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# The many roads traveled by *Helicobacter pylori* to NFκB activation

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**Many of the pathologies linked to *Helicobacter pylori* are caused by the ability of the bacteria to induce chronic inflammation in the stomach of the host. One of the major transcription factors that regulate inflammation is NFκB, which is constitutively activated in many cancers including some gastric cancers. *H. pylori* has been shown to activate NFκB using several different bacterial components and host signaling pathways in cell-type and strain-specific ways. Our recent studies demonstrate that *H. pylori* utilizes its virulence factor CagA to target signaling molecule TAK1 for the activation of NFκB. In this article, we will summarize our findings together with other recent progress in the *H. pylori*-mediated activation of NFκB and discuss the role of CagA and TAK1 in the *H. pylori*-mediated activation of NFκB and gastric diseases.**

**Key words:** Helicobacter pylori, NF-κB, CagA, TAK1, TRAF6, ubiquitination

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Approximately half of the world's population is infected with *H. pylori*, a gram-negative bacterium which colonizes the stomach and is linked to gastritis, gastric ulcers, MALT lymphoma and gastric adenocarcinoma.[1] Most infections with *H. pylori* are persistent, which causes a steady state of inflammation in the stomach. Chronic inflammation has been linked to many diseases, including numerous types of cancers.[2] NFκB, a master regulator of pro-inflammatory cytokines and anti-apoptotic signaling molecules, is one of the most well-studied transcription factors activated by *H. pylori* infection. There are many pathways that lead to the activation of NFκB, and many stimuli, including lipopolysaccharide (LPS), peptidoglycan and TNFα.[3] The binding of these ligands to their receptors leads to the activation of signaling pathways which converge upon the phosphorylation and activation of the IκB kinase (IKK) complex. This kinase complex in turn phosphorylates and induces the degradation of IκBα, which in unstimulated conditions binds and sequesters NFκB within the cytoplasm. NFκB, a heterodimer of p50 and RelA/p65, is released and moves into the nucleus, where it undergoes various post-translational modifications and transactivates its target genes.[4] Due to the important role of NFκB in inflammation-related diseases and cancer, how *H. pylori* activates NFκB has been a long-standing question.

*H. pylori* colonize the mucosal layer of the stomach, attaching themselves to the gastric epithelium via various bacterial adhesins and epithelial receptors.[5] The epithelium is therefore the first point of contact for the bacteria in the host. Immune cells attracted to the site of infection by cytokines released from the epithelial cells also respond to *H. pylori*.[1] This further amplifies the immune response from the infection and the resulting inflammation damages the mucosal layer, which can result in ulcers, gastritis and adenocarcinoma.[1] Although *H. pylori* effects responses from both epithelial cells and immune cells including monocytes and lymphocytes, the *H. pylori*-induced inflammatory response generated by gastric epithelial cells is the most studied.

Infection with *cag*PAI-positive *H. pylori* is associated with more severe outcomes in the inflammation-linked illnesses mentioned as compared to infection with *cag*PAI-negative *H. pylori*.[6,7] The approximately 40 kb cytotoxin-associated gene (*cag*) pathogenicity island contains genes which encode a type 4 secretion system

(T4SS) and a pathogenicity factor called cytotoxin-associated gene A, or CagA.[8] A protein ranging in size from 120–145 kDa, CagA is injected into the host epithelial cells via the T4SS, where it acts on a number of different signaling pathways leading to inflammation and cell scattering.[9]

## CagA-Dependent and Independent Activation of NFκB by *H. pylori*

A role for CagA in the activation of NFκB and the production of IL-8 has been suggested by many studies. For example, using an interleukin-8 (IL-8) promoter-reporter assay, Sharma et al. showed the requirement of CagA for NFκB activation.[10] It was also shown that ectopically expressed CagA induced NFκB translocation into the nucleus, and also induced IL-8 production in gastric epithelial cells.[11,12] Additionally, NFκB activation and inflammation was markedly less in the gastric antra of Mongolian gerbils infected with *cagA*-deficient *H. pylori* as compared to infection with wild-type *H. pylori*.[13] However, the exact function of CagA in NFκB activation is still unclear. Our recent studies demonstrate a clear dependence on the presence of CagA for NFκB activation by *H. pylori* in gastric epithelial cells.[14] We showed that wild-type *H. pylori*, but not the *cagA*-deficient isogenic mutant, was capable of inducing the degradation of IκBα and the phosphorylation of RelA, enhancing the DNA-binding of NFκB, and activating NFκB-driven expression of IL-8 and TNFα.[14]

