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## Composition, structure and function of the *Helicobacter pylori* *cag* pathogenicity island encoded type IV secretion system

Steffen Backert<sup>\*1</sup>, Nicole Tegtmeyer<sup>1</sup>, and Wolfgang Fischer<sup>2</sup>

<sup>1</sup>Department of Biology, Division of Microbiology, Friedrich Alexander University Erlangen-Nuremberg, Staudtstr. 5, D-91058 Erlangen, Germany

<sup>2</sup>Max von Pettenkofer-Institute for Hygiene & Medical Microbiology, Ludwig Maximilians-University, D-80336 Munich, Germany

### Abstract

Many Gram-negative pathogens harbor type IV secretion systems (T4SS) that translocate bacterial virulence factors into host cells to hijack cellular processes. The pathology of the gastric pathogen *Helicobacter pylori* strongly depends on a T4SS encoded by the *cag* pathogenicity island. This T4SS forms a needle-like pilus, and its assembly is accomplished by multiple protein–protein interactions and various pilus-associated factors that bind to integrins followed by delivery of the CagA oncoprotein into gastric epithelial cells. Recent studies revealed the crystal structures of six T4SS proteins and pilus formation is modulated by iron and zinc availability. All these T4SS interactions are crucial for deregulating host signaling events and disease progression. New developments in T4SS functions and their importance for pathogenesis are discussed.

### Keywords

$\beta$ -catenin; CagA; CagL; *cagPAI*; CagY; c-Met; cortactin; EGF receptor; gastric disease; GTPase; *Helicobacter pylori*; integrin; MARK2/PAR1b kinase; signaling; T4SS; type IV secretion; tyrosine kinases; VirB proteins

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*Helicobacter pylori* is a human-specific pathogen that colonizes the hostile environment of the stomach. Approximately half of the world's population carries this Gram-negative bacterium, which is associated with a spectrum of gastric pathologies ranging from chronic gastritis to peptic ulcers and even gastric cancer in a subset of individuals [1,2]. Infection commonly appears in early childhood and, if not treated, *H. pylori* can persist throughout life. Evolutionary analyses indicate that *H. pylori* has been associated with modern humans for at least 100,000 years, possibly after the bacterium was first acquired by a host jump

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\* Author for correspondence: Steffen.Backert@fau.de.

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from an unknown source [3]. During this long time of coexistence, *H. pylori* developed various mechanisms of host immune evasion, and is now considered a paradigm for chronic infections [2]. The clinical outcome of infections is controlled by complex host–pathogen interactions, and influenced by factors such as genetic predisposition of the host, bacterial genotype and environmental parameters. Worldwide *H. pylori* strains are genetically diverse, and this variability affects virulence factors generating different degrees of pathogenicity potential. Well-known pathogenicity-associated processes include urease-triggered neutralization of acidic pH, flagella-mediated motility, expression of multiple adhesins (BabA/B, SabA, OipA, AlpA/B, HopQ, HopZ and others), cell damage caused by the vacuolating cytotoxin VacA, cleavage of E-cadherin by the protease HtrA as well as induction of apoptosis and inhibition of T-cell proliferation by  $\gamma$ -glutamyl transpeptidase [2,4].

One virulence factor that clearly enhances the risk of gastric cancer development is the *cag* pathogenicity island (*cagPAI*), a typical chromosomal DNA insertion element [5]. The *cagPAI* was discovered in 1996 and its presence discriminates highly virulent *cagPAI*-positive (type-1) from less virulent *cagPAI*-negative (type-2) strains [6]. The *cagPAI* is about approximately 40 kb in size and contains 27–31 genes, including *cagA* and others which encode proteins forming a type IV secretion system (T4SS) [7]. The *cagPAI* was acquired by a single horizontal DNA transfer event about 60,000 years ago, and its maintenance until today suggests that the T4SS provided *H. pylori* with evolutionary advantages [8]. This T4SS forms a needle-like surface appendage (T4SS pilus), which is induced upon host contact [9]. The *cagT4SS* exports CagA from adherent *H. pylori* across the bacterial and epithelial membranes into the host cell cytoplasm [10,11]. CagA itself has originally been recognized as an immunodominant antigen [12–15]. Patients that are seropositive both for *H. pylori* and CagA harbor a 5.8-fold higher risk of developing intestinal and diffuse gastric adenocarcinoma compared with uninfected persons, whereas individuals infected with CagA-negative type-2 strains are only at a 2.2-fold higher risk of developing distal gastric adenocarcinoma compared with uninfected persons [16]. Thus, the *cagT4SS* is a strong predictor of severe disease outcome. Here we review recent developments in the composition, structure and function of the *cagT4SS* with an emphasis on its role in *H. pylori* pathogenesis.

