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Short communication

# *Helicobacter pylori* promotes eukaryotic protein translation by activating phosphatidylinositol 3 kinase/mTOR

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ABSTRACT

The innate immune response elicited by *Helicobacter pylori* in the human gastric mucosa involves a range of cellular signalling pathways, including those implicated in metabolism regulation. In this study, we analysed *H. pylori*-induced PI3K/Akt/mTOR signalling, which regulates glycolysis and protein synthesis and associates thereby with cellular energy- and nutrients-consuming processes such as growth and proliferation. The immunohistochemical analysis demonstrated that Akt kinase phosphorylation is abundant in gastric biopsies obtained from gastritis, gastric adenoma and adenocarcinoma patients. Infection with *H. pylori* led to the phosphorylation of Akt effectors mTOR and S6 in a type 4 secretion system (T4SS)-independent manner in AGS cells. We observed that the activation of these molecules was dependent on PI3K and the Src family tyrosine kinases. Furthermore, *H. pylori* induced the phosphorylation of 4E-BP1 and eIF4E and suppressed the phosphorylation of eEF2, which are important regulators of protein synthesis. Inhibition of PI3K and Akt kinase prevented the phosphorylation of 4E-BP1, suggesting that PI3K signalling is involved in the regulation of translation initiation during *H. pylori* infection. Metabolic labelling showed that infected cells had higher rates of [$^{35}$S]methionine/cysteine incorporation, and this effect could be prevented using LY294002, an PI3K inhibitor. Thus, *H. pylori* activates PI3K/Akt signalling, mTOR, eIFs and protein translation, which might impact *H. pylori*-related gastric pathophysiology.



## 1. Introduction

Phosphatidylinositol-3-kinase (PI3K) phosphorylates phosphatidylinositol in the plasma membrane and converts this molecule into polyphosphoinositides, including phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 interacts with the plekstrin homology domain of a serine/threonine kinase Akt for translocation to the plasma membrane, allowing Akt phosphorylation at Thr308 trough phosphoinositide-dependent kinase-1 (PDK-1) (Datta et al., 1999). This phosphorylation partially activates Akt, which is sufficient to induce the mammalian target of rapamycin (mTOR) complex 1 (mTORC1). Additional phosphorylation at Ser473, either through autophosphorylation or DNA-dependent protein kinase (the elusive PDK-2 before), mTORC2 and integrin-linked kinase (ILK), leads to the complete activation of Akt (Hemmings and Restuccia, 2012). Akt phosphorylates a network of the substrates involved in regulation of metabolism, the cell cycle, protein synthesis, proliferation and apoptosis (Manning and Cantley, 2007; Hers et al., 2011). PI3K/Akt signalling is activated downstream of tyrosine kinase- and G-protein-coupled receptors in response to growth factors (GFs) (Insulin-like GF, epidermal GF, platelet-derived GF and hepatocyte GF), cytokines (interleukins 2–5, 8), mitogenes and hormones (Datta et al., 1999).

PI3K/Akt activity is negatively regulated through PIP3 Phosphatase and Tensin Homolog (PTEN), protein phosphatase 2, SH2-containing inositol phosphatase and Carboxyl-Terminal Modulator Protein (Hemmings and Restuccia, 2012). The PI3K/Akt signalling pathway is hyperactive in some metastatic cancers, including gastric tumours (Cinti et al., 2008; Almhanna et al., 2011).

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Activated receptor tyrosine kinases, function-off *PTEN* mutations and *PKB*/*Akt* gene amplification are responsible for PI3K/Akt induction in gastric carcinomas (Michl and Downward, 2005; Tran et al., 2013).

Hyperactivation of PI3K/Akt signalling during cancer development alters the activity of downstream transduction pathways that control overall protein synthesis and translation of individual mRNAs (Schneider and Sonenberg, 2007). Because increased rates of protein synthesis positively correlate with growth and proliferation and, thus, participate in cancer biogenesis, components of the translational machinery might represent potential targets for tumour treatment strategies.

Diffuse and intestinal gastric cancer development is often associated with chronic *H. pylori* infections in humans (Peek and Crabtree, 2006). Here, we show that the activation of PI3K/Akt signalling in response to *H. pylori* regulates protein translation in infected cells.

## 2. Materials and methods

### 2.1. Reagents

Mouse anti-GAPDH antibody was purchased from Millipore, USA. All other antibodies were from Cell Signaling Technology Inc., USA. Akti1/2, AG1478/AG825, LY294002 and PP2 were purchased from Calbiochem/Merck KGaA, Germany. PD98059 was obtained from BioSource, Belgium. PHA665752 was kindly provided from Pfizer Global Pharmaceuticals, USA.

### 2.2. Cell culture and bacteria

AGS cells (ATCC) were cultured in RPMI-1640 medium (PAA Laboratories GmbH, Austria) supplemented with 10% foetal bovine serum (FBS) in a humid atmosphere at 37 °C with 5% $CO_2$. At 16 h before infection with *H. pylori*, the complete medium was replaced with fresh RPMI-1640 medium supplemented with 0.5% FBS.

