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Micronutrients: A double-edged sword in microbial-induced gastric carcinogenesis

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Abstract

Epidemiologic studies throughout the world have uniformly demonstrated significant relationships between the intake of dietary micronutrients and gastric cancer risk. An exciting concept that has gained considerable traction recently is that micronutrients modulate gene expression within *Helicobacter pylori*, the strongest identified risk factor for gastric carcinogenesis. We present evidence here that essential micronutrients have a direct effect on *H. pylori* virulence, which subsequently affects interactions at the host-pathogen interface, thereby facilitating the development of premalignant and malignant lesions in the stomach. Further, these fundamental concepts provide a framework for understanding mechanisms driving the development of other malignancies that arise from foci of gastrointestinal inflammation.

Keywords

Gastrointestinal cancer; gastric cancer; *Helicobacter pylori*; heme; iron; zinc

Gastrointestinal cancers

Recent data indicate that more than 14 million new cancer cases and over eight million cancer-related deaths occur annually worldwide [1,2]. This disease predominantly affects individuals residing in under developed countries, and currently account for approximately 57% of cases and 65% of all cancer-related deaths occur in developing nations [1,2].

Gastrointestinal malignancies represent a significant subset of the collective cancer burden,

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Conflict of Interest

The authors declare that no conflicts of interest exist.

accounting for nearly two million deaths annually [2–5]. Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer-related death, with an estimated 400,000 deaths each year [1,2]. Esophageal cancer is associated with high mortality and poor survival rates, and over 80% of all cases occur in developing countries [1,2]. Colorectal cancer is the third most common cancer and the fourth leading cause of cancer-related death in the world, accounting for approximately 700,000 deaths annually [1,2]. Unlike esophageal cancer, 55% of colorectal cancer cases occur in developed countries. Compared to esophageal and colorectal cancer, however, gastric cancer exerts the greatest global impact and represents the fifth most common malignancy and the third leading cause of cancer-related death worldwide, leading to over 700,000 deaths each year [1,2]. More than 70% of all cases occur in developing countries, and in some regions of the world, gastric adenocarcinoma is the most common malignancy. Specifically, in Eastern Asia, this malignancy accounts for more than half of the global burden and the highest cancer-related mortality, with rates five-ten times greater than those observed in North America [1,2]. The disparity in gastric cancer burden in different regions of the world reflects regional differences among populations, which are dependent upon host genetics, environmental factors, and, for gastric adenocarcinoma, defined and well-characterized infectious agents. Based on these modifiable risk factors, a substantial proportion of gastric cancer cases and deaths could be prevented by identifying specific environmental risk factors and microbial virulence determinants that augment cancer risk. These results would engender more effective screening practices and preventative measures targeting subpopulations at the greatest risk for cancer to reduce disease burden and mortality. This review will focus on the role of micronutrients, in particular the essential micronutrients iron and zinc, on the risk of gastrointestinal cancers. Gastric adenocarcinoma and the mechanisms by which micronutrients, in conjunction with *H. pylori* infection, facilitate disease progression and malignant transformation will be discussed more fully as a model that exemplifies the interplay between environmental elements and microbial virulence determinants as a means to identify individuals at the greatest risk for disease.

Essential micronutrients and cancer risk

Epidemiologic studies throughout the world have demonstrated significant relationships between diet and gastrointestinal cancer risk (Figure 1A, Key Figure). Within the last decade, essential micronutrients, such as heme, iron, and zinc, have gained particular attention in studies assessing the relationship between diet and various cancers.

Heme

Heme is an iron-containing molecule within hemoglobin that serves as the site for oxygen binding. Due to its potent reactivity, homeostasis of heme is tightly regulated and controlled via its sequestration into hemoproteins, such as hemoglobin. However, under conditions of oxidative stress, heme can be liberated from hemoproteins, and non-protein-bound heme is highly cytotoxic [6]. Carcinogenicity associated with heme has been widely demonstrated in cancers arising within the gastrointestinal tract, including esophageal, gastric, and colorectal cancer (Figure 1A, Key Figure) [7–14]. A recent study assessing the relationship between dietary heme intake and esophageal adenocarcinoma demonstrated a significant positive

association between heme intake and the risk of disease (Figure 1A, Key Figure) [7–9,11]. Similar to the associations observed in esophageal adenocarcinoma, positive associations between heme intake and gastric [8,10], as well as colorectal cancer [7,8,12–14], have also been demonstrated (Figure 1A, Key Figure). The mechanism by which heme iron increases cancer risk is likely linked to its ability to function as a nitrosylating agent, leading to formation of N-nitroso compounds that are known carcinogens [15]. Overall, existing data clearly indicate that heme is a significant risk factor for gastrointestinal carcinogenesis.

