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MINIREVIEW

Modulation of Host-Microbe Metabolism by Cholera Toxin

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ABSTRACT In order for successful fecal-oral transmission, enteric bacterial pathogens have to successfully compete with the intestinal microbiota and reach high concentrations during infection. *Vibrio cholerae* requires cholera toxin (CT) to cause diarrheal disease, which is thought to promote the fecal-oral transmission of the pathogen. Besides inducing diarrheal disease, the catalytic activity of CT also alters host intestinal metabolism, which promotes the growth of *V. cholerae* during infection through the acquisition of host-derived nutrients. Furthermore, recent studies have found that CT-induced disease activates a niche-specific suite of *V. cholerae* genes during infection, some of which may be important for fecal-oral transmission of the pathogen. Our group is currently exploring the concept that CT-induced disease promotes the fecal-oral transmission of *V. cholerae* by modulating both host and pathogen metabolism. Furthermore, the role of the intestinal microbiota in pathogen growth and transmission during toxin-induced disease merits further investigation. These studies open the door to investigating whether other bacterial toxins also enhance pathogen growth and transmission during infection, which may shed light on the design of novel therapeutics for intervention or prevention of diarrheal diseases.

KEYWORDS cholera, cholera toxin, diarrheal disease, enteric pathogens, gut microbiota, metabolism, transmission, *Vibrio cholerae*, host-pathogen interactions, pathogenesis

Diarrheal diseases are a leading cause of death in children under 5 years old globally (1). *Vibrio cholerae* is the causative agent of cholera, an acute diarrheal disease characterized by severe diarrhea, vomiting, and hypovolemic shock. Although most cases of cholera are mild, severe disease can lead to death within hours of the onset of symptoms, with death occurring in 50 to 70% of untreated patients (2). The World Health Organization estimates that there are between 3 and 5 million cholera cases and 100,000 to 120,000 deaths every year (3). Children under 5 years old are disproportionately at risk for cholera (1). It is estimated that about half of all cholera deaths occur in children under five (3). While oral cholera vaccines have shown some promise, they may provide less protection in children under 5 years old (4, 5). Due to the lack of cost-effective vaccination and poor vaccine efficacy in children, there is a need for alternative preventative and therapeutic strategies. Thus, further examination into the mechanisms that *V. cholerae* uses to colonize the gastrointestinal tract and transmit to a new host may shed light into designing novel therapeutic targets for intervention or prevention of the disease in children who are at higher risk. The ability for enteric bacterial pathogens to reach high concentrations in the gastrointestinal tract is required for efficient fecal-oral transmission (6). Conventional wisdom holds that the severe diarrheal disease caused by CT is important for the transmission of *V. cholerae*, since infected individuals can shed up to 20 liters of diarrheal fluid ("rice water stool") per day containing high concentrations of the pathogen (1). However, the molecular mechanisms by which CT promotes fecal-oral transmission of *V. cholerae* remain poorly understood. Recently, it has been found that during infection, the catalytic activity of CT promotes the

