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Review

The role of CEACAMs *versus* integrins in *Helicobacter pylori* CagA translocation: a systematic review

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ABSTRACT

The delivery of *Helicobacter pylori* CagA into host cells was long believed to occur through the integrin cell surface receptors. However, the role of CEACAM receptors has recently been highlighted, instead. Here, we have categorized the existing experimental evidence according to whether deletion, upregulation, downregulation, or inhibition of the target ligands (T4SS or HopQ) or receptors (integrins or CEACAMs), result in alterations in CagA phosphorylation, cell elongation, or IL-8 production. According to our analysis, the statistics favor the essence of most of the T4SS constituents and the involvement of HopQ adhesin in all three functions. Concerning the integrin family, the collected data is controversial, but yielding towards it being dispensable or involved in CagA translocation. Yet, regarding cell elongation, more events are showing β1 integrin being involved, than αvβ4 being inhibitory. Concerning IL-8 secretion, again there are more events showing α5, β1 and β6 integrins to be involved, than those showing inhibitory roles for β1, β4 and β6 integrins. Finally, CEACAM 1, 3, and 5 are identified as mostly essential or involved in CagA phosphorylation, whereasCEACAM 4, 7, and 8 are found dispensable and CEACAM6 is under debate. Conversely, CEACAM1, 5 and 6 appear mostly dispensable for cell elongation. Noteworthy is the choice of cell type, bacterial strain, multiplicity and duration of infection, as well as the sensitivity of the detection methods, all of which can affect the variably obtained results.

The type IV secretion system (T4SS) exists in both Gram-negative and -positive bacteria. In the former group, it consists of 12 components: 11 VirB (VirB1-VirB11) and VirD4 proteins. Their nomenclatures were deduced from the T4SS of *Agrobacterium tumefaciens* [1]. The *cag* pathogenicity island, *cag*PAI, contains 27–31 genes (encoding the T4SS proteins) and is considered as the main virulence determinant of *Helicobacter pylori*, causing various gastrointestinal complications, especially gastric cancer [2]. The core complex of the T4SS system is composed of CagT (VirB7), CagX (VirB9) and CagY (VirB10) and its inner velum is composed of CagE (VirB3/B4), CagW (VirB6) and CagV (VirB8). The outer membrane complex of the T4SS, consists of CagM and Cag3, which co-assemble with the mentioned core complex components [3]. In addition, there is a component named CagU, located in the inner membrane, the function of which remains to be determined [4]. CagH, CagC [5], CagL, and CagI [6] are believed to be involved in the pilus formation of the T4SS apparatus. CagF is a chaperon protein, which binds to CagA and facilitates its secretion [7]. CagL is an essential component of the T4SS system, which plays an important role in pilus formation and attachment to the host cell receptors [8]. Thus, CagL is considered as a key protein of *H. pylori,* involved in the translocation of CagA [9].

The integrin family is a group of cell adhesion receptors that consists of 24 members of αβ heterodimers, constituted of 18 α and 8 β subunits, which play a critical roles in the attachment of cells to their extracellular matrix (ECM) and are involved in cell-cell interactions [10,11]. Only 8 of the integrin heterodimers recognize the RGD motif in their ligands (α5β1, α8β1, αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, and αIIbβ3) [11]. The β1, β2, and αv integrins are the largest groups in integrin classification and no homology has been identified between α and β subunits [11]. Integrins are widely expressed on nearly every nucleated cell. However, their expression is dynamically regulated and changes rapidly, when cells leave their normal situation [11]. Each integrin dimer has its own specific function. CagL [8,12], CagY, CagI and CagA [13] have been shown to interact with integrin receptors on the host cells. CagL specifically binds to $\alpha_5\beta_1$, $\alpha_v\beta_5$ and $\alpha_v\beta_6$ integrin receptors on the cell surface

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[8,9,14]. CagA also interacts with $\alpha_5\beta_1$ integrin receptors, as demonstrated by surface plasmon resonance analysis [13].

H. pylori binding to gastric epithelial cells is the first step in bacterial colonization, which is carried out by the outer membrane proteins (OMPs) [15]. Following whole genome sequencing of *H. pylori* in 1997, 32 genes pertaining to bacterial OMPs were discovered [16]. These surface proteins include BabA, SabA, OipA, AlpA, AlpB, and HopQ, among others, which may play a major role in the cell binding of *H. pylori* and thereby support the transfer of virulence factors, including the CagA oncoprotein, into the interior of gastric epithelial cells *via* the T4SS syringe [17].

HopQ, an *H. pylori* OMP, has recently come into focus and is known to specifically interact with the carcinoembryonic antigen related cell adhesion molecules (CEACAMs), expressed on the human gastric epithelial cells [18]. Unlike other Hop proteins, the binding of HopQ to its receptor is glycan-independent [19,20]. There are two allelic types of HopQ; type I (1926 bp, 642 aa) and type II (1899 bp, 633 aa), which are ~70 % similar [21]. The HopQ type I allele, which has two subtypes (IA and IB) [22], is mostly present in *cagA*$^+$/*vacA s1 H. pylori* strains [21]. The extracellular domain of HopQ is sufficient to interact with the N-terminal domain of CEACAM1 [19]. The affinity (K_D value) of HopQ for the N-domain of CEACAM1, 3, and 5 are 23 ± 1, 268 ± 4, and 61 ± 3 nM, respectively, suggesting the highest affinity of HopQ for CEACAM1 [20]. HopQ I and HopQ II both interact with the same interaction site on CEACAM1, with the difference that HopQ II-C1ND forms fewer hydrogen bonds, as compared to HopQ I-C1ND [23]. However, the affinity of HopQ II (K_D of 69) for the C1ND domain of CEACAM1 is about six-fold higher than the affinity of HopQ I (K_D of 417) [23].

The CEACAM receptors are members of the carcinoembryonic antigen (CEA) family, belonging to the immunoglobulin superfamily, with diverse tissue distributions [24]. Twelve members of the CEACAM family have been characterized. The N-terminal immunoglobulin variable domain (IgV) of CEACAMs is the main constituent for ligand interaction [25]. CEACAM1 is expressed on epithelial and endothelial cells, as well as leukocytes and T cells [26,27]. CEACAM5 and CEACAM7, however, are solely expressed on epithelial cells and CEACAM6 is expressed on epithelial cells and granulocytes. Granulocytes also express CEACAM3 and CEACAM8 [24]. In *H. pylori*, HopQ binds strongly and specifically to the extracellular domain (N-terminal) of CEACAM1, CEACAM3, CEACAM5, and CEACAM6, but not to CEACAM4, 7 or 8 [19].

1. Goal

We have conducted this systematic review to explore the evidence supporting the roles of the above-mentioned ligands and receptors in: A) CagA translocation, B) cell elongation and C) IL-8 production.

2. Methods

To address our goal, we followed the criteria of the Preferred Reporting Items Statement for Systematic Reviews and Meta-Analysis (PRISMA) [28]. Thus, this study follows four main sections including, inclusion and exclusion criteria, search and data sources, study selection and data extraction.

2.1. Inclusion and exclusion criteria

The inclusion criteria for the selection of the extracted articles were that the study focused on: (1) *H. pylori* and (2) CagA translocation, cell elongation or IL-8 production, associated with HopQ, T4SS constituents, integrin or CEACAM whole gene or protein manipulations. The exclusion criteria included publications: (1) in non-English languages; (2) focusing on genes/proteins other than HopQ, T4SS, CEACAM or integrins; and (3) in which CagA translocation, cell elongation or IL-8 production were not studied.

2.2. Search and data sources

Search for the relevant papers consisted of identifying the keywords, formulating the search strategy, and selecting data sources. Keywords were identified based on our objectives, and searches were made using MeSH to find synonymous keywords. For the purpose of brevity, only articles published since the year 2000 were included.

2.3. Selection of the studies

EndNote X9 was used as reference management software for removing duplicate articles. Our inclusion/exclusion criteria resulted in 63 papers, which were used for data extraction.

2.4. Data extraction

The data of the selected papers were collected and categorized based on the impact of the deletion/addition of ligand (T4SS constituents/HopQ) or receptor (integrin/CEACAM) on CagA phosphorylation (CagA-P), cell elongation and IL-8 production. The resulting information was inserted into Tables 1–4

2.5. Cell line receptor expression data

The baseline RNA expression levels of integrin and CEACAM receptors on the studied cell lines were extracted from the Expression Atlas (https://www.ebi.ac.uk/gxa/home), and OncoExpress database in CrownBio (https://www.crownbio.com/). In brief, the name of each receptor in the species *Homo sapiens* and *Mus musculus* were used as queries in the Expression Atlas and the results for the normal stomach, gastric adenocarcinoma and the target cell lines, were all downloaded in FPKM format (fragments per kilobase of exon model per million mapped reads) and exported into an excel sheet. For the OncoExpress database in CrownBio, the name of each cell line was used as a query and then each receptor name was submitted. The expression levels were documented as Log2 FPKM, which were converted into FPKM. Missing information was extracted from pertinent reports, if available (Table 5). The receptor expression levels were categorized as 1) high (above 1000 copies), 2) medium (100–1000 copies), 3) low (10–100 copies) and 4) no (0–10 copies).

3. Results and discussion

Our strategy was to thoroughly analyze the studies, in which "inactivation" measures were used. These include: 1) the deletion of the "target ligand" (i.e., *cagL/I/M/H* or *HopQ* gene) from the infecting *H. pylori* strain or blocking/inhibiting the resulting gene product, or 2) knocking out, downregulating the expression of, or inhibition of the "target receptor" (i.e. integrins or CEACAMs) in the recipient cell line. The effects of these manipulations were further analyzed by "activation" studies: 1) complementation of the depleted strains, with the wild-type gene or 2) de novo/overexpression of integrins/CEACAMs in the deficient cell lines, while monitoring their effects on 1) CagA translocation, 2) cell elongation and/or 3) IL-8 production. It must be kept in mind that the cell elongation phenotype as a downstream function of CagA-P, can only be detected in AGS cells. Every *H. pylori* strain/cell line/time point, was considered as one event. The deduced roles were

classified as: a) "Essential" (lost or induced function, upon deletion/inhibition or addition of the target gene/protein, respectively), b) "Involved" (reduced or increased function upon deletion/inhibition or addition of the target gene/protein, respectively) c) "Dispensable" (no alterations) or d) "Inhibitory" (lost or induced function, upon addition or deletion/inhibition of the target gene/protein, respectively).

3.1. Ligands

3.1.1. The role of T4SS constituents in CagA phosphorylation, cell elongation and IL-8 production

T4SS apparatus is categorized as the core complex, the pilus, the energetic components, the translocation associated factors, lytic transglycosylase, as well as the substrate CagA [3].

The data presented in Table-1 and Figure-1 indicate that of the T4SS core complex, *cagH* (*hp0541*), *cagM* (*hp0537*), *cagT* (*hp0532*), *cagU* (*hp0531*), *cagV* (*hp0530*), *cagW* (*hp0529*), *cagX* (*hp0528*), *cagδ*/*cag3* (*hp0522*) and *cagY* (*hp0527*) are essential for CagA translocation (Fig. 1A). On the other hand, the data on the role of the membrane-associated *cagN* (*hp0538*) of the T4SS core complex in CagA-P, seems controversial. In other words, two studies found it involved in the 26695/AGS context [88,29,30], while another study declared it dispensable for CagA-P, as well as cell elongation in the G27/AGS context [31] (Figure-1A). The two studies on cell elongation have found *cagM* [89,32], *cagT* [32] and *cagY* [33] of the core complex essential for this function (Figure-1B).

Of the T4SS pilus components, *cagC* (*hp0546*), *cagI* (*hp0540*), *cagL* (*hp0539*), in addition to the previously mentioned *cagY* and *cagH*, are found essential for CagA-P (Fig. 1A). Interestingly, these three T4SS constituents (CagL, CagI, and CagH) are co-purified and together take part in pilus formation [6], while the former two create a late-stage functional complex [34]. Mutations in the critical amino acids (Y58/E59) of CagL, fully abrogate CagA delivery and may act as a molecular control switch [35]. However, this phenomenon was not further confirmed in Y58E59, D58K59, D58E59, N58E59 or N58K59 CagL isogenic mutant strains of *H. pylori* [36]. Interestingly, the RGD motif in CagL is inaccessible at low pH, which again becomes available at neutral pH to allow for receptor binding [37]. As for cell elongation, the data available on *cagI*, *cagH* and *cagL* finds them essential, except for one study, which has found reduced cellular elongation following infection of AGS cells with *cagL*-deleted G27 *H. pylori* strain [77,38] (Figure-1B). This is in accordance with the fact that an RGD helper sequence (RHS: FEAN), near the RGD sequence on CagL appears to be responsible for the cortactin-induced cell elongation, at early time points after infection [39].

Of the energetic components; *cagE* (*hp0544*), *cagα* (*hp0525*), and *cagβ*/*cag5* (*hp0524*) are essential for CagA-P (Fig. 1A). Regarding cellular elongation, two studies have found *cagE* [40] and *cagβ* [41] as essential (Fig. 1B).

Of the translocation-associated factors, *cagF* (*hp0543*), otherwise known as CagA-chaperone [29,42,43], in addition to the previously mentioned *cagβ*, was found essential for CagA-P. *cagZ* (*hp0526*) of the translocation-associated factors, was found essential for CagA-P and cell elongation in the P12/AGS context [41] and involved in CagA-P in the 26695/AGS context [29] (Table-1). And the limited evidence [18,29,80] on *cagγ*/*cag4* (*hp0523*) declares this lytic transglycosylase to be essential for CagA translocation (Fig. 1A). There is no available information on its role in cell elongation.