The *H. pylori* strain P1 wt and isogenic mutants *cagA* and *virB7* (Backert et al., 2000) were cultured for 48–72 h as previously described (Churin et al., 2003) and subsequently added to the epithelial cells at a multiplicity of infection of 100.

### 2.3. Tissue samples and immunohistochemistry

Stomach biopsy specimens were obtained from patients (age range 19–96 years) according to the recommendations of the updated Sydney System (Dixon et al., 1996) and were examined by the same experienced gastrointestinal pathologist who was blinded to the clinical and endoscopic data. *H. pylori* was detected through H&E and Warthin-Starry-silver staining. The histological features of the gastric mucosa, including inflammation and atrophy, were scored according to the updated Sydney System. Neoplasia was diagnosed according to the 2010 WHO classification criteria. The deparaffinised sections were stained with primary rabbit polyclonal antibodies (dilution 1:50) and biotinylated secondary antibody as previously described (Sokolova et al., 2013). The immunostaining was semiquantitatively evaluated according to the Remmele immunoreactive score (IRS) (Remmele and Stegner, 1987; Allred et al., 1998). Briefly, the percentage of positively stained epithelial cells was divided into five grades of 0–4 (0%, <10%, 10–50%, 51–80% and >80%) and multiplied by the intensity of the immunohistochemical reaction scaled from 0 to 3. The obtained IRS was interpreted as 0 to 1 = no expression; 2 to 3 = week expression; 4 to 8 = moderate expression; 9 to 12 = strong expression.

### 2.4. Cell lysates and western blotting

The cell lysates were prepared using modified RIPA buffer, and western blotting was performed as previously described (Sokolova et al., 2008, 2013).

### 2.5. Protein synthesis assay

At 6 h post infection (p.i.), the cell medium was replaced with Met/Cys-free RPMI-1640 (Sigma–Aldrich Chemie GmbH, Germany) supplemented with 2 mM L-glutamine (Sigma–Aldrich Chemie GmbH, Germany), 0.5% dialysed FBS (Invitrogen) and 4 mg/ml clarithromycin for 30 min. Then the cells were labelled with [$^{35}$S]Met/Cys using Redivue Pro-Mix Cell Labeling Mix (Amersham) at 0.05 mCi per 1 ml in fresh RPMI-1640/L-glutamine/FBS medium for 30 min. After washing with PBS, the labelled cells were scrapped from dishes in 0.3 ml of PBS supplemented with 1 mM AEBSF (AppliChem GmbH, Germany). Total proteins were precipitated from the 20-μl suspension by using 10% TCA. The precipitates were placed onto filters and washed twice with 5% TCA and twice with 95% ethanol. The dried filters were placed in 5 ml of scintillation cocktail, and the [$^{35}$S]Met/Cys incorporation was measured using LS 6000 Liquid scintillation counter (Beckman Coulter). The protein concentration in the cell suspension was estimated using the Pierce$^{R}$ BCA Protein Assay kit (Thermo Scientific, USA), and the radioactivity was calculated as CPM per 1 mg of protein.

### 2.6. Transfection

At 48 h before infection, AGS cells ($1 \times 10^5$ cells/35-mm dish) were transfected with 40 nM siRNA (Santa Cruz Biotechnology Inc., USA) using siLentFect$^{TM}$ Lipid Reagent (BioRad, USA) in Gibco Opti-MEM$^{TM}$ I culture medium (Life Technologies, UK) supplemented with 5% FBS. A scrambled siRNA was used as a control.

### 2.7. Statistical analysis

The statistical analysis of the results was done using a Student's *t*-test. $P < 0.05$ was considered significant.

## 3. Results and discussion

### 3.1. H. pylori activates PI3K, Akt, mTOR and S6

Many studies conducted in the past 4–5 years have revealed a prognostic and/or predictive role of Akt phosphorylation in breast, prostate and non-small cell lung cancers (Cicenas, 2008). Examining 145 biopsies obtained from the stomachs of healthy subjects and patients with different gastric pathologies showed that Akt was phosphorylated in gastric epithelial cells to varying extents (Fig. 1A). Strong Akt phosphorylation was observed in 11.4% of specimens without pathological changes and 23.3% of biopsies from patients with *H. pylori*-induced gastritis (Table 1). Furthermore, the biopsies from gastric carcinoma patients demonstrated staining intensity similar to that of patients with *H. pylori*-related gastritis: the number of specimens strongly positive for phosphorylated Akt was approximately 2 times greater than that in healthy subjects (Table 1, Fig. 1A). Notably, there were no differences in the abundance of total Akt in stomach biopsies from all investigated groups (data not shown). These findings are consistent with Cinti et al. (2008), who observed increased levels of phosphorylated Akt in gastric carcinoma.