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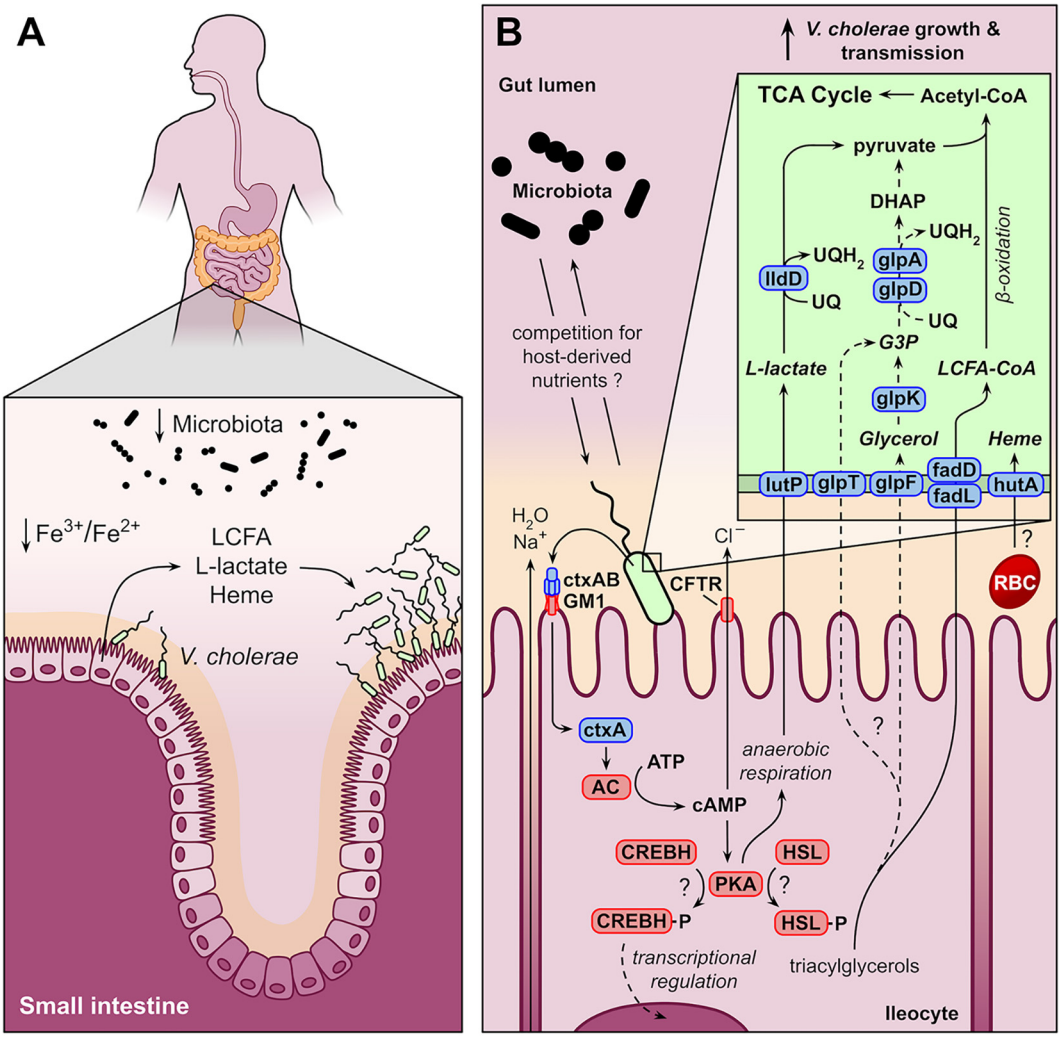

FIG 1 (A and B) Model for modulation of host-microbe metabolism by cholera toxin. The intestinal microbiota in the small intestine and host factors regulate intestinal metabolism, thereby maintaining gut homeostasis. During infection, *Vibrio cholerae* colonizes the ileum of the small intestine and produces high levels of cholera toxin (CT). CT activates adenylate cyclase, which increases cellular levels of 3′,5′-cyclic AMP (cAMP) and leads to changes in a plethora of metabolic functions that are regulated by the cAMP-dependent protein kinase (PKA). Increased cAMP induces lipolysis and can cause extensive breakdown of lipids in cells, leading to secretion of free long-chain fatty acids (LCFAs) and likely glycerol. CT-mediated increase in cAMP also induces anaerobic glycolysis, leading to reduced consumption of oxygen and increased production of L-lactate. Modulation of host metabolism causes dysbiosis of the small intestine microbiota and drives the growth *V. cholerae* during infection by acquisition of host-derived nutrients, including heme (from capillary congestion) when iron becomes limiting. These CT-induced processes drive luminal expansion and transmission of *V. cholerae* during infection. The role of the microbiota and other host factors, such as cAMP-responsive element-binding protein H (CREBH) in the context of CT-induced remodeling of host-pathogen and host-microbe metabolism remains poorly understood.

explosive growth of *V. cholerae* in the gut lumen through the acquisition of host-derived nutrients (Fig. 1). Furthermore, CT activity induces a distinct transcriptome signature in the pathogen that includes the upregulation of a suite of genes involved in virulence and metabolism (7). These recent findings represent a paradigm shift in our understanding of the function of CT as a modulator of both host and pathogen metabolism, which may have broad implications for how other toxins promote pathogen growth and transmission. Interestingly, *V. cholerae* shed in the stool during infection is in a "hyperinfectious" state that elevates the fecal-oral transmissibility potential of the pathogen (8). It has also been reported that specific members of the gut microbiota in humans are implicated in the recovery from *V. cholerae* infection (9). However, the role of modulation of host-microbe metabolism by CT in the fecal-oral transmission of *V. cholerae* remains poorly understood. Thus, unraveling the molecular mechanisms by which cholera toxin and other bacterial toxins

promote pathogen transmission during infection will be key to identifying future preventative and therapeutic targets for CT and other toxin-mediated diarrheal diseases.