As for IL-8 production, based on the limited data available, some T4SS elements, seem essential for IL-8 production, without which this function is impaired (Table-1, Fig. 1C). These include *cagH*, *cagU*, *cagV* and *cagW* from the T4SS core complex and cag*α* from the energetic components. In addition, there is another set, for which data is declaring them essential and/or involved in IL-8 production. These include *cagM*, *cagN*, *cagT*, *cagX* and *cagδ* of the core complex, *cagI* and *cagL* of the pilus components, *cagE* of the energetic components, and cagZ of the translocation-associated factors, as well as the lytic transglycosylase, cag*γ* (Fig. 1C).

The C-terminal coiled-coil region of the CagL protein appears to be essential for this function [44]. IL-8 production is also induced in primary human endothelial (HUVEC and EA.HJ926) cells, which normally produce an estimated 10–20 fold higher level of IL-8 upon *H. pylori* infection, as compared to gastric epithelial cells [45]. However, once *cagL* gene is inactivated in the infecting *H. pylori* (P12) strain, the level of IL-8 produced by these cells, drops significantly [45]. This observation was repeated in the bile duct (KKU-100 and KKU-M156) and kidney (HEK293) epithelial cells, infected with *cagL* [46] and *cagM*-mutant 251 *H. pylori* strains, respectively [47].

There is another set of T4SS constituents, for which there is controversial data, some declaring them essential or involved, yet others finding them dispensable for IL-8 production. These include *cagY* (core and pilus), *cagI* (pilus), *cagF* and *cagβ* (translocation-associated factors). Regarding *cagY*, 3 studies are finding it essential, and 11 studies declaring it involved. Yet one study has found it dispensable for IL-8 production, by one of the 3 oral epithelial cell lines (HN), infected with Cuz20 *H. pylori* strain [33]. As for *cagI*, there are 2 studies that have declared it essential [33,34], yet one study has demonstrated it to be dispensable in the production of IL-8, following infection of AGS cells with the 26695 *H. pylori* strain [29]. Similarly, for *cagF*, one study has provided evidence of it being involved in IL-8 production of P12-infected AGS cells [43]. Yet, two other studies found no role for it in IL-8 production of these same cells, following infection with the 26695 [29] and G27 [48] *H. pylori* strains. Finally, *cagβ* is found dispensable in IL-8 production, by 5 studies (Table-1), but 2 studies provide evidence in support of its role in IL-8 production of P12-infected AGS cells [41,49].

Of the remaining elements: *cagD* (*hp0545*) as an accessory factor was found essential [48] or involved [29] in CagA-P and involved in IL-8 production according to both studies (Table-1). As for *cagC* (*hp0546*), a potential pilus subunit, its absence resulted in the loss of CagA-P, as well as loss [29] or reduced [50] IL-8 production. There has only been one study that has evaluated the absence of *cagζ*/*Cag1*(*hp0520*), *cagε*/*cag2* (*hp0521*), *cagP* (*hp0536*), *cagS* (*hp0534*) and *cagQ* (*hp0535*) as accessory factors and found them dispensable for CagA-P and IL-8 production [29]. Whereas, the same study found *cagG* (*hp0542*) which is thought to be involved in pilus biogenesis, as essential for CagA-P and involved in IL-8 production [29]. There is no information on cell elongation for these T4SS constituents (Figure-1).

Amongst the above-mentioned studies on the role of T4SS constituents in CagA-P, cell elongation and IL-8 production, every wild type gene complementation experiment resulted in restoration of the lost or reduced function (Table-1).

3.2. The role of HopQ in CagA phosphorylation, cell elongation and IL-8 production

It has recently been established that HopQ strongly binds the amino-terminal IgV-like domain and dimerization interface of certain human CEACAM molecules, in a glycosylation-independent manner [19] and results in CEACAM monomerization [51]. The information collected regarding *hopQ* inactivation and its impact on CagA-P, cell elongation and IL-8 production by different cells following infection with various *H. pylori* strains is presented in Table-2 and Figure-2. The collection of these studies on CagA-P can be categorized into 3 groups, the first of which finds *hopQ* essential

Table-1
Manipulations of T4SS constituents and their effects on CagA phosphorylation, cell elongation and IL-8 production [75–96].

No.	Author Year (Ref)	Gene/Protein Manipulation	*H. pylori* MoI*/ Time PI*	*H. pylori* Strain	Cell line*	CagA Phosphorylation Wild Type	CagA Phosphorylation Manipulated	Cell elongation Wild Type	Cell elongation Manipulated	IL-8 production Wild Type	IL-8 production Manipulated
1	Tegtmeyer et al. 2022 [75]	***cagY*** deletion	1:25/ 24h	P12	AGS	Yes→	Reduced/Lost (WB[1], Fig. 4A)	ND[2]		Yes→	Reduced/Lost (ELISA, Fig. 4C)
		cagX deletion					Reduced/Lost (WB, Fig. 4A)				Reduced/Lost (ELISA, Fig. 4C)
		cagβ/cag5 deletion					Reduced/Lost (WB, Fig. 4A)				Unchanged (ELISA, Fig. 4C)
2	Shrestha et al. 2022 [62]	***cagβ/cag5*** deletion	1:100/ 7h	NCTC11 637	AGS	Yes→	Reduced/Lost (WB, Fig. 5)	ND		ND	
3	Lettl et al. 2021 [76]	***cagT*** deletion	1:100/ 4h	P12	AGS	Yes→	Reduced/Lost (WB Fig. 1C)	ND		ND	
4	Choi et al. 2021 [77]	***cagL*** deletion	1:100/ 5 & 20h	G27	AGS	Yes→	Reduced/Lost (WB, Fig. 3B)	Yes→	Reduced/Lost (IM[3], Fig 4)	Yes→	5h: Reduced/Lost (ELISA, Fig. 5)
											20h: Reduced/Lost (ELISA, Fig. 5)
		cagL complementation				Reduced/Lost→	Restored (WB, Fig. 3B)	Reduced/Lost→	Restored (IM, Fig 4)	Reduced/Lost→	5h: Restored (ELISA, Fig. 5)
										Reduced/Lost→	20h: Restored (ELISA, Fig. 5)
5	Sharafutdinov et al. 2021 [78]	***cagY*** deletion	1:50/ 24h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 4C)	ND		ND	
		cagL deletion				Yes→	Reduced/Lost (WB, Fig. 4C)				
6	Tegtmeyer et al. 2020 [33]	***cagY*** deletion	1:100/ 6h	Cuz20	AGS	Yes→	Reduced/Lost (WB, Fig. 2A)	Yes→	Reduced/Lost (PCM[4], Fig. 1E)	Yes→	Reduced/Lost (ELISA, Fig. 4A)
					HN	No→	Unchanged (WB, Fig. 2B)	No→	Unchanged (PCM, Fig. 1G)		Unchanged (ELISA, Fig. 4B)
					CAL-27		Unchanged (WB, Fig. 2C)		Unchanged (PCM, Fig 1F)		Reduced/Lost (ELISA, Fig. 4C)
					BHY		Unchanged (WB, Fig .2D)		Unchanged (PCM, Fig. 1H)		Reduced/Lost (ELISA, Fig. 4D)
7	Lin et al. 2020 [79]	***cagα*** deletion	1:100/ 5-6h	26695	AGS	Yes→	Reduced/Lost (WB, Fig. 2)	ND		Yes→	Reduced/Lost (ELISA, Fig. 3A)
		cagα complementation				Reduced/Lost→	Restored (WB, Fig 2)			Reduced/Lost→	Restored (ELISA, Fig. 3A)
		cagβ/cag5 deletion				Yes→	Reduced/Lost (WB, Fig. 2)	ND		Yes→	Unchanged (ELISA, Fig. 3A)
		cagβ/cag5 complementation				Reduced/Lost→	Restored (WB, Fig 2)			Unchanged→	Unchanged (ELISA, Fig. 3A)
		cagE deletion				Yes→	Reduced/Lost (WB, Fig. 2)			Yes→	Reduced/Lost (ELISA, Fig. 3A)
		cagE complementation				Reduced/Lost→	Restored (WB, Fig 2)			Reduced/Lost→	Restored (ELISA, Fig. 3A)
8	Dooyema et al. 2020 [57]	***cagE*** deletion	1:50/ 4h	26695	AGS	Yes→	Reduced/Lost (WB, Fig. 2D)	ND		Yes→	Reduced/Lost (ELISA, Fig. 2B)
				7.13			Reduced/Lost (WB, Fig. S6B)			Yes→	Reduced/Lost (ELISA, Fig. S6D)
9	Zhao et al. 2019 [80]	***cagγ/cag4*** deletion	1:100/ 6h	26695	GES-1	Yes→	Reduced/Lost (WB, Fig. 2B)	ND		Yes→	Reduced/Lost (ELISA, Fig. 3)
		cagL deletion					Reduced/Lost (WB, Fig .2B)				Reduced/Lost (ELISA, Fig. 3)
10	Kumari et al. 2019 [30]	***cagW*** deletion	1:100/ ?	26695	AGS	Yes→	Reduced/Lost (WB, Fig. 4B)	ND		ND	
		cagN deletion					Reduced/Lost (WB, Fig. 4B)				
11	Skoog et al. 2018 [81]	***cagY*** deletion	1:100/ 20-22h	J166	AGS	ND		ND		Yes→	Reduced/Lost (ELISA, Fig. 6)
				PMSS1							
				KUS13							
12	Dyer et al. 2018 [22]	***cagW*** deletion	1:100/ 3h	G27	AGS	Yes→	Reduced/Lost (WB, Fig. 3A)	ND		ND	
		cagW complementation				Reduced/Lost→	Restored (WB, Fig. 3A)				
13	Tafreshi et al. 2018 [45]	***cagL*** deletion	1:1-10/ 24h	P12	HUVEC	ND		ND		Yes→	Reduced/Lost (ELISA, Fig. 2A, 3A)
					EA. Hy926					Yes→	Reduced/Lost (ELISA, Fig. 2B)
		cagL complementation			HUVEC					Reduced/Lost→	Restored (ELISA, Fig. 2A, 3A)
					EA. Hy926					Reduced/Lost→	Restored (ELISA, Fig. 2B)
		cagβ/cag5 deletion			HUVEC					Yes→	Unchanged (ELISA, Fig. 3A)
14	Gall et al. 2017 [82]	***cagE*** deletion	1:10/ 6-24h	G27	AGS	ND		ND		Yes→	6h: Reduced/Lost (ELISA, Fig 1C)
											24h: Reduced/Lost (ELISA, Fig 1C)
					HCT116						6h: Reduced/Lost (ELISA, Fig 2C)
											24h: Reduced/Lost

												(ELISA, Fig 2C)
15	Alandiyjany et al. 2017 [83]	***cagE*** deletion	1:20/ 48-72h	AB31	MKN28	ND		ND		Yes→		Reduced/Lost (ELISA, Fig 3)
				10A								Reduced/Lost (ELISA, Fig 3)
				AB5								Reduced/Lost (ELISA, Fig 3)
				64								Reduced/Lost (ELISA, Fig 3)
16	Bönig et al. 2016 [84]	***cagL*** insertion/inactivation	1:50/ 22h	SU2	AGS	Yes→	Reduced/Lost (WB, Fig. S6)	ND		Yes→		Reduced/Lost (ELIZA, Fig 3)
17	Wiedemann et al. 2016 [44]	***cagL*** deletion	1:100/ 5h	B128	AGS	ND		Yes→	Reduced/Lost (IM, Fig. 3C)	Yes→		Reduced/Lost (ELISA, Fig. 1B, 2G)
		cagY deletion						ND		Yes→		Reduced/Lost (ELISA, Fig. 1B)
18	Varga et al. 2016 [38]	***cagE*** deletion	1:100/ 6h	J166	AGS	ND		ND		Yes→		Reduced/Lost (ELISA, Fig 2D)
				26695								Reduced/Lost (ELISA, Fig 2D)
19	Shariq et al. 2015 [40]	***cagE*** deletion	1:100/ 4h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. S4A)	Yes→	Reduced/Lost (IM, Fig. S4A)	Yes→		Reduced/Lost (ELISA, Fig. S4B)
		cagE complementation				Reduced/Lost→	Restored (WB, Fig. S4A)	Reduced/Lost→	Restored (IM Fig. S4A)	Reduced/Lost→		Restored (ELISA, Fig. S4B)
20	Hartung et al. 2015 [85]	***cagL*** deletion	1:50/ 6h	PMSS1	AGS	ND		ND		Yes→		Reduced/Lost (ELISA, Fig. 2L)
				P12								Reduced/Lost (ELISA, Fig. 2O)
		cagL complementation		PMSS1						Reduced/Lost→		Restored (ELISA, Fig. 2L)
				P12						Reduced/Lost		Partially Restored (ELISA, Fig. S2F)
		cagE deletion		PMSS1						Yes→		Reduced/Lost (ELISA, Fig. 2L)
		cagY deletion		G27								Reduced/Lost (ELISA, Fig. 2G)
21	Tafreshi et al. 2015 [36]	***cagL*** deletion	1:100/ 6-24h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 2B)	Yes→	Reduced/Lost (IM, Fig. 2A)	Yes→		6h: Reduced/Lost (ELISA, Fig. S3)
												24h: Reduced/Lost (ELISA, Fig. 1A)
				26695		Yes→	Reduced/Lost (WB, Fig. S4B)	Yes→	Reduced/Lost (IM, Fig. S4A)	Yes→		Reduced/Lost (ELISA, Fig. 1B)
22	Tegtmeyer et al. 2014 [35]	***cagL*** deletion	1:100/ 8h	26695	AGS	Yes→	Reduced/Lost (WB, Fig. 1C)	ND		ND		
		cagL complementation				Reduced/Lost→	Restored (WB, Fig. 1C)					
23	Johnson et al. 2014 [50]	***cagC*** deletion	1:100/ 4h	26695	AGS	ND		ND		Yes→		Reduced/Lost (ELISA, Fig. 4B)
		cagC complementation								Reduced/Lost →		Restored (ELISA, Fig. 4B)
		cagT deletion								Yes→		Reduced/Lost (ELISA, Fig. 6B)
		cagT complementation								Reduced/Lost →		Restored (ELISA, Fig. 6B)
		cagX deletion								Yes→		Reduced/Lost (ELISA, Fig. 6B)
		cagX complementation								Reduced/Lost →		Restored (ELISA, Fig. 6B)
		cagδ/cag3 deletion								Yes→		Reduced/Lost (ELISA, Fig. 8B)
		cagδ/cag3 complementation								Reduced/Lost →		Restored (ELISA, Fig. 8B)
		cagM deletion								Yes→		Reduced/Lost (ELISA, Fig. 8B)
		cagM complementation								Reduced/Lost →		Restored (ELISA, Fig. 8B)
		cagY deletion								Yes→		Reduced/Lost (ELISA, Fig. 8B)
24	Barrozo et al. 2013 [86]	***cagY*** deletion	1:100/ 22h	PMSS1	AGS	Yes→	Reduced/Lost (WB, Fig. 9C)	ND		Yes→		Reduced/Lost (ELISA, Fig. 9C)
				SS1		No→	Unchanged (WB, Fig. 9D)			No→		Unchanged (ELISA, Fig. 9D)
				J166		ND				Yes→		Reduced/Lost (ELISA, Fig. S1)
				J166	KATOIII	ND				Yes→		Reduced/Lost (ELISA, Fig. S1)
		cagY complementation		PMSS1	AGS	Reduced/Lost→	Restored (WB, Fig. 9C)			Reduced/Lost →		Restored (ELISA, Fig. 9C)