Despite the essential role of the *cag*PAI in the activation of NFκB and the induction of IL-8 in epithelial cells, the requirement for CagA, not just the T4SS, for the inflammatory response has been a point of contention nearly since the discovery of the pathogenicity island. It has been reported that the T4SS, but not CagA, is required for *H. pylori*-mediated production of IL-8 since T4SS mutants failed to induce IL-8.[15] However, as the T4SS translocates CagA, impaired NFκB activation by mutants in which the T4SS is defective cannot rule out the possibility that the important component is, in fact, CagA. Another concern regarding the requirement of CagA for *H. pylori*-mediated NFκB activation is the potential for "polar effects" from the deletion of the *cagA* gene.[16] Nevertheless, as *cagA* is monocistronic and transcribed in a different direction than other genes comprising the *cag* pathogenicity island (*cag*PAI),[8] it is unlikely that the *H. pylori* isogenic *cagA* mutant strains are exerting "polar effects" on other genes within the *cag*PAI. Concordantly, no "polar effects" have been reported for the *cagA*-deficient *H. pylori* strains in the literature.

While CagA has clearly been shown to be essential for the activation of NFκB, it must be noted that this requirement could be *H. pylori* strain-specific. The considerable variation in the sequences of CagA from different *H. pylori* strains led to the designation of "Western" and "Eastern" strains based on the sequences surrounding the phosphorylation domains in the C terminus of the gene.[17] These differences correlate with the ability of the CagA protein to associate with SHP-2 and induce the "hummingbird" phenotype.[17] Sequence variations within the CagA multimerization domain also influence these CagA-induced phenotypes within host cells.[18] In addition to affecting the "hummingbird" phenotype, strain variations have also been linked to variations in IL-8 production. Exchanging *cagA* genes allows low IL-8-inducing *H. pylori* strains to be converted into high inducing strains and vice versa.[11] We hypothesize that these effects may be caused by increased or decreased abilities of these CagA proteins to bind and activate upstream signaling components, such as TAK1, influencing the downstream activity of NFκB. However, the sequences required for CagA to induce NFκB activation remain elusive and need further investigation.

Additionally, the requirement for CagA in the activation of NFκB could also be cell-type specific. CagA seems to not be essential for the *H. pylori*-induced activation of NFκB in cells other than gastric epithelial cells. In macrophages, *H. pylori* activated NFκB via TLR2 (for induction of IL-6 and IL-1β) and TLR4 (for induction of IL-12, IL-10 and IL-8).[19,20] Findings similar to those of the activation in macrophages were reported in lymphocytes by Ohmae et al. In human B lymphocytes, *H. pylori* activates not only the NFκB classical pathway, but also the alternative pathway, which involves the processing of p100, likely by TLR4 recognition of *H. pylori* LPS.[21]

In addition to LPS, other bacterial components are also utilized by *H. pylori* for the activation of NFκB.[22] For example, recent papers have shown that bacterial peptidoglycan (PG) is delivered via outer membrane vesicles through the T4SS into host cells where it binds to pattern recognition receptor NOD1, which signals to activate NFκB.[23-25] It appears that CagA and PG target different cellular signaling molecules for the NFκB activation. However, one remaining question is why *H. pylori* utilizes various components for NFκB activation. One possibility is that CagA and PG synergistically activate NFκB to obtain the maximal inflammatory response in gastric epithelial cells. Another possibility is that CagA and PG might compensate for each other. In *H. pylori* strains where CagA is not a strong NFκB inducer, PG might play a dominant role in initiating the inflammatory response. Finally, it is also possible that CagA and PG selectively activate specific subsets of NFκB target genes. While CagA might be mainly responsible for the expression of proinflammatory cytokines, the PG-NOD1 pathway may be more important for the expression of antimicrobial peptides,[25] since the PG-NOD1-mediated inflammatory response might be due largely to the activation of AP-1-dependent expression of IL-8.[26] Further experiments will be needed to differentiate among these possibilities.

