Exploiting the Gastric Epithelial Barrier: *Helicobacter pylori*'s Attack on Tight and Adherens Junctions

Steffen Backert, Thomas P. Schmidt, Aileen Harrer and Silja Wessler

Abstract Highly organized intercellular tight and adherens junctions are crucial structural components for establishing and maintenance of epithelial barrier functions, which control the microbiota and protect against intruding pathogens in humans. Alterations in these complexes represent key events in the development and progression of multiple infectious diseases as well as various cancers. The gastric pathogen *Helicobacter pylori* exerts an amazing set of strategies to manipulate these epithelial cell-to-cell junctions, which are implicated in changing cell polarity, migration and invasive growth as well as pro-inflammatory and proliferative responses. This chapter focuses on the *H. pylori* pathogenicity factors VacA, CagA, HtrA and urease, and how they can induce host cell signaling involved in altering cell-to-cell permeability. We propose a stepwise model for how *H. pylori* targets components of tight and adherens junctions in order to disrupt the gastric epithelial cell layer, giving fresh insights into the pathogenesis of this important bacterium.

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S. Backert (✉) · A. Harrer
Division of Microbiology, Department of Biology, Friedrich Alexander University Erlangen-Nuremberg, Staudtstr. 5, 91058 Erlangen, Germany
e-mail: Steffen.Backert@fau.de

T.P. Schmidt · S. Wessler (✉)
Division of Microbiology, Department of Molecular Biology, Paris-Lodron University of Salzburg, Billroth Str. 11, 5020 Salzburg, Austria
e-mail: Silja.Wessler@sbg.ac.at

N. Tegtmeyer and S. Backert (eds.), *Molecular Pathogenesis and Signal Transduction by Helicobacter pylori*, Current Topics in Microbiology and Immunology 400, DOI 10.1007/978-3-319-50520-6_9

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1 Introduction

1.1 The Polarized Epithelium as the First Barrier for H. pylori *Colonization*

Helicobacter pylori colonizes the gastric mucosal epithelium in the hostile environment of the human stomach. This epithelium represents a highly organized and essential cell monolayer controlling important digestive, absorptive and secretory functions. However, this epithelium also forms a first barrier against the microbiota and pathogenic microbes such as *H. pylori*. The discovery of *H. pylori* in gastric biopsies by Robin Warren and Barry Marshall, more than 33 years ago, radically changed the view on understanding and treatment of gastric disorders as an infectious disease (Marshall and Warren 1984). Today, we know that about half of the human world population carries *H. pylori*, causing chronic gastritis in all carrying persons, and more severe gastric disease in about 10–15% of infected individuals (Amieva and El-Omar 2008; Atherton and Blaser 2009; Polk and Peek 2010; Salama et al. 2013; Yamaoka and Graham 2014). Colonization by *H. pylori* commonly appears early in childhood, and if not treated by antimicrobial therapy, the bacteria can persist lifelong. Although *H. pylori* colonization is frequently associated with a strong inflammatory reaction, which is controlled by the host innate and adaptive immune systems, the bacteria are not eliminated. Various mechanisms of immune evasion have been reported, and *H. pylori* became a prime example of a persistent bacterial pathogen causing chronic infections (Ramarao et al. 2000; Gobert et al. 2001; Wunder et al. 2006; Patel et al. 2013; Foegeding et al. 2016). Evolutionary analyses revealed that *H. pylori* has been associated with modern humans over at least 100,000 years, possibly after the bacterium was first acquired by a single host jump from a yet unknown carrier (Moodley and Linz 2009). Because of this long time of coevolution, it has been proposed that *H. pylori* colonization may have been beneficial for the human host and hence provided a selective advantage (Backert and Blaser 2016). In the modern world, however, these infections cause a heavy burden of morbidity and mortality as a consequence of peptic ulcer disease, gastric adenocarcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma (Correa and Houghton 2007; Atherton and Blaser 2009;

Salama et al. 2013). In the last few years, the cellular and molecular mechanisms utilized by *H. pylori* to subvert host defences have been studied thoroughly (Amieva and El-Omar 2008; Salama et al. 2013; Backert et al. 2015; Caron et al. 2015). These investigations showed that the clinical outcome of infection with *H. pylori* is controlled by a highly sophisticated host–pathogen crosstalk. Multiple factors determine disease outcome including the bacterial genotype, genetic susceptibility of the host as well as environmental parameters.

H. pylori strains are remarkably diverse both in their genetic content and pathogenicity. Dozens of factors were identified to affect the pathogenesis of *H. pylori.* These determinants can be classified as virulence factors and pathogenicity-associated factors. There are two classical virulence factors encoded by *H. pylori*, the vacuolating cytotoxin (VacA) and the effector protein CagA. VacA is categorized as a pore-forming toxin, and many of its activities are associated with the formation of membrane channels in target cells. The best studied VacA activity is vacuole formation. However, the toxin has many other effects on host cells including the induction of apoptosis in epithelial cells or blocking the proliferation of immune cells (Foegeding et al. 2016). VacA genes are harbored by virtually all *H. pylori* isolates worldwide, but exhibit considerable sequence variation. *VacA* is present in several alleles that have been identified in the signal region (s1 and s2), mid-region (m1 and m2) and others, occurring in multiple combinations. The type s1/m1 VacA molecules produce an extensive cell vacuolation phenotype, while S2-type VacAs are inactive in such assays (Foegeding et al. 2016). The second factor is CagA, which is encoded by the *cag* (cytotoxin-associated genes) pathogenicity island (*cag*PAI) present in highly virulent *H. pylori* strains and being absent in less virulent isolates. The *cag*PAI encodes a type IV secretion system (T4SS), which is induced upon host contact and forms a syringe-like pilus structure for the export of virulence factors such as the CagA effector protein into host target cells (Tegtmeyer et al. 2011a; Backert et al. 2015). After delivery, CagA becomes tyrosine phosphorylated at EPIYA motifs by Src and Abl kinases (Lind et al. 2014; 2016) and mimics a host cell factor for triggering intracellular signaling cascades affecting membrane dynamics, disruption of cell–cell junctions as well as pro-inflammatory, cell cycle-related and anti-apoptotic transcriptional responses (Tegtmeyer et al. 2011b; Mueller et al. 2012; Zhang et al. 2015). Other known pathogenicity-associated phenotypes include flagella-driven motility in the mucus layer, acid neutralization by the urease (UreA, UreB and accessory proteins), adhesion to gastric epithelial cells mediated by adhesins (BabA, SabA, OipA, AlpA/B, HopQ, HopZ and others) as well as proteolytic targeting of host receptors such as E-cadherin by the secreted serine protease HtrA (Aspholm et al. 2006; Dubois and Boren 2007; Roure et al. 2012; Posselt et al. 2013; Yamaoka and Graham 2014). In addition, specific polymorphisms in human genes involved in inflammatory and immune-regulatory processes such as interleukin-1β (IL-1β), Toll-like receptors (TLRs) or NOD (nucleotide oligomerization domain) receptors as well as lifestyle properties (diet, smoking, alcohol consumption, etc.) have also been linked to an increased risk of developing gastric disease including cancer (Amieva and El-Omar 2008; Polk and Peek 2010).

Disruption of the epithelial barrier and in particular opening the intercellular adhesions is an important hallmark of *H. pylori*-dependent inflammation and neoplastic transformation. In principle, these individual processes can interfere with the functional cell-to-cell contacts independently of each other, but the combination of diverse temporary and locally regulated mechanisms reveals a dynamic and complex network. Here, we summarize our current knowledge on the multiple functions of *H. pylori* factors on exploiting the epithelial barrier and discuss the multitude of involved host signaling cascades with focus on their importance in pathogenesis.