				SS1		Unchanged→	Unchanged (WB, Fig. 9D)			Unchanged→	Unchanged (ELISA, Fig. 9D)
25	Belogolova 2013 [55]	***cagE*** *deletion*	1:100/ 8h	G27	AGS		ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 2D)
26	Yeh et al. 2013 [87]	***cagL*** deletion	1:100/ 16-18h	Hp1033	AGS	Yes→	Reduced/Lost (WB, Fig. 2D)		ND	Yes→	Reduced/Lost (ELISA, Fig. 2E)
27	Boonyanugomol et al. 2013 [46]	***cagL*** deletion	1:1/ 6h	Hp251	KKU-100		ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 3B)
					KKU-M156					Yes→	Reduced/Lost (ELISA, Fig. 3B)
					AGS					Yes→	Reduced/Lost (ELISA, Fig. 3B)S
28	Gorrell et al. 2013 [49]	***cagL*** deletion	1:100/ 24h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 3B)		ND	Yes→	Reduced/Lost (ELISA, Fig. 1)
		cagβ/cag5 deletion				Yes→	Reduced/Lost (WB, Fig. S2A)			Yes→	Reduced/Lost (ELISA, Fig. 1)
29	Kumar et al. 2013 [88]	***cagI*** deletion	1:100/ 4h	26695	AGS		ND	Yes→	Reduced/Lost (IM, Fig. S4)	ND	
30	Pham et al. 2012 [34]	***cagI*** deletion	1:100/ 4h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 3A)	Yes→	Reduced/Lost (IM, Fig. 3A)	Yes→	Reduced/Lost (ELISA, Fig. 3B)
		cagI complementation				Reduced/Lost→	Restored (WB, Fig. 3A)	Reduced/Lost→	Restored (IM, Fig. 3A)	Reduced/Lost→	Restored (ELISA, Fig. 3B)
31	Conradi et al. 2012 [39]	***cagL*** deletion	1:50-100/ 2-4h	P12	AGS	Yes→	2h: Reduced/Lost (WB, Fig.7)	Yes→	2h: Reduced/Lost (IM, Fig. 8C)	ND	
							4h: Reduced/Lost (WB, Fig.7)		4h: Reduced/Lost (IM, Fig. 8C)		
		cagL complementation				Reduced/Lost→	2h:Restored (WB, Fig. 7)	Reduced/Lost→	2h:Restored (IM, Fig. 8C)		
							4h:Restored (WB, Fig. 7)		4h:Restored (IM, Fig. 8C)		
32	Ding et al. 2012 [89]	***cagT*** deletion	1:200/ 6-8h	26695	AGS	Yes→	Reduced/Lost (WB, Fig. 1A)	Yes→	Reduced/Lost (data not shown)	Yes→	Reduced/Lost (ELISA, Fig. 1B)
		cagM deletion					Reduced/Lost (WB, Fig. 1A)		Reduced/Lost (data not shown)		Reduced/Lost (ELISA, Fig. 1B)
33	Shaffer et al. 2011 [6]	***cagL*** deletion	1: 100/ 24h	26695	AGS	Yes→	Reduced/Lost (WB, Fig 1C)	ND		Yes→	Reduced/Lost (ELISA, Fig 1B)
		cagL complementation				Reduced/Lost→	Restored (WB, Fig 1C)			Reduced/Lost→	Restored (ELISA, Fig. 1B)
		cagH deletion				Yes→	Reduced/Lost (WB, Fig. 2F)			Yes→	Reduced/Lost (ELISA, Fig. 2E)
		cagH complementation				Reduced/Lost→	Restored (WB, Fig 2F)			Reduced/Lost→	Restored (ELISA, Fig. 2E)
		cagI deletion				Yes→	Reduced/Lost (WB, Fig. 2F)			Yes→	Reduced/Lost (ELISA, Fig. 2E)
		cagI complementation				Reduced/Lost→	Restored (WB, Fig. 2F)			Reduced/Lost→	Restored (ELISA, Fig. 2E)
34	Hutton et al. 2010 [47]	***cagM*** deletion	1: 10/ 24h	HP251	HEK-293		ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 2C)
35	Jurik et al. 2010 [41]	***cagY*** deletion	1:100 / 4h	26695	AGS		ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 1A)
		cagβ/cag5 deletion				Yes→	Reduced/Lost (WB, Fig. 1B)	Yes→	Reduced/Lost (PCM, Fig. 1B)	Yes→	Unchanged (ELISA, Fig. 1A)
		cagβ/cag5 complementation				Reduced/Lost→	Restored (WB, Fig. 1B)	Reduced/Lost→	Restored (PCM, Fig. 1B)	ND	
		cagβ/cag5 deletion		P12			ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 3C)
		cagZ deletion				Yes→	Reduced/Lost (WB, Fig. 3B)	Yes→	Reduced/Lost (PCM, Fig. 3B)	Yes→	Reduced/Lost (ELISA, Fig. 3C)
36	Jime´nez-Soto et al. 2009 [13]	***cagL*** deletion	1:60/ 2-4h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 3a)		ND	Yes→	Reduced/Lost (ELISA, Fig. 3B)
		cagL complementation				Reduced/Lost→	Restored (WB, Fig 3a)			Reduced/Lost→	Restored (ELISA, Fig. 3B)
37	Cendron et al. 2009 [48]	***cagF* (5)** deletion	1:100/ 3h-24h	G27	AGS	Yes→	Reduced/Lost (WB, Fig 6)		ND	Yes→	Unchanged (ELISA, Fig. 9)
		***cagD* (2)** deletion					Reduced/Lost (WB, Fig 6)				Reduced/Lost (ELISA, Fig. 9)
		cagD complementation				Reduced/Lost→	Restored (WB, Fig 6)			Reduced/Lost→	Restored (ELISA, Fig. 9)
		cagE deletion					ND			Yes→	Reduced/Lost (ELISA, Fig. 9)
38	Kowk et al. 2007 [8]	***cagL*** deletion	1:100/ 2-4h	P12	AGS	Yes→	Reduced/Lost (WB, Fig 3A, 3C)	Yes→	Reduced/Lost (IM, Fig 3C)	ND	
		cagL complementation				Reduced/Lost→	Restored (WB, Fig 3A, 3C)	Reduced/Lost→	Restored (IM, Fig 3B, 3C)		
39	Pattis et al. 2007	***cagY*** deletion	1:10 0/ 4h	P12	AGS		ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 1A)

	[43]	*cagF* deletion					Yes→	Reduced/Lost (WB, Fig. 1B)	Yes→	Reduced/Lost (IM, Fig. 1B)		Reduced/Lost (ELISA, Fig. 1A)
40	Bourzac et al. 2006 [31]	*cagN* deletion		1:100/ 8h	G27	AGS	Yes→	Unchanged (WB, Fig. 3A)	Yes→	Unchanged (IM, Fig. 3B)	ND	
41	Couturier et al. 2006 [42]	*cagX* deletion		1:100/ 4h	G27	AGS	Yes→	Reduced/Lost (WB, Fig. 5A)	ND		ND	
		cagF (3) deletion						Reduced/Lost (WB, Fig. 5A)	Yes→	Reduced/Lost (PCM, Fig. 5B)		
42	Al-Ghoul et al. 2004 [90]	*cagF* (2) deletion		1:100/ 4h	26695	AGS	ND		Yes→	Reduced/Lost (IM, Fig. 1)	ND	
		cagL deletion								Reduced/Lost (IM, Fig. 1)		
43	Savvides et al. 2003 [91]	*cagα* (2) deletion		1:100/ 4h	26695	AGS	Yes→	Reduced/Lost (WB, Fig. 4C)	ND		ND	
44	Fischer et al. 2001 [29]	*cagδ/cag 3* deletion	*cagY* (1) deletion	*cagU* (1) deletion	*cagE* deletion	*cagH* deletion	1:100/ 4h	26695	AGS	Yes→	Reduced/Lost (WB, Fig. 3)	ND	Yes→	Reduced/Lost (ELISA, Fig. 3)
		cagγ/cag4 (1) deletion	*cagX* (1) deletion	*cagV* (1) deletion	*cagM* deletion	*cagC* deletion								
		cagα (1) deletion	*cagW* deletion	*cagT* deletion	*cagL* deletion									
		cagZ deletion		*cagD* (1) deletion		*cagN* deletion					Reduced/Lost (WB, Fig. 3)			Reduced/Lost (ELISA, Fig. 3)
		cagβ/cag5 deletion			*cagI* deletion						Reduced/Lost (WB, Fig. 3)			Unchanged (ELISA, Fig 3)
		cagζ (1) deletion		*cagP* (1) deletion		*cagS* (1) deletion					Unchanged (WB, Fig 3)			Unchanged (ELISA, Fig 3)
		cagε (1) deletion		*cagQ* (1) deletion		*cagF* (1) deletion								
		cagG (1) deletion									Reduced/Lost (WB, Fig. 3)			Reduced/Lost (ELISA, Fig. 3)
45	Odenbreit et al. 2000 [92]	*cagE* deletion		1:100/ 4h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 1C)	ND		ND	

[1]WB: Western Blotting, [2]ND: Not Determined, [3]IM: Inverted Microscopy, [4]PCM: Phase Contrast Microscopy, *Cell line descriptions (Table-5), [+]MoI: Multiplicity of Infection, [x] PI: Post Infection

in the P12/AGS context [52,53] In the latter study, however, when given more time (105 min *versus* 30 min), other compensatory means seem to kick in and end up finding *hopQ* as involved, not essential [52]. As for the PMSS1 strain, with multiple *hopQ* (*IA*, *IB* and *II*) alleles, infecting AGS cells, only simultaneous deletion of the *IB* and *II* alleles results in total loss of CagA-P [16]. In other words, at least one copy of either *hopQ* type *IB* or *II* is found essential for CagA translocation [22]. The second and most populated group of studies find *hopQ* involved in CagA-P, in the AGS, Hela, KatoIII, and HL60 cells, infected with P12 *H. pylori* strain, as well as in the NCTC11637/AGS context (Table-2). Interestingly however, there are also studies, that have infected AGS or Hela cells with the *hopQ*-inactivated 26695, 7.13 (both copies) or P12 *H. pylori* strains and have found the process of CagA-P unaffected (Table-2). Paradoxically, there is only one study, that has detected increased CagA-P upon infection of AGS cells with *hopQ*-inactivated 26695 *H. pylori* strain, which occurred in a hyper-adherent mutant strain [54]. Thus, the authors concluded that it is the extent of bacterial adherence to the AGS cells that determines the rate of CagA-P [54]. The partial reduction in bacteria-cell adherence observed following *hopQ* inactivation was later supported by another study [18], but not others [22,52].

In regards to cell elongation following infection with *hopQ*-inactivated strains, the few available studies, again find it mostly involved in the P12/AGS context [19,53]. In the same context, however, one study finds it essential [55] for cell elongation, whereas bacterial motility and the extent of cell adherence were not affected. Yet, another study finds it dispensable [18].

