New ways of thinking about (and teaching about) intestinal epithelial function

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Barrett KE. New ways of thinking about (and teaching about) intestinal epithelial function. Adv Physiol Educ 32: 25–34, 2008; doi:10.1152/advan.00092.2007.—This article summarizes a presentation made at the Teaching Refresher Course of the American Physiological Society, which was held at the Experimental Biology meeting in 2007. The intestinal epithelium has important ion transport and barrier functions that are pivotal to normal physiological functioning of the intestine and other body systems. These functions are also frequently the target of dysfunction that, in turn, results in specific digestive disease states, such as diarrheal illnesses. Three emerging concepts are discussed with respect to ion transport: the complex interplay of intracellular signals that both activate and inhibit chloride secretion; the role of multiprotein complexes in the regulation of ion transport, taking sodium/hydrogen exchange as an example; and acute and chronic regulation of colonic sodium absorption, involving both sodium channel internalization and de novo synthesis of new channels. Similarly, recently obtained information about the molecular components of epithelial tight junctions and the ways in which tight junctions are regulated both in health and disease are discussed to exemplify ways to teach about intestinal barrier properties. Finally, both genetically determined intestinal diseases and those arising as a result of infections and/or inflammation are described, and these can be used as the means to enhance the basic and clinical relevance of teaching about intestinal epithelial physiology as well as the impact that the understanding of such physiology has had on associated therapeutics. The article also indicates, where relevant, how different approaches may be used effectively to teach related concepts to graduate versus medical/professional student audiences.

THE INTESTINAL EPITHELIUM represents a vast interface between the body and external environment. At this strategic location, it subserves a physiological imperative to permit the uptake of nutrients while restricting the uptake of undesirable substances into the body, such as toxins, as well as limiting the incursion of microorganisms. As such, both the transport and barrier properties of the epithelium are closely regulated (2). The challenge of preventing microbial uptake is also underscored by the fact that shortly after birth, the intestine becomes colonized with a complex microbiota, and the epithelium thereby establishes a lifelong, largely symbiotic relationship with these so-called commensal bacteria. We are beginning to realize that this relationship profoundly affects epithelial function and also limits the ability of pathogenic microorganisms to gain a foothold in the intestinal lumen or to invade the body in healthy individuals (17).

Two additional characteristics of the epithelium also merit note. First, the individual epithelial cells are turning over continually. Arising from stem cells anchored near the base of the crypts, daughter epithelial cells migrate out onto the villi of the small intestine or surface of the colon, with an accompanying alteration in their transport and barrier characteristics. Indeed, the intestinal epithelium is one of the most rapidly dividing tissues of the adult body, with cells eventually detaching from the basement membrane to be lost to the lumen (26). Second, the epithelium is pivotally involved in determining the fluidity of the luminal contents, which is required for proper digestion and absorption of ingested foodstuffs to take place. This function depends on absorption of nutrients but also on the active secretion and absorption of electrolytes. Disease states can arise if there is too much or too little fluid in the lumen (e.g., diarrhea and intestinal obstruction, respectively), implying that physiological control of the mechanisms that govern luminal fluidity are critical for homeostasis and wellbeing (2).

This article is based on a presentation delivered at the annual Teaching Refresher Course of the American Physiological Society, which focused on gastrointestinal (GI) topics in 2007. It was my goal to highlight emerging insights into two facets of intestinal epithelial biology that have seen rapid research advances in recent years and to provide guidance as to how such information could be incorporated in the physiological education of medical or graduate students. Accordingly, this article summarizes my remarks on mechanisms that account for the regulation of intestinal transport mechanisms to subserve physiological needs and also insights into the dynamic regulation of intestinal barrier properties. I also discuss how this information can be illuminated by discussion of specific digestive diseases.