MODULATION OF HOST INTESTINAL METABOLISM BY CHOLERA TOXIN

The CT subunits (CtxA and CtxB) are encoded on a lysogenic filamentous bacteriophage called CTXφ (10). Pathogenic isolates of *V. cholerae* can produce and secrete CT in the gut during the infectious cycle. *V. cholerae* transmits via the fecal-oral route by ingestion of contaminated food or water. Once in the small intestine, *V. cholerae* adheres to the intestinal epithelium using toxin-coregulated pilus (TCP), and it is there that the pathogen produces CT, which binds to the ganglioside GM1 on intestinal epithelial cells. The catalytic activity of CT activates adenylate cyclase, which increases cellular levels of 3′,5′-cyclic AMP (cAMP) in target cells, activating the eukaryotic cAMP-dependent protein kinase A (PKA) (11). The severe secretory diarrhea caused by CT is thought to occur when PKA phosphorylates and activates the cystic fibrosis transmembrane conductance regulator (CFTR), an apical ion channel that transports chloride out of epithelial cells, resulting in an electrolyte imbalance in the lumen of the intestine and massive water loss (12). CT-induced activation of PKA can also lead to numerous metabolic changes in target cells including the upregulation of hormone-sensitive lipase (HSL), which can mediate the lipolysis of triglycerides and ultimately the release of free long-chain fatty acids (LCFAs) and glycerol (13). The observation that CT induces lipolysis has been observed since the early 1970s in experiments using fat pads isolated from rats and measuring the enzymatic release of glycerol (14). Interestingly, lipolysis assays were commonly used as a method to detect the catalytic activity of CT before the introduction of more quantitative methods, such as enzyme-linked immunosorbent assay-mediated detection (13). However, the biological significance of lipolysis in the metabolism of *V. cholerae* during disease was overlooked. In the eukaryotic cells, lipids are primarily stored as triglycerides, nonpolar lipid molecules composed of one glycerol molecule and three fatty acid molecules, often within lipid droplets. In adipocytes, HSL, triglyceride lipase, and monoacylglycerol lipase are the major enzymes responsible for the breakdown of stored triglycerides (15). Recently, it was found that during infection, CT-induced disease leads to increased concentrations LCFAs in the lumen of the small intestine, and uptake of LCFAs by *V. cholerae* drives the pathogen's growth during infection (discussed below in "How Cholera Toxin Drives the Growth of *V. cholerae* in the Gut"). The mechanisms by which cellular LCFAs are transported to the lumen of the small intestine during CT-induced disease is unclear, but the well-known effects of CT on increasing intestinal permeability suggests that this is likely a passive mechanism. Leakage of albumin from the vasculature into the intestinal lumen has been reported in human cholera patients, as well as in CT-treated rabbits (16). Interestingly, albumin has a high binding affinity to LCFAs (17), which may represent a mechanism for trafficking of LCFAs into the lumen during disease. The CT-mediated increase in cAMP can also induce a metabolic switch to anaerobic glycolysis in host target cells, leading to reduced consumption of oxygen and production of L-lactate. Recently, it was also found that during infection, along with LCFAs, CT-induced disease also leads to increased concentrations of L-lactate in the lumen of the small intestine, consistent with previous observations of a PKA-dependent metabolic switch to anaerobic glycolysis in CT-treated cells. However, additional studies are needed to determine which host transcription factors are modulated in response to increased concentration of cAMP that leads to both lipolysis and production of L-lactate.

Although the activation of the host enzyme HSL has been implicated in lipolysis during CT-induced disease, the role of other lipases, including triglyceride lipase and monoacylglycerol lipase remains unclear. Furthermore, whether other host factors activated by CT are involved in promoting the bioavailability of host-derived nutrients during disease remains unexplored. Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor and transcription factor found in various tissues that controls the expression of genes playing key roles in lipid metabolism (18). In colonic epithelial cells (colonocytes), PPARγ binds the short-chain fatty acid butyrate and drives cellular metabolism toward β-oxidation (19). It was recently reported that PPARγ may mediate LCFA processing by small intestinal epithelial cells

(20). However, little is known about the function of PPARγ and the repertoire of positively regulated genes in the small intestine and whether CT-induced disease perturbs PPARγ to modulate cellular metabolism and promote lipolysis during *V. cholerae* infection. Another potential avenue for exploration relates to CT and its well-known immunomodulatory properties, both for A (cAMP-dependent) and B (GM1-binding dependent) subunits (21). CT (both A and B subunits) can mediate upregulation of T_H17 responses, which leads to increased IL-17A production and likely also IL-22 (22). IL-17 and IL-22 can cooperatively enhance mucosal barrier function (23), and IL-22 also plays a role in lipid metabolism by promoting lipolysis (24) and reducing expression of lipid transporters in the gut (25). Thus, the modulation of the immune system may be connected to alterations in host metabolism during CT-induced disease. Furthermore, the role of CT in activating cAMP production suggests transcriptional modifications to host factors regulating metabolism in the lumen of the small intestine. Transcription factors such as cAMP-responsive element-binding protein H (CREBH) have been shown to regulate the expression of genes maintaining LCFA, glucose, and iron homeostasis in the small intestine (26). Altogether, the potential downstream impacts of CT on host metabolism and transcription warrant further investigation to explore the importance of any potential uncharacterized host factors in host-derived nutrient acquisition by the *V. cholerae* during disease.

