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Commentary

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Pass the bicarb: the importance of HCO_3^- for mucin release

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Accumulation of thick, sticky mucus is a hallmark of the genetic disease cystic fibrosis (CF) and has a central role in CF pathophysiology. Mutations in the CF transmembrane regulator (CFTR) ion channel are known to result in abnormally thick and sticky mucus; however, why mucus accumulates in CF is still not completely understood. In this issue of the *JCI*, Garcia and colleagues show that mucin — the heavily glycosylated protein contained within mucus — requires CFTR and bicarbonate in order to be released from mouse intestine (see the related article beginning on page 2613). The authors propose a model whereby CFTR-mediated bicarbonate secretion must be concurrent with mucin exocytosis for proper mucin release.

Despite the fact that the gene responsible for cystic fibrosis (CF), CF transmembrane regulator (CFTR), was cloned in 1989 (1), the molecular pathophysiology of the disease is still controversial. Currently, two major areas of investigation are being pursued by researchers worldwide. One focuses on the extracellular effects of altered electrolyte transport in CF. One consequence ascribed to CFTR dysfunction is that excessive amounts of thick, sticky mucus accumulate in affected organs. This can obstruct the organ lumen and create a permissive environment for abnormal microbial colonization, which in turn leads to chronic inflammation and tissue damage. Why CF mucus is so different from normal mucus is not well understood. The other focus in CF research turns inward to the cell interior. Investigations in this area have identified numerous cellular proteins that interact with, and whose activities are affected by, CFTR. These intracellular activities of CFTR are often referred to as being cell autonomous, in contrast with extracellular effects. In many cases, the importance of these cell-autonomous effects of CFTR to disease pathology is not clear.

The basic defect in CF

CF is an autosomal recessive genetic disease that is caused by mutations in the CFTR gene. The best-established activity of CFTR is as a cAMP-regulated chloride

channel that is localized at the luminal plasma membrane of various epithelia. CFTR can also transport bicarbonate (HCO_3^-), especially under stimulated conditions (2). To complicate things, CFTR interacts with numerous cellular proteins, including other electrolyte transporters. Therefore, dysfunction of CFTR affects a variety of cellular functions including ion transport. The gastrointestinal system, especially the exocrine pancreas and intestinal tract, are strongly affected in CF. Most CF patients suffer from pancreatic insufficiency (inability of the exocrine pancreas to produce sufficient digestive enzymes to facilitate intestinal food breakdown and absorption) due to plugging of the pancreatic ducts and tissue destruction, and obstruction of the distal small intestine with mucofeculent material is not uncommon (3). However, the major life-shortening problem in CF is airway infection and eventual failure of respiratory function. Mucus also accumulates in CF airways and, similarly to its detrimental effects in the gut, this likely plays a central role in the pathogenesis of CF airway disease. Unfortunately, none of the various genetically engineered *Cftr* mutant mouse models of CF mimic the severity of spontaneous human airway pathologies (4). Although the gut and lung are very different, the relationship between CFTR dysfunction and mucus production in the gut is expected to be instructive for airway pathophysiology. It is useful then that, similar to the situation in the human CF gut, exuberant mucus accumulation in the intestine is a feature of most *Cftr* mutant

mice (Figure 1). Prior work in *Cftr*-knock-out mice has shown a strong association among mucus accumulation, gut hydration, bacterial load, and gut inflammation (5). However, even with maximal use of osmotic laxatives to improve gut hydration, mucus accumulation and other aspects of CF gut pathology are still not fully corrected.

Focusing on the extracellular effects of CF, in this issue of the *JCI*, Garcia and colleagues report on their investigation of the roles of HCO_3^- and CFTR in stimulated release of mucin from the mouse small intestine (6). Secreted mucin in the gut is largely released by goblet cells, which are interspersed among enterocytes in the lining epithelium (Figure 1), and goblet cells are also similarly situated in the airway epithelium. HCO_3^- secretion at the luminal surface of epithelia involves CFTR by at least two mechanisms (Figure 2). CFTR indirectly supports HCO_3^- transport by providing luminal chloride, which is then exchanged for HCO_3^- (by various anion exchangers) across the luminal plasma membrane (2). CFTR can also directly act as an HCO_3^- -conducting channel (7). HCO_3^- is utilized locally by epithelia to regulate their surface pH and that of luminal contents. The importance of HCO_3^- in gastrointestinal function is well established — it is required for neutralization of gastric acid as the stomach contents enter the small intestine for digestion. In the gut, HCO_3^- secretion is intimately associated with mucin release, as both processes are stimulated by many of the same signaling molecules (e.g., PGE_2).