Regulation of Epithelial Transport

General mechanisms of transporter regulation. A host of epithelial transport mechanisms are expressed in the GI tract (2). Quantitatively, fluid secretion is driven primarily by the active transepithelial secretion of chloride ions, whereas fluid absorption is provided for predominantly by the uptake of sodium in conjunction with digested nutrients when the latter are present during the postprandial period or by the coupled absorption of sodium and chloride during fasting. The distal colon also performs electrogenic sodium absorption, thought to be an important salvage mechanism that finally dehydrates the stool before the waste products of digestion are eliminated from the body. Each of these transport mechanisms, as well as others that contribute in a more minor way to intestinal fluid homeostasis, is acutely regulated to subserve local needs in the postprandial period and beyond. Similarly, it is emerging that intestinal epithelial transport mechanisms can be regulated more chronically to provide for long-term adaptation to environmental changes, such as the amount of sodium supplied in

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the diet. Finally, transport functions may be selectively modified in the setting of disease. Indeed, while the intestine is not a major contributor to whole body water and electrolyte status in health, when intestinal transport is deranged, such as by a secretory diarrheal pathogen, rapid and even life-threatening changes in circulating electrolyte concentrations can ensue given the very large surface area of the intestinal epithelium. Accordingly, insights into the precise mechanisms that regulate the secretion or absorption of electrolytes should be conveyed to students because of their physiological as well as pathological implications.

Epithelial transport of electrolytes involves specific membrane transport proteins, with the majority of these now identified at the molecular level. Similarly, there has been an explosion of new insights into how such transport proteins are both acutely and chronically modulated. As shown in Fig. 1, four main mechanisms of transporter regulation are now recognized (2). In the first of these, the transporter is posttranslationally (and usually reversibly) modified by the addition of a substituent that brings about a conformational change in the protein, altering its activity. Classically, as shown in Fig. 1, such regulation is brought about by covalent phosphorylation of the transporter itself, although other substituents likely can modify transport function in similar ways. However, acute regulation of transporters secondary to covalent binding of substituents other than phosphate is only beginning to be explored. Similarly, noncovalent interactions, such as with cytosolic calcium or other ions, are well known to increase the activity of specific transport proteins. Finally, as I discuss below, it is increasingly recognized that it may not be the transport protein itself that is modified to alter its function in response to changes in physiological conditions but rather another so-called “scaffolding” protein with which the transporter associates. Scaffolding may also allow for the assembly of a localized signaling complex that orients transporters and their modifying kinases, for example, in a way that maximizes the efficiency and specificity of transporter activation.

The second mechanism for the regulation of epithelial transporters, which can underlie both acute and more chronic modulation of activity, is brought about by the regulated insertion or retrieval of transporters from the plasma membrane, thereby influencing their overall abundance at the site needed for functional activity. We now recognize that a significant proportion of several transport proteins are kept in reserve in vesicles located close to the epithelial cell membrane, from where they can be recruited by a regulated process of vesicle fusion when increased transport activity is needed. Conversely, transporter levels can be reduced when such molecules are removed from the plasma membrane by endocytosis. In this latter scenario, the transporters may then be held in a recycling pool from where they can eventually return to the membrane, or may be targeted for degradation. This latter outcome implies that transporter abundance will thereby be reduced until new transporters are synthesized, providing for a more prolonged downregulation of transport capacity. Indeed, as implied in the third mechanism of transporter regulation shown in Fig. 1, the protein may associate with accessory proteins capable of modifying the transporter with substituents such as ubiquitin, which targets transporters for internalization and destruction in the proteosome.

The final mechanism for transporter regulation, which is predominantly called into play to support chronic, adaptive changes in transport function, relates to changes in the transcription and/or translation of specific transporter genes. For example, on a low-sodium diet, increased circulating levels of aldosterone result in upregulated expression of the three subunits that comprise the epithelial sodium channel (ENaC) not only in renal epithelial cells but also in the epithelial cells that line the colon, thus promoting the conservation of sodium from the intestinal lumen (25).

Examples of transport regulatory mechanisms. As alluded to above, intestinal epithelial transport function must be finely regulated on both minute-to-minute and more chronic time scales to provide for physiological needs. For example, reflexes may trigger short bursts of epithelial chloride secretion as the mucosa is deformed by the passage of a food bolus, thus providing for lubrication (27). Thereafter, secretion must be arrested to avoid dehydration. Similarly, colonic contents must be dehydrated in a controlled yet progressive fashion, via specific absorptive mechanisms, to permit their passage along the length of the large intestine without the loss of excessive amounts of body water. It is not surprising, therefore, that complex and integrative intracellular regulatory mechanisms have developed to provide for the exquisite regulation of intestinal epithelial transport in both positive and negative ways, which are triggered by cellular responses to the physical and chemical nature of the contents of the intestinal lumen. Three examples of regulatory mechanisms are therefore provided here to underscore our increasing understanding of such processes.