HOW CHOLERA TOXIN DRIVES THE GROWTH OF *V. CHOLERAE* IN THE GUT

The contribution of CT to the luminal growth of *V. cholerae* has been long overlooked. This is in part due to the long history of *V. cholerae* CT mutants as promising oral vaccines (27). Human volunteer studies over 30 years ago using CT mutants of *V. cholerae* El Tor N16961 for vaccine challenge found a reduced overall luminal growth of CT mutants in the gastrointestinal tract compared to the parent strain (28). Vaccine studies with mutant strains tended to emphasize the similar concentrations between vaccine and parent strains in the stool as an indication of robust colonization by the vaccine strain, since this would be important for a successful oral vaccine candidate in establishing adequate immune responses. However, when assessing total bacterial growth, the volume of diarrheal fluid that is shed during CT-induced disease must be considered. Humans with cholera can shed up to 20 liters of "rice water stool" in 24 h, often containing over 10^{11} organisms per L (1). Quantifying bacterial growth by concentration (CFU/g) is misleading when comparing CT-producing *V. cholerae* strains and CT mutants in both humans and animal models because of the dilution factor caused by the significant fluid accumulation and loss during CT-induced disease. When CT-induced fluid accumulation is corrected for, a clear growth benefit can be observed for *V. cholerae* strains capable of producing CT. A study in the 1980s reported that CT-producing *V. cholerae* had increased intestinal colonization compared to nontoxigenic strains, but the mechanism was unknown (29). Recently, it was reported that in both infant rabbits and suckling mice, CT-induced disease promotes the growth of *V. cholerae* in the lumen of the small intestine (7). Importantly, no fitness advantage is observed for the wild-type *V. cholerae* when animals are coinfected with the Δ*ctxAB* (isogenic CT) mutant, demonstrating that the Δ*ctxAB* mutant is not intrinsically defective for growth *per se* but rather that some aspect of CT-induced disease drives luminal growth of the pathogen. Furthermore, the luminal growth of the Δ*ctxAB* mutant can be restored by oral treatment of animals with a single dose of CT. In that same study, *in vivo* RNA-sequencing of *V. cholerae* during infection of infant rabbits revealed that a suite of metabolism genes is significantly upregulated in *V. cholerae* during colonization of the small intestine compared to the large intestine. These genes include genes involved in tricarboxylic acid (TCA) cycle metabolism, LCFA metabolism, and L-lactate utilization (7). Importantly, these metabolic pathways are significantly upregulated in the CT-producing wild-type *V. cholerae* strain relative to the isogenic Δ*ctxAB* mutant. Furthermore, the luminal concentrations of both LCFA and L-lactate were found to be significantly elevated in the small intestine of rabbits infected with the wild-type *V. cholerae* strain compared to animals infected with the isogenic Δ*ctxAB* mutant. Together, these findings were the first to show direct evidence that CT-induced

disease promotes the intestinal growth of *V. cholerae* during infection by inducing metabolic changes in the gut which enable pathogen acquisition of host-derived nutrients. It is important to emphasize that in these studies, the two strains used (wild-type *V. cholerae* strain El Tor C6706 and the Δ*ctxAB* mutant) differed only by a single deletion in the *ctxAB* operon, demonstrating the remarkable ability of CT to remodel both pathogen and host metabolism in the gut during infection.