It is clear that the model system and the strain of bacteria that are used to study the *H. pylori*-induced activation of NFκB can affect the requirement for various components of the bacteria (**Fig. 1**). From the other side, the host proteins utilized by the various components of *H. pylori* add another layer of complexity to the *H. pylori*-induced activation of NFκB.

## Host Signaling Molecules Utilized by *H. pylori* to Activate NFκB

While great efforts have been made to identify the *H. pylori* components for NFκB activation and inflammation, determining the molecules within the host cells that transduce NFκB signaling also attracts much attention. Many cellular

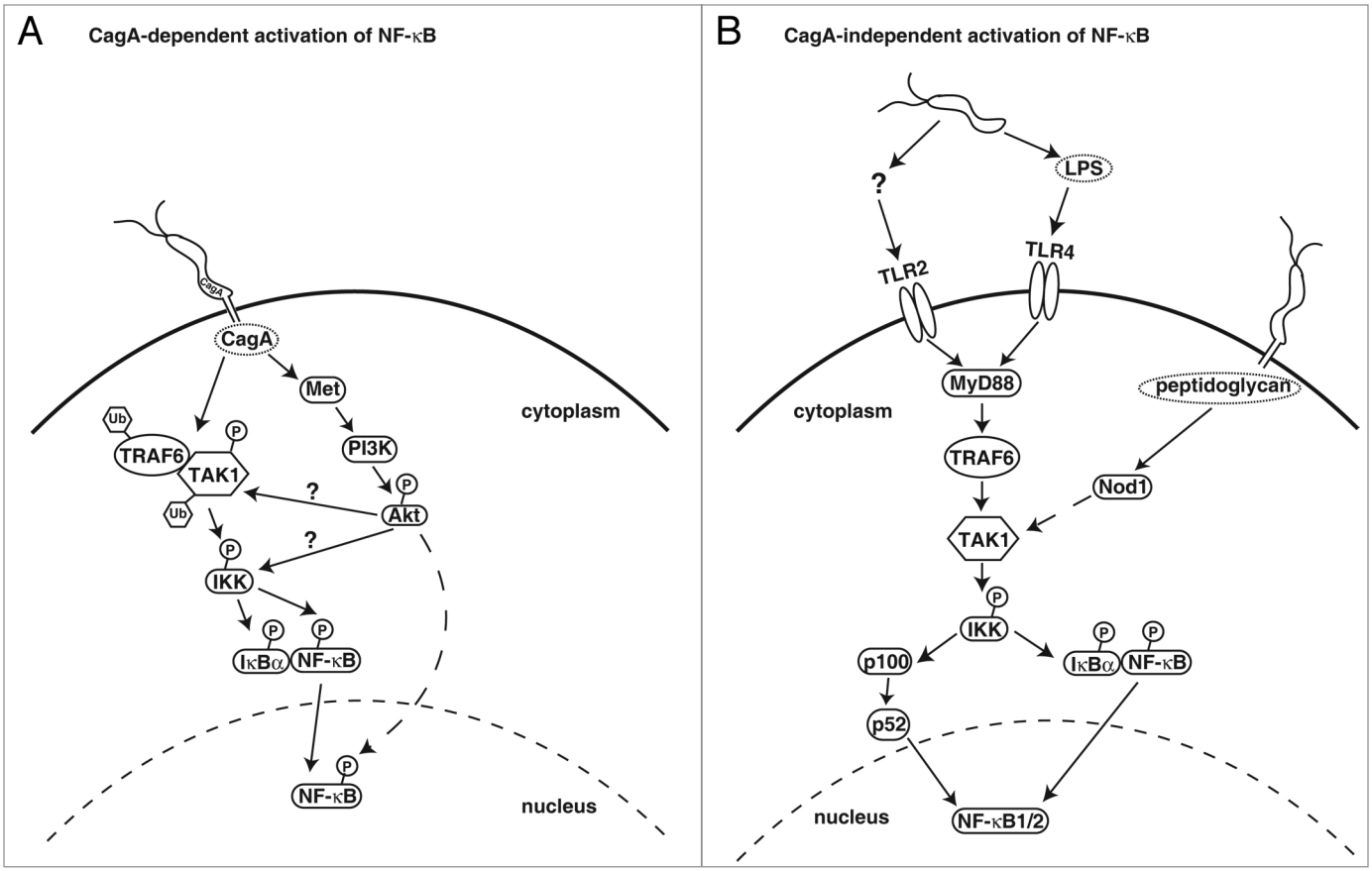


**Figure 1.** *H. pylori* activation of NFκB via CagA-dependent and CagA-independent pathways. (A) CagA-dependent activation of NFκB. CagA is injected into host epithelial cells where it interacts with TRAF6 and TAK1 or Met to activate NFκB through TAK1 or Akt, respectively. Akt then activates IKK directly or indirectly via TAK1. Akt could also activate NFκB by indirect phosphorylation of RelA. (B) CagA-independent activation of NFκB. Host immune cells are stimulated by *H. pylori* products, such as LPS, via TLR pathways for the activation of NFκB. Alternatively, peptidoglycan injected into cells via the T4SS stimulates Nod1 activity leading to NFκB activation. Solid arrow: known interaction or activation; solid arrow with question mark: proposed interaction or activation; dashed arrow: activation through multiple steps.

signaling molecules have been suggested to be involved in *H. pylori*-mediated NFκB activation in epithelial cells or in immune cells. Besides Nod1, which has been discussed above, *H. pylori* was shown to induce the interaction between PAK1 and NIK, a kinase which in turn activates the IKK complex for NFκB activation.[27] Also, interference with MyD88, TRAF2, TRAF6 or TAK1 signaling was shown to downregulate *H. pylori* activation of NFκB.[14,19,28] While roles for MyD88, TRAF6 and TAK1 have been validated by siRNA knockdown, some of these studies used dominant negative forms of the signaling molecules which are apt to have non-specific effects. Depletion of these signaling molecules using siRNA will be able to further confirm these findings. Furthermore, whether some of these signaling molecules are involved in the CagA-dependent or -independent *H. pylori*-mediated activation of NFκB is not clear.