## Composition of the *cagT4SS*

T4SSs are found in many Gram-negative as well as Gram-positive bacteria. They are evolutionarily related to DNA conjugation machineries, but are exploited by several pathogenic bacteria including *Agrobacterium*, *Bartonella*, *Bordetella*, *Brucella*, *Helicobacter* and *Legionella* to deliver DNA or protein substrates into a wide range of eukaryotic target cells [17]. A T4SS apparatus commonly consists of 11 VirB proteins, encoded by the *virB1–virB11* genes in the prototypical T4SS of *Agrobacterium tumefaciens*. By contrast, 15–16 different components of the *cagT4SS* are required for its two major phenotypes, induction of IL-8 secretion from epithelial cells and translocation of the CagA protein. The *cagT4SS* contains proteins with detectable sequence similarity to all VirB proteins (Table 1), and additionally five essential components without any similarity to

known proteins, making this system clearly distinct from canonical T4SSs in other bacteria [11].

Assuming that those proteins with sequence similarity to components of other T4SSs assemble in an analogous manner to the Trw T4SS of conjugative plasmid R388, for which the structure has recently been elucidated [31], we propose that the *cagPAI* secretion apparatus consists of a core complex assembled by CagT (VirB7), CagX (VirB9) and CagY (VirB10), and of an inner membrane complex comprising CagE (VirB3/B4), CagW (VirB6) and CagV (VirB8) (Figure 1A). However, the outer membrane-associated part of the *cagT4SS* additionally contains two essential *cagPAI*-specific components, CagM [32] and Cag3 [33], which would have to co-assemble with CagT, CagX and CagY into an extended core complex. Likewise, the inner membrane complex might additionally contain a *cagPAI*-specific protein, CagU, for which no protein interactions within the system have been described so far [32]. One further essential component with a predicted transmembrane helix, CagH, is instead considered to be involved in T4SS pilus formation, which might also involve other essential proteins such as CagC [34], CagL [9,29] or CagI [29] (see below). Further essential components are the putative peptidoglycan hydrolase Cag4/Cag $\gamma$ , and the cytoplasmic ATPase Cag $\alpha$  (VirB11) [30].

For translocation of the CagA protein, the VirD4-like coupling protein Cag5/Cag $\beta$  and the *cagPAI*-specific translocation factors CagF, CagZ (and possibly CagD), are required. Cag $\beta$  is thought to be responsible, probably together with its interaction partner CagZ, for recognition of the CagA T4SS signal sequence [11]. CagF is considered as a CagA secretion chaperone, and recent evidence shows that CagF forms a high-affinity, 1:1 complex with CagA, in which different domains of CagA contribute to binding [36].

## T4SS pilus formation

Most of the well-characterized T4SSs (particularly the DNA-transporting systems) produce surface pili that are typically composed of a major and a minor pilin subunit [17]. Therefore, it was no surprise that pilus-like surface structures associated with the *cagT4SS* could be identified by electron microscopy studies (Figure 1B). These structures are probably induced by contact with host cells and seem to be more irregular than classical pili, and there is some evidence that they are sheathed [9,26,29,37]. The *cagT4SS* pili could be stained with antibodies directed against several components, including CagY, CagX, CagT and CagL [9,26,37]. For CagY, CagX and CagT, this finding seems to contradict their involvement in the putative core complex, but it should be noted that all three proteins are considerably different from their canonical orthologs and probably contain additional domains with as yet unknown functions. Since CagL has been detected on T4SS pili and since it interacts with CagI and CagH [25,29], it is possible that in fact all three proteins are associated with T4SS pili, although neither CagI nor CagH localization to pili has been experimentally demonstrated. Deletion of *cagI* or *cagL* genes results in a loss of pilus formation, whereas a hyperpilated phenotype with longer and thicker pili has been described for the *cagH* mutant [29]. Finally, immunogold stainings also showed the presence of CagA at the tips of the T4SS pili, providing first direct evidence that CagA can be delivered through these surface appendages [9].