The uptake of exogenous LCFAs in bacteria requires the LCFA transporter protein FadL (30) and *in vivo* RNA-sequencing analysis of *V. cholerae* indicated that the *fadL* homolog VC1043 was highly expressed in the small intestine of neonatal rabbits, but only in the *V. cholerae* strain capable of producing CT (7). Competitive infections in mice with the wild-type strain and a *fadL* mutant demonstrated that LCFA uptake confers a fitness advantage to *V. cholerae* but not in strains lacking the ability to produce CT (i.e., Δ*ctxAB* mutant background). Furthermore, oral administration of purified CT rescues the growth and the fitness advantage of the Δ*ctxAB* mutant over a Δ*ctxAB fadL* double mutant, further demonstrating that the ability for *V. cholerae* to take up LCFAs during infection confers a fitness advantage to the pathogen but only in the context of the CT diseased gut where LCFA would be predicted to become available. Future studies will investigate whether this concept holds true for L-lactate metabolism in *V. cholerae* during infection, where CT-induced luminal availability of host L-lactate also participates in driving the intestinal growth of *V. cholerae* during infection. Interestingly, mammalian lactate dehydrogenases generate only L-lactate, while fermenting microbes in the gut microbiota produce both D-lactate and L-lactate enantiomers (31). *V. cholerae* specifically encodes the ability to take up L-lactate through its L-lactate permease (*lldP*; VCA0983) and oxidize it to pyruvate using L-lactate dehydrogenase (*lldD*; VCA0984), which can donate an electron to an electron acceptor for respiration. Both *lldP* and *lldD* were highly expressed in the ileum of the CT-producing wild-type *V. cholerae* strain relative to the Δ*ctxAB* mutant during colonization of the ileum. Furthermore, *V. cholerae* glycerol uptake and metabolic pathways, including the aerobic and anaerobic glycerol-3-phosphate dehydrogenases (*glpD* and *glpA*, respectively) were also found to be upregulated during CT-induced disease and colonization of both the ileum (small intestine) and cecum (large intestine) (7). However, the importance of glycerol utilization by *V. cholerae* in the context of CT-induced lipolysis during infection remains to be explored. RNA-sequencing (RNA-seq) data also suggested that *V. cholerae* may perform both aerobic and anaerobic respiration during infection, and indeed recent studies have demonstrated that the pathogen can utilize both nitrate and oxygen during colonization of the gut (32, 33). This suggests that there may exist temporal or niche-specific availability of oxygen and alternative electron acceptors in the gastrointestinal tract during infection. The role of CT-induced disease on availability of oxygen and nitrate (or other alternative electron acceptors) and the impact on *V. cholerae* respiration during infection remains unknown, but given the profound effects of the toxin on host and pathogen physiology, this warrants investigation.

It is important to understand the limitations of animal models of disease, particularly when studying a human-specific pathogen such as *V. cholerae*. In that regard, a previous study that analyzed the *V. cholerae* proteins present in the stools of 32 human cholera patients identified FadL (VC1043), LldD, and the heme uptake protein HutA as some of the highest expressed *V. cholerae* proteins, supporting the concept that these metabolic pathways are also important for the growth of *V. cholerae* in the human gut during disease (34).

HOW CT-INDUCED DISEASE LEADS TO IRON DEPLETION AND HEME UPTAKE BY *V. CHOLERAE*

Bacterial pathogens have evolved strategies to overcome the limitation of metals within the mammalian host, with iron being one of the most important metals withheld by the host during infection, as previously reviewed (35, 36). The genome of *V. cholerae* El Tor strain N16961 consists of two circular chromosomes, one large (chromosome I) and one small (chromosome II), encoding 2,775 and 1,115 open reading frames, respectively (37). A previous study using a rabbit ileal loop model of *V. cholerae* infection found that *in vivo*

growth of *V. cholerae* in the intestine results in an upregulation of genes encoded on chromosome II (38). Interestingly, a recent study found that the upregulation of *V. cholerae* genes found on chromosome II depends on CT, since a 16-fold induction of genes encoded in the small chromosome was observed in the wild-type *V. cholerae* relative to the Δ*ctxAB* mutant during colonization of the ileum (7). When free iron is abundant, the ferric uptake regulator (31) in *V. cholerae* binds to ferrous iron (Fe^{2+}) which acts as a corepressor to inhibit the transcription of iron-regulated genes (39). However, when iron is limiting, the reduced bioavailability of Fe^{2+} leads to the derepression of genes involved in iron acquisition, including genes involved in the transport and utilization of heme and the *V. cholerae* siderophore vibriobactin (40). Interestingly, most *V. cholerae* genes regulated by the Fur regulon that would be predicted to be upregulated when iron is limiting were found to be significantly upregulated in wild-type *V. cholerae* relative to the Δ*ctxAB* mutant during colonization of the ileum of the small intestine (7). Consistent with this, it was also found that total iron concentrations were indeed significantly lower in the lumen of the small intestine of wild type-infected animals relative to animals infected with a Δ*ctxAB* mutant. This CT-dependent iron depletion is not due to increased inflammation and host iron sequestration during CT-induced disease as a stable marker of inflammation, Lipocalin-2, was not elevated in the lumen of infant rabbits infected with the CT-producing wild-type strain. Furthermore, genes involved in the uptake of heme, including the heme uptake receptor, *hutA*, were among the highest expressed iron-regulated genes in the wild-type *V. cholerae* during colonization of the small intestine. Cholera is not an inflammatory disease (1). However, *V. cholerae* infection causes a pathology known as "capillary congestion" in the ileum of the small intestine, defined as an accumulation of red blood cells in the mucosa (41). Previous studies in *V. cholerae*-infected infant rabbits have demonstrated that capillary congestion requires CT (42). Recently, it was demonstrated that capillary congestion also occurs in the small intestine of suckling mice infected with the wild-type *V. cholerae* or in mice treated orally with a single dose of CT but not in Δ*ctxAB* mutant-infected animals (7). Importantly, heme (measured as hemin) was detected in the ileum (where CT is expressed) and was found at higher concentrations relative to the cecum of infant rabbits infected with the wild-type *V. cholerae*. Furthermore, infant rabbits that were infected with wild-type *V. cholerae* had elevated concentrations of heme in the lumen of the ileum compared to the ileum of animals infected with the Δ*ctxAB* mutant, consistent with coupled RNA-seq data showing higher expression of heme-utilization genes in the wild-type *V. cholerae* relative to the Δ*ctxAB* mutant and in the ileum relative to the cecum. These data indicate that infection with *V. cholerae* leads to a CT-mediated increase in heme in the gut lumen. Although the source of heme remains unclear, the CT-induced capillary congestion of red blood cells appears to be the most likely source. Intriguingly, *V. cholerae* encodes a hemolysin (HlyA), located on chromosome II, which exerts hemolytic activity (43) and is highly expressed in the small intestine of infant rabbits during infection (7). However, the role of HlyA in the availability of heme during infection with *V. cholerae* remains unknown.