The activation of PI3K and Akt in *H. pylori* infection *in vitro* has been previously described (Churin et al., 2003; Sokolova et al., 2008). Studying the role of CagA and the type 4 secretion system (T4SS), we observed that the infection of AGS cells with the wt

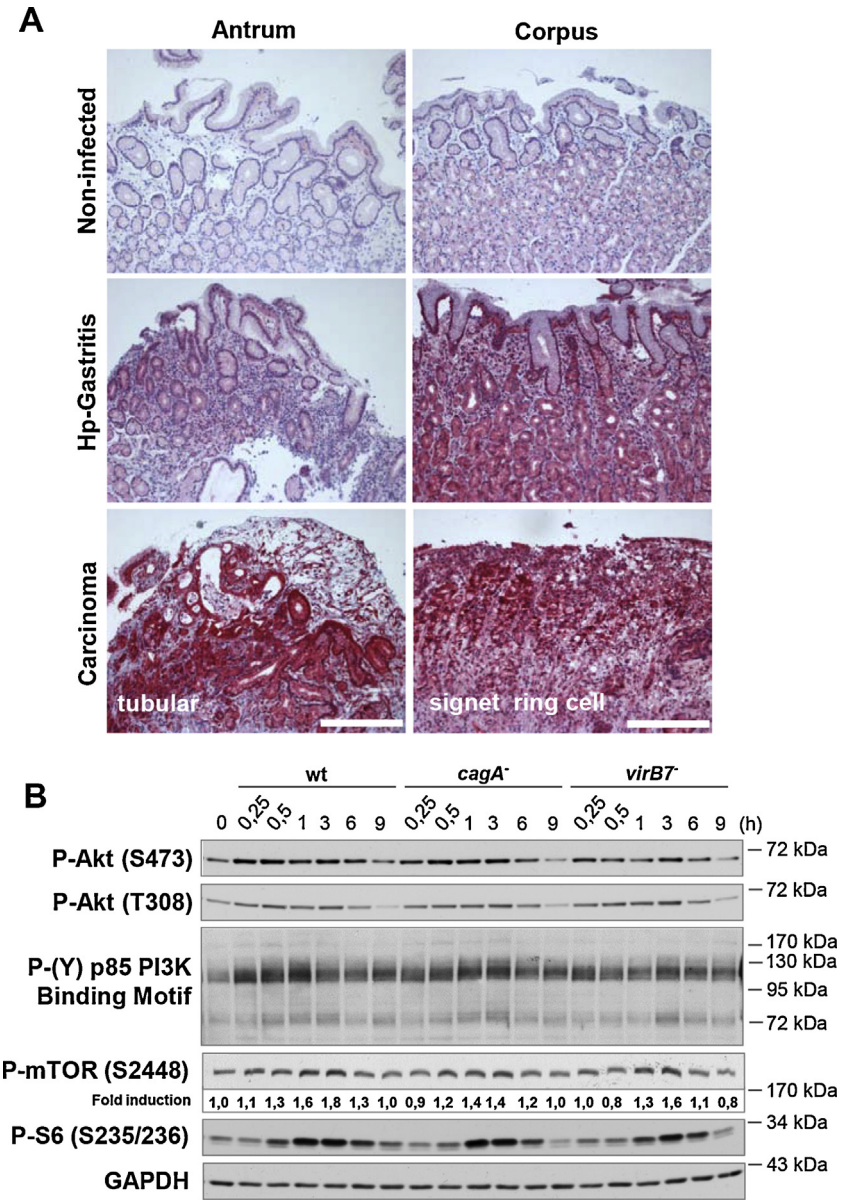


**Fig. 1.** *H. pylori* induces the phosphorylation of Akt, mTOR and S6.
(A) Akt S473 phosphorylation is abundant in the gastric biopsy samples from gastritis and gastric carcinoma patients. High-power field confocal images are shown. Magnification, 20×. Scale bars = 200 μm. (B) Cells were infected with the wt or *cagA* and *virB7* mutants of *H. pylori* strain P1 for various times, and cell lysates were prepared. The western blot analysis was performed using the indicated antibodies.

P1 strain of *H. pylori* or with the isogenic CagA- or VirB7-deficient mutants induces rapid and sustained phosphorylation of Akt within the C-terminal hydrophobic motif at Ser473 and the kinase domain at Thr308 (Fig. 1B). VirB7 is required for the assembly of the pilus-like T4SS, which functions in the transport of CagA and other yet unknown bacterial effector molecules into epithelial cells (Backert et al., 2000). Therefore, neither functional T4SS nor CagA is required for Akt phosphorylation in AGS cells. This is consistent with the data of Nagy et al. (2009) and Tabassam et al. (2009), obtained using *H. pylori* strains 7.13 and ATCC26695, respectively. In these