1.2 Mechanisms of H. pylori*-Triggered Reprogramming of Epithelial Cell Differentiation*

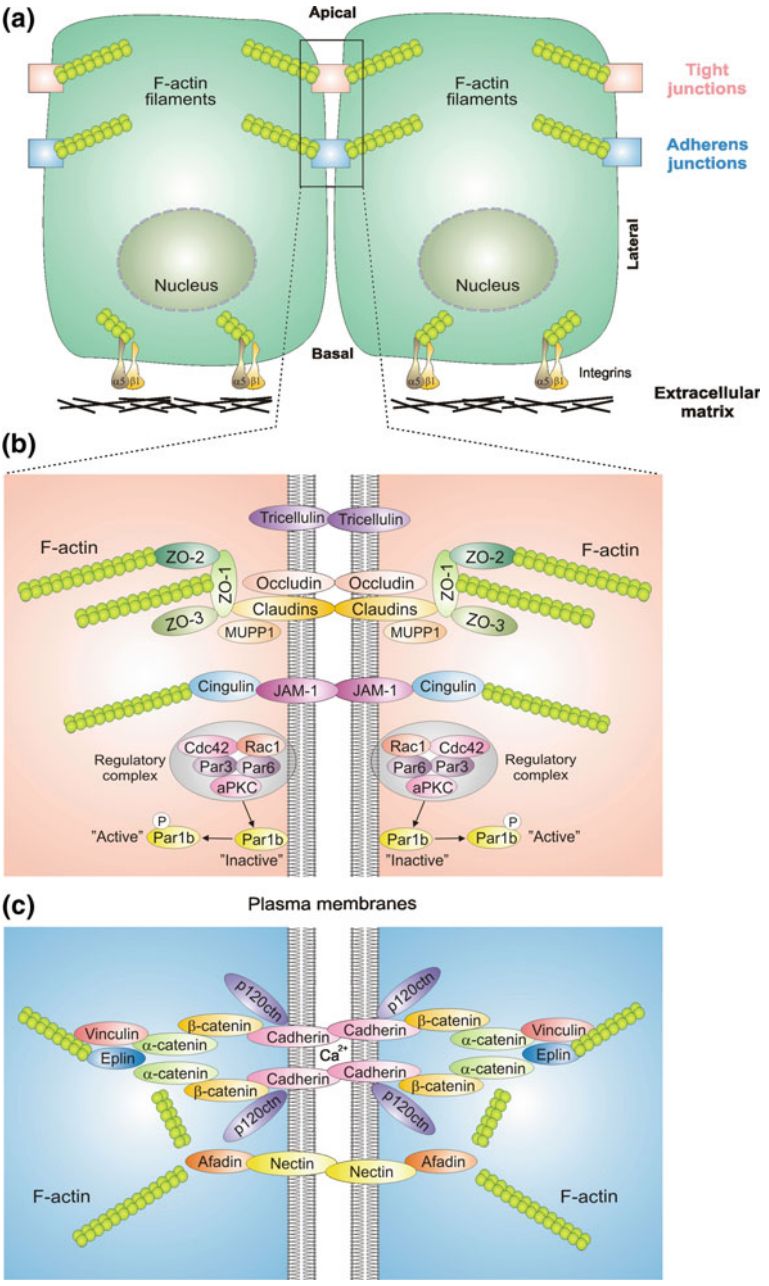
One virulence factor that clearly determines a high risk of gastric cancer development is the *cag*PAI encoding CagA. CagA itself has originally been recognized as an immuno-dominant antigen. Patients who are sero-positive for *H. pylori* and CagA exhibit a 5.8-fold higher risk of developing intestinal and diffuse gastric adenocarcinoma as compared to uninfected persons, whereas individuals infected with CagA-negative strains are only at a 2.2-fold higher risk of developing distal gastric adenocarcinoma compared to uninfected control persons (Parsonnet et al. 1997). Thus, the *cag*T4SS was established as a strong predictor of severe disease outcome. CagA-positive wild-type *H. pylori* can induce pre-malignant and malignant pathologies in the Mongolian gerbil infection model system. Four weeks after infection, almost each animal exhibited gastric dysplasia, and by eight weeks about two-thirds revealed gastric adenocarcinoma (Franco et al. 2005). Another straightforward experiment confirming the role of CagA in gastric cancer progression in vivo was provided by establishing CagA-expressing transgenic C57BL/6 J mice (Ohnishi et al. 2008). After 72 weeks, these transgenic mice developed gastric epithelial hyperplasia, while some animals revealed polyps and adenocarcinomas in the stomach. Moreover, systemic expression of CagA in mice leads to the development of leukocytosis with IL-3/GM-CSF hypersensitivity, while various animals displayed B-cell lymphomas and myeloid leukemias (Ohnishi et al. 2008). Based on the above findings, CagA was described as the first bacterial oncoprotein. These studies were confirmed by two other transgenic model organisms. A transgenic drosophila model demonstrated that CagA functions as a mimetic for the eukaryotic adaptor protein Gab1 (Botham et al. 2008). Expression of CagA in drosophila and zebrafish exhibited significantly enhanced levels of downstream c-Jun N-terminal kinase (JNK) phosphorylation and Wnt target gene induction, leading to proliferation of intestinal epithelial cells and growth of small cell carcinoma and adenocarcinoma (Wandler and Guillemin 2012; Neal et al. 2013). Altogether, the above studies established conclusively that *H. pylori* can induce the generation of gastric adenocarcinoma in gerbils and other model organisms. In addition, transgenic expression

of CagA alone appears to be sufficient for the development of severe malignant lesions in various transgenic animals.

A hallmark of gastric cancer development is the strong inflammatory response phenotype (Backert and Naumann 2010). Chronic inflammation and late stages of cancer are often accompanied by disruption of the proper architecture in the gastric epithelium, and *H. pylori* have been found intercellular and intracellular in gastric cancer biopsy samples (Necchi et al. 2007). In fact, infection with CagA-positive *H. pylori* as an important disease-associated feature has been clearly implicated in the disruption of the epithelial layer contributing to inflammatory gastric diseases (Polk and Peek 2010; Salama et al. 2013; Yamaoka and Graham 2014; Caron et al. 2015). Based on histology parameters, gastric cancer can be categorized into diffuse or intestinal types and both are associated with chronic infection by *H. pylori*. While we know very little about the pathogenesis of diffuse-type carcinoma, the intestinal type usually comprises a set of well-known steps. In the latter model, chronic active inflammation induced by *H. pylori* corresponds to the initial phase of disease progression followed by the loss of gastric glands, development of atrophy and hyperproliferation (Amieva and El-Omar 2008; Polk and Peek 2010). This might be dominated by specific alterations in cell cycle, apoptosis rates and cell proliferation. Finally, there is a progressive loss of differentiation leading to the invasive growth of individual neoplastic cells.

2 Structure and Composition of Polarized Cell Monolayers in the Healthy Epithelium

In order to understand how *H. pylori* can disrupt epithelial barrier functions, we must first take a look at the organization of healthy epithelia. An intact epithelial barrier requires tightly controlled cell architecture to provide effective protective functions. A complex network of diverse regulatory structures is necessitated in the establishment and maintenance of the protective epithelium. Besides a strictly regulated intracellular cytoskeleton and cell-to-ECM (extracellular matrix) interactions, epithelial cell-to-cell adhesions are crucially important and involve tight junctions (TJs) and adherens junctions (AJs) (Yu and Elble 2016; Sumigray and Lechler 2015; Rodriguez-Boulan and Macara 2014). The structure of the healthy epithelium is normally maintained by the integrity of the apical–basal polarity, a highly organized actin cytoskeleton and junctional complexes exhibiting tumor-suppressive and/or anti-metastasis properties (Fig. 1a). Importantly, junctional complexes are based on the extensively studied lateral cell-to-cell contacts including TJs (Fig. 1b) and E-cadherin-based AJs (Fig. 1c) as well as desmosomes, gap junctions and others, which are discussed elsewhere (Wei and Huang 2013). As shown in Fig. 1, TJs mark the apical–basolateral border of polarized cells and establish a highly selective barrier to prevent leakage and paracellular diffusion of small molecules. Importantly, functional TJs build up cell polarity through


(a)
Apical
F-actin filaments
F-actin filaments
Tight junctions
Adherens junctions
Lateral
Nucleus
Nucleus
Basal
Integrins
Extracellular matrix
(b)
Tricellulin
Tricellulin
F-actin
ZO-2
ZO-1
Occludin
Occludin
Claudins
Claudins
ZO-3
MUPP1
ZO-1
ZO-2
F-actin
ZO-3
MUPP1
Cingulin
JAM-1
JAM-1
Cingulin
Regulatory complex
Cdc42
Rac1
Par3
Par6
aPKC
Rac1
Cdc42
Par6
Par3
aPKC
Regulatory complex
"Active"
P
Par1b
Par1b
"Inactive"
Par1b
P
Par1b
"Active"
"Inactive"
(c)
Plasma membranes
p120ctn
β-catenin
Cadherin
Vinculin
α-catenin
Eplin
Ca2+
p120ctn
β-catenin
Cadherin
Vinculin
α-catenin
Eplin
α-catenin
Cadherin
β-catenin
Cadherin
α-catenin
β-catenin
p120ctn
p120ctn
Afadin
Nectin
Nectin
Afadin
F-actin
F-actin