REGULATION OF CHLORIDE SECRETION. My laboratory and those of others have elucidated a variety of signaling pathways by which intestinal chloride secretion is either activated or inhibited. In general, three main triggers of chloride secretion have been identified, consisting of increases in intracellular...
cAMP, cGMP, or calcium. The cyclic nucleotide-dependent responses are characterized by their large magnitude and relatively sustained kinetics, with the response likely terminated in vivo by degradation or reuptake of the inciting agonist, downregulation of cognate receptors, or some combination of these. Calcium-dependent chloride secretion, on the other hand, is of relatively small magnitude and is transient, even in the face of continued exposure to the relevant agonist or, indeed, in the face of sustained increases in intracellular calcium. It has been speculated that calcium-dependent chloride secretion plays a key role in fine tuning the extent of epithelial secretion as physiological circumstances dictate or in providing for short bursts of reflex secretion (most commonly produced by the release of acetylcholine from enteric nerves) as needed. Thus, an understanding of the precise mechanisms that terminate calcium-dependent chloride secretion is likely of importance to understanding normal physiology as well as a variety of disease states (5).

Chloride secretion can thus be activated by stimulating a variety of receptors coupled to Gq, such as the muscarinic receptor that can be activated by carbachol (CCh). The stimulatory phase of the response is mediated by the resulting increase in cytosolic calcium, which is believed to activate both basolateral potassium channels and a class of apical calcium-activated chloride (CLCA) channels. Activity of these channels, in concert with the chloride uptake activity of a basolateral Na-K-2Cl cotransporter (NKCC1) and with energy provided by basolateral Na-K-ATPase, allows for the flow of chloride from the bloodstream to the intestinal lumen. Water and sodium are then believed to follow passively via the paracellular route. However, as shown in Fig. 2, almost as soon as the secretory response is initiated, the increase in cytosolic calcium also recruits an additional downstream signaling cascade that eventually turns off the secretory response. First, calcium activates calmodulin-dependent protein kinase (CaMK), which, in turn, can sequentially stimulate the soluble tyrosine kinases Pyk-2 and Src. These effectors are then implicated in triggering the activity of a membrane-bound matrix metalloproteinase capable of releasing the mature form of transforming growth factor (TGF)-α from its transmembrane precursor, thereby freeing TGF-α to bind to and activate its receptor, the EGF receptor (EGFR). Src also can phosphorylate the EGFR directly. In either case, the EGFR, in turn, recruits downstream effectors, including the ERK isoforms of MAPKs, to ultimately inhibit apical chloride secretion via actions (likely indirect) on apical CLCA channels (6, 18, 21).

Interestingly, an alternate ligand for EGFR, EGF itself, is also capable of inhibiting intestinal epithelial chloride secretion, although unlike CCh it does not act initially as a stimulus for this transport process. Moreover, while the inhibitory effects of both EGF and CCh on chloride secretion both intersect at the level of EGFR activation, the downstream effectors and targets that are responsible for the inhibitory effects are distinct. When the EGFR is activated by EGF itself, it recruits another EGFR family member, ErbB2, to an EGFR/ErbB2 heterodimer, thereby redirecting signaling to the recruitment of phosphatidylinositol 3-kinase (PI3K), activation of the ε-isoform of PKC, and ultimately leading to the inhibition of a basolateral potassium channel (6, 8, 33, 34). The differential effects of EGF versus CCh on EGFR-associated signaling in intestinal epithelial cells are likely attributable to the fact that each ligand promotes the phosphorylation of a specific subset of the available tyrosine residues on the cytoplasmic tail of the EGFR, thereby recruiting distinct adaptor proteins and associated effectors (23). Ultimately, however, both signaling pathways lead to an inhibition/termination of calcium-dependent chloride secretion, albeit by interrupting ion flow through different channels.