Despite the association of different proteins with *cagT4SS* pili, the major subunit of these pili is still unknown. Since one essential component of the *cagT4SS*, CagC, is an ortholog of the canonical major pilin VirB2, this protein has been assumed to fulfil a similar function, and immunogold stainings showed its presence at the bacterial surface [34]. However, a recent study in which a *cagC* mutant was found to produce similar amounts of pili as the wild type, has challenged the notion that CagC is the major pilin [28]. While pilus production has been reported to be independent of CagY as well [27], Cag3, CagX, CagT, CagV and CagM were described to be necessary for pilus formation [28]. These findings cannot be accommodated in a standard model of T4SS pilus composition and assembly. Further studies are therefore required to elucidate the constituents and the assembly of *cagT4SS* pili.

Formation of pili seems to be regulated by the host and environmental conditions. For instance, iron limitation induces the *cagT4SS* to produce more pili and to translocate more CagA [38]. Interestingly, injection of CagA into polarized cells by an active T4SS has been shown to facilitate apical surface colonization by recruiting iron [39]. On the contrary, a recent study has shown that depletion of zinc ions, as it would occur in the presence of the neutrophil effector calprotectin, reduces the number of T4SS pili and thus inhibits CagA translocation [40].

### Interaction of T4SS proteins with host cell receptors

Comparative analysis of *cagPAI* sequences from different geographic *H. pylori* populations showed a high overall gene conservation, but suggested that some genes (*cagY*, *cagQ*, *cagN*, *cagL*, *cagI*, *cagC* and *cagA*) have most likely evolved under diversifying selection [8], suggesting a surface localization of the corresponding proteins and their recognition by the immune system. Such a selective pressure, possibly exerted by the immune response, could be more directly demonstrated for recombination events in CagY with impact on its proinflammatory (IL-8 inducing) activity [27]. Interestingly, some of the *cagPAI* proteins under evolutionary pressure, including CagL [9,41], CagY, CagI and CagA [42], have also been identified as proteins capable of interacting with integrin molecules as receptors on host cells (Figure 2). Specifically, CagL has been shown to bind to integrins  $\alpha_5\beta_1$  and  $\alpha_V\beta_5$  [9,43]; for CagY, CagI and CagA, interactions with integrin  $\alpha_5\beta_1$  have been demonstrated [42]. For binding to integrin  $\alpha_5\beta_1$  and to epithelial cells, an RGD motif within CagL is necessary [9,21], but the requirement of this motif for T4SS functionality is controversial. Consequences of integrin binding by CagL are an activation of the nonreceptor host cell kinases FAK and c-Src in the case of integrin  $\alpha_5\beta_1$  [41] or an upregulation of gastrin expression in the case of integrin  $\alpha_V\beta_5$  (Figure 2) [43].

The finding of CagA at the bacterial surface or at the pilus tips [9,42] suggested that surface-associated CagA might be a translocation intermediate, although the CagA protein does not enter cells autonomously in the absence of *H. pylori*. In support of this notion, recombinant CagA fragments, which bind to integrin, can block CagA translocation by *H. pylori* when added to bacteria-epithelial cell cocultures [23]. As a further aspect, CagA has also been shown to bind to phosphatidylserine exposed to the outer leaflet of the target cell membrane upon infection, and site-specific CagA mutants that are unable to bind this phospholipid are

translocated at very low levels [45]. It remains to be determined, however, how integrin binding and phospholipid interaction contribute to translocation of CagA across the target cell membrane.