As for the role of *hopQ* in IL-8 production, one study provides evidence for its essence in the NCTC11637/AGS context [19], others for its involvement in the P12/AGS [18,19,53,55] and P12/MKN45, G27/MKN45 and P12/NUGC-4 [56] contexts (Table-2). Yet, as stated above when the one or both copies of the *hopQ I* allele in the 26695 and 7.13 *H. pylori* strains are deleted, respectively, IL-8 production remains unaffected [57]. Collectively, every complementation study with wildtype *hopQ* gene resulted in complete restoration of the originally lost or reduced CagA-P, cell elongation or IL-8 production (Table-2).

It has been demonstrated that the thiol oxidoreductase HP0231 of *H. pylori* is crucial for the proper functioning of HopQ in mediating CagA translocation and phosphorylation [53], though this finding was not later confirmed [58]. The crystal structure of HopQ I and II isotypes reveals that they both target the trans-dimerization interface of CEACAMs, leading to their monomerization and mediating CagA translocation [23]. Moreover, the disulfide bond in the loop CL1 (connecting C103 to C132) of HopQ, is essential for this function [58]. It was found that a β-hairpin insertion (HopQ-ID) in the HopQ's extracellular 3 + 4 helix bundle domain, in particular, is responsible for this function. Such that the application of the corresponding peptide (HopQ-ID) or α-HopQ antibody reduces CagA-P and cell elongation [19].

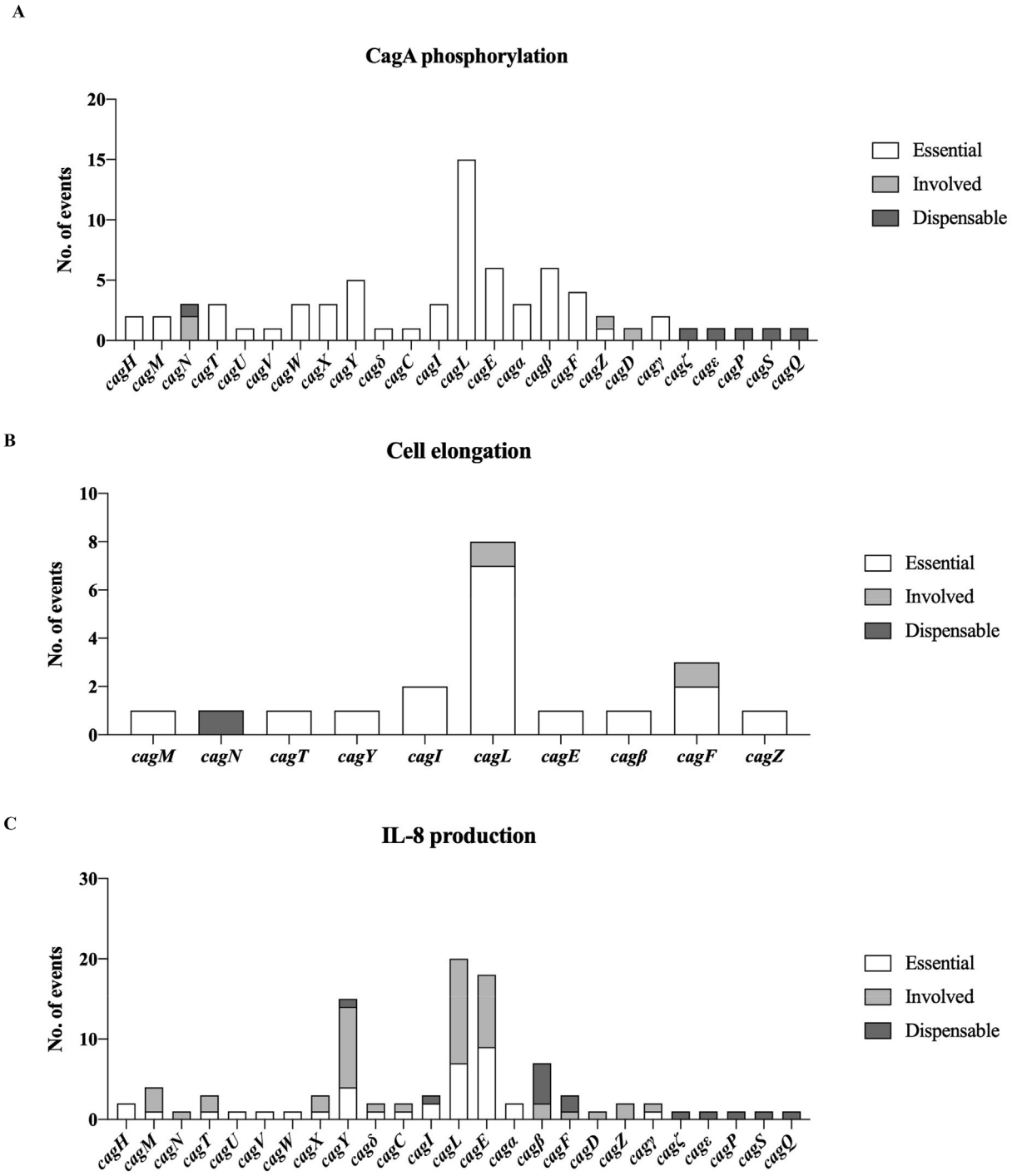

Figure-1. The role of the T4SS constituents in the downstream functions, following *H. pylori* infection. A) CagA phosphorylation, B) cell elongation and C) IL-8 production. The deduced roles were classified as: "Essential" (lost or induced function, upon deletion/inhibition or addition of the target gene/protein, respectively), "Involved" (reduced or increased function upon deletion/inhibition or addition of the target gene/protein, respectively) or "Dispensable" (no alterations).

3.3. Receptors

3.3.1. The role of integrin receptors in CagA phosphorylation, cell elongation and IL-8 production

The previous dogma regarding the host cell receptor responsible for T4SS formation, mediating CagA translocation was the integrin family and in particular the α5β1 heterodimer [44]. However, the information accumulated herein on the manipulation of cell surface integrins and their effects on CagA translocation suggests otherwise (Table 3).

Amongst these, the information obtained on the essence of β1 integrin in CagA-P, is somewhat controversial (Figure-3A). In other words, there are studies that find it essential for this function, such that upon the exogenous expression (complementation) of β1 integrin in the otherwise deficient murine embryonic fibroblast (GD25) and epithelial (GE11) cells, induced CagA-P is observed

Table-2
Manipulations of HopQ and its effects on CagA phosphorylation, cell elongation and IL-8 production [75–96].

No.	Author Year (Ref)	Gene/Protein manipulation	*H. pylori* MoI*/ Time PI$	*H. pylori* Strain	Cell line^	CagA phosphorylation Wild Type	CagA phosphorylation Manipulated	Cell elongation Wild Type	Cell elongation Manipulated	IL-8 production Wild Type	IL-8 production Manipulated
1	Taxauer et al 2021 [56]	***hopQ*** deletion	1:10/ 8h	P12	MKN45	ND[1]		ND		Yes→	Reduced/Lost (ELISA, Fig. 1B)
				G27							Reduced/Lost (ELISA, Fig. 1B)
				P12	NUGC-4						Reduced/Lost (ELISA, Fig. 3B)
2	Letti et al. 2021 [76]	***hopQ*** deletion	1:25/ 150min	P12	AGS	Yes→	Reduced/Lost (TEM-CagA[2], Fig. 3B)	ND		ND	
		hopQ complementation				Reduced/Lost→	Restored (TEM-CagA, Fig. 3B)				
3	Maubach, G. et al. 2020 [70]	***hopQ*** deletion	1:100/ 150 min	P12	AGS	Yes→	Reduced/Lost (IP[3]/WB[4], Fig. 2H)	ND[3]		ND	
					Hela		Reduced/Lost (IP/WB, Fig. 2H)				
4	Dooyema et al. 2020 [57]	***hopQ*** deletion	1:10, 1:25, 1:50, 1:100, 1:200/ 4h	26695	AGS	Yes→	Unchanged (WB, Fig. 2D)	ND		Yes→	Unchanged (ELISA, Fig. 2B)
		hopQ complementation				Unchanged	Unchanged (WB, Fig. S6B)			Unchanged →	Unchanged (ELISA, Fig. S6D)
		hopQ deletion		7.13		Yes→	Unchanged (WB, Fig. S6A)			Yes→	Unchanged (ELISA, Fig. S6C)
5	Zhao, Q. et al. 2018 [18]	***hopQ*** deletion	1:60/ 2-5-4h	P12	AGS	Yes→	Reduced/Lost (TEM-CagA/ WB, Fig. 3A/C)	Yes→	Unchanged (IM, Fig. S5)	Yes→	Reduced/Lost (WB, Fig. S6)
					KatoIII	Yes→	Reduced/Lost (TEM-CagA/ WB, Fig. 5B/C)	ND		ND	
		hopQ complementation			AGS	Reduced/Lost →	Restored (BGF[5]/ WB, Fig. 3A/C)	Unchanged→	Unchanged (IM, Fig. S5)	Reduced/Lost→	Restored (WB, Fig. S6)
					KatoIII	Reduced/Lost→	Restored (TEM-1-CagA/ WB, Fig. 5B/C)	ND		ND	
6	Grzeszczuk, M. J. et al. 2018 [53]	***hopQ*** deletion	1:100/ 3h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 2)	Yes→	Reduced/Lost (CLSM[6], Fig. 1)	Yes→	Reduced/Lost (qRT-PCR[7], Fig. 3)
		hopQ complementation				Reduced/Lost→	Restored (WB, Fig. 8)	Reduced/Lost→	Restored (CLSM, Fig. 7)	Reduced/Lost→	Restored (qRT-PCR, Fig. 9)
7	Feige, M. H. et al. 2018 [52]	***hopQ*** deletion	1:100- 1:300/ 30 & 105 min	P12	AGS	Yes→	Reduced/Lost In 30 min (IP/WB, Fig. 3C)	ND		ND	
							Reduced/Lost In 105 min (IP/WB, Fig. 3C)				
					Hela	Yes→	Unchanged In 30 min (IP/WB, Fig. S2C)				
							Unchanged In 105 min (IP/WB, Fig. S2C)				
		hopQ complementation			AGS	Reduced/Lost→ In 30 min	Restored In 30 min (IP/WB, Fig. 3C)				
						Reduced/Lost→ In 105 min	Restored In 105 min (IP/WB, Fig. 3C)				
					Hela	Unchanged→ In 30 min	Unchanged In 30 min (IP/WB, Fig. S2C)				

						Unchanged→ In 105 min	Unchanged In 105 min (IP/WB, Fig. S2C)					
8	Dyer, V. et al. 2018 [22]	***hopQ*** deletion	IA	1:100/ 3h	PMSS1	AGS	Yes→	Unchanged (WB, Fig. 5C)	ND		ND	
			IB				Yes→	Unchanged (WB, Fig. 5C)				
			II				Yes→	Unchanged (WB, Fig. 5C)				
			IA IB				Yes→	Unchanged (WB, Fig. 5C)				
			IA II				Yes→	Unchanged (WB, Fig. 5C)				
			IB II				Yes→	Reduced/Lost (WB, Fig. 5C)				
			IA IB II				Yes→	Reduced/Lost (WB, Fig. 5C)				
9	Bonsor, D. A. et al. 2018 [51]	***hopQ*** deletion		1:100/ 4h	P12	HEK293	No→	Unchanged (TEM-CagA, Fig. 6D)	ND		ND	
		hopQ complementation					Unchanged→	Unchanged (TEM-CagA, Fig. 6D)				
		hopQ deletion				AGS	Yes→	Reduced/Lost (TEM-CagA, Fig. 6E)				
		hopQ complementation					Reduced/Lost→	Restored (TEM-CagA, Fig. 6E)				
10	Königer, V. et al. 2016 [20]	***hopQ*** deletion		1:60/ 4h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. S5B)	ND		ND	
						KatoIII		Slightly Reduced/Lost (WB, Fig. S5E)				
		hopQ complementation				MKN45		Reduced/Lost (WB, Fig. S5D)				
						AGS	Reduced/Lost→	Restored (WB, Fig. S5B)				
						KatoIII	Slightly Reduced/Lost→	Restored (WB, Fig. S5E)				
						MKN45	Reduced/Lost→	Restored (WB, Fig. S5D)				
11	Javaheri, A. et al. 2016 [19]	***hopQ*** deletion		1:50/ 6h	NCTC 11637	AGS	Yes→	Reduced/Lost (WB, Fig. 5D)	ND		Yes→	Reduced/Lost (ELISA, Fig. 5E)
					P12		Yes→	Reduced/Lost (WB, Fig. 5D)	Yes→	Reduced/Lost (CLSM, Fig. 5G/H)	Yes→	Reduced/Lost (ELISA, Fig. 5E)
		hopQ complementation					ND		Reduced /Lost→	Restored (CLSM, Fig. 5G/H)	ND	
		α-HopQ antibody					Yes→	Reduced/Lost (WB, Fig .5J)	Yes→	Reduced/Lost (CLSM, Fig. 5J)		
		HopQ-derived peptide (HopQ-ID)					Yes→	Reduced/Lost (WB, Fig. 5K)	Yes→	Reduced/Lost (CLSM, Fig. 5K)		
12	Busch, B. et al. 2015 [93]	***hopQ*** deletion		1:60/ 4h	P12	HL-60	Yes→	Reduced/Lost (WB, Fig. 6A)	ND		ND	
		hopQ complementation					Reduced/Lost→	Restored (WB, Fig 6A)				
13	Belogolova, E. et al. 2013 [55]	***hopQ*** deletion		1:100/ 3-6h	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 5C)	Yes→	Reduced/Lost (IF^{s}, Fig. 5A)	Yes→	Reduced/Lost (ELISA, Fig. 4C)
		hopQ complementation					Reduced/Lost→	Restored (WB, Fig. 5C)	ND		ND	
14	Loh, J. T. et al. 2008 [54]	***hopQ*** deletion		1:100-20/ 5h	26695	AGS	Yes→	Increased (WB, Fig. 2B)	ND		Yes→	Unchanged (ELISA, Fig. 3)