Of course, the level of detail I have described above is almost certainly in excess of that which is relevant for medical or other professional students, although even for such audiences I believe it is important to stress that chloride secretion can both be stimulated or inhibited as needed during the course of responding to the ingestion of a meal. On the other hand, the signaling paradigm described exemplifies several principles likely of interest to graduate students in physiology and other biomedical disciplines, including the concept that a single agonist can both stimulate and then inhibit a given cellular function, the idea that information may traverse the cell membrane several times before signaling outcomes are interpreted, and the principle that a single receptor may transmit distinct signaling information depending on the mechanism by which it is activated, and the specifics of the molecular changes that thereby ensue.

**Fig. 2.** Signaling pathways involved in the positive and negative regulation of calcium-dependent chloride secretion by intestinal epithelial cells. The muscarinic agonist carbachol (CCh) binds to an M3 receptor (M3R) on the basolateral membrane, leading to an increase in cytoplasmic calcium that initially stimulates chloride secretion via the activation of apical calcium-activated chloride (CLCA) channels (as well as basolateral calcium-activated potassium channels; not shown). However, subsequently, there is sequential recruitment/activation of calmodulin-dependent protein kinase (CaMK), soluble tyrosine kinases Pyk2 and Src, release of transforming growth factor (TGF)-α, and phosphorylation of the receptor for EGF (EGFR). In turn, this recruits downstream effectors that ultimately can inhibit the activity of apical CLCA channels. EGF itself can also inhibit chloride secretion, but via an alternate pathway that involves recruitment/activation of the orphan receptor tyrosine kinase ErbB2, phosphatidylinositol 3-kinase (PI3K), and the ε-isoform of PKC, which produce inhibition of a basolateral potassium channel whose function is normally required to sustain the driving force for chloride that exists across the opposite membrane. In either case, an inhibition of transepithelial chloride secretion ensues. [Adapted from Ref. 4 and used with permission.]
specificity with which they are activated. Figure 3 summarizes
a large body of work by Donowitz and coworkers (10) that
elegantly illustrates this concept.

The predominant sodium/hydrogen exchanger (NHE) ex-
pressed in the intestine and involved in electroneutral NaCl
(and thus fluid) absorption between meals is the NHE3 isoform
(40). Previous studies have revealed that agonists known to be
capable of stimulating or inhibiting NHE3 activity in intact
intestinal tissues did not necessarily do so when the transporter
was expressed exogenously in model cell lines. We now know
that this failure likely relates to the absence of accessory
proteins that are absent in such models but yet interact with
NHE3 in the native intestine. One such protein, designated as
NHE regulatory factor-2 (NHERF2), is shown in Fig. 3 and
serves as a scaffold to direct the interaction of NHE3 with other
signaling moieties. In the specific example shown, NHE3,
localized to the brush-border membrane of villous epithelial
cells, is found in association with cGMP-dependent protein
kinase II (cGKII), which is itself localized to the plasma
membrane by virtue of the fact that it is myristoylated (myris-
toylation is a posttranslational modification that adds myristic
acid to a protein, thereby increasing its ability to interact with
hydrophobic domains, such as the plasma membrane). How-
ever, the association of these two proteins is more specifically
promoted by NHERF2, which directly associates with not only
NHE3 and cGKII but also, via its ability to interact with a protein
known as ezrin, with the actin cytoskeleton, providing
further structural stability and localization for the complex.
Ezrin is a member of the so-called ezrin-radixin-moesin family
of proteins, which contributes to the overall integrity of all
cells.

The sum of these interactions produces a signaling complex,
scaffolded by NHERF2, that is primed to respond to extracel-
lar signals. In this specific example, the signal is supplied by
either endogenous (guanylin) or exogenous [STa, the heat-
stable toxin of enterotoxigenic Esherichia coli (ETEC)] ligands
of apically localized guanylyl cyclase C. When these ligands
are encountered, therefore, they produce high concentrations of
cGMP, which readily can activate cGKII. The latter enzyme is
likewise perfectly positioned to phosphorylate the COOH ter-
minus of NHE3, which ultimately reduces the activity of this
transporter and the ability to reclaim fluid from intestinal
contents in the period between meals. The pathophysiological
relevance of this process is found in the observation of diarrhea
in patients infected with ETEC, the major causative agent of
traveler’s diarrhea (13). STa also simultaneously stimulates
chloride secretion, further worsening the disease and likely
involving the assembly of similar protein complexes in af-
ected epithelial cells.