V. cholerae encodes three receptors for heme uptake, and *hutA* was found to be the most highly expressed Fur-regulated gene in the wild-type *V. cholerae* relative to the Δ*ctxAB* mutant during infection (7). Previous studies have shown that a double mutant strain of *V. cholerae* that lacks *hutA* and the vibriobactin receptor, *viuA*, has a growth defect in both suckling mice and infant rabbits (44, 45). A recent study demonstrated that competitive infections in mice with the wild-type strain and a *hutA viuA* mutant confers a fitness advantage to *V. cholerae* but not in strains lacking the ability to produce CT (i.e., Δ*ctxAB* mutant background). Furthermore, oral administration of purified CT rescues the growth and the fitness advantage of the Δ*ctxAB* mutant over a Δ*ctxAB hutA viuA* triple mutant, further demonstrating that the ability for *V. cholerae* to take up heme during infection confers a fitness advantage to the pathogen but only in the context of the CT diseased gut where heme would be predicted to become available. It is not clear why the HutA receptor seems to be the most important receptor *in vivo*, but previous studies have demonstrated that HutA is required for the use of hemoglobin as an iron source, suggesting that hemoglobin, as well as free heme, may be available to the pathogen during disease (44). The mechanism by which the lumen of the small intestine becomes iron depleted

during *V. cholerae* infection and why this depends on CT-induced diseased remains unclear. However, RNA-seq data suggest that CT-induced disease leads to upregulation of the TCA cycle, including iron/sulfur cluster-containing enzymes such as succinate dehydrogenase, the iron/sulfur cluster assembly protein cysteine desulfurase, and other iron-binding proteins involved in metabolism. Thus, it is likely that during infection with *V. cholerae*, the accompanied CT-induced boost in TCA cycle-mediated growth leads to a rapid utilization of iron, which is exacerbated by loss of water (and iron-carrying bacteria).

WHAT IS THE ROLE OF THE GUT MICROBIOTA IN CT-DEPENDENT SUSCEPTIBILITY TO *V. CHOLERAE* INFECTION AND DISEASE?

