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Changing the locks on intestinal signaling

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summary

Endogenous peptides and structurally similar bacterial heat-stable enterotoxins (ST) bind guanylate-cyclase C (GC-C), resulting in fluid homeostasis or diarrhea, respectively. In this issue of *Cell Host & Microbe*, Carey et al. (2021), show how bats have evolutionarily maintained homeostatic signaling while avoiding pathogenic effects of ST.

Mammals have highly conserved mechanisms to maintain fluid homeostasis in the intestine. Guanylate cyclase-C (GC-C), a protein dimer localized to the membrane of intestinal enterocytes plays a central role by serving as a receptor for small cysteine-rich peptides produced by intestinal epithelia, guanylin and uroguanylin (Figure 1A). Binding of these ligands to the receptor domain of GC-C stimulates the conversion of GTP to cGMP by the intracellular catalytic domain. The resulting increases in cGMP activate cGMP-dependent protein kinase II (PKG) which in turn modulates the activity of cellular ion channels. This includes the sodium-hydrogen ion exchanger NHE3 and the CFTR chloride channel resulting in net export of salt and water into the intestinal lumen. These peptide hormones, in concert with their GC-C receptor, act in a paracrine fashion to maintain critical fluid efflux into the intestinal lumen.

Heat stable toxins (ST) are cysteine-rich short (18-19 amino acid) peptides secreted by Gram-negative enteric pathogens, particularly enterotoxigenic *Escherichia coli* (ETEC). They share sequence homology and overall structure with the endogenous peptide hormones, and bind GC-C with high affinity (Schulz et al., 1990) resulting in robust stimulation of the cGMP-PKG axis and pronounced secretory diarrhea (Figure 1B). Mutations in GC-C which preserve binding of peptide hormones at the expense of ST could mitigate the diarrheal effects of the toxins and offer a selective advantage.

Interestingly, in studies reported here by Carey, *et al.* (Carey et al., 2021), phylogenetic analysis of primate and bat GC-C sequences provides evidence for positive selection of sites confined to the ligand binding domain of the receptor, potentially selected by pressure from

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ST. Of note, variation in the ligand-binding domain was particularly intense in bats. These mutations in GC-C conferred a differential ability to respond to ST peptides, mitigating the impact of some while enhancing signaling by others. Remarkably, the investigators discovered that some species of bats had developed compensatory mutations in uroguanylin, offsetting the variation in GC-C and permitting continued paracrine signaling through the cGMP-PKG axis (Figure 1C). In effect, bats have evolved to change the lock to bar toxin engagement, while keeping the new keys to themselves.

Although precisely which toxins, or which pathogens, might be driving the evolutionary changes to GC-C in bats and their corresponding endogenous peptides remains unclear, the studies illustrate the potential for extraordinary co-evolution under microbial selection. An open question is whether it is actually ST-induced diarrhea *per se* that has driven the selection. Activation of cGMP production in the host is likely to have a multitude of effects that extend beyond modulation of ion channels to export NaCl and water. In fact, signaling through this critical “second messenger” pathway may alter transcription of multiple genes (Zhao et al., 2005), and impact other key elements of physiology, including intestinal epithelial cell proliferation (Basu et al., 2014), and stem cell maintenance vital to development of the mucosal surface architecture required for nutrient absorption (Steinbrecher and Cohen, 2011).

Elucidation of the impacts of enterotoxins on the host that may have provided the evolutionary forces underlying the positive selection of the mutations reported here is an important avenue of investigation. Enterotoxigenic *Escherichia coli* (ETEC) produce both heat-stable toxins (ST-H and ST-P) as well as heat-labile toxin (LT). LT also signals through a major “second messenger” cyclic nucleotide system by activation of adenylate cyclase to increase intracellular cAMP, which in turn activates protein kinase A (PKA). Like PKG, PKA modulates the activity of NHE3 and CFTR with similar fluid efflux into lumen of the small intestine. In addition, signaling through PKA can affect the transcription of thousands of genes (Zambon et al., 2005), suggesting that like ST the impact of heat-labile toxin likely extends beyond the episode of acute, typically self-limited diarrhea.

Indeed, ST-mediated signaling through GC-C has a number of other important implications for human health. Intriguingly, the proportionately low incidence of colon cancer in regions where ETEC is prevalent prompted earlier investigations of the anti-proliferative effects of ST on colonic neoplasms where GC-C signaling along the crypt-villus axis is altered in favor of epithelial proliferation (Pitari et al., 2003). The molecular mimicry of ST for endogenous peptides also underlies development of peptide drugs linaclotide and plecanatide used in treatment of irritable bowel syndrome and chronic constipation.

ETEC astoundingly account for hundreds of millions of estimated cases of diarrheal illness each year, particularly in young children in low-middle income countries that lack fundamental human needs of clean water and basic sanitation (Khalil et al., 2018). Increasingly, these pathogens have been associated with important sequelae including pathologic changes to the small intestine accompanied malnutrition and stunted growth. Malnutrition, consequently, places children at substantially increased risk of death from diarrheal disease and other infections. While the molecular pathogenesis of these sequelae

has yet to be elucidated, they almost certainly are the result of repeated exposure to one or both of the toxins produced by these ubiquitous pathogens.