◂**Fig. 1** Model for the organization of polarized epithelial cells and composition of intercellular junctions. **a** Simplified schematic presentation of the polarized cell layer in a healthy epithelium. Two important types of intercellular junctions, the tight junctions (TJ, *orange*) and adherens junctions (AJ, *blue*), are indicated and exhibit specific localization at the apical lateral borders. The basal focal adhesions are composed of integrins such as α5β1 integrin and connect the extracellular matrix with the intercellular actin cytoskeleton. Gap junctions and (hemi)desmosomes are other examples, but they are not discussed in this chapter. **b** TJs are key protein complexes in establishing and maintaining epithelial cell polarity. They are crucial for the tight sealing of the cellular sheets, which control the paracellular ion flux and maintain tissue homeostasis. TJs are localized at the apical side of the lateral membrane keeping barriers between the apical and basal compartments of the plasma membranes as indicated. TJs comprise at least four types of transmembrane proteins: junctional adhesion molecules (JAMs), claudins, occludin, tricellulin and various indicated cytoplasmic proteins. While the transmembrane proteins mediate cell-to-cell adhesion, the cytosolic TJ platform bears several other protein types (e.g., PDZ proteins, such as the zonula occludens (ZO) protein-1/-2/-3, MUPP1 or cingulin) which connect the TJ transmembrane proteins to the associated cytoskeleton. These molecules can also engage other signaling factors, including small GTPases, kinases, phosphatases and transcription factors. The integrity of TJs is maintained by a regulatory complex including atypical PKC (aPKC), Cdc42, Rac1, Par3 and Par6. aPKC can phosphorylate and activate Par1b kinase at threonine residue 595. Activated Par1b specifically localizes to the basal and lateral membranes to regulate cell polarity. **c** AJs are positioned immediately below TJs and form a complex of membrane proteins and associated factors which ensure the mechanical adhesion between two neighboring cells. AJs assemble via calcium-dependent homophilic interactions between the extracellular domains of E-cadherin connecting the cells as indicated. E-cadherin not only acts as an adhesive molecule, but also plays important roles as a suppressor of growth development and carcinogenesis. The calcium-dependent integrity of AJs is stabilized by binding of E-cadherin to intracellular catenins. The proximal carboxy-terminal domain of E-cadherin interacts with the cytoplasmic protein β-catenin. $p120^{ctn}$ can interact with the juxtamembrane part of E-cadherin, further stabilizing the entire adherens junction platform. The E-cadherin–β-catenin complex is connected to the actin cytoskeleton via binding to α-catenin, EPLIN (epithelial protein lost in neoplasm) and vinculin as shown. Nectin and afadin contribute to the organization of E-cadherin-mediated AJ functions in epithelial cells

impeding lateral diffusion of membrane proteins between the apical and basolateral domains of the epithelium leading to special characteristics and functions of the cell surface. Further functions include the control of epithelial proliferation and differentiation (Balda and Matter 2016; Martin 2014; Aijaz et al. 2006). The structure of TJs involves different transmembrane proteins (e.g., occludin, claudins, junctional adhesion molecules [JAMs]) connecting adjacent cells (Fig. 1b). Occludin consists of four transmembrane domains, two extracellular loops and two intracellular domains. Their functions appear to be crucially important in epithelial differentiation, but not in the establishment of the barrier (Schulzke et al. 2005). Although no significant sequence similarity to occludin exists, claudin proteins also contain four transmembrane domains, two extracellular loops and two intracellular domains (Krause et al. 2015). The human claudin family contains 27 members (Krause et al. 2015) and is important in the establishment and maintenance of the barrier function (Inai et al. 1999). Members of the JAM family include JAM-A, JAM-B, JAM-C and JAM4/JAML (Garrido-Urbani et al. 2014) and have a single transmembrane domain, an extracellular domain with two Ig-like motifs, and a

cytoplasmic domain (Kostrewa et al. 2001). JAM proteins are required for intercellular adhesion as well as for maintaining cell polarization (Garrido-Urbani et al. 2014). In the cytoplasm, TJs form an intracellular plaque that is composed of a complex network of scaffolding and adaptor proteins connecting the actin cytoskeleton and intracellular signaling molecules (Fig. 1b). Zonula occludens-1 (ZO-1), ZO-2 and ZO-3 are scaffolding proteins that interact directly with claudins and occludin (Runkle and Mu 2013). Other cytoplasmic proteins are cingulin, Rab13, afadin, membrane-associated guanylate kinase with inverted orientation-1 (MAG proteins) and multi-PDZ domain protein 1 (MUPP-1), etc. A variety of signaling molecules, such as small GTPases of the Rho family (Rho, Rac and Cdc42), ZO-1-associated kinase, PKCζ, etc., complete the functional structure of TJs (Denker and Nigam 1998).

In a given polarized epithelium, AJs are located directly beneath TJs. The main function of AJs is the formation of intercellular adhesions. The integrity of AJs is established by the homophilic interactions of members of the cadherin family of proteins (Niessen and Gottardi 2008; Gumbiner 2005). E-cadherin (Cdh1) is an N-glycosylated transmembrane protein composed of an extracellular (EC) domain, transmembrane domain and an intracellular (IC) domain. The extracellular domain contains five repetitive amino acid sequences (EC1–EC5) with calcium-binding motifs located between the individual EC domains (Ringwald et al. 1987; Harrison et al. 2010). Binding of calcium ions is a requirement for the formation of interactions between the EC domains. The flexible three-dimensional E-cadherin structure is pushed into a rigid, while rod-like assembly upon calcium binding allowing interactions of EC1 and EC3 in *cis* and in *trans* (Niessen and Gottardi 2008; Ozawa et al. 1990; Takeda et al. 1999; Pokutta et al. 1994). As shown in Fig. 1c, the IC domain of E-cadherin interacts with members of the catenin family, in particular β-catenin and p120-catenin ($p120^{ctn}$). Binding of β-catenin to the IC bridges E-cadherin to the actin cytoskeleton through the interaction with α-catenin. In turn, α-catenin can bind actin either directly or via other proteins like EPLIN (epithelial protein lost in neoplasm) or vinculin (Abe and Takeichi 2008; Hazan et al. 1997; Meng and Takeichi 2009). Together with β-catenin, interaction of $p120^{ctn}$ with the juxtamembrane part of E-cadherin stabilizes the integrity of AJs and contributes to the regulation of the turnover of E-cadherin (Yap et al. 1998; Gooding et al. 2004).

3 Depolarization of Epithelial Cells by *H. pylori* Involves Alterations in Tight and Adherens Junctions

Infections with *H. pylori* are accompanied by alterations of the cell architecture leading to depolarization of the epithelium. In particular, the intercellular junctions are direct targets. The functionality of AJs can be disrupted by multiple mechanisms. Importantly, gastric cancer of the diffuse type is strongly correlated with

severe interference of AJ function through loss of function mutations or (epi)genetically downregulation of the *cdh1* gene. Additionally, ectodomain shedding of E-cadherin by upregulated matrix metalloproteases (MMPs) is intensively discussed in the literature to dysregulate E-cadherin and to increase the malignancy of gastric cancer as the consequence of the loss of adhesive properties of AJs to increase metastasis of tumor cells (Margineanu et al. 2008; Chan 2006). Upon disruption of AJs, the catenins can aggravate this phenomenon. Besides their function in the stability and integrity of AJs, β-catenin and $p120^{ctn}$ are implicated in the tumor-suppressive function of E-cadherin. Destabilization of the AJ complex can lead to the release of β-catenin and $p120^{ctn}$. Non-junctional cytoplasmic β-catenin is constantly degraded by the proteasome after phosphorylation by a multiprotein complex comprising glycogen synthase kinase 3β (GSK-3β), casein kinase 1 (CK1), adenomatous polyposis coli (APC) and axin to prevent nuclear functions of β-catenin (He et al. 2004). An activated Wnt signaling pathway can result in an inhibition of β-catenin phosphorylation followed by the stabilization of β-catenin (MacDonald et al. 2009). Accumulated β-catenin can translocate into the nucleus, where it interacts with T-cell-specific transcription factor/lymphoid enhancer-binding factor (TCF/LEF) family to activate the expression of cancer-associated Wnt target genes, such as *c-myc* or *cyclin d1* (MacDonald et al. 2009; McCrea and Gottardi 2016). Similarly, nuclear $p120^{ctn}$ protein physically interacts with Kaiso to relieve Kaiso-mediated inhibition transcription of canonical Wnt target genes, including *cyclin d1* or *mmp-7* (Daniel and Reynolds 1999; Park et al. 2005; Spring et al. 2005). In conclusion, intact AJ complexes are crucially important in the establishment of intercellular adhesions and in tumor suppression. However, *H. pylori* developed fascinating mechanisms to disrupt intercellular adhesions (Wessler and Backert 2008), which are summarized in Table 1 and discussed in the following text.