**Table 1**
Akt phosphorylation in human gastric mucosa tissue.

| Diagnosis | Number of specimens | Age | Gender | | Distribution based on staining intensity, number of specimens (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | M | F | Negative | Weak | Moderate | Strong |
| Without pathological changes | 35 | 19–72 | 21 | 14 | 11/31.4 | 7/20.0 | 13/37.1 | 4/11.4 |
| Hp-Gastritis | 30 | 32–82 | 19 | 11 | 3/10.0 | 6/20.0 | 14/46.7 | 7/23.3 |
| Adenoma (low grade dysplasia) | 21 | 31–82 | 11 | 10 | 0/0 | 5/23.8 | 6/28.6 | 10/47.6 |
| Adenocarcinoma | 59 | 34–96 | 27 | 32 | 6/10.2 | 11/18.7 | 26/44.0 | 16/27.1 |
| Well & moderate differentiated adenocarcinoma | 16 | 59–85 | 9 | 7 | 1/6.3 | 4/25 | 5/31.3 | 6/37.5 |
| Poorly differentiated adenocarcinoma | 43 | 34–96 | 18 | 25 | 5/11.6 | 7/16.3 | 21/48.8 | 10/23.3 |

*Note*: M, male; F, female.

studies, the outer membrane protein OipA and peptidoglycan were responsible for Akt phosphorylation.

The infection of AGS cells with the *H. pylori* P1 wt strain or the *cagA* and *virB7* mutants induced tyrosine phosphorylation in the consensus motif YXXM (Fig. 1B), which exists in many tyrosine kinase receptors and their substrates, including epidermal GF receptor (EGFR), fibroblast GF receptor, insulin receptor (IR) substrate IRS-1, BCAP and GRB-associated binder 1. This phosphorylation creates docking sites for the SH2 domains of PI3K, leading to the activation of the PI3K/Akt signalling (Liu et al., 2012). Thus, the phosphorylation of PI3K upstream regulators within their PI3K-binding motifs occurs in a CagA- and T4SS-independent manner.

Akt regulates cell growth and protein synthesis through the activation of the serine-threonine kinase mTOR (phosphorylation at Ser2448) and p70 ribosomal protein S6 kinase (p70S6K) (Navé et al., 1999; Averous and Proud, 2006). As shown in Fig. 1B, the wt and mutant P1 strains promoted phosphorylation of mTOR at Ser2448 within 1–3 h in AGS cells. mTOR activation is a common feature of many viral and some bacterial infections, which supports pathogen replication and survival in the host cell (Martin et al., 2012). Some bacterial exotoxins, e.g., α-hemolysin of *S. aureus*, deactivate mTORC1, thereby initiating autophagy (von Hoven et al., 2012).

Next, we examined the phosphorylation of the S6 ribosomal protein (S6), a direct substrate of mTOR-regulated p70S6K and to a lesser extent, ERK-regulated p90 ribosomal protein S6 kinase (p90S6K) (Ruvinsky and Meyuhas, 2006). The infection of AGS cells with *H. pylori* induced a prominent transient phosphorylation of S6 at priming phosphorylation sites within 0.5–3 h p.i., independently of CagA and T4SS (Fig. 1B).

To confirm that the phosphorylation of mTOR and S6 is directed through Akt kinase and to identify other potential upstream mediators, we either transfected AGS cells with siRNA specifically targeting Akt or pretreated AGS cells with several inhibitors prior to infection with *H. pylori*. The depletion of Akt diminished the phosphorylation of mTOR and S6 in the infected cells (Fig. 2A). The PI3K inhibitor LY294002 (20 μM) and the Akt inhibitor Akti1/2 (2 μM) completely inhibited Akt phosphorylation and affected the *H. pylori*-induced phosphorylation of mTOR and S6, with LY294002 being more efficient in inhibiting mTOR phosphorylation (Fig. 2B). Therefore, either separate from Akt, another kinase contributes to mTOR phosphorylation in infected cells, or mTOR may also act upstream of Akt. To exclude the possibility that suppression of S6 phosphorylation by LY294002 was due to unspecific inhibition of mTOR, we used also a lower concentration of LY294002. 2-μM LY294002, which should not affect autokinase activity of mTOR (Brunn et al., 1996), completely inhibited phosphorylation of Akt and S6 (Supplementary Fig. 1).

The src family tyrosine kinase inhibitor PP2 (2.5 μM) reduced the *H. pylori*-induced phosphorylation of Akt, mTOR and S6 (Fig. 2B). Thus, src family tyrosine kinases are important regulators of Akt signalling in *H. pylori* infection.

*H. pylori*-induced EGFR activation mediates Akt Ser473 phosphorylation in several cell lines (Sokolova et al., 2008; Tabassam et al., 2009). Interestingly, pretreatment of AGS cells with the specific EGFR tyrosine kinase inhibitor AG1478 together with HER-2 inhibitor AG825 (5 μM) completely abrogated Akt phosphorylation at Ser473, but phosphorylation at Thr308 was only slightly affected (Fig. 2B). Additionally, pretreatment with EGFR/HER-2 inhibitors slightly decreased S6 phosphorylation but did not affect mTOR phosphorylation. Considering the current model of Akt regulation, we concluded that EGFR regulates PDK-2, mTORC2 and ILK, leading to the complete activation of Akt through phosphorylation at Ser473. This Akt phosphorylation is undistinguished for downstream regulation of mTOR and S6.