The concept to convey to (graduate) students is that cells are
not amorphous bags of randomly interacting chemicals but
rather that receptors, kinases, and the like, as well as the
substrates that they ultimately target to influence cell function,
are organized within specific subdomains of the cell, and,
without such organization, signaling is curtailed. Medical/
professional students will likewise be interested in learning that
STa is a microbial mimic of the endogenous ligand guanylin (in
fact, knowledge of the existence of the former led to discovery
of the latter) and is likely used by ETEC to highjack normal
epithelial cell signaling pathways, thereby disseminating the
infection to additional hosts secondary to the induction of
diarrhea. On the other hand, diarrheal disease may also repre-
sent a primitive adaptation/defense mechanism designed to
protect the host, as discussed in greater detail below.

REGULATION OF ELECTROGENIC SODIUM TRANSPORT. Sodium
transport mediated by ENaC occurs in the distal colon of most
mammalian species, likely as a final mechanism whereby fluid
can be salvaged from the lumen prior to loss of feces from the
body. The importance of this transport process in the renal
tubular epithelium has long been recognized, but its relevance to
intestinal physiology is also now emerging given evidence that
downregulation of colonic sodium absorption may contribute to
diarrheal symptoms in specific digestive disorders such as
the inflammatory bowel diseases (IBDs) of Crohn’s disease
and ulcerative colitis. Accordingly, there is increasing interest
in the signals that regulate electrogenic sodium absorption,
although as yet most of the available information has been
derived from renal, as opposed to colonic, experimental mod-
els. Nevertheless, the broad details of ENaC regulation, as
shown in Fig. 4, are likely to apply to the intestine as well as the
kidneys.

Like chloride secretion, electrogenic sodium absorption can
be stimulated by an increase in intracellular cAMP (29). The
cyclic nucleotide influences channel opening in two ways.
First, it has a stimulatory effect on the channel itself. Second,
it inhibits the activity of a ubiquitin ligase, Nedd 4-2, which
otherwise can target ENaC for internalization and degradation
via the ubiquitin/proteosomal pathway. Inhibition of Nedd 4-2
would therefore be expected to increase the membrane abun-
dance of ENaC and thus sodium uptake into colonic epithelial
cells. Sodium is thus accumulated in the cytosol and can be
exported across the basolateral membrane into the bloodstream
via the energy-dependent activity of the Na-K-ATPase pump.

Students may ask why it makes sense that the same intracel-
lar signal promotes both chloride secretion and sodium ab-
sorption in the distal colon, which on the face of things would
seem to be a futile cycle that promotes no net fluid movement.
In fact, the explanation for this conundrum lies in the fact that
chloride secretion is localized primarily to crypt epithelial cells

![Fig. 3. Model of cGMP inhibition of Na/H exchanger (NHE) isoform 3
(NHE3) in intestinal epithelial cells. NHE regulatory factor-2 (NHERF2) acts
as a G kinase-anchoring protein (GKAP), forming a complex between NHE3,
NHERF2, and cGMP-activated protein kinase II (cGKII). cGKII is also fixed
as a G kinase-anchoring protein (GKAP), forming a complex between NHE3,
NHERF2, and cGMP-activated protein kinase II (cGKII). cGKII is also fixed
as a G kinase-anchoring protein (GKAP), forming a complex between NHE3,
NHERF2, and cGMP-activated protein kinase II (cGKII).](http://advan.physiology.org/DownloadedFrom/10.220.32.246 on June 18, 2017)
in the colon, whereas sodium absorption is a property of the surface colonocytes. Thus, when the epithelium encounters an endogenous agonist that elevates cAMP, fluid can be secreted into the crypts to hydrate them and flush out luminal constituents, including potential toxins as well as host products such as antimicrobial peptides supplied by Paneth cells. On the other hand, once this flushing action has been fulfilled, the fluid can be reclaimed selectively across the surface epithelium to keep the crypts clean without losing excessive fluid to the stool. The physiological relevance of this process is underscored by evidence that diminished colonic chloride secretion has been associated with colonic barrier dysfunction (see below).