3.1 Direct Targeting of Tight Junction Factors by H. pylori *Effector Proteins*

3.1.1 Selective Opening of Tight Junctions by *H. pylori* VacA

Early studies have provided hints that *H. pylori* can target TJs in the gastric epithelium. The first implicated bacterial factor was VacA. Papini et al. (1998) reported that treatment of various polarized cell lines with acid-activated VacA increased the cell permeability for low molecular weight (<350–440 Dalton) molecules and ions such as iron (Fe^{3+}) and nickel (Ni^{2+}). In accordance with these data, the transepithelial electrical resistance (TER) was decreased by VacA treatment. However, high-resolution immunofluorescence analyses of VacA-treated cells failed to reveal alterations of junctional proteins, including ZO-1, occludin and E-cadherin. The authors proposed that VacA induces a selective permeabilization of

Table 1 Reported activities of *H. pylori* factors targeting tight and adherens junction proteins

Bacterial factor	Proposed function	Interaction partner	Experimental evidence	Host cells used	*H. pylori* strain infection	Applied methods	References
VacA	Increase of cell permeability	n.d.	Treatment with enriched VacA	MDCK, T84, epH4, HeLa	None	CLSM, TER, WB, NRU, ^{125}I-EGF degradation	Papini et al. (1998)
VacA	Increase of cell permeability	n.d.	Infection	MDCK	CCUG 17874, SPM326, 95-54	TER, WB, QAB, NRU, PF	Pelicic et al. (1999)
VacA	Cell cytoskeletal disruption	n.d.	Treatment with VacA-containing supernatants	AGS, HeLa, RK13	J116, 60190, 60190v1, M99, Tx30a, M99v1	BFM, WB, CLSM, AA, TEM, PCR	Bebb et al. (2003a, b)
VacA	Apical membrane disruption	Calpain, ezrin	Treatment with enriched VacA	Rabbit gastric primary cells	None	CLSM, WB, EM, APUA, Ca^{2+} influx, IVP, PCR, PA	Wang et al. (2008)
CagA	Disruption of tight junctions	ZO-1, JAM	Infection	MDCK, AGS	G27	CLSM, MF, WB, TEM, LBSAB, CSA, DA	Amieva et al. (2003)
CagA	Activation of β-catenin	n.d.	Gerbil infection, transfection, proteomics	AGS, MG-262	7.13, B127, J166, J68	CLSM, IHC, WB, PCR, LA, ELISA	Franco et al. (2005)
CagA	Loss of cell polarity	ZO-1	Transfection of CagA-GFP	MDCK, AGS, HEK-293	None	CLSM, MIA, PCR, TLIA, IP	Bagnoli et al. (2005)
CagA	Activation of β-catenin	E-cadherin	Transfection of CagA	MKN28, MKN45	None	CLSM, IP, WB, LA, cDNA-MA, FC, NB	Murata-Kamiya et al. (2007)
CagA	Loss of cell polarity	MARK2/ Par1b	Transfection of CagA	COS-7, AGS, MDCK	None	CLSM, TER, MS, in vitro KA, WB, IP	Saadat et al. (2007)
CagA	Loss of cell polarity	MARK2/ Par1b	Infection, transfection of CagA-GFP	MDCK, AGS	G27	CLSM, IP, WB, CF, RT-PCR, iTRAQ, COA, SCX, LC-MS/MS, CSA, DRMP	Zeaiter et al. (2008)

(continued)

Table 1 (continued)

Bacterial factor	Proposed function	Interaction partner	Experimental evidence	Host cells used	*H. pylori* strain infection	Applied methods	References
CagA	Regulation of MMP-7 expression	E-cadherin, p120	Infection, siRNA knockdown	MKN28, Phoenix 293	SS1, 7.13	CLSM, RT-PCR, RT, ChIP, SCF, WB, LA, QAB, DA	Ogden et al. (2008)
CagA	Invasion of epithelial cells	E-cadherin, c-Met, p120	Infection, transduction of human E-cadherin	AGS, NCI-N87, IPA220	26695, 60190	CLSM, IP, WB, MIA, ICC	Oliveira et al. (2009)
CagA	Increase of claudin-2 expression	Cdx2	Infection	AGS	P1	SEM, TEM, WB, EMSA, qPCR, sqPCR, CLSM, MIA	Song et al. (2013)
CagA	Snail mediates EMT via GSK-3 depletion	GSK-3	Infection, transfection of CagA	MKN28, AGS, 293	60190	CLSM, IP, WB, IHC, in vitro KA, qPCR, RGA, MIA, PCR, IMA, IFM	Lee et al. (2014)
CagA	Targeting of claudin-7	β-Catenin, Snail	Infection, microinjection	Gastroids, MKN28	7.13, 60190	CLSM, FC, IHC, WB, CF, LA, siRNA, EdUA, DA, RT-PCR	Wroblewski et al. (2015)
CagA	MMP activation	n.d.	Infection, siRNA knockdown	AGS, MKN45, MKN28, KATO-III	P12, 26695, 60190, 84183	CLSM, microarray, WB, qPCR, siRNA, MIA, CZ, PCR	Sougleri et al. (2016) Costa et al. (2016)
Urease	Targeting of occludin via NH_3	n.d.	Infection, treatment with ammonium	Caco-2, MDCK, Jurkat	P12	CLSM, WB, TER, PF, RT-PCR, DA	Lytton et al. (2005)
Urease	Targeting TJs via MLCK	n.d.	Infection, MLCK inhibitor treatment	MKN28	7.13, 60190	CLSM, IHC, WB, TER, CF, AA, DA	Wroblewski et al. (2009)

(continued)

Table 1 (continued)

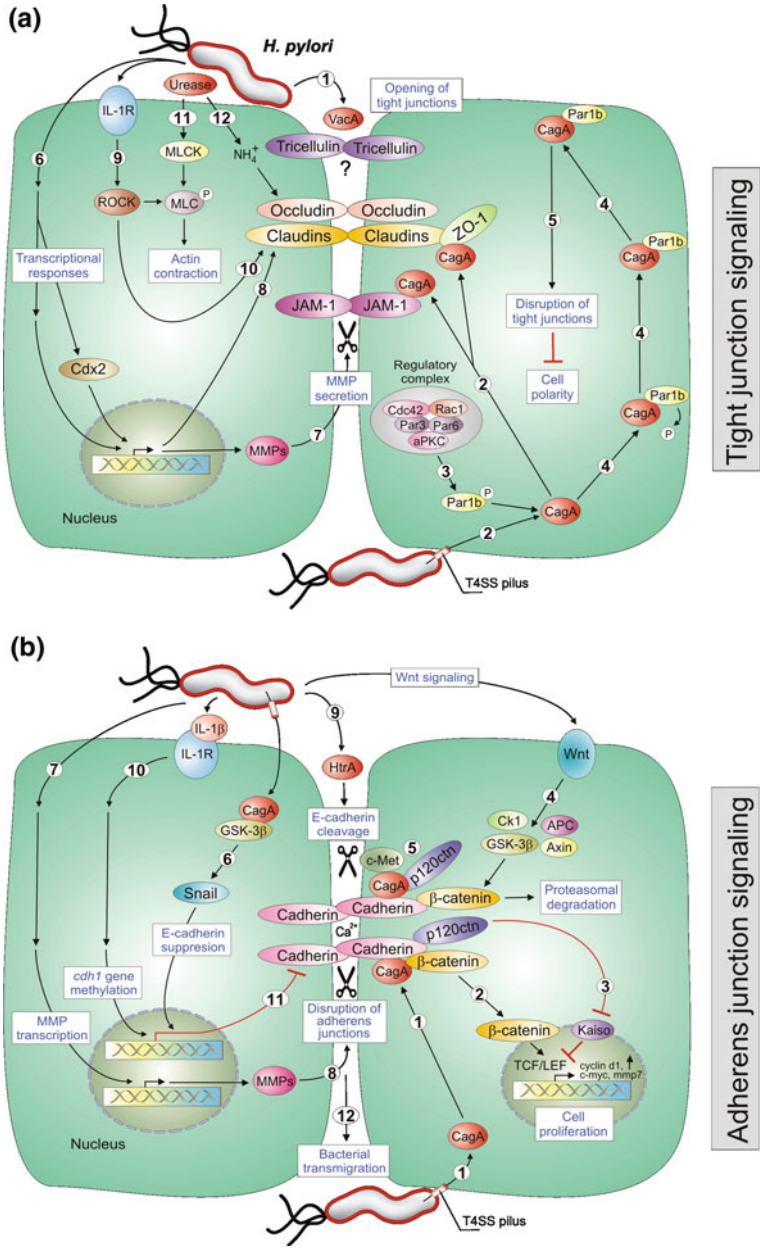
Bacterial factor	Proposed function	Interaction partner	Experimental evidence	Host cells used	*H. pylori* strain infection	Applied methods	References
HtrA	Opening of AJs	E-cadherin	Infection and in vitro cleavage	MKN28, MDCK, INT-407	P12, 26695	CLSM, WB, CZ, PCR, GPA, MS	Hoy et al. (2010, 2012)
HtrA	Opening of AJs	E-cadherin	Infection and in vitro cleavage	MKN28	SS1, B38, G27, J99, B8, 35A+ 15 clinical strains	WB, CZ, PCR	Tegtmeyer et al. (2016)
HtrA	Opening of AJs	E-cadherin	Infection and in vitro cleavage	MKN28, AGS, NCI-N87	P12, 26695	SPR, TER, WB, MS, HPLC, PS, NTS	Schmidt et al. (2016a, b)