Iron

Iron is an essential micronutrient for virtually all organisms, and plays a fundamental role in critical cellular functions and processes. As a result, host iron metabolism is tightly regulated to avoid both cellular toxicity associated with iron overload and anemia associated with iron deficiency. Iron content represents a double-edged sword in that iron excess is associated with chronic diseases, such as atherosclerosis, cardiovascular disease, type 2 diabetes, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, neurodegenerative diseases, and cancer, while iron deficiency, the most common nutritional deficiency in the world (World Health Organization), is also associated with an increased risk of malignancy [16,17]. Similar to heme, iron is positively associated with the risk of colorectal cancer (Figure 1A, Key Figure) [8,18], potentially due to its redox potential and the generation of reactive oxygen species. Iron excess results in oxidative stress, lipid peroxidation, and DNA damage, all of which contribute to oncogenesis [8,18]. In contrast to the association between heme/iron and colorectal cancer, inverse relationships have been reported between iron content and esophageal and gastric cancer (Figure 1A, Key Figure) [8,19]. Experimental data from *in vivo* models have demonstrated that iron supplementation is protective against carcinogen-induced esophageal and gastric cancer [20], and, conversely, that iron deficiency accelerates carcinogen-induced gastrointestinal cancer and metastasis [17], results confirming the associations demonstrated in humans.

Zinc

Similar to iron, zinc is another essential micronutrient that plays a vital role in metabolism and serves as a cofactor for the function of host proteins and transcription factors that regulate key cellular function, such as response to oxidative stress, DNA damage repair, cell cycle progression, and apoptosis [21]. Zinc harbors antioxidant properties and plays a critical role in host defense against the initiation and progression of cancer, including cancers within the gastrointestinal tract [21,22]; however, similar to associations observed with iron, risk varies depending on the specific gastrointestinal niche (Figure 1A, Key Figure). Epidemiological studies of esophageal cancer have demonstrated an inverse relationship between zinc intake and the risk of malignancy (Figure 1A, Key Figure) [21–23], and experimental data have demonstrated that zinc deficiency promotes esophageal tumor progression in mice [24]. Similar to esophageal cancer, an inverse relationship exists between zinc intake and colorectal cancer risk (Figure 1A, Key Figure) [13,21]. One study demonstrated that zinc induces growth arrest in colon cancer cells *in vitro*, while *in vivo* studies have demonstrated that zinc supplementation confers a chemoprotective effect against chemically-induced colonic carcinogenesis in rats [25], supporting the role of zinc as a protective antioxidant. In contrast to esophageal and colorectal cancers, a positive

association exists between levels of zinc intake and the risk of gastric cancer (Figure 1A, Key Figure), particularly among Asian populations [21,22].

One possible explanation for the disparity in risk for cancer exerted by essential micronutrients is the microbial burden within the gastrointestinal tract at different sites. The microbial biomass and diversity differs throughout the gastrointestinal tract, ranging from the esophagus to the colon (Figure 1A, Key Figure), and the effects of micronutrients at specific sites may be due to specific microbial species residing at these sites [26,27,28]. The stomach represents a unique niche to delve into such relationships as it is dominated by an alpha-species, *Helicobacter pylori*.

Gastric adenocarcinoma and *Helicobacter pylori*

The strongest known risk factor for the development of gastric adenocarcinoma is colonization by the bacterial pathogen, *Helicobacter pylori*, a Gram-negative microorganism that colonizes greater than 50% of the world's population. The prevalence of *H. pylori* varies substantially throughout the world and is significantly higher in developing versus developed countries, consistent with increased gastric disease burden in these regions.