Bats appear to be evolving to avoid either the toxin-induced acute diarrheal illness, or perhaps subsequent intestinal pathology, while maintaining homeostatic mechanisms essential to regulation of fluid balance in the intestine. Humans will need effective toxoid vaccines (Zegeye et al., 2019) to counter the effects of these pathogens rather than waiting to evolve under their selective pressure.

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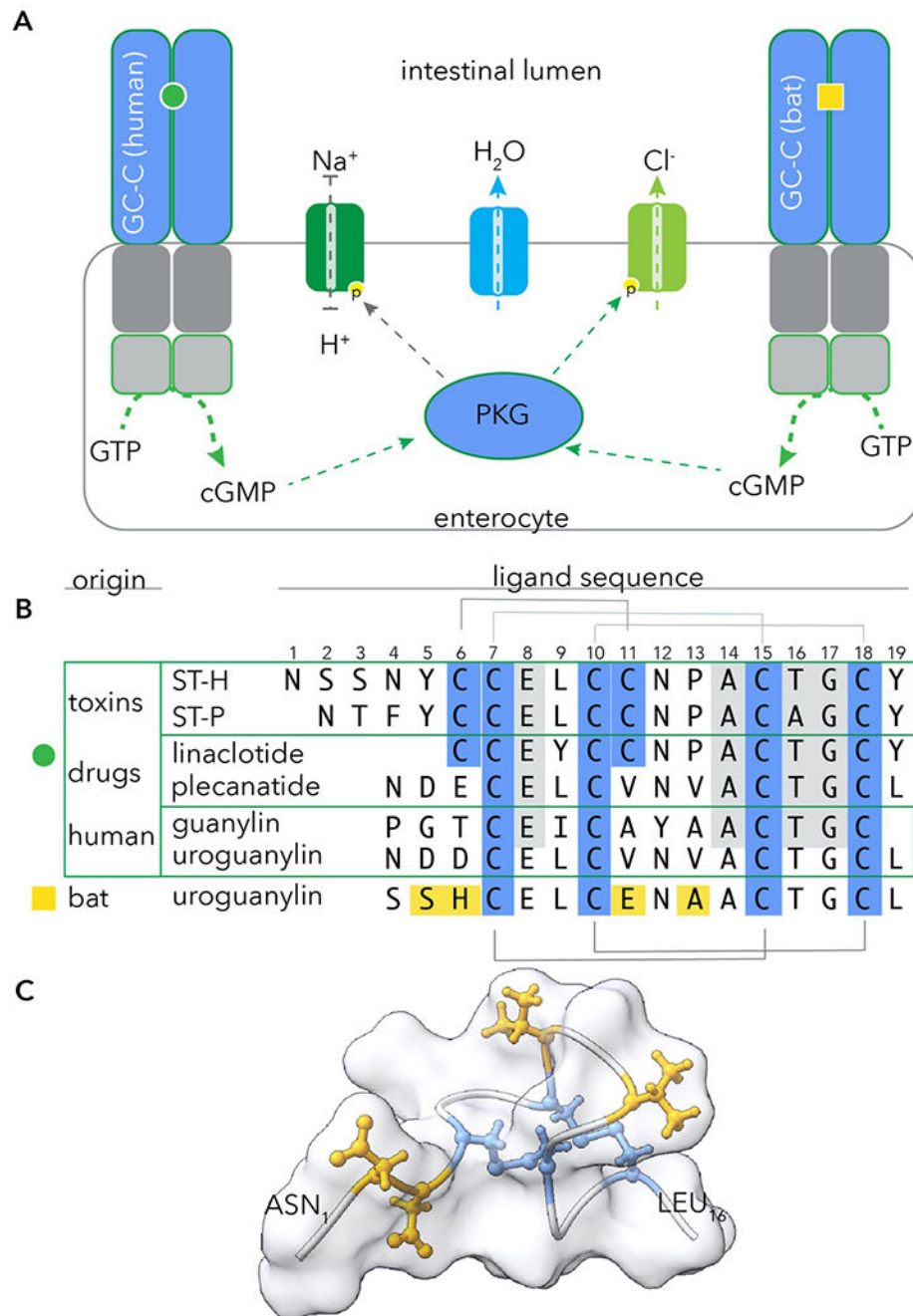


Figure 1. signaling through guanylate cyclase C (GC-C) maintains intestinal fluid homeostasis. A. (left) canonical GC-C-cGMP-PKG signaling pathway in which endogenous peptide ligands guanylin and uroguanylin, or heat stable toxins bind the GC-C receptor domain to signal conversion of GTP to cGMP via the intracellular catalytic domain. Upon activation by cGMP, PKG phosphorylates cellular targets including the NHE3 sodium/hydrogen ion exchanger and (CFTR), driving osmotic release of fluid into the intestinal lumen. B. Amino acid alignment of peptides including ETEC heat-stable toxins ST-H and ST-P, peptide drugs

linaclotide and plecanatide, and human guanylin and uroguanylin. Cysteines are shown in blue with inter-cysteine disulfide bridges in toxins at top and in endogenous peptides at bottom. Sequence of bat uroguanylin from *M. lucifugus*, which preferentially activates GC-C (figure A right) from that species is shown at bottom. Amino acids in yellow correspond to mutations relative to consensus peptide. C. predicted structure of human uroguanylin (PDB 1UYA, (Marx et al., 1998)). Cysteines are depicted in blue; amino acids in yellow correspond to those altered in *M. lucifugus*.

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