[a]**Abbreviations:** ^{125}I-EGF degradation (^{125}I-iodination epidermal growth factor degradation); AA (apoptosis assay); APUA (aminopyrine uptake assay); BFM (bright field microscopy); cDNA-MA (cDNA microarray); CF (cell fractionation); ChIP (chromatin immunoprecipitation); CLSM (confocal laser scanning microscopy); COA (collagen overlay assay); CSA (calcium switch assay); CZ (casein zymography); DA (densitometric analysis); DRMP (detergent-resistant membrane preparation); EdUA [EdU(5-ethynyl-2′-deoxyuridine)-assay]; ELISA (enzyme-linked immunosorbent assay); EM (electron microscopy); EMSA (electrophoretic mobility shift assay); FC (flow cytometry); GPA (gentamicin protection assay); HPLC (high-performance liquid chromatography); ICC (immunocytochemistry); IFM (immunofluorescence microscopy); IHC (immunohistochemistry); IMA (invasion and migration assay); in vitro KA (kinase assay); IP (immunoprecipitation); iTRAQ (isobaric tags for relative and absolute quantitation); IVP (in vitro proteolysis); LA (luciferase assay); LBSAB (leakage of bovine serum albumin biotin); LC-MS/MS (liquid chromatography-mass spectrometry/mass spectrometry); MF (membrane fractionation by iodixanol gradients, OptiPrep™); MIA (Matrigel™ invasion assay); MS (mass spectrometry); n.d. (not determined); NB (northern blotting); NRU (neutral red uptake); NTS (N-terminal sequencing); PA (permeabilization assay); PCR (polymerase chain reaction); PF (paracellular flux); PS (peptide synthesis); QAB (quantification of adherent bacteria assay); qPCR (quantitative real-time PCR); RGA (reporter gene assay); RT (retroviral transduction); RT-PCR (real-time reverse transcriptase PCR); SCF (subcellular fractionation); SCX (strong cation exchange chromatography); SEM (scanning electron microscopy); siRNA (small interfering RNA); SPR (surface plasmon resonance); sqPCR (semiquantitative PCR); TEM (transmission electron microscopy); TER (transepithelial electrical resistance); TLIA (time-lapse imaging analysis); WB (Western blotting)

the paracellular epithelial route to certain molecules and ions, which may serve to acquire nutrients to support *H. pylori* survival and growth in vivo (Papini et al. 1998). Further work showed that infection of Madin-Darby canine kidney (MDCK) monolayers with *H. pylori* also resulted in a decrease in TER, while isogenic Δ*vacA* mutants did not (Pelicic et al. 1999). A similar effect was observed with various VacA-producing isolates, including those expressing m2-type toxins that exhibit no vacuolating activity, suggesting that vacuole formation per se is not required for the response. Later, it was postulated that VacA permeabilizes the apical membrane of gastric parietal cells and induces hypochlorhydria (Wang et al. 2008). Using freshly isolated rabbit gastric glands and cultured parietal cells, it was shown that VacA induces an influx of extracellular calcium (Ca^{2+}), followed by activation of the protease calpain and subsequent cleavage of ezrin, a regulator of filamentous actin (F-actin) in cell junctions (Selbach et al. 2004), which results in the liberation of ezrin from the apical membrane of parietal cells (Wang et al. 2008). Electron microscopic examination revealed that VacA treatment disrupts the radial arrangement of F-actin filaments in apical microvilli due to the loss of ezrin integrity in parietal cells (Wang et al. 2008). Further studies showed that VacA-containing culture supernatants disrupt the actin cytoskeleton of epithelial cell lines, leading to cell rounding and apoptosis through anoikis (Bebb et al. 2003a). However, the role of VacA is not yet fully clear as other data have shown that isogenic Δ*vacA* mutants behaved like wild-type *H. pylori* and changed TER during infection (Wroblewski et al. 2009).

3.1.2 Disruption of Tight Junctions by Ectopic CagA

Another bacterial effector protein involved in the alteration of TJs by *H. pylori* is CagA. A pioneering study has shown that CagA mediated the redistribution of TJ proteins in MDCK cells. Amieva et al. (2003) showed that translocated CagA associates with ZO-1 and JAM, causing an ectopic assembly of TJ components at sites of bacterial adherence, thus altering composition and function of the apical–junctional complex. Further studies have indicated that ectopic expression of CagA can disrupt the cell-to-cell junctions and this depends on the phosphorylation state of CagA and specific CagA domains (Bagnoli et al. 2005). Transfection experiments showed that CagA expression is not only sufficient to disrupt the apical junctions, but also perturbs epithelial cell differentiation. CagA-expressing cells lose their polarity and cell-to-cell adhesion, acquiring an invasive cell phenotype (Bagnoli et al. 2005). Expression of the carboxy-terminal EPIYA-containing CagA domain stimulates pseudopodial activity, but is not sufficient to trigger cell migration. Importantly, the amino terminus of CagA targets the protein to the apical cell junctions. However, neither domain alone is sufficient to change cell polarity or cell adhesion, but when coexpressed in *trans*, the amino terminus determines the localization of both polypeptide chains (Bagnoli et al. 2005). It appears that the first 200 amino acids of CagA are implicated in an inhibition of certain CagA functions, in particular diminishing cell elongation and apical surface constriction (Pelz et al. 2011).


(a)
H. pylori
Urease
IL-1R
MLCK
ROCK
MLC
Transcriptional responses
Actin contraction
Cdx2
Nucleus
MMPs
MMP secretion
VacA
Opening of tight junctions
Tricellulin
Occludin
Claudins
JAM-1
ZO-1
CagA
Par1b
Regulatory complex
Cdc42
Rac1
Par3
Par6
aPKC
Disruption of tight junctions
Cell polarity
T4SS pilus
Tight junction signaling
(b)
Wnt signaling
IL-1β
IL-1R
HtrA
Wnt
CagA
GSK-3β
E-cadherin cleavage
Ck1
APC
Axin
c-Met
p120ctn
Snail
Cadherin
β-catenin
Proteasomal degradation
E-cadherin suppresion
Ca2+
cdh1 gene methylation
MMP transcription
Disruption of adherens junctions
Kaiso
TCF/LEF
cyclin d1, t c-myc, mmp7
Cell proliferation
Bacterial transmigration
Adherens junction signaling