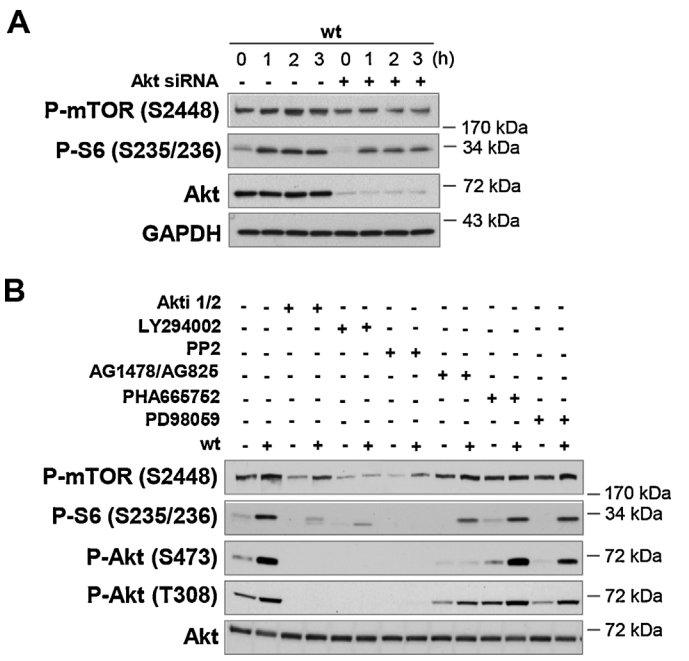


**Fig. 2.** PI3K/Akt and src kinases play a key role in the regulation of mTOR and S6 in *H. pylori*-infected AGS cells.
AGS cells were transfected with siRNA against Akt (A), or were pretreated with different inhibitors for 30 min (B). The cells were subsequently infected with *H. pylori* P1 wt for 3 h or for the times indicated, and cell lysates were prepared. The western blot analysis was performed using the indicated antibodies.

Akt and the tyrosine kinase receptor c-Met play a role in *H. pylori* infection through the induction of gastric epithelial cell motility (Churin et al., 2003; Nagy et al., 2009). Moreover, PI3K/Akt pathway is activated upon c-Met stimulation by the agonist hepatocyte GF (Zhou and Wong, 2006). However, the c-Met inhibitor PHA665752 (0.2 μM) had no effect on phosphorylation of Akt and its downstream targets in *H. pylori*-infected cells (Fig. 2B).

In contrast to AG1478/AG825 and PHA665752, the receptor tyrosine kinase inhibitor genistein (150 μM) prevented *H. pylori*-induced phosphorylation of Akt at Thr308 (Supplementary Fig. 2). These results suggest that tyrosine kinases other than EGFR and c-Met could contribute to PI3K/Akt activation in infected cells. The depletion of major PI3K/Akt inducers, such as insulin tyrosine kinase receptor, insulin-like GF tyrosine kinase receptor (IGF-IRα/β) and transforming GF β receptor type II (TGFβ RII), with specific siRNAs did not affect Akt phosphorylation at Thr308 at 1–3 h p.i. in AGS cells (Supplementary Fig. 2).

The MEK selective inhibitor PD98059 (10 μM) had no effect on *H. pylori*-induced mTOR phosphorylation (Fig. 2B). A slight reduction in Akt phosphorylation at Ser473 was observed, potentially reflecting interactions between MEK/ERK and Akt signalling as described above for EGFR. These data also demonstrate that S6 phosphorylation is not regulated by ERK in the infected cells (Fig. 2B). Thus, S6 phosphorylation does not occur through ERK-regulated p90S6K but rather is mediated by p70S6K. Hence, although the influence of ERK on mTOR activity has also been proposed (Ma et al., 2005), this kinase does not mediate the phosphorylation of mTOR and S6 in infected AGS cells.

