kinase signaling complex. Gut 61:986–996

Yamaoka Y (2010) Mechanisms of disease: *Helicobacter pylori* virulence factors. Nat Rev Gastroenterol Hepatol 7:629–641

Yeh YC, Chang WL, Yang HB, Cheng HC, Wu JJ, Sheu BS (2011) *H. pylori* cagL sequence polymorphism Y58E59 induces a corpus shift of gastric integrin $\alpha_5\beta_1$ related with gastric carcinogenesis. Mol Carcinog 50:751–759

Yeo HJ, Savvides SN, Herr AB, Lanka E, Waksman G (2000) Crystal structure of the hexameric ATPase from *H. pylori*. Mol. Cell 6:1461–1472

Yokoyama K, Higashi H, Ishikawa S, Fujii Y, Kondo S, Kato H, Azuma T, Wada A, Hirayama T, Aburatani H, Hatakeyama M (2005) Functional antagonism between *Helicobacter pylori* CagA and vacuolating toxin VacA in control of the NFAT signaling pathway in gastric epithelial cells. Proc Natl Acad Sci U S A 102:9661–9666

Zeaiter Z, Cohen D, Müsch A, Bagnoli F, Covacci A, Stein M (2008) Analysis of detergent-resistant membranes of *Helicobacter pylori* infected gastric adenocarcinoma cells reveals a role for MARK2/Par1b in CagA-mediated disruption of cellular polarity. Cell Microbiol 10:781–794

Zhang X, Perez-Perez G, Tegtmeyer N, Sticht H, Backert S, Blaser M (2015) A specific A/T polymorphism in Western tyrosine phosphorylation B-motifs regulates *Helicobacter pylori* CagA epithelial cell interactions. PLoS Pathog 11:e1004621