◂**Fig. 2** Model of *H. pylori*-induced epithelial barrier disruption by specific targeting of tight and adherens junction proteins. Schematic presentation of two junctional complexes and particular signaling pathways, which are induced during infection with *H. pylori* and/or treatment with purified proteins. For simplification, only targeted factors are displayed. **a** TJs are hijacked by purified VacA, which can selectively open the TJs by a yet unknown mechanism (*1*). *H. pylori* translocates CagA proteins into the host cell cytoplasm via the T4SS pilus. CagA has been shown to colocalize with ZO-1 and JAM proteins (*2*). The overall integrity of TJs is maintained by a regulatory complex (gray circle) including atypical PKC (aPKC), Cdc42, Rac1, Par3 and Par6. aPKC can phosphorylate Par1b kinase at threonine residue 595. Activated Par1b specifically localizes to the basal and lateral membranes to regulate cell polarity (*3*). Transfected or translocated CagA binds Par1b and thereby inhibits aPKC-mediated phosphorylation of Par1b (*4*). The CagA-Par1b complex mislocalizes to TJs and apical membranes (*4*). This signaling results in the disruption of TJs and loss of cell polarity (*5*). *H. pylori* also stimulates host nuclear responses (*6*). In this way, various matrix metalloproteases (MMPs) are transcriptionally upregulated, which can be secreted and cleave TJ proteins (*7*). Another target is transcription factor Cdx2, which upregulates the expression of claudin-2 (*8*). *H. pylori* can also induce IL-1 receptor (IL-1R) phosphorylation, playing a role in ROCK kinase activation (*9*) and subsequently claudin-4 disruption (*10*). The *H. pylori* urease enzyme also affects TJ proteins in two ways, first by activating myosin light chain (MLC) phosphorylation by MLC kinase (*11*) or by elevating free $NH4^+$ levels resulting in occludin fragmentation by a yet unknown mechanism (*12*). **b** AJs are targeted by *H. pylori* in multiple ways. The bacteria translocate CagA proteins into the host cell which may interact with E-cadherin directly (*1*). This interaction results in the release of β-catenin from the E-cadherin complex and subsequently translocation of β-catenin into the nucleus. In this way, β-catenin acts as cofactors for TCF/LEF transcription factors to stimulate the expression of various proliferative target genes such as the proto-oncogenes *cyclin d1* and *c-myc* (*2*). This response can be enhanced by p120-catenin ($p120^{ctn}$) translocating to the nucleus where it interacts with Kaiso to relieve Kaiso-mediated inhibition of TCF/LEF transcription (*3*). Deregulation of the Wnt pathway including glycogen synthase kinase 3 beta (GSK-3β), adenomatous polyposis coli (APC), casein kinase 1 (CK1) and Axin feeds into the same pathway, affecting β-catenin phosphorylation, nuclear localization or degradation (*4*). Another report showed that CagA forms a complex with E-cadherin, c-Met and $p120^{ctn}$ affecting cell migration and invasion (*5*). Intracellular CagA can also bind GSK-3, resulting in reduced GSK-3 activity. In this way, CagA stabilizes Snail, a transcriptional repressor of E-cadherin expression (*6*). *H. pylori* also activates MMP transcription (*7*), leading to elevated MMP secretion and AJ protein cleavage (*8*). In addition, *H. pylori* secretes the serine protease HtrA, which can cleave E-cadherin directly (*9*). It was also shown that *H. pylori* activates the IL-1 receptor (IL-1R) by upregulating IL-1β, which results in E-cadherin gene (*cdh1*) methylation (*10*), suppression of E-cadherin translation and downregulation at the protein level (*11*). The result of these processes is a local epithelial disruption allowing some *H. pylori* entering the intercellular space and reaching basal membranes (*12*). In this manner, the bacteria could probably access the basal integrin receptor and translocate CagA. The position of CagA translocation and the sequence of various indicated events are hypothetical in this model and were not yet proven experimentally

The detailed interplay between the inhibitory N-terminal part of CagA and the tyrosine-phosphorylated C-terminal part needs to be investigated in future. Further studies showed that CagA specifically interacts with PAR1/MARK kinase, which has an essential role in controlling epithelial cell polarity (Saadat et al. 2007; Nesic et al. 2010; Zeaiter et al. 2008). Association of CagA inhibits PAR1 kinase activity and prevents atypical protein kinase C (aPKC)-mediated PAR1 phosphorylation, which dissociates PAR1 from the membrane, collectively causing junctional and polarity defects (Fig. 2a). Taken together, these data suggest that CagA induces a

morphogenetic program in polarized epithelial cells resembling an epithelial mesenchymal transition (EMT) phenotype, which may be an early event in *H. pylori*-induced carcinogenesis. Further support for these ideas came by studies on gastroids that developed into a self-organizing differentiation axis. Infection of these gastroids showed that *H. pylori* induced the mislocalization of claudin-7 and increased cell proliferation in a CagA- and β-catenin-dependent fashion (Wroblewski et al. 2015). In another publication, it was reported that CagA targets Cdx2 (caudal-related homeobox 2) during an infection of AGS cells with *H. pylori* wild type and Δ*cagA* as control (Song et al. 2013). Cdx2 is an intestine-specific transcription factor highly expressed in multistage tissues of dysplasia and cancer. One specific target of Cdx2, claudin-2, is involved in the regulation of TJ permeability. It was shown that Cdx2 upregulated the expression of TJ factor claudin-2 both at transcriptional and at translational levels (Song et al. 2013). However, AGS cells do not form polarized cell monolayers due to the lack of proper junctions; thus, further studies with polarized cell lines are required. In another study, it was demonstrated that *H. pylori* can disrupt claudin-4 by a Rho kinase (ROCK)-dependent pathway in human HGE-20 gastric epithelial cell monolayers, but this occurs independently of CagA and VacA and without altering claudin-4 transcription (Lapointe et al. 2010). Additional experiments revealed that *H. pylori* induced IL-1 receptor type I (IL-1RI) phosphorylation playing a role in ROCK activation and claudin-4 disruption. Taken together, these findings identify a novel pathophysiological mechanism by which *H. pylori* disrupts gastric epithelial barrier structure via IL-1RI-dependent activation of ROCK, which in turn mediates claudin-4 disruption in TJs (Lapointe et al. 2010).

3.1.3 Urease-Dependent Targeting of Tight Junctions via MLCK and MLC

Urease is another effector protein of *H. pylori*, which has been shown to target TJs, presumably by two independent mechanisms. The urease produces ammonium (NH_3/NH_4^+), which is elevated in the gastric aspirates of *H. pylori*-infected patients and has been implicated in the disruption of TJ functional integrity and the induction of gastric mucosal damage during infection. Lytton et al. (2005) have reported that acute exposure to ammonium salts or NH_3/NH_4^+ derived from urea metabolism by wild-type *H. pylori* resulted in a 20–30% reduction in TER. In contrast, cultures that were exposed to supernatants derived from Δurease mutant *H. pylori*, showed no significant decrease in TER. Occludin-specific immunoblots revealed the expression of a low molecular weight form of occludin at 42 kDa after NH_3/NH_4^+ treatment, but its origin is yet unknown (Lytton et al. 2005). A few years later Wroblewski et al. (2009) demonstrated that phosphorylation of myosin regulatory light chain (MLC) by MLC kinase (MLCK) regulates TJ function during *H. pylori* infection. MLCK was activated by *H. pylori* and the progressive loss of barrier function that was attenuated by inactivation of *ureB*, but not *cagA*, *cagE* or *vacA* genes (Wroblewski et al. 2009). Decrease in TER was also dependent on functional urease activity, and this was significantly decreased by inhibition of

MLCK or Rho kinase or by loss of UreB. In addition, *H. pylori* infection of either cultured monolayers or hypergastrinemic INS-GAS mice induced occludin endocytosis, reflecting the disruption of TJs. Taken together, these results indicate that modulation of TJ functions by *H. pylori* involves various bacterial factors which target individual TJ components by different pathways.

3.2 H. pylori *Actively Disrupts Adherens Junctions to Induce an EMT-like Phenotype*

The loss of E-cadherin functionality is associated with the EMT process through which epithelial cells can convert to motile and invasive growing cells during the progression of gastric carcinogenesis (Yilmaz and Christofori 2010; Huang et al. 2015). Early studies indicated that *H. pylori* infections are significantly associated with the loss of E-cadherin expression and/or functions. Several modes of action have been described that trigger both intracellular signal transduction through the translocated CagA effector and extracellular modifications of E-cadherin through proteases epithelial and bacterial origin. Implicated bacterial factors and altered signaling pathways are highlighted in Fig. 2b and summarized in the following section.