Therefore, the use of a range of inhibitors revealed that PI3K/Akt, mTOR and the S6 ribosomal protein are activated through tyrosine kinases of the src family, and mTOR and the S6 ribosomal protein are downstream targets of PI3K/Akt in *H. pylori*-infected cells. The upstream signalling events that direct *H. pylori*-mediated activation of PI3K and the src tyrosine kinases still remain to be investigated. The possibility that Akt is regulated following

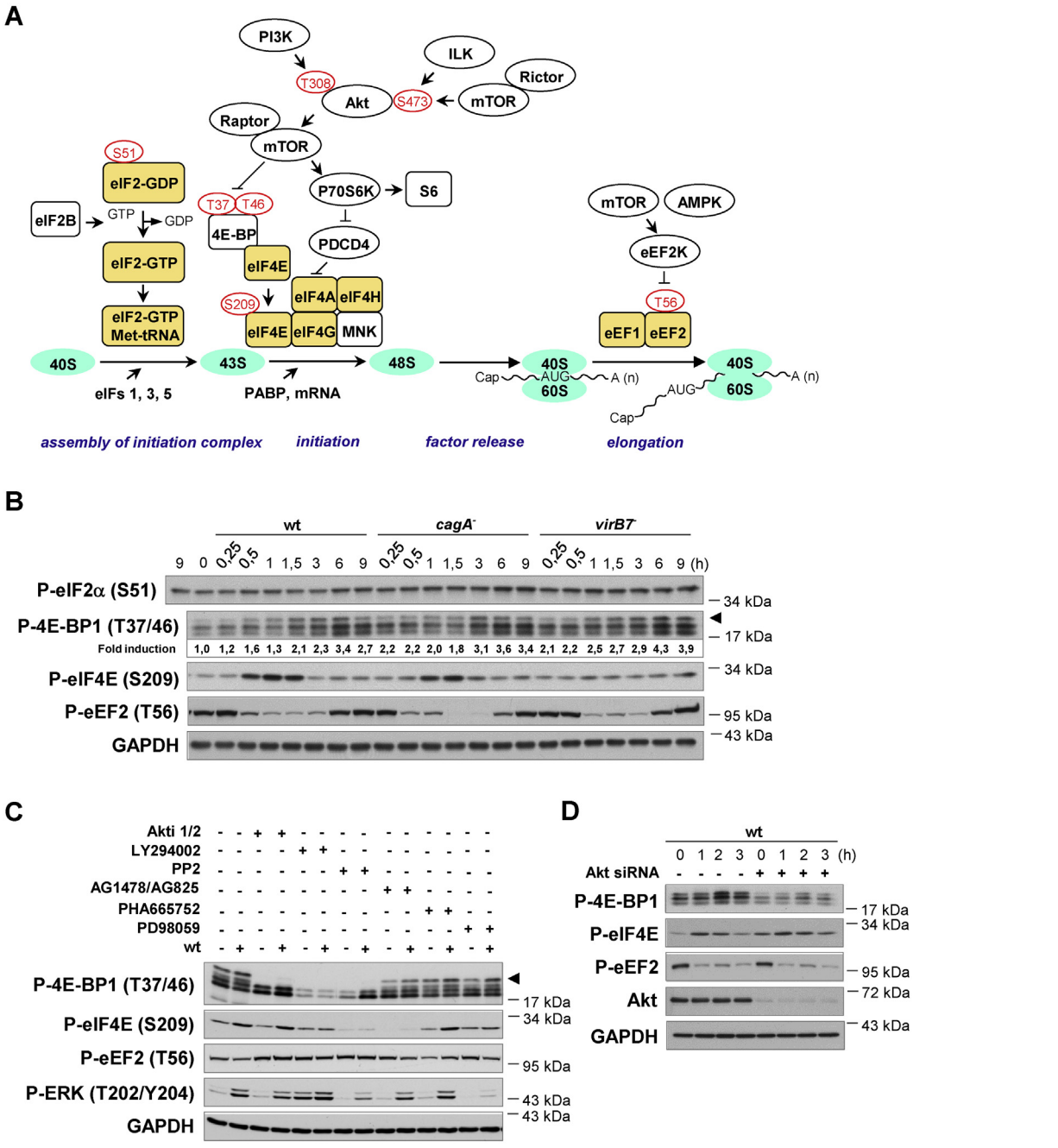


**Fig. 3.** PI3K/Akt and src kinases play a key role in regulation of 4E-BP1, eIF4E and eEF2 in *H. pylori* infection. (A) Schematic diagram depicting main molecular regulators of mRNA translation. (B) The cells were pretreated with different inhibitors for 30 min and subsequently infected with *H. pylori* P1 wt for 3 h. (C) At 48 h before infection with *H. pylori* P1 wt, the cells were transfected with siRNA against Akt. The cell lysates were analysed by western blotting using the indicated antibodies.

activation of several plasma membrane receptors cannot be excluded.