There may also be circumstances where it would be desirable to activate colonic chloride secretion without an accompanying increase in sodium absorption. Indeed, agonists that elevate intracellular calcium do just that, since ENaCs are inhibited by calcium, whereas, as described above, calcium is at least a weak agonist of chloride secretory responses. Sodium absorption can also be acutely downregulated homeostatically. Increased concentrations of intracellular sodium stimulate the activity of Nedd 4-2, which would otherwise cause ENaC internalization and degradation. Both calcium and sodium in the cytosol, on the other hand, cause an inhibition of ENaC activity. (Modified from a figure in Ref. 2 and used with permission.)

Emerging Aspects of Epithelial Barrier Function

Another key function of the intestinal epithelium is to serve as a selective barrier, permitting the uptake of nutrients and other desired solutes while excluding toxins, microorganisms, and the like. There has been a very significant acceleration of our understanding of epithelial tight junctions at a molecular level as well as the ways in which their permeability is regulated in health and disease (32, 35). Two concepts that have recently “permeated” my own teaching include the idea that specific molecular components of the tight junction may either seal or form paracellular pores as well as the idea that tight junctions are not static structures but may be dynamically regulated in both health and disease by a variety of mechanisms.

Molecular composition of tight junctions. As alluded to above, recent years have seen a substantial increase in the number of proteins that have been shown to contribute to the tight junctions that seal the apical poles of epithelial cells, including in the intestine. We now know that the space between cells is occupied by the interlocking extracellular loops of two types of transmembrane proteins: occludins and claudins. The claudin family was discovered when it was observed that a mouse deficient in occludin expression had no obvious defect in epithelial barrier function, at least in the intestine (30). In addition, the cytoplasmic domains of these proteins interact extensively with scaffolding and regulatory proteins, including zonula occludens (ZO)-1 and a host of kinases. Ultimately, the tight junction is linked to the cellular cytoskeleton and, particularly, to the actomyosin ring that encircles the cell at the level of the junction, in much the same way that a strip of elastic can cinch the waist of a dress.

Focusing specifically on the transmembrane claudins, the classical view is that as more of these molecules are inserted into the junction, the permeability of the epithelium to paracellular tracers will decrease as the junction is progressively occluded. However, it has become increasingly appreciated that this model is too simplistic (35). For example, the claudins that have now been cloned, many of which are apparently expressed in the intestine, have different properties such that upregulated expression of some, but not all, decreases epithelial resistance and/or reduces the transepithelial flux of larger tracer molecules (Table 1). Similarly, claudins may selectively retard the paracellular movement of some, but not other, ionic species, often depending on charge and/or size. The situation is complicated further by the fact that claudins on adjacent cells can interact in a heterotypic as well as homotypic fashion, further diversifying the properties of the resulting tight junctions. Finally, some claudins can increase, rather than decrease, permeability.

Table 1. Effects of specific intestinal claudins on epithelial barrier function

<table>
<thead>
<tr>
<th>Protein</th>
<th>Transepithelial Resistance</th>
<th>Sodium Permeability</th>
<th>Flux of Paracellular Tracers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Claudin 1</td>
<td>Increased</td>
<td>Not reported</td>
<td>Decreased</td>
</tr>
<tr>
<td>Claudin 2</td>
<td>Decreased</td>
<td>Increased</td>
<td>Not reported</td>
</tr>
<tr>
<td>Claudin 4</td>
<td>Increased</td>
<td>Decreased</td>
<td>No change</td>
</tr>
<tr>
<td>Claudin 5</td>
<td>Increased</td>
<td>Decreased</td>
<td>No change</td>
</tr>
<tr>
<td>Claudin 7</td>
<td>Increased</td>
<td>Increased</td>
<td>No change</td>
</tr>
<tr>
<td>Claudin 8</td>
<td>Increased</td>
<td>Decreased</td>
<td>No change</td>
</tr>
</tbody>
</table>

[Adapted from Ref. 35.]
the permeability of a given tight junction. This seemingly paradoxical observation has led Anderson and coworkers (36) to propose a model where adjacent claudin molecules can form not only seals but also pores with specific permeability characteristics (Fig. 5). Claudin-2, for example, increases the rate of permeation of sodium ions via the paracellular route, and other claudins may similarly form selective pores for other biologically relevant ionic species, such as magnesium.

