The intestinal consequences of cholera enterotoxin are caused by activation of the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel through the actions of an as-yet-unknown adenylate cyclase. A new study hunts down this elusive enzyme, showing that mouse and human intestinal epithelium functionally and structurally pair adenylate cyclase isoform 6 (AC6) with CFTR. These findings provide important insights into the molecular mechanisms underlying the robust pathological activation of CFTR activity and promise new opportunities to treat cholera.

Cholera is an acute intestinal infection that affects both children and adults and is caused by ingestion of food or water contaminated with the bacterium *Vibrio cholerae*. Infection leads to large volume losses (up to 500–1000 ml/h in adults) of rice-watery stool, which can further lead to rapid dehydration, shock, and death. Untreated, the lethality of this pandemic disease may be ∼50% (1). Even though treatments are available, the World Health Organization estimates that 3–5 million cholera cases still lead to 100,000 to 120,000 deaths every year (http://www.emro.who.int/health-topics/cholera-outbreak/index.html, accessed August 2, 2018).

The mechanism by which *Vibrio cholerae* causes these physiological outcomes is well understood, with the exception of one important detail. Infection starts when *Vibrio cholerae* secretes cholera enterotoxin (CTX), a hexameric protein consisting of an enzymatic A subunit and five B subunits that bind to the surface of intestinal cells to mediate endocytosis. The toxin reaches the lumen of the endoplasmic reticulum via retrograde trafficking, where the enzymatic active domain of the A subunit (CTA1) is released and translocated into the cytosol. There, its enzymatic activity is allosterically activated by a host ADP-ribosylation factor and causes ADP-ribosylation of the $G_{\alpha_c}$ subunit of heterotrimeric G proteins.

The ADP-ribosylated $G_{\alpha_c}$ cannot hydrolyze GTP to GDP and thus remains in an activated state, which binds and stimulates membrane-bound adenylate cyclases (ACs) to catalyze the conversion of ATP to cAMP and pyrophosphate, so this stimulation of AC catalytic activity dramatically increases CAMP concentrations in the cell. This, in turn, activates protein kinase A, which phosphorylates and thereby activates the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel, increasing Cl$^-$, HCO$_3^-$, and fluid secretion into the intestinal lumen. So, what is the missing piece? At this time, nine types of mammalian membrane-bound ACs are known. Which AC regulates CFTR in the intestine and is responsible for the CTX-induced diarrhea?

Thomas et al. (9) approached this question by first determining the relative expression of ACs in mouse and human intestinal epithelial cells using mRNA sequencing. They found AC6 to be the predominant AC isoform in both species. To study the cellular location of AC6 and its role in CFTR regulation, they generated an epithelium-specific AC6 knockout (Acety6Δ/Δ mice) as a control. Like CFTR, AC6 was predominantly found in the apical compartment in the mouse ileum and absent in Acety6Δ/Δ mice. The authors next considered the possibility that CFTR and AC6 might interact with each other. Using co-immunoprecipitation and proximity ligation assays, they detected a CFTR–AC6 complex at the apical membrane of mouse and human intestinal epithelial cells, but not in the epithelium of Acety6Δ/Δ mice. They further corroborated their results by performing co-immunoprecipitation experiments with recombinant AC6 fragments. These experiments indicated that amino acids 917–1165 within the cytosolic domain of AC6 are required for the interaction with CFTR.

Given these results, Thomas et al. (9) then tested the hypothesis that AC6 is the major physiological driver of CFTR function in the intestinal epithelium. First, they found that both the AC agonist forskolin and CTX induced spectacular increases in CAMP levels in mouse intestinal crypt cells that expressed AC6, but that these responses were dramatically reduced in AC6 knockout intestinal epithelial cells. Next, they investigated fluid secretion into the mouse intestine upon CTX challenge in the presence and absence of AC6. Although CTX induced significant intestinal fluid secretion in the presence of AC6, it failed to do so in the AC6 knockout. CAMP-independent CFTR activation, for example by raising cGMP levels in the intestinal cells, was not affected by the loss of AC6. These results indicate that AC6 is required for CTX-induced diarrhea. To show that this conclusion is equally valid for mice and humans, the authors compared fluid secretion of murine and human intestinal...
organoids/enterospheres. Enterospheres from both species showed robust fluid secretion in the presence of forskolin or CTX. However, enterospheres from AdecyN/A mice and human intestinal organoids transduced twice with AC6 lentiviral shRNA to knock down AC6 expression showed negligible fluid secretion in response to forskolin and CTX.

The work of Thomas et al. (9) highlights the compartmentalization of both cAMP signaling and CFTR activity regulation. The close proximity of CFTR to the catalytic center of AC6, mediated via a direct interaction, may explain why CTX induces such large CFTR-dependent intestinal fluid losses. The findings further support the concept that protein kinase A is anchored within the vicinity of the CFTR–AC6 complex to be able to translate the compartmentalized rise in cAMP into CFTR activation (10). In addition, disruption of the interaction of CFTR with AC6 by disease-associated mutations could contribute to the loss of CFTR function in cystic fibrosis, a possibility that will need to be investigated further.

The study of Thomas et al. (9) also introduces AC6 as a potential target for the development of new approaches to treat cholera that should be explored further. Current treatment is oral and intravenous rehydration therapy (1). However, large volume fluid replacement is not always possible. It has limitations during large outbreaks or in disaster areas where cholera can occur. Based on the authors’ data, inhibition of AC6 through a small-molecule drug could effectively reduce CTX-induced diarrhea. AC6 inhibition would counteract CTX-induced elevations of intracellular cAMP levels and mitigate additional pathogenic or cytotoxic effects caused by the activation of other signaling pathways via this important cellular second messenger. However, it might be challenging to develop highly selective AC6 inhibitors that do not affect the AC isoforms with critical physiological roles in other cells and tissues.

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Figure 1. CTX-induced opening of CFTR channels in intestinal epithelia cells via AC6. The CTA1 domain of the CTX A subunit translocates into the cytosol. Its enzymatic activity is allosterically augmented by a host ADP-ribosylation factor (ARF) (4) and results in ADP-ribosylation of Gαs. Gαs is a component of a heterotrimeric G protein (with β and γ subunits) that is associated with a heptahelical G protein–coupled receptor (dark blue line). When Gαs is activated, it binds GTP and dissociates from the complex, binding to and activating the CTX-associated adenylate cyclase 6 (AC6). Because ADP-ribosylated Gαs remains in the activated state, this process results in a significant raise in cAMP, activation of protein kinase A (PKA), and subsequent “unlocking” of CFTR channel activity as described in the main text. Based on studies conducted with mainly nonintestinal cells (reviewed in Ref. 10), PKA is likely anchored to the immediate vicinity of the apical membrane and the CFTR–AC6 complex though A-kinase–anchoring proteins (AKAPs) (not shown in this figure). However, the detailed complexes in intestinal cells have yet to be defined.