Mucin release requires CFTR and HCO_3^-

In their study in this issue of the *JCI*, Garcia et al. (6) describe the development of a new system for studying mucin release. Mucin release is operationally measured as glycoprotein levels present in the solution that is perfused through a segment of mouse small intestine ex vivo. The PAS base reaction is used to measure glycoproteins (mainly mucins) that undergo exo-

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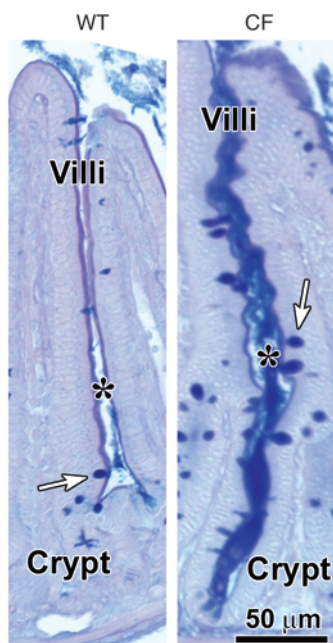


Figure 1

Mucus accumulates in the *Cftr*-knockout mouse (a model of CF) small intestine. Intestinal tissue was fixed in Carnoy's solution, and mucus was stained with PAS base plus Alcian blue, pH 2.5, as described in ref. 5. In contrast with what occurs in wild-type animals, mucus released from goblet cells (arrows) in CF mice accumulates in the crypt lumen and along the villous surfaces (*).

The current study (6) shows that the presence of HCO_3^- in the medium is crucial for efficient stimulated release of mucin. When HCO_3^- was omitted from the serosal bathing medium (Figure 2), stimulated mucin release was reduced by about half. Furthermore, pharmacological inhibition of CFTR totally abolished stimulated mucin release. The authors proposed a model (see Figure 11 in ref. 6) whereby luminal HCO_3^- secretion is CFTR-dependent and HCO_3^- rapidly displaces bound Ca^{2+} and H^+ from mucins upon exocytosis, thereby allowing their proper expansion and disaggregation to form normal mucus (Figure 2).

Remaining questions

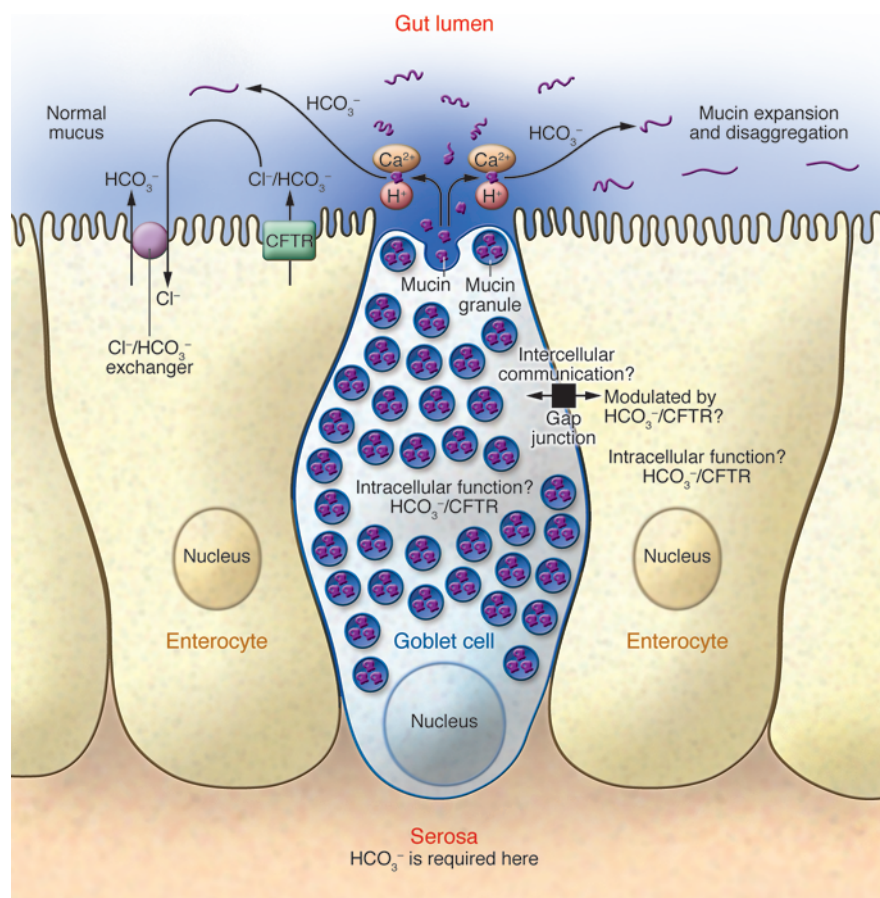
The model (6) proposes that release of mucin from goblet cells is assisted by HCO_3^- secreted from neighboring entero-