The gut microbiota is an important factor in the susceptibility to enteric pathogens (46). The intestinal microbiota maintains gut homeostasis by regulating a suite of host metabolic processes throughout the gastrointestinal tract. In the small intestine, the gut microbiota regulates fat storage (47) and metabolic processes in response to dietary lipids (48). During *V. cholerae* infection, *V. cholerae* colonizes the small intestine of both humans and animals, where the pathogen secretes high concentrations of CT. Specific members of the gut microbiota can mediate susceptibility to *V. cholerae* infection (49, 50) and are implicated in the recovery of humans infected with *V. cholerae* infection (9). However, little is known about the specific roles of small intestinal microbiota in susceptibility to enteric pathogens of the small intestine, such as *V. cholerae*, and whether toxins produced by these pathogens during infection, such as CT, alter the microbiota to promote pathogen growth. Neonatal animals are susceptible to infection by *V. cholerae*, whereas adult animals are not. Therefore, neonatal animals must be used as a model for cholera in humans. The infant rabbit model of cholera recapitulates many of the CT-dependent disease symptoms observed in humans (51, 52). Neonatal (suckling) mice also exhibit CT-dependent diarrheal disease and are considered the "gold standard" for evaluating intestinal colonization by *V. cholerae* (53). However, susceptibility to *V. cholerae* colonization and disease changes with age in animals. Children under the age of 5 comprise half of all cholera cases and deaths (3). In addition, susceptibility to *V. cholerae* wanes in mice: 10-day-old mice experience reduced *V. cholerae* colonization and disease compared to suckling mice, and adult mice experience no colonization and disease (53). However, *V. cholerae* is able to colonize the intestine of germfree adult mice (54, 55) or conventionally raised adult mice treated with antibiotics (56, 57). Interestingly, the gut microbiota composition changes with age from low diversity and predominantly facultative anaerobic *Lactobacillus* species in early life to high diversity comprised of predominantly obligate anaerobes (58). Recent studies suggest the composition of the microbiota affects susceptibility to *V. cholerae* (50). Neonatal susceptibility is not unique to *V. cholerae*. For example, suckling mice are also more susceptible to the enteric pathogen *Salmonella enterica* serovar Typhimurium, and the addition of prominent adult commensals, specifically *Clostridia* species, to suckling mice increases survival and decreases the growth of *S.* Typhimurium (59). Conventionally raised adult mice depleted of obligate anaerobe commensals, through antibiotic treatment, have increased susceptibility to *V. cholerae* colonization and disease. Interestingly, the addition of *Bacteroides* species can rescue the resistance to *V. cholerae* in adult mice and confers resistance to *V. cholerae* in suckling mice (57).

Enteric pathogens and the gut microbiota can interact through a variety of mechanisms including direct competition over nutrients, changes to the environment through secretion of metabolites, or direct antagonism. Recent studies have explored the variety of interactions between *V. cholerae* and the gut microbiota. *V. cholerae* senses a variety of gut microbiota-derived metabolites, including short-chain fatty acids and antimicrobial peptides, that inhibit pathogen growth (57, 60). In addition, the relative proportion of secondary and deconjugated secondary bile salts in the intestinal lumen can impact *V. cholerae* colonization. Secondary bile salts activate CT expression as well as a suite of other virulence factors (61), while deconjugated bile salts inhibit pathogen virulence and growth (50, 62). The gut commensal bacterium *Blautia obeum* produces bile salt hydrolase that deconjugates bile salts and lowers the concentrations of secondary bile salts in the gut, reducing

virulence of *V. cholerae* (50). Furthermore, *V. cholerae* can antagonize closely related members of the gut microbiota during colonization using its type VI secretion system (T6SS) (63), which also enhances pathogen growth in the gut. The composition of the gut microbiota changes during *V. cholerae* infection as humans infected with *V. cholerae* have been reported to harbor lower levels of *Bacteroides* species than those who are recovering from cholera (9). It is still unclear how CT-induced disease and modulation of intestinal metabolism in conjunction with the T6SS lead to specific changes to the gut microbiota. Future studies will focus on determining whether modulation of the gut microbiota by CT plays a role in pathogen susceptibility as well as intestinal growth during infection.

HOW CHOLERA TOXIN PROMOTES FECAL-ORAL TRANSMISSION

Mathematical models suggest that pathogenic bacteria cause disease because the harm that they induce is somehow coupled to infectious transmission (64). CT is thought to be important for the transmission of *V. cholerae* as cholera victims develop severe diarrheal disease, which enables the physical dissemination of the pathogen. However, recent findings suggest that besides causing diarrheal disease, CT enhances the growth of *V. cholerae* and induces a distinct transcriptome signature in the pathogen that includes the upregulation of a suite of genes involved in virulence and metabolism (7). The ability of other enteric bacterial pathogens, such as *S.* Typhimurium, to reach high concentrations in the gastrointestinal tract is required for efficient fecal-oral transmission (6, 65). Furthermore, studies have found that *S.* Typhimurium virulence factors enable the pathogen to acquire host-derived electron acceptors during infection, and that the ability of this pathogen to acquire these nutrients is required for efficient fecal-oral transmission (66). Furthermore, *V. cholerae* shed during infection is in a "hyperinfectious" state that elevates the fecal-oral transmissibility potential of the pathogen (8). Recently, *in vivo* RNA-seq analyses of *V. cholerae* during infection of infant rabbits identified 243 *V. cholerae* genes that are significantly upregulated in the wild-type *V. cholerae* compared to the Δ*ctxAB* mutant during colonization of the gut, including genes involved in metabolism and virulence (7). Interestingly, while 101 genes were upregulated during colonization of the ileum where CT is expressed, 118 unique genes were upregulated in *V. cholerae* after transition to the large intestine (cecum). Genes found to be upregulated in a CT-dependent manner in the large intestine included genes involved in biofilm formation and chemotaxis. Previous studies have demonstrated that growth in a biofilm induces a hyperinfectious phenotype in *V. cholerae* (67, 68). It is tempting to speculate that CT-induced genes in the large intestine, such as those promoting biofilm formation, may contribute to the fecal-oral transmission of *V. cholerae*. *V. cholerae* depends on specific signals during its infectious life cycle in order to accurately express CT and other virulence factors in the small intestine. Upon ingestion, *V. cholerae* encounters bile salts in the stomach and the proximal small intestine, which leads to the transcriptional activation of CT and TCP (61). Interestingly, host-derived nutrients, including LCFAs and L-lactate, have been found to inhibit ToxT-dependent production of CT and TCP (69, 70). It is also tempting to speculate that *V. cholerae* may have evolved a mechanism for sensing that disease has been accomplished by using LCFAs and L-lactate as "late disease" signals and dampening virulence and transitioning to a "transmission ready" state. Notwithstanding the CT-induced growth and induction of *V. cholerae* genes, the diarrheal disease itself remains an important factor in the fecal-oral transmission of the pathogen. Indeed, CT has proven to be a multifunctional protein that is capable of causing diarrheal disease, modulating the immune system, and altering host-pathogen metabolism. Thus, it is possible that during the evolution of *V. cholerae* as a pathogen, CT has coevolved to promote transmission by a multifactorial mechanism, involving rapid growth in the small intestine ("phase 1"), induction of "transmission genes" in the large intestine ("phase 2"), and finally, physical dissemination of the pathogen through diarrheal disease ("phase 3").