Our recent studies give biochemical evidence to show that CagA activates NFκB by hijacking TAK1 in gastric epithelial cells. We demonstrate that *H. pylori* activation of NFκB requires TAK1, and that CagA increases the TAK1-dependent activation of NFκB.[14] In our findings, CagA is injected into the host epithelial cells, where it binds to TAK1 and enhances its TRAF6-dependent lysine (K) 63-linked ubiquitination. Emerging evidence suggests that activation of TAK1 and IKK is regulated by TRAF6-mediated K63-linked ubiquitination of TRAF6 itself, IRAK1 and NEMO.[29] Different from K48-linked polyubiquitination, which represents a signal for degradation by the proteasome, K63-linked polyubiquitination acts as scaffolding to bring signaling molecules in a pathway into contact to aid in the transference of a signal.[30] K63-linked ubiquitination is important for the kinase activity of TAK1, since inhibiting its ubiquitination abolished its ability to activate NFκB. Also, we found that CagA enhances this activity as measured by TAK1 autophosphorylation and by the ability of TAK1 to activate the downstream kinase complex IKK, which is directly phosphorylated and activated by TAK1.[14]

Consistently, two other studies also show that TRAF6-mediated K63-linked ubiquitination is essential for the activation of TAK1 and NFκB.[31,32] The ubiquitinated lysine residue(s) of TAK1 in response to *H. pylori* infection remains unidentified. It has been shown that lysine 34 of TAK1 is ubiquitinated by TRAF6 in response to TGFβ, and ubiquitination

of this lysine correlates with the activity of TAK1.[33] However, this lysine does not seem to be involved in the *H. pylori*-induced activation of NFκB, since mutation of lysine 34 to arginine did not affect TAK1 ubiquitination or its ability to activate NFκB, indicating that another unidentified lysine residue(s) might also undergo K63-linked ubiquitination leading to the activation of IKK and NFκB.[14] In this regard, two lysine residues in TAK1 have recently been identified to be ubiquitinated by TRAF6, and the K63-linked ubiquitination of these two sites are important for the activation of TAK1 and NFκB.[31,32] It would be of great interest to determine whether these two sites are involved in the *H. pylori*-mediated ubiquitination of TAK1.