**Acute regulation of tight junctional permeability.** In addition to the regulation of tight junctional permeability that is brought about gradually by changes in the relative expression levels of specific tight junctional components, we now recognize that the integrity of the intestinal epithelial barrier can be regulated more acutely secondary to intracellular signaling mechanisms. In some cases, such regulation relates to physiological processes. For example, there is evidence that the transcellular absorption of glucose may simultaneously increase the rate at which the sugar can be transferred through the paracellular pathway, increasing the efficiency of nutrient uptake (31). However, acute regulation of intestinal barrier function is more commonly invoked as a pathophysiological event, contributing to disease pathogenesis when the intestine is challenged by microbes or inflammatory insults.

One mechanism whereby the permeability of the intestinal barrier can be increased is via the internalization and/or degradation of specific junctional components, both the transmembrane constituents and those regulatory proteins that bind to these on their cytoplasmic face (32). Changes in the phosphorylation status of specific proteins such as occludin or ZO-1, brought about by kinases or phosphatases, may cause them to be relocated within the cell such that they can no longer function to maintain barrier integrity. Tight junction function can also be influenced by tension within the actomyosin ring. Simplistically, if the ring contracts, this literally can pull the junctions between adjacent cells apart if the forces generated exceed those produced by the affinity of claudins and/or occludin for their partners on neighboring cells. For example, binding of the inflammatory cytokine TNF-α to its receptor on the basolateral membrane of intestinal epithelial cells induces signals that increase both the expression and activation status of myosin light chain kinase (MLCK), an enzyme that phosphorylates myosin and leads to contraction of myosin-based filaments (Fig. 6). TNF-α levels increase in the mucosa when the intestine is inflamed, such as in the setting of IBD. Subepithelial immune effector cells can likewise be stimulated to secrete TNF-α in response to signals released by luminal or invading pathogens. The reduction in epithelial integrity brought about by MLCK activity allows additional permeation of pathogen effector molecules into the lamina propria, setting up a cycle whereby barrier integrity is further compromised until the threat can be resolved.

**Regulation of Epithelial Function in Specific Disease States**

Particularly for medical students, it is helpful to teach about epithelial function by describing how such function is deranged in specific disease states. Some of these are “experiments of nature” in which mutations in specific transport proteins show why such proteins are normally required for intestinal homeostasis. More common diseases, such as diarrheal illnesses, can likewise be used as excellent illustrations of epithelial physiology. Such examples may also provide insights into the mechanism of action of established and emerging therapies.

**Genetic disorders involving intestinal epithelial dysfunction. Cystic fibrosis.** Cystic fibrosis is a relatively common genetic disorder, particularly in Caucasian populations. While the pul-

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**Fig. 5. Speculative model of tight junction pores produced by claudins.** Continuous rows of claudin particles show homotypic adhesion, and charged residues in their extracellular domains exert electrostatic influences on paracellular ion movement. The data support a model in which claudins seal and provide selective pores in the paracellular pathway. The cytoplasmic tails of claudin molecules bind scaffolding proteins, including multi-PDZ domain protein-1 (MUPP1) and zonula occludens (ZO)-1, which in turn link them to the cytoskeleton. This may provide for the physiological regulation of tight junctions by changes in the cytoskeleton. [Reproduced with permission from Ref. 36.]
The GI complications of this disease are the most well known, as survival of patients with cystic fibrosis has increased into adulthood as a result of improved therapies, GI and liver diseases are increasingly seen. Many newborns with cystic fibrosis may also present with the condition of meconium ileus, where thickened, dehydrated intestinal secretions cause intestinal obstruction shortly after birth. Cystic fibrosis is attributable to any one of hundreds of mutations in the gene encoding for CFTR, which is a cyclic nucleotide-gated channel for chloride and likely bicarbonate (39).