## Structural analyses of *cagT* 4SS proteins

A major contribution to our current understanding of T4SS transporters came from unraveling the structure of a T4SS core assembly from conjugative plasmid pKM101, a large approximately 1-MDa complex composed of three structural VirB/D proteins, which are embedded in the bacterial double membrane [46]. Moreover, recent determination of the structure of approximately 3-MDa assembly of eight T4SS components from plasmid R388 shows intriguing details and revolutionized our view of molecular T4SS architecture [31]. In addition to these major findings, the crystal structures of six *H. pylori* T4SS proteins have been reported (Table 1). Cag $\alpha$  (VirB11) forms a hexameric ring in conjunction with the HP 1451 regulator [18]. This complex operates as a gating factor in the inner bacterial membrane, which cycles between ATP binding/hydrolysis resulting in closed and open VirB11 conformations [18]. The crystal structure of CagD unraveled a covalent dimer in which each monomer folded as a single domain comprising three  $\alpha$ -helices and five  $\beta$ -strands [22]. The structures of CagZ, a 23-kDa protein involved in the translocation of CagA, and CagS, a 23-kDa protein encoded by a conserved *cagPAI* gene whose function remains elusive, have also been solved [19,20]. In addition, CagL forms an elongated four- $\alpha$ -helix bundle, but revealed substantial flexibility in seven crystallographically independent structures [21]. The RGD-motif is surface exposed but located within a long  $\alpha$ -helix, which is unprecedented as previously characterized integrin binding RGD motifs are found within extended or flexible loops. Intramolecular disulfide bonds engineered to reduce CagL flexibility resulted in a more stable protein, but were unable to support cell adhesion, suggesting that CagL may partly unfold for integrin  $\alpha_5\beta_1$  receptor binding [21].

Moreover, two groups reported crystal structures of an approximately 100-kDa amino-terminal fragment of the effector protein CagA [23,24]. Three distinct domains were identified, giving insights into intramolecular and intermolecular interactions. Domain-I constitutes a mobile N-terminus, whereas domain-II tethers CagA to the plasma membrane through binding of phosphatidylserine [24] or facilitates integrin  $\alpha_5\beta_1$  engagement [23]. Domain-III inter-acts intramolecularly with the intrinsically disordered C-terminal domain, and this interaction potentiates the pathogenic scaffold/hub function of CagA [24]. In addition, the co-crystal structure of a subdomain of CagA with the human kinase MARK2/PAR1b was solved, showing that a CagA peptide (amino acids 885–1005) mimics substrates of this kinase family, resembling eukaryotic protein kinase inhibitors [47]. Further studies showed that CagA-MARK2 binding is necessary to induce cell polarity defects [48]. Co-crystals were also obtained for the association of CagA with apoptosis-stimulating protein of p53-2 (ASPP2), a human tumor suppressor [49]. A specialized three-helix bundle at the N-terminus of CagA binds to a 7-kDa proline-rich sequence of ASPP2 and disruption of this interaction decreased survival of *H.pylori*-infected cells [49]. These reports underscore that structural data are crucially important for understanding the functions of CagA and other T4SS proteins (Figure 2).

## Delivery & function of T4SS effectors

Clinical studies and animal infection models have shown that the presence of *cag*T4SS and CagA is associated with the development of gastric disease [1–2,16]. Interestingly, Mongolian gerbils infected with wild-type *H. pylori* exhibit gastric pathology similar to infected humans and approximately 75% of the animals developed gastric adenocarcinomas after only 8 weeks of infection, while this was not the case with isogenic *cagA* or T4SS mutants [38,50]. In addition, the first direct causal link between CagA and oncogenesis was reported in CagA-expressing transgenic mice, which developed polyps and adenocarcinomas in the stomach after 72 weeks [51]. Thus, CagA and the T4SS play central roles during *H. pylori* pathogenesis *in vivo*. Most of the functional studies, however, were performed with infected cultured gastric cells *in vitro*. These studies revealed profound T4SS activities that were either CagA dependent or CagA independent, and provided fundamental insights in disease-associated signaling events [4,52]. Some of these important signaling pathways are highlighted in Figure 2.