3.2.1 Interference of *H. pylori* CagA with the Integrity of Adherens Junctions

The early observation that *H. pylori* induces a CagA-dependent EMT-like scattering phenotype led to the initial suggestion that CagA is directly responsible for the disruption of lateral AJs through the regulation of intracellular signal transduction pathways. Correspondingly, a physical interaction between ectopically expressed CagA and the IC domain of E-cadherin was shown. CagA binding to the IC domain of E-cadherin was proposed to compete with β-catenin interaction in a CagA phosphorylation-independent manner leading to an increase of cytoplasmic β-catenin (Murata-Kamiya et al. 2007). Mislocalization of β-catenin and internalization of E-cadherin in the cytoplasm upon infection with *H. pylori* wild type, but not with the Δ*cagA* mutant strain, indicated a pleiotropic effect by CagA through binding to Crk adaptor proteins (Suzuki et al. 2005). In addition to the CagA/E-cadherin interaction, it was further postulated that CagA can bind to $p120^{ctn}$ and c-Met, which leads to a suppression of the *H. pylori*-driven cell-invasive phenotype (Oliveira et al. 2009). Ectopically expressed CagA requires the EPIYA motif-containing sequence for binding to E-cadherin, which has been also identified as a multimerization domain within the CagA molecule (Kurashima et al. 2008; Ren et al. 2006). However, it is not entirely clear if there is a direct interaction betweeen CagA and the IC domain of E–cadherin, but may involve additional signaling

molecules such as the PAR1 kinase (Kurashima et al. 2008; Saadat et al. 2007). These data are partly in accordance with a previous work showing the release of β-catenin from the membrane after infection with a CagA-positive *H. pylori* strain in the *Mongolian gerbil* infection model (Franco et al. 2005). Once released from the AJ complex, β-catenin is constantly degraded by the proteasome after phosphorylation by GSK-3β/CK1/APC/axin complex (He et al. 2004). In *H. pylori*-colonized MDCK cells, suppression of β-catenin phosphorylation and degradation was observed as a CagA-independent process that was regulated by GSK-3β and Akt1 kinases (Sokolova et al. 2008), indicating that β-catenin is not only released from the AJ complex, but exhibits additional functions in the nucleus as a cofactor of TCF/LEF transcription factors, which are discussed in Sect. 3.3.

The intracellular CagA-mediated deregulation of AJ functions is controversial in the literature. Analysis of the EMT-like phenotype of *H. pylori*-infected cells revealed that cell motility and elongation are induced via different signal transduction pathways. Obviously, cell motility and loss of cell-to-cell adhesion are independent of CagA delivery, while cell elongation requires CagA translocation and tyrosine phosphorylation (Moese et al. 2004, Tegtmeyer et al. 2009). Biopsy samples of *H. pylori*-positive patients also demonstrated a *cag*PAI-independent reduction of junctional β-catenin expression (Bebb et al. 2006). Accordingly, the disintegration of E-cadherin-mediated AJs was shown as a CagA-independent process as demonstrated by isogenic *H. pylori* mutants (Sokolova et al. 2008; Weydig et al. 2007). These data imply that CagA-independent mechanisms must exist which can deregulate AJ functions in gastric epithelial cells in response to *H. pylori* infections. Further studies are necessary to clarify these important questions.

3.2.2 Disruption of E-Cadherin-Mediated Adherens Junctions by Secreted HtrA

Apart from possible destabilization of the E-cadherin complex by intracellular CagA (Murata-Kamiya et al. 2007; Oliveira et al. 2009), downregulation of E-cadherin expression or promoter hypermethylation (Chan et al. 2003), also proteolytic cleavage of E-cadherin upon infection with *H. pylori* has been consistently reported (Weydig et al. 2007; Schirrmeister et al. 2009). Ectodomain shedding represents an important mechanism in E-cadherin regulation of the healthy epithelium, but is also a very frequent event in cancer progression and is often associated with a poor prognosis due to the high capability to metastasize. Several host cell proteases have been described to cleave E-cadherin on the cell surface, including matrix metalloprotease (MMP)-3, 7, 9 and ADAM (a disintegrin and metalloprotease)-10 and 15 as well as plasmin and kallikrein 7 (Maretzky et al. 2005; Ryniers et al. 2002; Johnson et al. 2007; Davies et al. 2001; Noe et al. 2001; Covington et al. 2005; Symowicz et al. 2007). In fact, *H. pylori* has been shown to upregulate several E-cadherin proteases, including MMP-1 (Pillinger et al. 2007), MMP-3 (Sougleri et al. 2016), MMP-7 (Ogden et al. 2008; Yin et al. 2010; Bebb et al. 2003b), MMP-9 (Kundu et al. 2006), MMP-10 (Costa et al. 2016) or

ADAM-10 (Hoy et al. 2010; Schirrmeister et al. 2009). Correspondingly, an increase in serum level of the extracellular domain of E-cadherin has been detected in *H. pylori*-infected patients by O'Connor et al. (2011), which might serve as a biomarker or prognostic marker of gastric cancer.

It is unequivocally clear that host proteases are upregulated and activated in response to *H. pylori* infection, which are implicated in E-cadherin shedding. However, siRNA-mediated downregulation and pharmacological inhibition of various MMPs and ADAM proteases revealed that additional proteases must be involved in this process (Hoy et al. 2010; Schirrmeister et al. 2009). Interestingly, it was found that a soluble factor of *H. pylori* is sufficient to efficiently disrupt E-cadherin-based AJs (Weydig et al. 2007). The serine protease high-temperature-requirement A (HtrA) of *H. pylori* was finally identified as a secreted serine protease that directly and selectively targets E-cadherin in vitro and on gastric epithelial cells (Hoy et al. 2010). Generally, HtrA is expressed as a periplasmic protein, but is also secreted into the environment and was found in outer membrane vesicles (Bumann et al. 2002; Lower et al. 2008; Hoy et al. 2010; Olofsson et al. 2010; Boehm et al. 2013; Turner et al. 2015). Interacting with E-cadherin at the molecular level, HtrA targets amino acid stretches containing the [VITA]↓[VITA]-x-x-D-[DN] motif within the E-cadherin molecule as preferred cleavage positions (Schmidt et al. 2016b). HtrA cleavage sites are positioned between the five individual extracellular repeats (EC1-EC5), which are important calcium-binding motifs. Functional E-cadherin-mediated AJs require calcium binding to form functional homophilic interactions between the EC domains in *cis* and *trans*. Therefore, in a physiological context, these sites are only partially accessible in calcium-bound E-cadherin. It is hypothesized that calcium binding to the HtrA-targeted E-cadherin cleavage sites limits the HtrA-mediated E-cadherin and explains why a stable 90-kDa fragment is observed during infection with *H. pylori* instead of a fragment ladder (Schmidt et al. 2016a). These data point to a tightly controlled E-cadherin shedding mechanisms probably involving both host and bacterial proteases.

If HtrA-mediated E-cadherin shedding does not only open intercellular adhesion, but also destabilize the intracellular E-cadherin complex to release β-catenin, $p120^{ctn}$ has not been investigated yet. However, the consequences of the disruption of AJs by *H. pylori* are potentially substantial for the gastric epithelium and might be one aspect to permit *H. pylori* transmigration across this barrier (Hoy et al. 2010, 2012; Schmidt et al. 2016b). In *H. pylori*, HtrA showed a remarkable stability under extreme conditions (high temperature, high salt concentrations, etc.), which is certainly beneficial in the gastric environment (Hoy et al. 2013). The HtrA protease is highly conserved in *H. pylori* strains across the world and is absolutely essential for *H. pylori* survival (Tegtmeyer et al. 2016) underlining the importance of HtrA in *H. pylori* physiology and pathogenesis. In contrast to many other bacteria, it was not yet possible to create an *htrA*-deletion mutant in *H. pylori* (Tegtmeyer et al. 2016; Salama et al. 2004). Hence, the development and optimization of HtrA inhibitors are currently of high interest (Geppert et al. 2011; Lower et al. 2011; Perna et al. 2014, 2015). The application of an HtrA-specific small molecule

inhibitor indicated that HtrA significantly contributes to *H. pylori*-mediated E-cadherin ectodomain shedding and consequently, bacterial transmigration across an intact epithelial barrier (Hoy et al. 2010; Boehm et al. 2012). Based on the above HtrA cleavage sites in E-cadherin, a substrate-derived peptide inhibitor was also developed that selectively bound and inhibited HtrA, thereby blocking transmigration of *H. pylori* (Schmidt et al. 2016b). These studies imply that HtrA-mediated E-cadherin cleavage is a crucial step in the infection of *H. pylori* by opening intercellular adhesions locally allowing *H. pylori* access to the basolateral membranes of a polarized epithelium, where it can interfere with different host factors, which are necessary to promote the infection.