[1]ND: Not Determined, [2]TEM-CagA: β-lactamase reporter system (TEM-1 reporter assay), [3]IP: Immunoprecipitation, [4]WB: Western Blotting, [5]Blue Green Fluorescence, CLSM: [6]Confocal Laser Scanning Microscopy, [7]qRT-PCR: Quantitative Real Time PCR, *Cell line descriptions (Table-5), ⁻MoI: Multiplicity of Infection, ˢPI: Post Infection

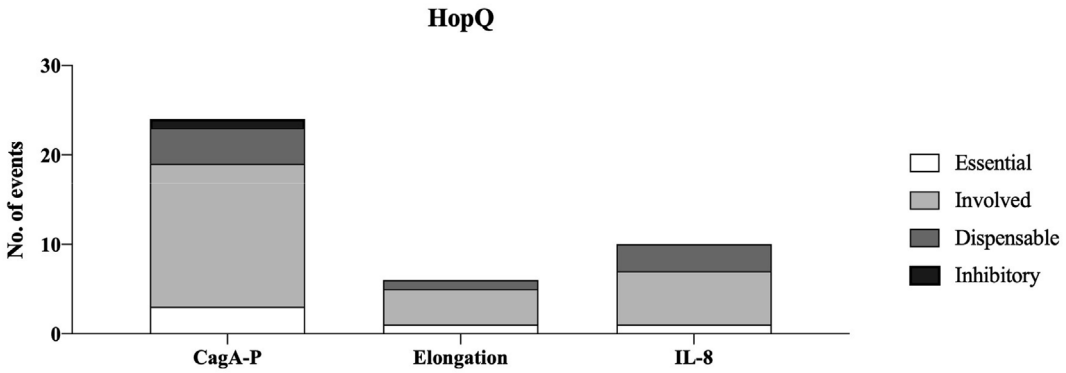

Figure-2. The role of HopQ in CagA phosphorylation, cell elongation and IL-8 production, following *H. pylori* infection. The deduced roles were classified as: "Essential" (lost or induced function, upon deletion/inhibition or addition of the target gene/protein, respectively), "Involved" (reduced or increased function upon deletion/inhibition or addition of the target gene/protein, respectively) "Dispensable" (no alterations) or d) "Inhibitory" (lost or induced function, upon addition or deletion/inhibition of the target gene/protein, respectively).

[8,13]. Accordingly, downregulation of β1 integrin [59] in AGS cells, or treatment of these cells with anti-β1 antibody results in reduced or lost CagA-P, following infection with P12 [8,60] and P1 [52] *H. pylori* strains, respectively. According to surface plasmon resonance measurements, CagA binding to α5β1 heterodimer is fairly strong, for which the extracellular part of the β1 integrin is crucial, such that antibody (9EG7)-mediated stabilization of β1 integrin open active conformation, proved this segment to act as a control switch in CagA translocation [60].

Nevertheless, there is evidence supporting the dispensable role of β1 integrin in CagA-P. Such that β1 integrin knock-out AGS cells, infected with a wide geographic panel of *H. pylori* strains, including the well-known P12, G27 and PMSS1 strains, were as effective in CagA-P, as their parent cell lines [18,32]. Similarly, even though confocal laser scanning microscopy confirms the co-localization of *H. pylori* and β1 integrin heterodimers, the β-lactamase reporter system revealed that CagA-P is not affected upon β1 integrin downregulation [61]. Accordingly, β1 integrin overexpression in AZ-521 cells infected with various *H. pylori* (P12, P310 and P420) strains had no impact on CagA-P [59] (Table-3). Furthermore, pre-infection with *H. pylori* was found to reduce CagA translocation in a dose-dependent manner, but this phenomenon was *hopQ*-dependent and β1-integrin-independent upon infection with a second strain [60]. In regards to the role of β1 integrin in cell elongation following *H. pylori* infection, the data finds it mostly essential/involved (Figure-3B). For instance, the above-mentioned study [32] finds it abrogated in β1-knockout AGS cells, infected with a wide geographic panel of *H. pylori* strains [P12, GAM94-24 (Gambia), K88 (Germany), G27 (Italy), TN2-GF4 (Japan), PMSS1 (Australia), 7.13 (USA), SJM180 (Peru)] [32]. This study concluded that β1 integrin is responsible for cell attachment and focal adhesion formation, which leads to cell elongation, upon *H. pylori*-induced cell dispersion [32]. As for IL-8 production, the data mostly supports its involvement (Fig. 3C), except for one study, in which down-regulation of β1 integrin in AGS cells, had no effect on IL-8 production following infection with the B128 *H. pylori* strain [44].

Regarding β4 integrin, its inactivation in AGS and KatoIII cells infected with P12 *H. pylori* strain, has indicated this integrin to be dispensable for CagA-P, with no information on the other two functions [18] (Figure-3A and Table-3).

Concerning the β6 integrin, once this integrin is transfected into the SW480 colon carcinoma cell lines, increased CagA-P is observed, in response to infection with four (P12, G27, NCTC 11637 and N6) *H. pylori* strains. IL-8 production was also increased under infection with P12, NCTC 11637 and N6 strains, but reduced upon infection with the G27 *H. pylori* strain [9]. These authors concluded that *H. pylori* CagL binds strongly to the α_v/β_6, rather than the $\alpha_5\beta_1$ heterodimer, in an RGD-dependent manner [9].

Total loss of CagA-P and cell elongation was demonstrated upon antibody-mediated inhibition of α5 integrin in AGS cells, infected with P12 strain [8] (Figure-3A). Accordingly, infection of AGS cells treated with anti-α5 antibody underwent significantly reduced and lost cell elongation when infected with HP251 [47] and P12 [8] *H. pylori* strains, respectively (Figure-3B). However, concerning the impact of this integrin on IL-8 production following *H. pylori* infection, the available data all votes for its involvement (Table-3, Fig. 3C).

There is only one study that has studied the impact of αv inactivation in AGS and KatoIII cells on CagA-P, following P12 *H. pylori* strain infection, and has found it dispensable in the former and inhibitory in the latter cells [18].

Simultaneous inactivation of multiple integrin genes and evaluation of their impact on CagA-P was carried out on AGS [18,32] and KatoIII [18] cells, for: 1) αv/β4, 2) αv/β1 2) β1/β4, and 3) αv/β1/β4. No matter the combination, there was no observed impact on CagA-P in these cells (Figure-3A). However, assessment of cell elongation, found αv/β4 inhibitory in one study [32], whereas both αv/β4 and β1/β4 combinations were found dispensable in another study [18]. Interestingly, though, in the same study, assessment of IL-8 production, found αv/β4 dispensable and β1/β4 inhibitory for this function [18] (Figure-3C).

Antibody-mediated blocking of the integrin heterodimers identified αvβ6 as involved and , α5β1 and αvβ3 as dispensable for IL-8 production of endothelial (HUVEC and EA.Hy926) cells [45], all following P12 strain *H. pylori* infection.

3.4. The role of CEACAM receptors in CagA phosphorylation, cell elongation and IL-8 production

As mentioned above, HopQ strongly binds to the amino-terminal IgV-like domain and dimerization interface of certain human CEACAM molecules, in a glycosylation-independent manner [19] and results in CEACAM monomerization [51]. According to Table 4, induced CagA-P is demonstrated following de novo expression of CEACAM1, CEACAM5 and CEACAM6 in oral epithelial (HN, CAL-27 and BHY) [33] cell lines. In a study on mouse gastric epithelial cells (YTN16 and YTN2), the expression of CEACAM1, CEACAM5 but not CEACAM6 resulted in induced CagA-P [62]. The mentioned studies in human oral and mouse gastric epithelial cells may help explain the resistance of the

Table-3
Manipulations of integrin receptors and their effects on CagA phosphorylation, cell elongation and IL-8 production [75–96].

No.	Author Year (Ref)	Gene / Protein Manipulation	*H. pylori* MoI+/ Time PIx	*H. pylori* Strain	Cell line*	CagA phosphorylation Wild Type	CagA phosphorylation Manipulated	Cell elongation Wild Type	Cell elongation Manipulated	IL-8 Production Wild Type	IL-8 Production Manipulated
1	Tegtmeyer et al. 2020 [32]	β1 integrin knockout	1:50/ 6h	GAM94-24 (Gambia)	AGS	Yes→	Unchanged (WB[1], Fig. 1B)	Yes→	Reduced/Lost (IM[2], Fig. 1)		ND[3]
				K88 (Germany)		Yes→	Unchanged (WB, Fig. 1B)	Yes→	Reduced/Lost (IM, Fig. 1)		
				G27 (Italy)		Yes→	Unchanged (WB, Fig. 1B)	Yes→	Reduced/Lost (IM, Fig. 1)		
				TN2-GF4 (Japan)		Yes→	Unchanged (WB, Fig. 1B)	Yes→	Reduced/Lost (IM, Fig. 1)		
				PMSS1 (Australia)		Yes→	Unchanged (WB, Fig. 1B)	Yes→	Reduced/Lost (IM, Fig. 1)		
				7.13 (USA)		Yes→	Unchanged (WB, Fig. 1B)	Yes→	Reduced/Lost (IM, Fig. 1)		
				SJM180 (Peru)		Yes→	Unchanged (WB, Fig. 1B)	Yes→	Reduced/Lost (IM, Fig. 1)		
				P12		ND		Yes→	Reduced/Lost (IM, Fig. 1E)		
		αv/β4 integrin knockout		GAM94-24 (Gambia)		Yes→	Unchanged (WB, Fig. 1C)	Yes→	Increased (IM, Fig. 1)		
				K88 (Germany)		Yes→	Unchanged (WB, Fig. 1C)	Yes→	Increased (IM, Fig. 1)		
				G27 (Italy)		Yes→	Unchanged (WB, Fig. 1C)	Yes→	Increased (IM, Fig. 1)		
				TN2-GF4 (Japan)		Yes→	Unchanged (WB, Fig. 1C)	Yes→	Increased (IM, Fig. 1)		
				PMSS1 (Australia)		Yes→	Unchanged (WB, Fig. 1C)	Yes→	Increased (IM, Fig. 1)		
				7.13 (USA)		Yes→	Unchanged (WB, Fig. 1C)	Yes→	Increased (IM, Fig. 1)		
				SJM180 (Peru)		Yes→	Unchanged (WB, Fig. 1C)	Yes→	Increased (IM, Fig. 1)		
				P12		ND		Yes→	Increased (IM, Fig. 1F)		
2	Tegtmeyer et al. 2019 [59]	β1 integrin overexpression	1:100/ 9h-18h	P12	AZ-521	No→	Unchanged (IP[4], Fig. 5A)	ND		ND	
		β1 integrin downregulation			AGS	Yes→	Reduced/Lost (WB, Fig. 8A, B)				
3	Tafreshi et al. 2018 [45]	Anti-α5β1 integrin antibody	1: 1/24 h	P12	HUVEC	ND		ND		Yes→	Unchanged (ELISA, Fig.5A)
		Anti-αvβ3 integrin antibody			EA.Hy926					Yes→	Unchanged (ELISA, Fig.5B)
4	Buß et al. 2019 [9]	β6 integrin expression	1:100/8h	P12	SW480	Yes→	Increased (WB, Fig. 5B)	ND		Yes→	Increased (ELISA, Fig.6)
				G27		Yes→	Increased (WB, Fig. 5B)			Yes→	Reduced/Lost (ELISA, Fig.6)
				NCTC		Yes→	Increased			Yes→	Increased

				11637			(WB, Fig. 5B)				(ELISA, Fig.6)
				N6		Yes→	Increased (WB, Fig. 5B)			Yes→	Increased (ELISA, Fig.6)
		Anti-αvβ6 integrin antibody	1:50/3h	P12	AGS	Yes→	Reduced/Lost (WB, Fig.7A, B)				ND
5	Feige et al. 2018 [52]	Anti-β1 integrin antibody	1:100/3h	P1	AGS	Yes→	Reduced/Lost (IP, Fig.2B)		ND		ND
6	Zhao et al. 2018 [18]	β1 integrin knockout	1:60/2.5h	P12	AGS	Yes→	Unchanged (TEM-CagA[5], Fig. 3A)		ND		ND
		β4 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3A)		ND		ND
		β1/β4 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3A)	Yes→	Unchanged (IM, Fig. S5)	Yes→	Increased (ELISA, Fig S6)
		αv integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3A)		ND		ND
		αv/β4 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3A)	Yes→	Unchanged (IM, Fig. S5)	Yes→	Unchanged (ELISA, Fig S6)
		β1 integrin knockout			KatoIII	Yes→	Unchanged (TEM-CagA, Fig. 3B)				
		β4 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3B)		ND		ND
		β1/β4 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3B)				
		αv integrin knockout				Yes→	Increased (TEM-CagA, Fig. 3B)				
		αv/β4 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3B)				
		αv/β1 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3B)				
		αv/β1/β4 integrin knockout				Yes→	Unchanged (TEM-CagA, Fig. 3B)				
7	Nakano et al. 2016 [61]	β1 integrin downregulation	1:100/9h	ATCC 43504	AZ-521	Yes→	Unchanged (IP, Fig. 6A)		ND		ND
8	Wiedemann et al. 2016 [44]	β1 integrin downregulation	1:100/5h	B128	AGS		ND		ND	Yes→	Unchanged (ELISA, Fig4c)
9	Hartung et al. 2015 [85]	β1 integrin downregulation	1:50/6h	G27	AGS		ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 3R)
10	Zhang et al. 2015 [94]	Anti-integrin β1 antibody	1:100/48h	NCTC 11637	AGS		ND		ND	Yes→	Reduced/Lost (ELISA, Fig. 4)
				T494						Yes→	Reduced/Lost (ELISA, Fig. 4)
				SS1						Yes→	Reduced/Lost (ELISA, Fig. 4)
				T049						Yes→	Reduced/Lost (ELISA, Fig. 4)