### 3.2. PI3K/Akt and Src family kinases play a key role in the regulation of 4E-BP1, eIF4E and eEF2 in H. pylori infection

PI3K/Akt, mTOR (within mTORC1) and its effector p70S6K are important regulators of protein synthesis (Holz et al., 2005). We examined whether the translational machinery is regulated during infection with *H. pylori*. The earliest step of translation is the formation of a Met-tRNA-eukaryotic translation initiation factor (eIF) 2 (eIF2)-GTP ternary preinitiation complex through the delivery of methionyl-tRNA to the 40S ribosome via eIF2 (Fig. 3A). The phosphorylation of the α subunit of eIF2 retards translation through the stabilisation of the eIF2-GDP-eIF2B complex, inhibiting the turnover of eIF2B and the exchange of GDP for GTP (Clemens, 1996). Under endoplasmic reticulum stress, amino acid deprivation, viral infection and exposition to bacterial pore-forming toxins, eIF2α becomes phosphorylated, promoting autophagy (von Hoven et al., 2012). *H. pylori*, despite secreting the pore-forming toxin VacA, does not trigger eIF2α phosphorylation in AGS cells (Fig. 3B), suggesting no inhibition of 43S complex formation, and thus, no inhibition of the initiation complex assembly in infected cells. p70S6K impacts the initiation step of translation through the phosphorylation of the negative regulator of eIF4A, PDCD4, and targeting this protein for subsequent degradation (Fig. 3A) (Dorrello et al., 2006). In

addition to signalling via p70S6K, mTOR regulates cap-dependent translation through controlling eIF4E via eIF4E binding protein 1 (4E-BP1) phosphorylation. The infection of AGS cells with the wt or *cagA* and *virB7* mutants of *H. pylori* P1 strain led to an enhanced phosphorylation of 4E-BP1 at the Thr37/46 priming site at 0.5 h p.i. (Fig. 3B). In resting cells, binding of 4E-BP1 to eIF4E inhibits the formation of the eIF4F complex (comprising eIF4E, eIF4G and eIF4A) (Fig. 3A). The hyperphosphorylation of 4E-BP1 leads to the release and recruitment of eIF4E to selected mRNAs and subsequent formation of the eIF4F complex for translation initiation (Mamane et al., 2006). Additionally, the phosphorylation of eIF4E at Ser209 by ERK- and p38-regulated MAPK-interacting kinases 1 and 2 (Mnk1, Mnk2) enhances translation initiation (Averous and Proud, 2006). Infection with *H. pylori* induced a transient (at 1 h p.i.) phosphorylation of eIF4E, and this effect was less prominent in *cagA*- and *virB7*-infected cells (Fig. 3B). This phosphorylation pattern might reflect the fact that upstream MAP kinases are regulated differently by bacteria, e.g., p38 phosphorylation requires functional T4SS, whereas ERK does not (Sokolova et al., 2013).

Another important step of translation is the elongation of the peptide chain (Fig. 3A). The phosphorylation of eEF2 at Thr56 via eEF2 kinase suppresses the elongation process (Averous and Proud, 2006). mTOR regulates phosphorylation of eEF2 kinase, thereby inhibiting the activity of this enzyme. The infection of AGS with the wt or the *cagA* and *virB7* mutants of *H. pylori* suppressed eEF2 phosphorylation at 1–3 h p.i. (Fig. 3b).

Thus, our data demonstrate that *H. pylori* regulates key effectors of the translational machinery, including 4E-BP1, eIF4E and eEF2. To confirm the role of PI3K/Akt in this process, we used the kinase inhibitors or Akt-targeting siRNA. As expected, the inhibition of PI3K and Akt through LY294002 (20 μM) and Akti1/2 (2 μM), respectively, and Akt depletion using specific siRNA abrogated the *H. pylori*-induced phosphorylation of 4E-BP1 and prevented de-phosphorylation of eEF2 (Fig. 3C, D; Supplementary Fig. 1). Inhibition of PI3K, but not Akt, led to decrease of eIF4E phosphorylation, with no inhibitory effect on ERK phosphorylation. Therefore, PI3K might contribute to eIF4E activation independently of Akt and ERK. The src family kinases inhibitor PP2 (2.5 μM) efficiently abrogated the effects of *H. pylori* on the phosphorylation of 4E-BP1, eIF4E and eEF2 (Fig. 3C). Surprisingly, the inhibition of EGFR abolished eIF4E phosphorylation, with no effect on ERK phosphorylation. This result demonstrates that i) EGFR is not a major ERK activator, and ii) factors other than ERK kinase mediate EGFR-dependent eIF4E phosphorylation in infected cells.

PD98059 (10 μM) showed no effect on 4E-BP1 and eEF2, and interestingly, caused only slight decrease in eIF4E phosphorylation (Fig. 3C). It confirms the only contributory role of ERK in eIF4E regulation.

The preincubation of the cells with the c-Met inhibitor PHA665752 (0.2 μM) did not affect the phosphorylation of the investigated molecular targets (Fig. 3C).

Thus, in *H. pylori* infection, the src kinases, PI3K and Akt regulate 4E-BP1 and eEF2, which represent mTOR targets and important players in translation initiation and elongation. In contrast, eIF4E phosphorylation in *H. pylori*-infected cells is primarily mediated through EGFR and the src kinases, and only slightly regulated through ERK; however Akt is not implicated in this process. The phosphorylation of eIF4E is mediated through Mnk, downstream effectors of ERK and p38. The activation of MEK/Erk in *H. pylori* infection has been previously described (Rieke et al., 2010), and EGFR and the src kinases have been shown to participate in ERK activation (Du et al., 2007; Tabassam et al., 2008). p38 MAPK is also induced through *H. pylori* infection (Pomorski et al., 2001; Sokolova et al., 2013) and might, therefore, contribute to Mnk/eIF4E phosphorylation.