First, upon delivery, CagA localizes to the plasma membrane and undergoes phosphorylation (CagA<sup>PY</sup>) by c-Src and c-Abl tyrosine kinases targeting EPIYA-sequence motifs [53]. Intracellular CagA can then interact with at least 24 host proteins in phosphorylation-dependent or -independent manners [10,54]. Besides the two binding partners (ASPP2 and MARK2/PAR1b) discussed above, other studies have shown that CagA<sup>PY</sup> also binds to and activates phosphatase SHP-2, triggering gastric epithelial cell elongation by stimulating the Rap1→B-Raf→ERK1/2 signaling pathway [55] and serine-phosphorylation of the actin-binding protein cortactin [56]. CagA can also recruit the tight junction proteins ZO-1 and JAM leading to disturbed cell junctions [57]. Injected CagA has been also found to bind the c-Met receptor which is activated in this scenario, and triggers cell scattering involving the small Rho GTPases Rac1 and Cdc42 [58]. This signaling also requires the activity of c-Abl, the adapter protein CrkII and CagA<sup>PY</sup> which form a trimeric complex upstream of the GTPases [59,60]. Furthermore, CagA has been reported to promote proliferative and antiapoptotic gene regulation via activation of growth factor receptors,  $\beta$ -catenin, and other signaling molecules (Figure 2). Consequently, the uncontrolled cellular growth in combination with the disruption of epithelial integrity by CagA can potentiate pre-cancerous changes in the stomach and explains the high incidence of gastric adenocarcinoma in patients infected with CagA-positive *H. pylori*.

Second, the T4SS-dependent but CagA-independent effects mainly comprise the induction of transcription factor NF- $\kappa$ B [61]. *H. pylori* induces a transient activation of the inhibitor of NF- $\kappa$ B (I $\kappa$ B $\alpha$ ) kinase (IKK) complex [62]. IKK $\alpha$  and IKK $\beta$  expression stabilizes the regulatory IKK complex subunit NF- $\kappa$ B essential modulator, and this is tightly controlled by two kinases, MEKK3 and TAK1 [62]. The activation of NF- $\kappa$ B finally results in the induction of proinflammatory cytokines such as IL-8 and TNF- $\alpha$  (Figure 2). However, the activation of NF- $\kappa$ B also suppresses gastric acid secretion. The mechanism of the latter involves CagL binding to integrin  $\alpha_5\beta_1$  which induces the dissociation of the metalloprotease ADAM-17 from this receptor complex [63]. This resulted in the activation of ADAM-17 and EGFR, followed by NF- $\kappa$ B-dependent transcriptional suppression of H, K-ATPase ( $\alpha$ -subunit), the central enzyme controlling gastric acid secretion (Figure 2).



Indeed, hypochlorhydria is commonly diagnosed during acute phase of infections, and thus, CagL-triggered cell signaling significantly contributes to *H. pylori* pathogenesis. Together, these reports validate that *H. pylori* subvert multiple cellular key processes by structural T4SS proteins and/or yet unknown effector(s).

## Conclusion & future perspective

Research on the *H. pylori* T4SS has provided us with fascinating insights into its biology and function, however, many open questions remain. It was established that the *cagPAI* plays a crucial role in gastric disease development and various involved mechanisms have been clarified over the years. The structure of the *cagT4SS* pilus has been visualized and recombination in genes like *cagY* was shown to ‘turn on’ or ‘turn off’ T4SS functions [27]. However, the composition of the *cag* pilus is still not clear. The surprising finding that *cagC* mutants still produce T4SS pili like wild-type *H. pylori* [28] is not compatible with current T4SS models in other bacteria [17]. Thus, the molecular assembly of the *cagT4SS* pilus needs to be rigorously reinvestigated. In addition, several T4SS-associated adhesins for integrin  $\alpha_5\beta_1$  were discovered, including CagL and CagY. It will be important to examine how these proteins are transported to the pilus surface and in which way the various integrin  $\alpha_5\beta_1$  interactions are regulated. Furthermore, much remains to be learned about when and how CagA is delivered into cells. The discovery of a T4SS receptor, suggests the existence of a sophisticated control mechanism for CagA injection. Importantly, integrins are normally present at basal cell surfaces and not apical sites. Thus, we need to investigate how *H. pylori* can make contact with integrins in polarized cells. The situation becomes even more complex considering very recent reports indicating that various factors encoded outside of the *cagPAI* (such as *hopQ* and LPS genes) are also necessary for T4SS-dependent delivery of CagA or NF- $\kappa$ B activation [64]. How this works is fully unclear yet and requires further investigation. It will also be interesting to find out if the *cagT4SS* translocates substrates other than CagA. Delivery of peptidoglycan is an intriguing possibility [65], but follow-up studies on T4SS-dependent peptidoglycan transportation are required. Finally, the evolutionary advantage of the T4SS for *H. pylori* needs to be uncovered. Potentially, reduction of gastric epithelial cell turnover by CagA and getting access to iron could be adaptive advantages for *H. pylori*, but this should be further studied. It therefore appears that the *cagT4SS* will continue to be an attractive research topic in future.