11	Eucker et al. 2014 [95]	β1 integrin downregulation	1:?/5h	43504	INT 407	ND	ND	Yes→	Reduced/Lost (ELISA, Fig. 1B)		
12	Gorrell et al. 2013 [49]	Anti-β1 integrin antibody	1:100/24h	P12	AGS	ND	ND	Yes→	Reduced/Lost (ELISA, Fig. S3)		
				P1					Reduced/Lost (ELISA, Fig. S3)		
13	Boonyanugomol et al. 2013 [46]	Anti-β1 integrin antibody	1:1/6h	HP251	KKU-100	ND	ND	Yes→	Reduced/Lost (ELISA, Fig. 4)		
					KKU-M156			Yes→	Reduced/Lost (ELISA, Fig. 4)		
					AGS			Yes→	Reduced/Lost (ELISA, Fig. 4)		
		Anti-α5 integrin antibody			KKU-100			Yes→	Reduced/Lost (ELISA, Fig. 4)		
					KKU-M156			Yes→	Reduced/Lost (ELISA, Fig. 4)		
					AGS			Yes→	Reduced/Lost (ELISA, Fig. 4)		
14	Hutton et al. 2010 [47]	Anti-β1 integrin antibody	1:10/4 or 6h	HP251	AGS	ND	Yes→	Reduced/Lost (IM, Fig.4A.B)	Yes→	Reduced/Lost (ELISA, Fig. 5B)	
		Anti-α5 integrin antibody					Yes→	Reduced/Lost (IM, Fig.4A.B)	Yes →	Reduced/Lost (ELISA, Fig. 5B)	
15	Jiménez-Soto et al. 2009 [13]	β1 integrin complementation	1:60/2 & 4 h	P217	GE11	No→	Induced (WB, Fig. 1B)	ND		ND	
					GD25	No→	Induced (WB, Fig. 1B)				
		Anti-β1 antibody (mAb 9EG7)		P12	AGS	Yes→	Reduced/Lost (WB, Fig. 5B)				
16	Snider et al. 2008 [96]	Anti-β1 antibody (AIIB2)	1:100/18h	60190	AGS	ND	Yes→	Reduced/Lost (Fig. 3B)	ND		
17	Kwok et al. 2007 [8]	β1 integrin expression	1:100/2-4 h	P12	GD25	No →	Induced (WB, CLSM[6]) (Fig. 1A, B)	ND		ND	
		Anti- α5 integrin antibody			AGS	Yes→	Reduced/Lost (WB, Fig. S2b)	Yes→	Reduced/Lost (IM, Fig. S2b)		
		Anti-β1 integrin antibody			AGS	Yes→	Reduced/Lost (WB, Fig. S2b)	Yes→	Reduced/Lost (IM, Fig. S2b)		

[1]WB: Western blotting, [2]IM: Inverted Microscopy, [3]ND: Not Determined, [4]IP: Immunoprecipitation, [5]TEM: β-lactamase reporter system (TEM-1 reporter assay), [6]CLSM: Confocal Laser Scanning Microscopy, * Cell line descriptions (Table-5), [+]MoI: Multiplicity of Infection, [x]PI: Post Infection

human oral niche and the mouse stomach to *H. pylori*-induced pathogenesis [33]. An interesting experiment has identified the role of human CEACAMs in humanized mouse myeloid cells [63]. Amongst these cells, human CEACAM1, 3 and 6 expression in mouse polymorphonuclear, but not macrophages or dendritic cells, induces CagA translocation and phosphorylation, in a *hopQ*-dependent manner [63]. Overexpression of CEACAM1 [18–20,23,59] and CEACAM5 [19,20,59] receptors in CEACAM-deficient human gastric epithelial (HEK293, MKN28 and AZ521) cell lines [59], also resulted in CagA-P gain of function. On the other hand, simultaneous knocking out or knocking down of these CEACAM 1, 3 and 5 receptors, in KatoIII [18] and AGS [20] cells, resulted in reduced CagA-P. Similarly, the only study, that has investigated the effect of anti-CEACAM1 antibody on AGS cells, observed reduced CagA-P as well as reduced cell elongation [19].

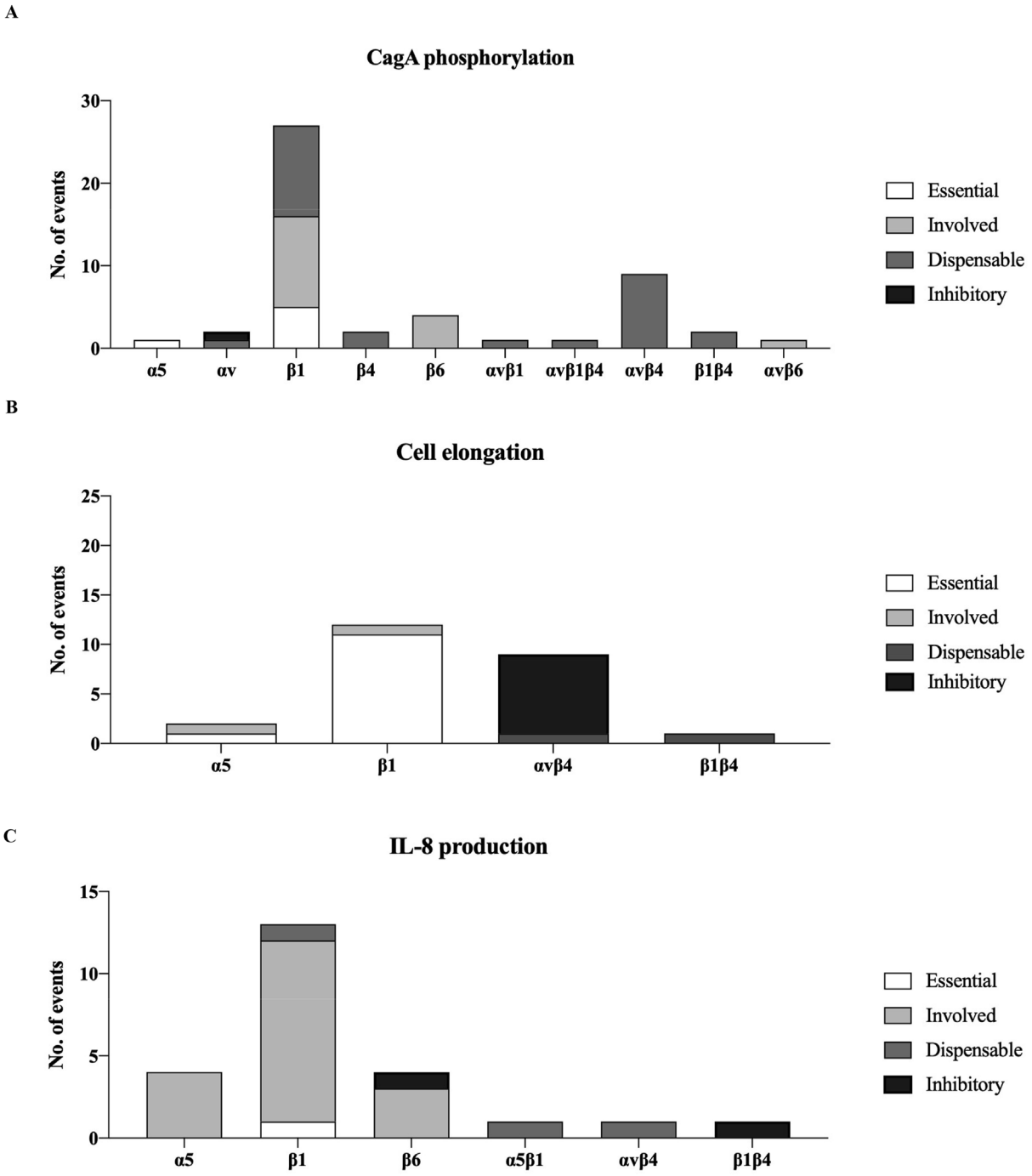

Figure-3. The role of the integrin receptors in the downstream functions, following *H. pylori* infection. A) CagA phosphorylation, B) cell elongation and C) IL-8 production. The deduced roles were classified as: "Essential" (lost or induced function, upon deletion/inhibition or addition of the target gene/protein, respectively), "Involved" (reduced or increased function upon deletion/inhibition or addition of the target gene/protein, respectively) "Dispensable" (no alterations) or d) "Inhibitory" (lost or induced function, upon addition or deletion/inhibition of the target gene/protein, respectively).

As mentioned above, the existing data on CEACAM6 is controversial (Figure-4). Such that upon expression of CEACAM6 in AZ-521 cells, CagA-P was not induced following P12 *H. pylori* strain infection of these cells [59], similar to what was observed in mouse gastric epithelial cells [62]. This was in contrast with CagA-P gain of function following NCTC11637 *H. pylori* infection of HEK293 cells, with exogenous expression of CEACAM6 [19]. The same study, found CEACAM 4, 7 and 8, dispensable for CagA-P [19]. In regards to cell elongation, the limited data available, finds CEACAM 1 5, and 6, dispensable for this purpose, in the three evaluated oral epithelial cell lines [33] (Figure-4B). We found no study, which has evaluated the loss or gain of IL-8 production, following CEACAM manipulation (Table-4).

3.5. Integrins versus CEACAMs

Intriguingly, integrins, the previously thought anchor of the T4SS apparatus, are not normally found at the apical membrane.

Table-4
Manipulations of CEACAM receptors and their effects on CagA phosphorylation, cell elongation and IL-8 production [75–96].

No.	Author Year (Ref)		Gene/ Protein manipulation	*H. pylori* MoI*/ Time PI$	*H. pylori* Strain	Cell line^	CagA phosphorylation Wild Type	CagA phosphorylation Manipulated cell line	Cell elongation Wild Type	Cell elongation Manipulated	IL-8 production Wild Type	IL-8 production Manipulated
1	Shrestha R. et al 2022 [62]	De novo expression	CEACAM1	1:100/ 7h	NCTC 11637	YTN16	No→	Induced (WB[1], Fig. S3)	ND[2]		ND	
						YTN2		Induced (WB, Fig. 4A)				
			CEACAM5			YTN16		Induced (WB, Fig. S3)				
						YTN2		Induced (WB, Fig. 4A)				
			CEACAM6			YTN16		Unchanged (WB, Fig. 4A)				
						YTN2		Unchanged (WB, Fig. S5)				
2	Tegtmeyer N.et al. 2020 [33]	De novo expression	CEACAM1	1:100/ 6h	HPAG1	HN	No →	Induced (WB, Fig. 6A)	No →	Unchanged (not shown)	ND	
			CEACAM5				No →	Induced (WB, Fig. 6A)	No →	Unchanged (not shown)		
			CEACAM6				No →	Induced (WB, Fig. 6A)	No →	Unchanged (not shown)		
			CEACAM1			CAL-27	No →	Induced (WB, Fig. 6B)	No →	Unchanged (not shown)		
			CEACAM5				No →	Induced (WB, Fig. 6B)	No →	Unchanged (not shown)		
			CEACAM6				No →	Induced (WB, Fig. 6B)	No →	Unchanged (not shown)		
			CEACAM1			BHY	No →	Induced (WB, Fig. 6C)	No →	Unchanged (not shown)		
			CEACAM5				No →	Induced (WB, Fig. 6C)	No →	Unchanged (not shown)		
			CEACAM6				No →	Induced (WB, Fig. 6C)	No →	Unchanged (not shown)		
3	Zhao, Q. et al. 2018 [18]		CEACAM1/5/6 Knock out	1:60/ 2.5h	P12 G27 1-20A TN2GF4	Kato III	Yes→	Lost/Reduced (TEM-CagA[3]/ WB, Fig. 5B,5D & 5C)	ND		ND	
4	Tegtmeyer N, et al. 2018 [59]	De novo expression	CEACAM1	1:100/ 9h	P12	AZ-521	No→	Induced (WB, Fig. 7A)	ND		ND	
			CEACAM5				No→	Induced (WB, Fig. 7B)				
			CEACAM6				No→	Unchanged (WB, Fig. 7C)				
5	Bonsor, D. A. et al. 2018 [51]	De novo expression	CEACAM1	1:100/ 4h	P12	HEK293	No→	Induced (TEM-CagA, Fig. 6D)	ND		ND	
6	Moonens, K. et al 2018 [23]	De novo expression	CEACAM1	1:5/50/100/ 6h	P12 Ka88 HPAG1	MKN28	No→	Induced (WB, Fig. 1E) Induced (WB, Fig. 5C) Induced (WB, Fig. 5C)	ND		ND	
7	Königer, V. et al. 2016 [20]	De novo expression	CEACAM1	1:60/ 4h	P12	HEK293	No→	Induced (WB, Fig. 3B)	ND		ND	
			CEACAM5					Induced (WB, Fig. 3C)				
		knockdown	CEACAM1,5,6			AGS	Yes→	Reduced/Lost (WB, Fig s5a)				
8	Javaheri, A. et al. 2016 [19]	De novo expression	CEACAM1	1:50/ 6h	P12	HEK293	No→	Induced (WB, Fig. 5F,S5F)	ND		ND	
			CEACAM1		NCTC 11637		No→	Induced (WB, Fig. S5G,F)				
			CEACAM3				No→	Induced (WB, Fig. S5G)				