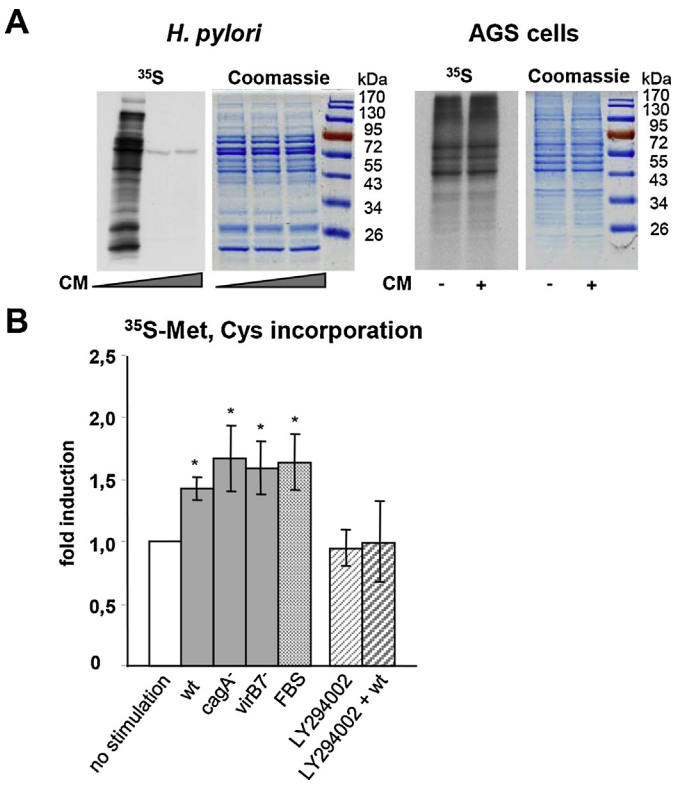


**Fig. 4.** *H. pylori* intensifies protein synthesis.
(A) AGS cells or the *H. pylori* P1 wt suspension were treated with clarithromycin for 30 min before adding [$^{35}$S]Met/Cys to the cultures. $^{35}$S incorporation into the newly synthesised proteins was detected in whole cell lysates through radioautography. Coomassie staining shows equal amounts of total protein in the samples. (B) Intact or pretreated with LY294002 AGS cells were infected with the wt or *cagA* and *virB7* mutants of *H. pylori* strain P1 or stimulated with 10% FBS and then labelled with [$^{35}$S]Met/Cys. The graphs represent mean fold changes of $^{35}$S incorporation in mg of protein from 3 separate experiments ± SD, with the value for unstimulated cells arbitrarily presented as 1. CM, clarithromycin; * $p < 0.05$, relative to non-stimulated cells.

### 3.3. *H. pylori promotes protein synthesis through PI3K*

Next, we examined the protein synthesis rate in AGS cells infected with *H. pylori*. To prevent the metabolic labelling of adherent bacteria with labelled amino acids, the cell cultures were treated with clarithromycin, which binds the 50S subunit of the bacterial ribosome and inhibits the translation of bacterial peptides (Fig. 4A). As a positive control, AGS cells were stimulated with 10% FBS. Similar to FBS, the infection of cells with wt or the *cagA* and *virB7* mutants of *H. pylori* increased the incorporation rate of [$^{35}$S]Met/Cys in *de novo* synthesised proteins in eukaryotic cells at 6 h p.i. (Fig. 4B). Pretreatment with the PI3K inhibitor LY294002 (20 μM) prevented the increase of [$^{35}$S]Met/Cys incorporation in *H. pylori*-infected cells. It is not clear whether the detected intensification of protein synthesis is part of the host cell defence programme, which includes the rapid production of cytokines and other proteins in e.g. an AP-1- and NF-κB-driven transcription-dependent manner, or whether the increase of protein synthesis in the host cell is a bacteria survival strategy.

Translation of specific mRNAs rather than global translation is tightly regulated, for example, through the availability of eIF4E. An increase in the amount or activity of eIF4E promotes translation of mRNAs with strong secondary structure which require excess of eIF4F complex for translation, e.g., mRNAs for Myc, fibroblast GF, ornitine decarboxylase, vascular endothelial GF (Graff and Zimmer, 2003; Richter and Sonenberg, 2005). Therefore, the activation of

particular components of translational machinery might specify the host cell response to bacterial infection.

In conclusion, PI3K/Akt signalling is not only involved in the generation and maintenance of the gastric cancer phenotype but also might play a role in the *H. pylori*-induced pro-inflammatory response and the development of gastritis.

## Conflict of interest

The authors declare that they have no conflict of interest.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocel.2014.08.023.

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