The way in which CagA enhances the ubiquitination of TAK1 is not yet understood. CagA is associated with the membrane when it enters the host cell, and has been shown to oligomerize.[34,35] It is possible that its oligomerization recruits TRAF6, an interaction which we showed in vitro, which enhances its E3 ligase activity and thus the ubiquitination of TAK1. Further supporting this, our data shows that oligomerization-deficient CagA does not activate NFκB.[14] CagA may also act as a connecting protein to bring TRAF6 and TAK1 into proximity to enhance the ubiquitination of TAK1. Another possibility is that CagA may inhibit deubiquitination by A20 or CYLD, two de-ubiquitinases shown to remove ubiquitin chains from TRAF6 and TAK1,[36-38] thereby enhancing the overall ubiquitination of TAK1.

How the ubiquitination of TAK1 activates the kinase is also unclear. It is expected that, like other K63-linked ubiquitinated signaling molecules, the K63-linked polyubiquitin chain functions as a scaffold to recruit other signaling molecules which are essential for the activation of TAK1. For example, MEKK3 has been shown to be recruited to the ubiquitinated TAK1, and this recruitment is required for the activation of TAK1.[31] Also, TAK1 ubiquitination may aid in its activity by recruiting substrates, such as the IKK complex. The ubiquitin-binding domain of the regulatory subunit NEMO has been shown to be important in the activation of IKK,[29] and it may be that it binds to the ubiquitin chains on TAK1.

While our studies clearly demonstrate the importance of TAK1 and TAK1 ubiquitination in CagA-dependent *H. pylori*-mediated NFκB activation, studies from others also suggest that some other molecules might be targeted by CagA and involved in the CagA-dependent NFκB activation. Suzuki et al. reported that CagA interacted with the hepatocyte growth factor receptor Met, resulting in the activation of PI3K and Akt, which led to the activation of NFκB and β-catenin.[39] Since CagA binds Met, an intramembrane protein, and is also known to oligomerize,[35] this binding and oligomerization may also lead to the recruitment of TRAF6. TRAF6 is recruited to the membrane by the dimerization of other membrane receptors, and it dimerizes in turn and becomes an active E3 ubiquitin ligase,[30] which we found to be required for the ubiquitination of TAK1.[14] Akt was also recently found to be ubiquitinated by TRAF6, which proved important for its phosphorylation and activation.[40] Although a role for Akt has been suggested in the activation of NFκB by *H. pylori*,[39,41] how Akt activates NFκB is not clear. Akt might activate IKK directly or indirectly through TAK1, or Akt might activate NFκB by inducing the phosphorylation of RelA[39,41] (**Fig. 1**). While TAK1 is activated in vitro by binding to unanchored ubiquitin,[42] Akt might function as a kinase for the in vivo phosphorylation and activation of TAK1. It is also possible that Akt may act in conjunction with TAK1 to fully activate the IKK complex via phosphorylation of IKK1/2.

Interestingly, TAK1 is involved in nearly every pathway that is activated by *H. pylori*, including the JNK, ERK and p38 MAPK pathways.[43] We suggest that our CagA-dependent mechanism of activation for TAK1 in NFκB activation may also contribute to the downstream activations of these other pathways, furthering our understanding of the role which CagA plays in the host cells. Determining the precise way by which CagA stimulates the TRAF6-dependent ubiquitination and activation of TAK1 would clarify the *H. pylori*-induced inflammatory response, and would provide new information that could be used to combat development of the diseases linked to *H. pylori* infection.


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