Two histologically distinct forms of gastric adenocarcinoma have been well-defined, diffuse-type and intestinal-type. Diffuse-type cancer is characterized by wide-spread neoplastic cell infiltration throughout the gastric mucosa, while intestinal-type cancer progresses through a series of well-defined pathological steps from normal gastric mucosa to superficial gastritis, chronic gastritis, atrophic gastritis, intestinal metaplasia, and finally dysplasia and adenocarcinoma (Figure 1B, Key Figure) [29]. Chronic *H. pylori* infection induces gastric inflammation and thus increases the risk of progression through each of these well-defined steps of gastric transformation, including atrophic gastritis, intestinal metaplasia, and dysplasia, which are considered precursors to the development of gastric adenocarcinoma (Figure 1B, Key Figure). In contrast to histologic stratification of gastric cancers, a recent study conducted comprehensive molecular analyses of gastric cancer cases to further classify this cancer into subtypes specifically associated with distinct clinical outcomes [30]. This classification yielded four subtypes, including MSI, TP53⁺, TP53⁻, and epithelial-to-mesenchymal transition (EMT) [30]. MSI or microsatellite-unstable tumors exhibited the best overall prognosis and the lowest frequency of recurrence [30]. The TP53 or tumor protein 53 classifications of active (TP53⁺) or inactive (TP53⁻) yielded intermediate prognosis and recurrence rates, with the inactive form yielding worse prognosis than the active form [30]. Finally, the EMT or epithelial-to-mesenchymal transition type classification exhibited the worse prognosis and the highest recurrence frequency [30]. Overall, these new molecular classifications of gastric cancer will greatly improve future screening practices.

Despite more than half of the world's population being colonized with *H. pylori*, only a fraction of infected individuals ever develop gastric dysplasia or adenocarcinoma. The discrepancy in overall *H. pylori* infection rates versus disease outcome is governed by specific relationships among host inflammatory responses, strain-specific bacterial virulence

determinants, and environmental factors, which ultimately influence interactions between *H. pylori* and its human host.

The *H. pylori* *cag* pathogenicity island

One extensively studied *H. pylori* strain-specific virulence determinant that significantly augments the risk for gastric cancer is the *cag* pathogenicity island (*cag* PAI). The *cag* island is a 40 kB DNA insertion element, containing approximately 32 genes that encode a bacterial type IV secretion system (T4SS) [17,31]. The *cag* PAI is present in approximately 60–70% of Western *H. pylori* strains and virtually 100% of East-Asian *H. pylori* strains [31]. Although all *H. pylori* strains induce gastritis, strains that harbor the *cag* PAI increase the risk for the development of severe gastritis, atrophy, dysplasia, and gastric adenocarcinoma, compared to strains that lack the *cag* island.

The *cag* island encodes a type IV secretion system (T4SS) that functions as a molecular syringe to facilitate the delivery of bacterial effector molecules into host gastric epithelial cells (Figure 2A). The terminal gene product of the *cag* island, CagA, is translocated into host cells by the type IV *cag* secretion system following bacterial attachment (Figure 2A). Following its injection into epithelial cells, CagA undergoes tyrosine phosphorylation by members of the Abl and Src family of kinases (Figure 2A) [17,31,32]. Intracellular phosphorylated-CagA, in turn, activates a eukaryotic phosphatase SHP-2 and Erk1/2, leading to morphologic changes, such as cell elongation and motility through robust actin cytoskeletal rearrangements, and other morphologic changes reminiscent of unrestrained stimulation by growth factors (Figure 2A) [17,31]. CagA also leads to activation of NF- κ B, which mediates robust pro-inflammatory responses, such as the induction of IL-8 (Figure 2A). Non-phosphorylated CagA also exerts numerous effects within gastric epithelial cells that contribute to pathogenesis. Non-phosphorylated CagA binds to epithelial tight-junction proteins ZO-1 and JAM-A (Figure 2A), leading to disruption of tight junctions at ectopic sites of bacterial attachment [17,31]. In addition, non-phosphorylated CagA disrupts adherens junctions leading to mislocalization and aberrant activation of β -catenin (Figure 2A) [17,31], a response associated with oncogenesis. In addition to CagA, there are also other bacterial factors that contribute to disruption of apical-junctional complexes, including VacA, urease, and HtrA [33]. Cumulatively, disruption of tight and adherens junctions lead to an overall loss of barrier function and cellular polarity, alterations that play an important role in carcinogenesis. Interestingly, transgenic mice overexpressing CagA develop gastric epithelial cell hyperproliferation and gastric adenocarcinoma [17], implicating this molecule as a bacterial oncoprotein that directly contributes to *H. pylori*-induced malignant transformation.