FUTURE OUTLOOK: BACTERIAL TOXINS IN MODULATION OF HOST-MICROBE METABOLISM

The recent findings that CT remodels both host and pathogen metabolism represent a paradigm shift in our understanding of the function of CT and may have broad implications

for how other bacterial toxins promote pathogen growth and transmission. For example, enterotoxigenic *Escherichia coli* (ETEC) can secrete heat-labile enterotoxin (LT), a heat-stable enterotoxin (ST), or both. The genes encoding LT share 78% nucleotide sequence identity to the *ctxAB* operon encoding CT, and the catalytic activity of LT is identical to that of CT (71). ST is also functionally similar to CT, but its catalytic activity leads to cyclic GMP-dependent activation of CFTR (72). Interestingly, a study reported that ETEC strains capable of producing LT had a colonization advantage over nontoxigenic ETEC strains in a mouse model of infection (73). Thus, it is very likely that both LT and ST also promote the intestinal growth of ETEC during infection through inducing metabolic changes in the gut and promoting acquisition of host-derived nutrients. Furthermore, various other bacterial toxins induce the cellular production of cAMP and other cyclic nucleotides in target cells. For example, the pathogen *Bacillus anthracis* secretes edema factor (EF), a adenylate cyclase that converts ATP to cAMP (74). The pathogen *Bordetella pertussis*, the causative agent of pertussis (74), encodes an adenylate cyclase toxin-hemolysin (CyaA) that elevates host cell cAMP (75). *Pseudomonas aeruginosa* secretes an adenylate cyclase, ExoY, by its type III secretion system (T3SS) that elevates intracellular accumulation of cAMP, cGMP and cUMP, that can activate PKA, as well as protein kinase G (76), which would be predicted to lead to changes in host cell metabolism. There is also evidence that other bacterial toxins, beyond those that elevate cellular cyclic nucleotides, may remodel host-pathogen metabolism and promote pathogen acquisition of host-derived nutrients. For instance, recent studies have found that *Clostridium difficile* uses toxin-mediated disease to remodel the nutritional environment to promote its own growth during disease (35, 77). Thus, the idea that bacterial toxins have evolved to couple disease and remodeling of host-pathogen metabolism is an emerging new concept in the field of host-microbe interactions.

CONCLUDING REMARKS

Since the enterotoxin activity of CT was first reported in supernatants of *V. cholerae* cultures over 63 years ago, we are just beginning to understand how the diarrheal disease caused by this toxin benefits the pathogen. The findings described in this review define a new function for cholera toxin as a virulence factor that enhances pathogen growth by remodeling host-microbe metabolism. These recent findings also provide a possible explanation for why cholera toxin genes are so highly selected for in the evolution of epidemic strains of *V. cholerae* and open the door for investigating novel mechanisms of how cholera toxin and other bacterial toxins enhance fecal-oral transmission. These recent advances represent a paradigm shift in our understanding of how microbial toxins function to enhance bacterial fitness during infection and will likely have broad implications for other bacterial toxins.

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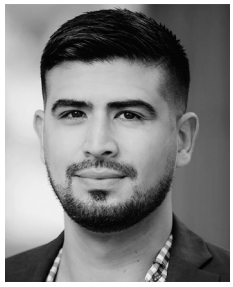

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