cytes, because goblet cells are not known to express CFTR whereas enterocytes do. A major question that arises from this work is this: where does HCO_3^- act in the release of mucin? HCO_3^- must be supplied to the serosal side of the tissue, and active CFTR is also required. These results suggest that HCO_3^- is transported across the epithelium and secreted into the intestinal lumen by CFTR (Figure 2). Surprisingly, direct addition of HCO_3^- to the solution perfusing the luminal surface does not support efficient mucin release. This appears to contradict the authors' proposed model – why should it matter how the HCO_3^- reaches the luminal surface in order to expand mucins? A possibility is that the local concentration of HCO_3^- at the surface of the epithelium may be much higher than the concentration tested experimentally (25 mM). The

cytosis and become sufficiently soluble to be released from the epithelial surface into the perfusate. The pioneering work of Verdugo and colleagues over 20 years ago showed that mucins rapidly expand upon exocytosis, as the shielding Ca^{2+} ions are displaced when mucins are exposed to the extracellular environment (8).

Figure 2

Schematic model summarizing potential sites of action of CFTR and HCO_3^- in mucin release. HCO_3^- is taken up from the serosal space and transported into the gut lumen in a CFTR-dependent manner by the enterocyte. CFTR acts as an HCO_3^- channel to directly transport HCO_3^- and also acts indirectly by supplying Cl^- to the lumen, which is then exchanged for HCO_3^- (arrow) by $\text{Cl}^-/\text{HCO}_3^-$ exchangers. The study in this issue by Garcia et al. (6) reports that HCO_3^- and CFTR are required for mucin secretion. The authors propose that, upon mucin secretion from goblet cells into the lumen, the mucins (previously condensed into mucin granules) rapidly disassociate from Ca^{2+} and H^+ ions, which allows mucin expansion and disaggregation and the formation of a normal mucus layer. However, Garcia et al. also report that direct addition of HCO_3^- to the gut lumen is insufficient to support mucin release. This suggests the possibility that HCO_3^- does not act at the luminal surface in mucin release. Are there intracellular functions of HCO_3^- and CFTR, such as fostering intercellular communication between the enterocyte and the goblet cell (e.g., intracellular pH regulation and gap junction communication), that potentiate mucin granule exocytosis?





most HCO_3^- -rich fluid produced in the body is pancreatic juice, in which HCO_3^- concentration can reach 150 mM. Thus, it is conceivable that a high concentration of HCO_3^- can be generated locally by enterocytes where it is accessible to the interior of mucin granules as they fuse with the goblet cell luminal membrane.

Another possibility that needs to be considered is that HCO_3^- and CFTR act intracellularly, either in the goblet cell or in the enterocyte (Figure 2). Mucin released from CF mouse gallbladder cells expands less rapidly than that released from wild-type cells (9). In the same prior study, it was suggested that CFTR was intracellular, possibly in mucin granules. In other work, it was shown that fluid and exocytic mucin release from an intestinal goblet-like cell line are coupled by the presence of anion channels in the mucin granule membrane (10). However, it was concluded that the anion channel involved in this goblet-like cell was not CFTR. So if CFTR and HCO_3^- act intracellularly in mucin release, it is unlikely that CFTR directly mediates transport of HCO_3^- through the mucin granule membrane.

A speculative possibility is that there is intercellular communication between enterocytes and the adjacent goblet cells that involves CFTR and HCO_3^- (Figure 2). It is known that communication through gap junctions potentiates stimulated exocytic protein release (11), which could operate in enterocytes and goblet cells in the intestinal epithelium. Also, CFTR and HCO_3^- can modulate cytosolic pH (12), and intracellular pH affects gap junction communications (13). Furthermore, CFTR has been shown to interact at the molecular level with gap junctions, and it modulates their ability to couple neighboring cells (14). Hence, the notion should be entertained

that CFTR and HCO_3^- enhance mucin release by acting in a cell-autonomous manner, perhaps involving gap junction-mediated intercellular communication from enterocytes to goblet cells that potentiates mucin granule exocytosis rather than by simply affecting luminal HCO_3^- secretion.

Conclusion

Paul Quinton hypothesized that HCO_3^- is a crucial player in CF, and he advocated revival of the term *mucoviscidosis* to describe this disease that so strongly involves mucus (15). In the current study (6), he and his colleagues have provided a test of this hypothesis and have shown that HCO_3^- and CFTR are needed for efficient mucin release. It remains to be determined by what mechanism and at what cellular/subcellular site HCO_3^- and CFTR act. While many believe that the majority of CF pathophysiology can be explained by perturbed electrolyte transport and its extracellular consequences on mucus and other exocrine secretions, it is good to keep an open mind. Perhaps these novel results from Garcia, Quinton, and colleagues have given us a glimpse of a cell autonomous activity of CFTR that is crucial for efficient mucin release and normal mucus formation.

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