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### EXECUTIVE SUMMARY

- The type IV secretion system encoded by the *cag* pathogenicity island of *Helicobacter pylori* represents a major disease-associated factor involved in gastric cancer development.
- The *cag*T4SS forms a specific T4SS pilus structure which is induced upon host cell contact.
- T4SS assembly is accomplished by multiple *cag* pathogenicity island protein-protein interactions.
- Delivery of CagA in host target cells requires the expression of a host cell receptor, which was identified as the integrin member  $\alpha_5\beta_1$
- Several pilus-associated proteins (CagA, CagI, CagL and CagY) were identified to interact with integrin  $\alpha_5\beta_1$ , which is required for delivery of CagA into target cells.
- Translocated CagA is phosphorylated and activated by oncogenic c-Src and c-Abl tyrosine kinases of the host.
- Intracellular CagA can interact with at least 24 host cell proteins in a phosphorylation-dependent and -independent fashion.
- In this way, CagA mimics several host cell proteins to hijack intracellular signaling cascades.
- Studies in transgenic mice showed that CagA is the first bacterial oncoprotein.
- The route of CagA delivery in polarized gastric epithelial cells remains unclear.
- More work needs to be done to clarify if CagA is delivered at apical and/or basolateral surfaces and the evolutionary advantage of the T4SS.









delivery of CagA into the cells occurs at apical and/or basolateral sites, the integrin receptors were indicated at both locations. The various bacterial effectors and host signaling molecules are explained in the bottom box. For more details see text.

T4SS: type IV secretion system.

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**Table 1**  
 Nomenclature, localization and functional importance of T4SS proteins encoded by the *cagPAI* of *Helicobacter pylori*.

Gene name <sup>†</sup>	Protein name <sup>‡</sup>	Size (kDa) <sup>†</sup>	Subcellular localization <sup>§</sup>	Structure data	Phospho CagA <sup>¶</sup>	IL-8 secretion <sup>#</sup>	T4SS pilus formation <sup>††</sup>	Proposed function <sup>§</sup>
<i>hpa0520</i>	CagZ/Cag1	13	IM	n.d.	+++	+++	n.d.	AF
<i>hpa0521</i>	CagE/Cag2	10	Cyt	n.d.	+++	+++	n.d.	AF
<i>hpa0522</i>	CagD/Cag3	50	OM	n.d.	-	-	-	OM complex
<i>hpa0523</i>	CagV/Cag4	18	Per	n.d.	-	-	n.d.	VirB1, PGN hydrolase
<i>hpa0524</i>	CagB/Cag5	80	IM	n.d.	-	+++	+++	VirD4, coupling factor
<i>hpa0525</i>	CagO/VirB11	35	Cyt, IM	Yes [18]	-	-	+++	VirB11, NTPase
<i>hpa0526</i>	CagZ	21	Cyt, IM	Yes [19]	++	++	n.d.	CagB stabilization
<i>hpa0527</i>	CagY	220	IM, OM, SA	n.d.	-	-	-	VirB10, core complex, integrin binding
<i>hpa0528</i>	CagX	55	IM, OM, SA	n.d.	-	-	+++	VirB9, core complex
<i>hpa0529</i>	CagW	55	IM	n.d.	-	-	n.d.	VirB6, IM channel
<i>hpa0530</i>	CagV	27	IM	n.d.	-	-	-	VirB8, core complex
<i>hpa0531</i>	CagU	23	IM	n.d.	-	-	n.d.	AF
<i>hpa0532</i>	CagT	30	OM, SA	n.d.	-	-	-	VirB7, core complex, OM lipoprotein
<i>hpa0534</i>	CagS	21	Cyt	Yes [20]	+++	+++	n.d.	AF
<i>hpa0535</i>	CagQ	14	IM	n.d.	+++	+++	n.d.	AF
<i>hpa0536</i>	CagP	13	IM	n.d.	+++	+++	n.d.	AF
<i>hpa0537</i>	CagM	40	OM	n.d.	-	-	-	OM complex
<i>hpa0538</i>	CagN	32	Per, IM	n.d.	+	+	n.d.	Accessory factor
<i>hpa0539</i>	CagL	26	Per, SA	Yes [21]	-	-	-	Pilus biogenesis, VirB5-like, integrin targeting
<i>hpa0540</i>	CagI	40	Per, SA	n.d.	-	-	-	Pilus biogenesis, integrin targeting
<i>hpa0541</i>	CagH	39	IM	n.d.	-	-	+++	Pilus biogenesis
<i>hpa0542</i>	CagG	16	Per	n.d.	-	++	n.d.	AF
<i>hpa0543</i>	CagF	29	Cyt, IM	n.d.	-	+++	n.d.	Chaperone of CagA