CEACAM4			No→	Unchanged (WB, Fig. S5G)			
CEACAM5			No→	Induced (WB, Fig. S5G)			
CEACAM6			No→	Induced (WB, Fig. S5G)			
CEACAM7			No→	Unchanged (WB, Fig. S5G)			
CEACAM8		—	No→	Unchanged (WB, Fig. S5G)			
α-CEACAM1 Antibody	P12	AGS	Yes→	Reduced/Lost (WB, Fig. 5I)	Yes→	Reduced/Lost (IM[4], Fig. 5I)	ND

[1]WB: Western blotting, [2]ND: Not Determined, [3]TEM-CagA: β-lactamase reporter system (TEM-1 reporter assay), [4]Inverted Microscopy, *Cell line descriptions (Table-5), [†]MoI: Multiplicity of Infection, [§]PI: Post Infection

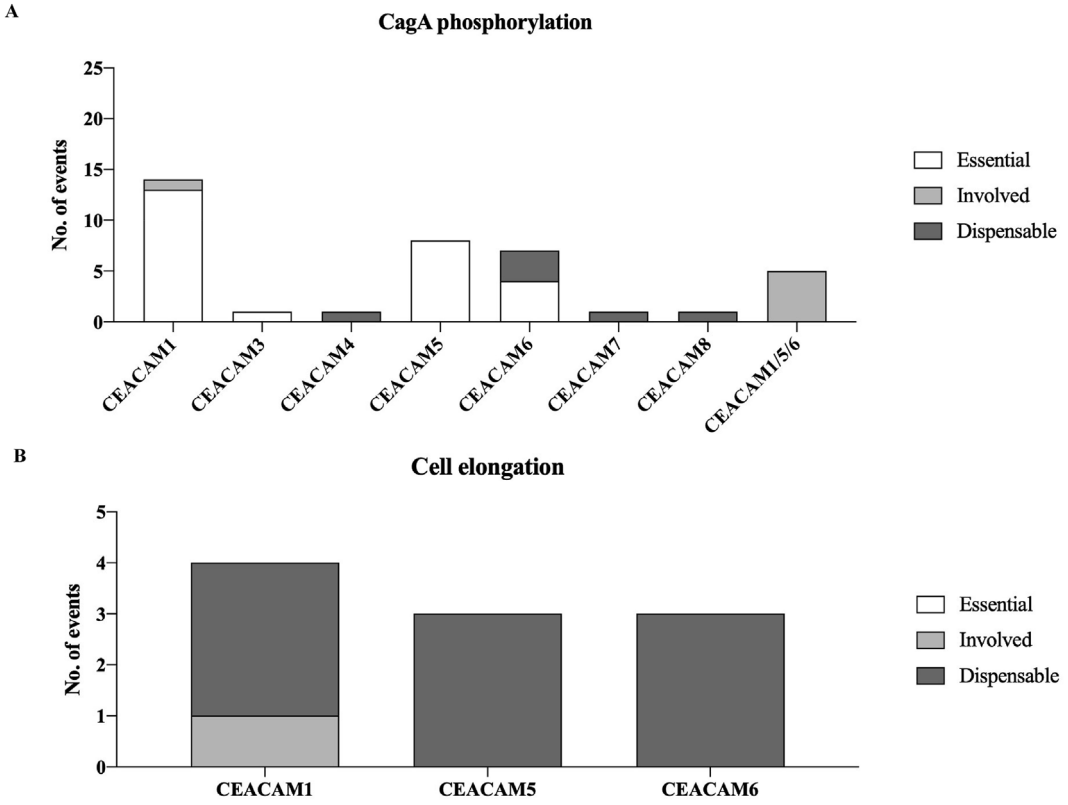

Figure-4. The role of the CEACAM receptors in the downstream functions, following *H. pylori* infection. A) CagA phosphorylation and B) cell elongation. The deduced roles were classified as: "Essential" (lost or induced function, upon deletion/inhibition or addition of the target gene/protein, respectively), "Involved" (reduced or increased function upon deletion/inhibition or addition of the target gene/protein, respectively) or "Dispensable" (no alterations).

Instead, they are localized at the basolateral membrane of polarized gastric epithelial cells [8]. If integrins play any role in CagA translocation, CagA might either be injected *via* the basolateral membrane and/or following basal-to-apical transcytosis of integrin molecules [64]. On the other hand, CEACAMs, are located on the lateral or apical sites, depending on the location of cell-cell contacts [65]. Accordingly, stimulation of CEACAM receptors positively affects the cell-matrix adhesion of epithelial cells [66]. In the case of CEACAM1, a phosphorylation-dependent interaction with integrin β3 has been reported [67]. CEACAM1 is also found colocalizing with the integrin β1 receptor on MCF7 cells, grown in Matrigel, both suggesting potential interactions between CEACAMs and integrins [68]. Gene expression studies have demonstrated that attachment of the bacteria to the CEACAM molecules results in the expression of an array of molecules, including CD105 (endoglin), within 1–3 h following *H. pylori* infection [66,68]. CD105 expression initiates the re-distribution of zyxin (focal adhesion protein), which binds to the cytoplasmic domain of CD105, without any impact on the level of integrin expression [69]. CEACAM stimulation, CD105 expression and ultimately rearrangement of the adhesion molecules, may indicate a remote monitoring of integrin-mediated cell adhesion, by CEACAMs [68]. It is worth mentioning, however, that the normal gastric lining is not abundant in CEACAM expression, and it is only

after *H. pylori* colonization and/or histopathologic changes of the stomach, that the expression of these receptors is upregulated [19].

3.6. Choice of cell lines

As is evident from the different experimental settings presented in Tables 1–4, there are several variables that may be responsible for the varying and sometimes controversial results and the subsequent interpretations. One of the determining factors may be the histopathologic stage of the employed experimental cell lines, ranging from healthy cells to fully transformed cancer cells. Accordingly, the expression levels of the target receptors (integrins and CEACAMs) on these cells, may vary as well. Furthermore, as depicted in Table-5, depending on the source of the cell lines, be it epithelial, endothelial, fibroblast, etc. and the species origin (human or mouse), and the organ from which it was derived (oral cavity, gastric, kidney, intestine, etc.), the type and intensity of the expressed receptors may differ. Therefore, we will primarily discuss the diversity of receptor expression levels on the more commonly used cell lines.

AGS is a human gastric adenocarcinoma cell line, which is most frequently used as the *in vitro* model for *H. pylori* infection of gastric epithelial cells. Analysis of RNA levels of CEACAMs and integrin receptors, identified expression levels of CEACAM1 and CEACAM5 at low, and CEACAM6 at medium levels (Table-5). Interestingly, at protein levels, AGS cells have no expression of CEACAM1, 5 and 6 at the logarithmic phase and it is only at the confluency stage, that they express a very small amount of CEACAM5 and 6 [19]. The expression of integrin β1, β4 and αv are at medium and β6, and α5 at low levels, in this cell line (Table-5). It can, thus, be concluded that AGS cells are better equipped with integrin receptors, than with CEACAMs. The manipulation of CEACAMs in this cell line was limited to the simultaneous downregulation of CEACAM 1/5/6 [20] and the use of α-CEACAM1 antibodies [19]. Considering the very limited expression of CEACAM1 on AGS cells, it seems unlikely that this molecule is in the driver's seat of the process of CagA translocation in AGS cells, and receptors other than CEACAMs or integrins, attaching to ligands other than HopQ may also be involved [18]. On the other hand, in examining the status of integrins, it seems antibody inhibition of integrin β1 [8,13] and α5 [8] leads to the loss of CagA translocation and this inhibition holds, even after 24 h of *H. pylori* infection [13]. Interestingly, if the expression of β1 is downregulated by siRNA treatment, CagA translocation is also reduced, but not fully abolished [59]. However, this observation was made at 9 h post infection. It seems that during the early hours there is no transfer of CagA protein, but takes place over time [59]. This could be a reason for the bacteria to compromise and find alternative means to translocate the CagA protein. It appears that only after the complete elimination of β1 integrin, and not its partial impairment or inhibition, it is that other receptors may come into play and compensate for its absence. This hypothesis may also hold true for partial antibody-mediated inhibition of αvβ6 integrins [9]; such that masking these receptors, only results in reduced CagA translocation at 3 h post infection. In contrast, the removal of αv/β4, β4, β1/β4, αv, and αv/β4 integrins, as seen for β1 integrin, does not affect protein transport during 2.5–6 h following infection [18,32].

MKN45 is another human gastric adenocarcinoma cell line, established from the poorly differentiated adenocarcinoma of the stomach of a 62-year-old woman, which is also frequently used as an *in vitro* model for *H. pylori* infection of gastric epithelial cells. Although this cell line is similar to AGS in terms of the expression of CEACAM 1 and 3, the expression of CEACAM 5 and 6 is at high levels (Table-5). In terms of the expression of integrins, it is similar to AGS, except for the lower expression of αv. Hence, this cell line may be considered as CEACAM-dominant and somewhat integrin-deficient. Unfortunately, there is no study on the effect of targeting or disrupting the CEACAMs or integrins of this cell line. The only two studies on this cell line, in this regard, have focused on its infection with a *hopQ*-inactivated *H. pylori* strain, which resulted in reduced CagA translocation [20] and IL-8 production [56]. This event clearly shows that the process of CagA translocation, even in CEACAM-dominant cells, is not exclusively dependent on HopQ/CEACAM interaction.

KatoIII is a human gastric carcinoma cell line, derived from the pleural effusion of gastric carcinoma in a 55-year-old male subject. According to the results of the RNA sequence analysis (Table-5), the expression of CEACAM 1, 5 and 6 is higher on these cells than that of AGS. In terms of the expression of integrins, KatoIII cells have similar expressions of β1, β4 and β6, as compared to AGS and lower expression of αv and minute amount of α5 (Table-5). Hence, collectively, this cell line is better equipped with CEACAM receptors and weaker in terms of integrin expression. There is only one report of the simultaneous deletion of CEACAM 1, 5, and 6 in this cell line, which has the highest expression [18]. Despite this genetic manipulation, *H. pylori* are still able to translocate CagA in a short time period (2.5 h), albeit to a lesser extent than in the wildtype KatoIII [18]. In other words, as detected by Western blotting, it could be concluded that in the absence of CEACAMs (1, 5 and 6), *H. pylori* is still able to translocate CagA, but at a very low rate. This hypothesis is however, refuted by the highly sensitive beta-lactamase reporter assay, which shows minimal levels of CagA transport in this context [18]. On the other hand, inactivation of β1, β4, β1/β4, αv, αv/β4 and αv/β1 integrins in this cell line, has no effect on CagA translocation, even in a short period (2.5 h) of co-incubation [18]. One hypothesis is that by removing one integrin, the expression of other integrins increases, and this process may lead to the replacement of these molecules in regards to CagA translocation. It could also be that due to the high expression of CEACAMs in this cell line, integrins are not involved in CagA translocation.

MKN28 is a well differentiated human gastric adenocarcinoma cell line, which is distinguished from the above-mentioned three cell lines, (AGS, MKN54 and KatoIII) by its lack of expression of the two key CEACAM molecules (1 and 5) and the low expression of CEACAM6 (Table-5). Therefore, it is a proper model for exogenously expressing these molecules and examining their effects on CagA translocation. This cell line has moderate to low expression levels of αv and does not express β6 integrin. However, only the role of CEACAM/HopQ interaction in CagA translocation, has been investigated in this cell line [23,58]. Such that upon infection of the MKN28 cells exogenously expressing CEACAM1, CagA translocation is induced [23]. The point about MKN28 cells is their inability to bind *H. pylori*, which seems to be due to the general low or lack of integrin and CEACAM expression. Hence, bacteria are not able to colonize and infect these cells and the sole expression of CEACAM1 seems to provide this capacity.

AZ-521 is a derivative of the human duodenal carcinoma cell line, HuTu-80, which lacks the expression of all CEACAMs and is deficient in most integrins, except for αv and β1 integrins, which are expressed at medium levels (Table-5). Studies have shown that no CagA translocation occurs in this cell line, during 9 h of infection with different (P12, P310 and P420) *H. pylori* strains [59]. Furthermore, overexpression of β1 integrin does not induce this function [59]. On the other hand, AZ-521 cells transfected with CEACAM1/5, were able to translocate CagA, when infected with the P12 *H. pylori* strain, during the same time course. Intriguingly, however, β1 integrin downregulation by siRNA, in this CEACAM1/5 expressing AZ-521 cell line, reduced CagA translocation by ~70 % [59]. On the contrary, in another study, siRNA-mediated gene silencing of β1

Table-5
CEACAM and integrin receptor expression levels of the studied cell lines.