Iron and *Helicobacter pylori*

Recent evidence has supported the concept that iron deficiency contributes to the risk of gastric cancer, and experimental data suggest that *H. pylori* contributes to iron depletion and deficiency in its host. To evaluate whether *H. pylori* infection *per se* is sufficient to induce iron deficiency, several studies have examined the effects of chronic *Helicobacter* infection on iron status using rodent models of infection. One model that is extensively utilized to study *Helicobacter*-induced pathogenesis is the transgenic INS-GAS mouse model. INS-

GAS mice overexpress human gastrin and spontaneously develop gastric cancer, but this takes the virtual lifetime of the animal [34]. However, concurrent infection with *Helicobacter* dramatically accelerates this process, suggesting that persistently elevated gastrin levels synergize with *Helicobacter* to augment the progression to gastric cancer [34]. A recent study demonstrated that infection of INS-GAS mice with a related *Helicobacter* species, *H. felis*, results in decreased serum iron concentrations and altered expression of host genes important in iron homeostasis [35], indicating that *Helicobacter* can alter iron homeostasis in the host. Unfortunately, *H. felis* is a rodent-specific pathogen and does not contain many of the virulence determinants present in the human pathogen *H. pylori*, including the *cag* pathogenicity island.

A recent study analyzed the effects of dietary iron deficiency on the development of *H. pylori*-induced malignancy in a different model of gastric cancer, Mongolian gerbils [36]. The Mongolian gerbil model recapitulates *H. pylori*-induced inflammatory responses and disease progression observed in humans, and, therefore, represents an excellent system to assess mechanisms of *H. pylori*-induced gastric carcinogenesis within the context of iron-deficiency. In gerbils maintained on an iron-depleted diet, *H. pylori* infection significantly increased the severity of gastritis and augmented and accelerated the development of gastric dysplasia and adenocarcinoma, responses that were not observed in iron-replete animals [36]. Importantly, gastritis, dysplasia, and adenocarcinoma did not develop in animals infected with an isogenic *H. pylori cag*⁻ mutant, which lacks a functional *cag* secretion system, under either iron-depleted or iron-replete conditions [36]. These data demonstrated that dietary iron deficiency significantly increased the severity of gastric inflammation and accelerated the development of *H. pylori*-induced premalignant and malignant lesions in a rodent model of gastric cancer. Further, this was dependent on the presence of the key virulence determinant, the *cag* type IV secretion system and CagA.

To define the mechanism through which iron depletion may directly influence the pathogenic potential of *H. pylori*, proteomic profiles of strains isolated from animals maintained on a low iron diet or a regular diet were compared [36]. Numerous protein differences were detected, including proteins that mediate survival, microbial adherence, and function of the *cag* type IV secretion system. *H. pylori* strains isolated from iron-deficient animals also expressed significantly higher levels of the bacterial oncoprotein, CagA (Figure 2B) [36].

To evaluate the *cag*-dependent virulence potential of *H. pylori* strains isolated from iron-deficient or iron-replete animals, *H. pylori* isolates were co-cultured with gastric epithelial cells *in vitro*. *H. pylori* strains isolated from iron-deficient animals exhibited an enhanced capacity to assemble the *cag* type IV secretion system and translocate CagA directly into host cells (Figure 2B) [36]. Increased CagA translocation under conditions of iron deficiency subsequently led to increased proinflammatory host cell responses, such as induction of IL-8 (Figure 2B) [36]. Collectively, these results indicate that exposure of *H. pylori* to low iron conditions enhances the virulence potential of this pathogen and its ability to modulate gastric epithelial cell signaling and pro-inflammatory responses, phenotypes that predispose the gastric epithelium to malignant transformation.

These *in vitro* and *in vivo* results were also extended into a human population of patients residing in a geographical region with a high risk for gastric cancer [36]. Consistent with findings in the rodent model of infection, *H. pylori* strains isolated from patients with the lowest iron levels induced more robust pro-inflammatory responses than strains isolated from patients with the highest iron levels [36], indicating that iron status of the host affects the virulence potential of *H. pylori* through amplification of the host inflammatory response. These studies have demonstrated that environmental factors, specifically nutritional iron deficiencies, dramatically alter *H. pylori* virulence and its direct ability to augment and accelerate gastric carcinogenesis. Another recent study revealed that CagA is partially responsible for inducing aberrant redistribution of the transferrin receptor from the basolateral cell surface to the apical cell surface, where *H. pylori* resides. These data raise the possibility that an unintended consequence of iron deficiency is increased CagA delivery into host cells as a mechanism of iron acquisition [37], thereby predisposing epithelial cells to prolonged exposure to intracellular CagA.