3.3 Direct Targeting of Tight and Adherens Junction Proteins by Changing Nuclear Responses

In *H. pylori*-infected patients with early-onset gastric cancer, somatic *cdh1* gene mutations and cytoplasmic β-catenin localization were observed indicating that E-cadherin-mediated AJs in gastric cancer patients were disrupted (Saito et al. 1999). Mislocalization of E-cadherin and catenins was also found in cultured and primary human epithelial cells through the detection of E-cadherin, β-catenin, α-catenin and $p120^{ctn}$ in intracellular vesicles upon infection with *H. pylori* leading to a destabilization of cell adherence (Conlin et al. 2004; Weydig et al. 2007; Krueger et al. 2007). This might be further supported by alterations of the E-cadherin expression. In patients, downregulation of E-cadherin expression was significantly associated with *H. pylori* infection in antral biopsy sections (Terres et al. 1998). Similar observations were made for α-catenin. Associated with an infection with *H. pylori*, mRNA levels of α-catenin were reduced in gastric cancer tissues (Yu et al. 2000). Together with additional cell adhesion molecules, E-cadherin expression was downregulated as monitored by RT-PCR and Western blot analyses (Lim et al. 2003). The downregulation of E-cadherin was further connected with promoter methylation in gastric mucosae from intestinal metaplasia, primary and metastatic cancer indicating that *H. pylori*-mediated promoter methylation might occur early in carcinogenesis (Chan et al. 2003; Perri et al. 2007). Eradication of *H. pylori* by antibiotics reverted hypermethylation in patients with chronic gastritis (Chan et al. 2006) and gastric cancer (Leung et al. 2006). The transcriptional downregulation of E-cadherin expression was also observed in cells, which were infected for 24 h with *H. pylori* in vitro (Lee et al. 2014b). Long-term infections stabilized the zinc-finger transcription factor and EMT marker protein Snail, which has been implicated in E-cadherin suppression via its binding to E-cadherin proximal promoter (Lee et al. 2014a). In MKN28 cells, *H. pylori* induced the suppression of claudin-7 that was regulated by elevated β-catenin and Snail levels. Comparably, Snail expression was elevated and claudin-7 levels were downregulated in *H. pylori*-infected gastric patients (Wroblewski et al. 2015).

Mechanistically, the transcriptional repressor Snail is stabilized in response to CagA/GSK-3 interaction through which GSK-3 activity is inactivated (Ngo et al. 2016; Sougleri et al. 2016).

Besides the transcriptional control of E-cadherin expression, impairing the E-cadherin-mediated AJ complex function activates cancer-associated signal transduction pathways. Cytoplasmic accumulated β-catenin can translocate into the nucleus where it can function as a cofactor for TCF/LEF transcription factors. Nuclear localization of β-catenin in *H. pylori*-infected AGS cells and after over-expression of CagA indicated that CagA might have a regulating effect in β-catenin-dependent signal transduction (Franco et al. 2005). Whether this reflects a physiological effect is questionable as AGS cells do not express E-cadherin, but show abnormal β-catenin localization. However, enhanced transactivation of *cdx1* in MKN28 cells (Murata-Kamiya et al. 2007) or *cyclin d1* in MDCK cells (Sokolova et al. 2008) was described acting as proliferation markers indicating that *H. pylori* infection can induce β-catenin-mediated TCF/LEF transactivation in E-cadherin-positive cells. However, the majority of reports indicate that CagA is not implicated in this process. Accordingly, β-catenin-mediated up-regulation of TCF/LEF-dependent transcription in MDCK cells was described as T4SS dependent, but CagA independent (Sokolova et al. 2008). A similar observation was made in a study investigating $p120^{ctn}$ functions in *H. pylori*-infected cells. Nuclear translocation of $p120^{ctn}$ relieved Kaiso-mediated transcriptional repression of *mmp-7*. This mechanism required a functional *cag*PAI, but not CagA expression (Ogden et al. 2008). If the increase of MMP-7 expression enhances E-cadherin shedding needs to be investigated in future, but represents a possible scenario.

In summary, these data imply that not only the function of E-cadherin within the AJ complex can be affected, but also its expression at the transcriptional level. Loss of function and downregulated expression of E-cadherin are strongly associated with invasive growth of gastric tumor cells, and *H. pylori* can deregulate various factors controlling E-cadherin and catenin functions. These findings indicate a coordinated deregulation of E-cadherin expression and function in *H. pylori*-infected gastric epithelial cells.

4 Multistep Model for *H. pylori*-Induced Epithelial Barrier Disruption

Through the expression of manifold pathogenic and virulence factors, *H. pylori* can colonize the gastric epithelial cells and interfere with host cell functions at several levels. Early events, such as bacterial motility, adherence and colonization, are indispensable as very first steps in the infection process and are discussed in other excellent reviews (Caron et al. 2015; Aspholm et al. 2006; Dubois and Boren 2007). Importantly, the progression of gastric cancer in *H. pylori*-infected patients is accompanied by the loss of cell polarity and the disruption of the epithelial

architecture, processes which are closely linked with cancer-associated signal transduction pathways. In this context, disruption of TJs and AJs is a hallmark of *H. pylori* infections and involves a combination of different bacterial factors that interact with host cell elements in a locally coordinated and phased manner assembling a complex multistep infection process.

Depolarization of *H. pylori*-infected epithelial cells requires efficient T4SS-mediated translocation of the oncoprotein CagA. The question whether CagA translocation occurs apically or basolaterally is still not answered. In vivo CagA translocation is difficult to prove. Hence, most studies describe an epidemiological association between CagA presence and *H. pylori*-dependent disorders. The knowledge of CagA-triggered depolarization and disruption of the epithelial barrier function mainly came from a series of studies on cultured non-polarized or only partially polarized tumor cells limiting the functional investigations of CagA. Until very recently, it was assumed that *H. pylori* can translocate CagA at the apical surface of non-polarized epithelial tumor cells without the requirement of a host receptor. In agreement with this idea, apical CagA delivery via phosphatidylserine and cholesterol was suggested as a possible mechanism (Murata-Kamiya et al. 2010) that together with infection and transfection studies in vitro implicated that CagA can directly target and disrupt intercellular junctional functions (Fig. 2). However, tyrosine-phosphorylated CagA reflecting its intracellular occurrence was primarily connected with the interaction of the T4SS adhesin CagL with the basolateral receptor $\alpha_5\beta_1$-integrin leading to the hypothesis that *H. pylori* needs to contact the basolateral membrane (Kwok et al. 2007; Saha et al. 2010; Tegtmeyer et al. 2010, 2014; Conradi et al. 2012a, b; Barden et al. 2013, 2014). Later on, additional T4SS components (CagY, CagI and CagA) were identified as β_1-integrin-interacting proteins (Jimenez-Soto et al. 2009) further supporting this hypothesis. However, whether apical and/or basolateral CagA translocation occurs in combination remains yet unknown. In this review, we proposed a complex multistep model covering several sophisticated processes to open intercellular TJs and AJs allowing the access of *H. pylori* to $\alpha_5\beta_1$-integrins summarizing CagA-dependent and CagA-independent mechanisms contributing to the loss of intercellular adhesions and epithelial depolarization.

5 Concluding Remarks

Gastric carcinogenesis is associated with *H. pylori*-induced signaling leading to depolarization of the epithelium. According to the multistep model, we hypothesize that the loss of intercellular adhesion is an important event and facilitates invasive growth of tumor cells. Mechanistically, disruption of TJs and AJs might allow efficient injection of the bacterial effector and oncoprotein CagA to derail cancer-associated signal transduction. Of course, *H. pylori*'s attack on intercellular adhesion is only one piece in the complex pathogenesis scenario, and the knowledge about the mechanisms is steadily increasing. It would also import to investigate in future whether and how other TJ and AJ components such as ZO-2, ZO-3,

cingulin, MUPP1, α-catenin, EPLIN, nectin or afadin may be affected during infection (Fig. 1). A combination of different bacterial factors is involved in gastric barrier disruption, and the direct interference of soluble *H. pylori* factors with components of the TJs and AJs came to attention in the last years as they represent highly attractive drug targets for novel intervention strategies to combat *H. pylori* infections. In addition to the apical–junctional complex, desmosomes and gap junctions are also important constituents, which impact to cell-to-cell interactions (Wei and Huang 2013). Future studies should also consider the investigation of these junctional protein platforms and their potential role in *H. pylori* infections.

Acknowledgements This work was supported by grants of the German Science Foundation (project B10 in CRC-796 and A4 in CRC-1181) to SB. The work of SW was supported by the grants P-24074 and P-24315 from the Austrian Science Fund (FWF).

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