	Cells	Description	CEACAMs[a]							Integrins[a]					Receptor Expression Levels
			1	3	4	5	6	7	8	β1	β4	β6	αv	α5	
1	Normal stomach	-	Low	Neg	Neg	Med	Low	Neg	Neg	Med	Med	Low	Low	Low	(CEACAM1/6^{Lo} 5^{Med}- Integrin β1/β4^{Med}- β6/αv/α5^{Lo})
2	Gastric Adenocarcinoma	-	Low	-	-	Med	Med	-	-	Med	Med	Low	Low	Low	(CEACAM1^{Lo} 6/5^{Med}- Integrin β1/β4^{Med}-β6/αv/α5^{Lo})
3	AGS	Human gastric adenocarcinoma	Low	Neg	Neg	Low	Med	Neg	Neg	Med	Med	Low	Med	Low	(CEACAM1/5^{Lo} 6^{Med}- Integrin β1/4/αv^{Med}-β6/α5^{Lo})
4	AZ-521	Human duodenal adenocarcinoma	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Med	Low	Neg	Med	Low	(CEACAM1/5/6^{Neg}- Integrin β1/αv^{Med}-β4/α5^{Lo})
5	SW480	Human colorectal adenocarcinoma	Low	Neg	Neg	Low	Low	Neg	Neg	Med	Low	Neg	Low	Low	(CEACAM1/5/6^{Lo}- Integrin β1^{Med} - β4/αv/α5^{Lo})
6	HUVEC	Human umbilical vein endothelial	Neg	Neg	Neg	Neg	Neg	Neg	-	Med	Neg	Neg	Med	High	(CEACAM1/5/6^{Neg}- Integrin β1/αv^{Med}-β4/β6^{Neg}/α5^{Hi})
7	KatoIII	Human gastric carcinoma	Med	Neg	Neg	High	High	Low	Low	Med	Med	Low	Low	Neg	(CEACAM1^{Med} -5/6^{Hi}- Integrin β1/β4^{Med}-β6/αv ^{Lo}/α5^{Neg})
8	HEK293	Human embryonic kidney	Low	Low	Low	Low	Low	Low	Low	-	Low	Low	High	High	(CEACAM1/5/6^{Lo} — Integrin β1$^{?}$/β4/β6^{Lo} - α5/αv ^{Hi})
9	HL60	Homo sapiens/ neutrophil-like	Neg	Neg	Low	Neg	Neg	Neg	Low	Low	Neg	Neg	Neg	Low	(CEACAM1/5/6^{Neg} — Integrin α5/β1^{Lo} -β4/β6/αv ^{Neg})
10	Hela	Human cervical cancer	Neg	Neg	Neg	-	Neg	Neg	Neg	Med	Low	Neg	Med	Med	(CEACAM1/6^{Neg} -5$^{?}$ — Integrin β1/αv/α5 ^{Med} -β4^{Lo} -β6^{Neg})
11	MKN28	Human gastric tubular adenocarcinoma	Neg	-	-	Neg	Low	Low	-	Med	Med	Neg	Low	Low	(CEACAM1/5^{Neg} -6^{Lo} — Integrin β1/β4^{Med}- β6^{Neg} -αv/α5^{Lo})
12	MKN45	Human Gastric adenocarcinoma	Low	Neg	Neg	High	High	Neg	Neg	Med	Med	Low	Low	Low	(CEACAM1^{Lo} -5/6^{Hi} — Integrin β1/β4^{Med} - β6/αv/α5^{Lo})
22	HCT116	Human colorectal carcinoma cells	Neg	Neg	NA	Neg	Neg	Neg	Neg	Med	Med	Neg	Low	Low	(CEACAM1/3/5/6/7/8^{Neg} -4NA — Integrin β2/β6^{Neg}- αv/α5^{Low}- β1/β4^{Med})
23	NUGC-4	Human gastric cancer cell	Neg	Neg	NA	Neg	Neg	Neg	Neg	Med	Med	Neg	Low	Neg	(CEACAM1/3/5/6/7/8^{Neg} -4NA — Integrin β2/β6/α5 ^{Neg} - αv^{Low}-β1/β4^{Med})
13	HN	Human oral epithelial cells	Neg	-	-	Neg	Neg	-	-	-	-	-	-	-	(CEACAM1/5/6^{Neg})
14	CAL-27	Human oral epithelial cells	Neg	-	-	Neg	Neg	-	-	-	-	-	-	-	(CEACAM1/5/6^{Neg})
15	BHY	Human oral epithelial cells	Neg	-	-	Neg	Neg	-	-	-	-	-	-	-	(CEACAM1/5/6^{Neg})
16	KKU-100	Human cholangiocarcinoma	-	-	-	-	-	-	-	Med	-	-	Med	-	(Integrin αv^{Med}- β1^{Med})
17	KKU-M156	Human cholangiocarcinoma	-	-	-	-	-	-	-	Med	-	-	Med	-	(Integrin αv^{Med}- β1^{Med})
18	GE11	Murine embryonic epithelial	-	-	-	-	-	-	-	Neg	-	-	-	-	(Integrin β1^{Neg})
19	GD25	Murine embryonic fibroblast like	-	-	-	-	-	-	-	Neg	-	-	-	-	(Integrin β1^{Neg})

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Uhlen's Lab: RNA-seq of coding RNA from tissue samples of 122 human individuals representing 32 different tissues.
Hallstrom et al., 2014 - Organism part: RNA-seq of coding RNA from tissue samples of 95 human individuals representing 27 different tissues in order to determine tissue-specificity of all protein-coding genes.
NIH Epigenomics Roadmap: RNA-seq of coding RNA of 19 human tissues from fetuses with congenital defects (NIH Roadmap Epigenomics Mapping Consortium)/.
FANTOM5 project — fetal: RNA-Seq CAGE (Cap Analysis of Gene Expression) analysis of human tissues in RIKEN FANTOM5 project.
Cell lines - CCLE - gastric adenocarcinoma.
675 Genentech - stomach, gastric adenocarcinoma: RNA-seq of 934 human cancer cell lines from the Cancer Cell Line Encyclopedia.
Cell lines - CCLE - colon adenocarcinoma: RNA-seq of 934 human cancer cell lines from the Cancer Cell Line Encyclopedia.
Cancer Genome Project - hematopoietic system, acute lymphoblastic leukemia.
Crownbio database.
Cell lines - colorectal cancer.
ENCODE - long polyA RNA, whole cell: RNA-seq of long poly adenylated RNA and long non poly adenylated RNA from ENCODE cell lines.
ENCODE - long non-polyA RNA, whole cell.
ENCODE - long polyA RNA, nucleus.
ENCODE - long polyA RNA, cytosol.
ENCODE - long non-polyA RNA, nucleus.
ENCODE - long non-polyA RNA, cytosol.
Cell lines - CCLE - signet ring cell gastric adenocarcinoma.
Proteomics - Cell Line - Comparative analysis of common cell lines.
Cell lines - CCLE - adult acute myeloid leukemia.
675 Genentech - blood, leukemia.
Cancer Genome Project - uterine cervix, cervical carcinoma.
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[a] The receptor expression levels were categorized as: 1) high (above 1000 copies), 2) medium (100-1000 copies), 3) low (10-100 copies) and 4) Neg (0-10 copies).

integrin in AZ-521 cells infected, with ATCC 43504 *H. pylori* strain, with the same multiplicity of infection and time course did not affect the residual CagA translocation, as detected by immunoprecipitation [61]. Whereas, deletion of *hopQ* from the *H. pylori* strain, infecting a CEACAM1-expressing AZ-521 cell line, found this adhesin to be essential for CagA translocation [39,59]. Altogether, these results suggest that the sole presence of β1 integrin in a cell line, lacking CEACAM molecules, cannot mediate the process of CagA translocation.

HEK293, an immortalized human embryonic kidney cell line, when compared to AGS cells, expresses higher levels of CEACAM3, α5 and αv and lower levels of CEACAM6 and β4 integrin (Table-5). Therefore, this cell line is also suitable for studying the role of the already existing α5 and αv integrins or exogenous expression of β integrins and CEACAM molecules, in the process of CagA translocation. In this regard, several studies have demonstrated that the de novo expression of CEACAM1 [19,20,23,51], CEACAM3 [19], CEACAM5 [20]and CEACAM6 [19], but not CEACAM 4, 7 and 8 [19], enables this cell line to allow for CagA translocation, during 4–6 h of infection. To confirm this process, *hopQ* inactivation in the infecting *H. pylori* strain reduced, but did not eliminate the protein transfer [19,20,51], hence, again hinting towards other molecules (ligands and/or receptors) potentially being also involved in this process, as well.

3.6.1. hopQ alleles

Another potential determinant may be the types of the infecting strains and their *hopQ* alleles. In this regard, very little has been discussed despite the observed CEACAM binding preferences for different strains [19] and different *hopQ* alleles [23]. In other words, amongst the strains presented in Tables 1–4, most (P12, 26695, G27, 7.13, NCTC11637, TN2, SJM180, and B128) carry the type I and some (i.e. GAM94-24) the type II allele. There are other strains, such as SS1, PMSS1 and J166, which carry both alleles. The only study that has examined such details has used the PMSS1 strain with *hopQ IA/IB* and *hopQ II* alleles [22]. This study has demonstrated that simultaneous inactivation of both *hopQ IB* and *hopQ II* alleles in this strain, results in loss of CagA translocation in AGS cells, whereas the *hopQ IA* allele seems to be redundant in this process [22].

3.7. The duration of and multiplicity of infection

The duration of the co-culture and multiplicity of infection (MOI), may also be responsible for the varying observations. Interestingly these variables were quite divergent among the reviewed studies and those who have studied multiple settings [18,23,52] have realized their impact on the obtained results. For instance, complete inactivation of the *hopQ* gene led to the loss of CagA translocation, only at the early (30 min) time point following *H. pylori* (P12) infection of AGS cells. However, at 103 min following infection residual CagA translocation is again observed [52]. Accordingly, at longer time periods (150 min–6 h), CagA is translocated despite *hopQ* inactivation, albeit at a lower extent than that observed for the wild type strain [18–20,51,55,70]. It seems that the longer the co-incubation of the bacteria and the cells, the higher the probability of other compensatory molecules on the part of the bacteria or the host cells to come into play, though instances to disprove this hypothesis, also exist. Another point of concern, is the multiplicity of infection, the impact of which has hardly been discussed.

3.8. The method of detection

Another determinant may be the method of choice used for CagA-P detection (immunoprecipitation, western blotting, microscopy, etc.), the sensitivity and accuracy of which may well impact the obtained results. For instance, the immunoprecipitation method, which enriches the target protein and collects immunocomplexes, is more precise than solid-based western blot analysis, which detects the expression of a specific protein in the cell lysate. This is evidenced by the fact that western blotting found AZ-521 cells, incapable of CagA-P [59], whereas in two other studies [61,71], CagA-P was detected using immunoprecipitation technique on the same cell line. However, the most sensitive and accurate method at hand seems to be the beta-lactamase reporter assay [18]. Nevertheless, it should also be kept in mind that other routes of CagA translocation, such as *via* the extracellular vesicles [72], may bypass the classic route of CagA transfer and may create certain confusion upon interpretation of the obtained results [73].

As expected, these multiple variations amongst the presented experimental settings, have yielded controversial and even at times contradictory results. Accordingly, an informative exchange regarding the role of integrins in CagA-P and the downstream cell elongation (hummingbird phenotype) in AGS cells, took place between two leading laboratories [32,74]. Tegtmeyer and Backert [32] commented on the paper by Zhao et al. [18], in which the roles of β1, β4, αv and αvβ4, were found dispensable in the process of CagA phosphorylation and cell elongation following *H. pylori* (P12) infection of deficient AGS cells. These authors [32] repeated the aforementioned experiments, with several *H. pylori* strains and confirmed the former findings regarding CagA-P. However, in regards to cell elongation, β1 and αvβ4 were found essential *versus* inhibitory, respectively. In response, Fischer and Haas [74] stated that the choice of the infecting strain, their level of adherence to the cells, and in turn the adherence of the cells to the tissue culture plates, can all be amongst the determinants explaining the two sets of somewhat contradictory results, regarding *H. pylori*-induced hummingbird phenotype.

4. Conclusion

Taken together, our detailed analysis of the limited evidence revealed that in the process of CagA phosphorylation and cell elongation, the T4SS constituents, mostly seem essential or involved, except for the role of *cagN* (core complex), which is controversial. However, for IL-8 production, those tested are mostly involved, whereas the roles of *cagβ* (energetic component) and *cagF* (translocation-associated factors) seem mainly dispensable. Concerning the ligand of current interest, HopQ, the data is controversial, but mostly voting for it being involved in CagA phosphorylation, cell elongation and IL-8 production. As for the previous longstanding receptor, the integrin family, the existing data on their essence in CagA phosphorylation is highly controversial, but yielding it dispensable or involved. Yet, regarding cell elongation, more events are showing β1 integrin being involved, than αvβ4 being inhibitory. Concerning IL-8 secretion, again there are more events showing α5, β1 and β6 integrins to be involved, than those showing inhibitory roles for β1, β4 and β6 integrins. Finally, of the recently highlighted CEACAM receptors, CEACAM 1, 3, and 5 are identified as mostly essential or involved in CagA phosphorylation. Yet, CEACAM 4, 7, and 8 are dispensable and CEACAM 6 is under debate. However, CEACAM 1, 5 and 6 appear mostly dispensable for cell elongation. To our knowledge, there is no

information on the essence of CEACAMs in IL-8 production. We would also like to hereby emphasize that assessment of the existing data while controlling for the cell type (source, receptor expression levels, adherence properties, etc.), bacterial strain (genetic composition and adherence levels), multiplicity and duration of infection, as well as the sensitivity and accuracy of the applied method of detection, would help strengthen the resulting interpretations.

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