Zinc and *Helicobacter pylori*

In addition to iron, the role of zinc in *H. pylori* pathogenesis and virulence has also been studied. This study analyzed the effect of zinc deprivation on *H. pylori* virulence, demonstrating that sequestration of zinc by the host represses formation of the *cag* type IV secretion system and CagA translocation, a phenotype that is reversed following addition of exogenous zinc (Figure 2B) [38]. Decreased formation of the *cag* secretion system and reduced delivery of CagA also led to diminished NF- κ B activation and IL-8 secretion induced by *H. pylori* (Figure 2B) [38]. The seemingly disparate relationship between levels of iron and zinc clearly indicate the complex contribution of micronutrients on the regulation of *H. pylori* virulence factors. However, there are other examples of reciprocal regulation of bacterial virulence factors governed by varying levels of micronutrients, in particular iron and zinc. Recent studies have demonstrated that zinc deficiency alters the virulence and host response to other gastrointestinal pathogens, including enteroaggregative (EAEC) and enteropathogenic (EPEC) *Escherichia coli* [39–43]. Further, expression and secretion of virulence proteins by EPEC are repressed by zinc and induced by iron [38]. The reciprocity in effects of zinc and iron on *H. pylori* pathogenesis likely underpins the differences observed in gastric cancer risk that are exerted by these two essential micronutrients.

Concluding Remarks

Several potential mechanisms exist by which dietary constituents either protect against or predispose patients to the development of gastrointestinal cancers, in particular gastric cancer (Outstanding Questions Box). One possibility is that dietary factors exert direct effects on the host. For instance, the direct effects of dietary constituents on the gastric epithelium may alter inflammatory responses or damage the gastric mucosa, thereby allowing increased entry of carcinogens into gastric tissue. Another possibility is that micronutrients may influence the composition of the gastric microbiota, which would have direct effects on *H. pylori*-induced disease. Interestingly, microbial species contribute to the development of cancer, with greater than 15% of cancers attributable to infectious agents [44]. However, little is known regarding the contribution of the gastrointestinal microbiome

to the development of cancers of the gastrointestinal tract. Studies in humans and rodent models of malignancy have identified specific microbial species and relationships among members of the microbial community that increase the risk for malignancy in the stomach and colon, and possibly the esophagus [44]. Therefore, defining the role of the microbiome and how this population evolves with respect to alterations in nutritional micronutrients will be an important future area of investigation. Finally, micronutrients can modulate gene expression in bacterial pathogens such as *H. pylori*, resulting in increased or decreased production of bacterial virulence factors. These potential mechanisms are not mutually exclusive, and it seems highly plausible that several mechanisms are in play. Although this process is likely multifactorial, evidence presented here indicates that within the context *H. pylori* infection, essential micronutrients have a direct effect on *H. pylori* virulence potential, which directly affects interactions at the host-pathogen interface and facilitates increased pro-inflammatory responses and malignant transformation.

There are also clinical ramifications that stem from understanding relationships between micronutrients and microbially-induced cancer. Numerous studies have now linked the availability of micronutrients, such as iron and zinc, to the risk of various gastrointestinal cancers. With respect to gastric cancer, among the billions of persons who are colonized with *H. pylori*, fewer than 3% will ever develop gastric cancer. Despite this, the majority of patients are subjected to eradication therapy; however, there are beneficial effects of *H. pylori* in humans, including protection from gastroesophageal reflux disease and esophageal cancer [45], which may be related to micronutrient intake. In addition, universal antibiotic treatment may promote the development of antibiotic resistance among both *H. pylori* and other commensal bacteria within the gastrointestinal tract, which preclude the use of large-scale indiscriminant *H. pylori* eradication campaigns unless the prevalence rates of gastric cancer are extremely high. Thus, there is great interest in developing techniques to identify sub-populations of people at high risk for developing disease. Levels of micronutrients may serve as measurable biomarkers for gastrointestinal cancer risk. Future studies regarding micronutrient availability in the context of *H. pylori*-mediated carcinogenesis are needed, not only to develop more effective means for preventing and treating this cancer, but to also serve as a paradigm for understanding the role of micronutrient availability and the pathogenesis of gastrointestinal malignancies.