Gene name <sup>†</sup>	Protein name <sup>‡</sup>	Size (kDa) <sup>†</sup>	Subcellular localization <sup>§</sup>	Structure data	Phospho CagA <sup>¶</sup>	IL-8 secretion <sup>#</sup>	T4SS pilus formation <sup>††</sup>	Proposed function <sup>§</sup>
<i>hpo544</i>	CagE	100	IM	n.d.	-	-	_##	VirB3/VirB4, NTPase
<i>hpo545</i>	CagD	22	IM, Per, SA	Yes [22]	+	+++	n.d.	Accessory factor
<i>hpo546</i>	CagC	13	IM, OM, SA	n.d.	-	-	+++¶¶	VirB2, pilus subunit?
n.d.	CagB	8	Cyt	n.d.	n.d.	n.d.	n.d.	AF
<i>hpo547</i>	CagA	125	Cyt, SA	Yes [23,24]	-	+++	n.d.	Translocated effector

<sup>†</sup> Gene designations and corresponding protein sizes are according to *H. pylori* strain 26695 [GenBank:AE000511.1].

<sup>‡</sup> Cag protein designations are according to a previous study [11].

<sup>§</sup> Subcellular localization of *cagPAI* proteins and proposed functions were reviewed by a previous article [11].

<sup>¶</sup> Phosphorylation of CagA and IL-8 induction in the corresponding *cag* gene knockout mutants of strain 26695 are listed according to a previous study [23]. Abbreviations used for the observed responses: -: Abolished; +: Weak; ++: Moderate; +++: Strong (like wild-type).

<sup>#</sup> Corrected data with regard to requirement for IL-8 induction [25].

<sup>††</sup> T4SS pilus formation in the corresponding knockout mutants are according to numerous previous studies listed below. Abbreviations used for pilus formation compared with wild-type *H. pylori*: -; Abolished; +: Weak; ++: moderate; +++: Unchanged; ++++: Hyperpilated.

<sup>¶¶</sup> T4SS pilus formation in strain NCTC11637 and corresponding knockout mutants are according to Tanaka and coworkers [26].

<sup>§§</sup> Conflicting data exist with regard to T4SS pilus formation in the corresponding *cagY* knockout mutant in strain J166 [27].

<sup>¶¶¶</sup> T4SS pilus formation in strain 26695 and corresponding knockout mutants are according to Johnson and coworkers [28].

<sup>###</sup> T4SS pilus formation in strain 26695 and corresponding knockout mutants are according to Shaffer and coworkers [29].

<sup>†††</sup> T4SS pilus formation in strain P12 and the corresponding *cagL* knockout mutant are according to Kwok and coworkers [9].

<sup>††††</sup> Conflicting data exist with regard to requirement for CagA phosphorylation [22,30].

AF: Accessory factor with yet unknown function; Cyt: Cytoplasmic; IM: Inner membrane; n.d.: Not determined; OM: Outer membrane; Per: Periplasmic; PGN: Peptidoglycan; SA: Surface associated or secreted into the supramatant; T4SS: Type IV secretion system.