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Trends Box

- Iron intake is negatively associated with gastric cancer risk, while zinc intake is positively associated with gastric cancer risk
- Iron deficiency augments assembly and function of the *Helicobacter pylori* *cag* type IV secretion system, while zinc sequestration abrogates formation and deployment of the *cag* type IV secretion system
- Micronutrients differentially modulate bacterial virulence, which directly contributes to microbial-induced inflammation, disease, and the development of cancer
- Monitoring *H. pylori* status in conjunction with micronutrient availability may represent measureable biomarkers to identify individuals at the highest risk for gastric cancer

Outstanding Questions Box

- How does zinc availability in the gastric mucosa directly contribute to *H. pylori*-induced inflammation and injury *in vivo*?
- How do specific dietary micronutrients alter the composition of the gastric microbiota and how does this contribute to the development of cancer?
- How do dietary micronutrients directly affect the virulence of specific gastrointestinal pathogens and how does this contribute to microbial-induced inflammation, disease, and cancer?
- What other essential micronutrients may contribute to microbial-induced inflammation, disease, and the development of cancer?
- How will associations between micronutrient levels and cancer risk directly impact screening practices and treatment in populations at the highest risk for disease?

Figure 1 (Key Figure). Essential micronutrients and gastrointestinal cancer risk

(A) Epidemiologic studies throughout the world have demonstrated significant relationships between diet and gastrointestinal cancer risk. Heme is positively associated with esophageal, gastric, and colorectal cancer risk. Iron is inversely associated with esophageal and gastric cancer risk, while positively associated with colorectal cancer risk. Zinc is inversely associated with esophageal and colorectal cancer risk, while positively associated with gastric cancer risk. Thus, micronutrient levels and gastrointestinal cancer risk are niche-specific. Microbial species contribute the development of cancer, with greater than 15% of

cancers attributable to infectious agents; however, little is known regarding the contribution of the gastrointestinal microbiome to the development of gastrointestinal cancers. The shading in this schematic represents the biomass and unique microbial species present in each gastrointestinal niche, which is dependent on the gastrointestinal pH at each site, raising the possibility that these populations may be affected by alterations in nutritional micronutrients. **(B)** Intestinal-type gastric cancer progresses through a series of well-defined pathological steps from normal gastric mucosa to superficial gastritis, chronic gastritis, atrophic gastritis, intestinal metaplasia, and finally dysplasia and adenocarcinoma. Chronic *H. pylori* infection induces gastric inflammation and thus increases the risk of progression through each of these well-defined steps of gastric transformation.

Figure 2. Molecular signaling alterations induced by intracellular delivery of CagA

(A) Translocation of CagA by the *H. pylori* *cag* type IV secretion system (T4SS) leads to activation of host signaling pathways that promote gastric epithelial responses with carcinogenic potential. Once delivered into host cells, CagA is phosphorylated by Src and Abl kinases, and phosphorylated CagA activates SHP2 and Erk1/2 leading to morphological changes, such as cellular elongation and scattering. Unphosphorylated CagA associates with the tight junction (TJ) proteins ZO-1 and JAM-A, resulting in disruption of cellular tight junctions. CagA also interacts with the adherens junction (AJ) protein E-cadherin, leading to disruption of cellular adherens junctions and release of β-catenin (β), a response associated with oncogenic outcomes. CagA signaling also leads to activation of NF-κB, which ultimately results in the induction of pro-inflammatory immune responses, such as induction of IL-8. (B) Under conditions of altered micronutrient levels, such as iron deficiency or excess zinc, *H. pylori* increases expression of CagA, formation of the *cag* type IV secretion system (T4SS), translocation of CagA into host cells, NF-κB activation, and host cell pro-inflammatory responses, such as induction of IL-8. These phenotypes associated with iron deficiency or high zinc are reversible following iron supplementation or zinc sequestration, respectively. Ultimately, the micronutrients iron and zinc dramatically affect the formation and function the *cag* type IV secretion system, leading to increased virulence of *H. pylori* and increased *H. pylori*-induced inflammation and malignant transformation.