The GI complications of cystic fibrosis reveal the role of CFTR, in addition to, perhaps, other chloride channels, in normal intestinal physiology (39). Thus, obstruction of the intestine itself, as well as biliary and pancreatic ducts, likely reflects an inability to appropriately hydrate the contents/secretions, secondary to a loss of chloride secretion that normally drives the movement of water into the lumen. Furthermore, duodenal, pancreatic, and biliary secretion of bicarbonate are also deficient in patients with cystic fibrosis. While many patients with severe cystic fibrosis experience destruction of their exocrine pancreas either before or shortly after birth, in those who retain some measure of pancreatic function, the accompanying decrease in duodenal pH compromises the activity of digestive enzymes and further compromises nutrition. CFTR can cooperate with apical anion exchangers in intestinal and ductular epithelial cells to drive bicarbonate secretion. There is also evidence to suggest that CFTR may itself function as a bicarbonate channel under specific electro-physiological conditions, although its affinity for bicarbonate is much less than that for chloride (24). In any event, the GI complications of cystic fibrosis in summary can be ascribed to defects in chloride, bicarbonate, and fluid secretion.

Some researchers have wondered why CFTR mutations are so prevalent in certain populations, when homozygosity (or compound heterozygosity) for such mutations results in such a devastating disease state that was almost uniformly fatal in childhood before the advent of modern therapies. Likely the most convincing model for this is the concept of “heterozygote advantage,” where the carriage of one mutant allele is favored by an advantage it confers against some other, more prevalent condition. Initially, it was hypothesized that possession of one mutant CFTR allele rendered individuals relatively resistant to toxigenic diarrheal illnesses such as cholera, but this was not borne out in animal models. Instead, it is now believed that wild-type CFTR is somehow involved in promoting the ability of Salmonella typhi to invade the body via the intestinal tract. Since typhoid fever was once relatively common in Europe, this may have provided the selection pressure for retention of mutant cystic fibrosis alleles (37). The concept of heterozygote advantage may additionally be useful to students as they study other genetic diseases.

Another interesting facet of cystic fibrosis biology is the presence of marked differences in the disease phenotype in some cohorts of patients, despite similar or identical genotypes (28). The loss of CFTR is uniformly deleterious for lung function in almost all patients. However, some have mild, if any, GI symptoms. It has been speculated that variable expression of other chloride channels or regulatory proteins may serve to modify disease severity and mitigate against the effect of CFTR absence on normal GI function.

**GLUCOSE-GALACTOSE MALABSORPTION.** Intestinal absorption of glucose involves its coupled transport with sodium, thereby linking glucose uptake to the favorable sodium gradient established by basolateral Na-K-ATPase in intestinal epithelial cells. The transporter responsible for glucose and sodium uptake is termed SGLT1. The discovery of rare infants who poorly tolerate glucose, galactose, or carbohydrates containing these sugars nicely illustrated the critical role of SGLT1, which was the first membrane transporter to be cloned using the technique of expression cloning (15). This method involves the injection of pools of cRNA molecules into *Xenopus* oocytes and then screening for the transport process of interest (in this case, sodium-dependent uptake of radiolabeled glucose). The cloning of SGLT1 is thus an excellent example to illustrate strategies used for cloning of an unknown protein to graduate students. Medical and other health professional students, on the other hand, are usually more interested in understanding the basis of glucose-galactose malabsorption, as described above. The disease, in fact, has been ascribed to a variety of missense and trafficking mutants of SGLT1, resulting in inadequate levels of the transporter in the apical membrane of small intestinal epithelial cells (20). Infants with glucose-galactose malabsorption, who experience severe osmotic diarrhea and failure to thrive on a normal diet, can nevertheless live healthy...
lives if provided with carbohydrate calories exclusively in the form of fructose, which does not require SGLT1 for its absorption.

Glucose-galactose malabsorption is a rare disorder arising from essentially a total absence of functional SGLT1 in the gut. However, many individuals harbor mutations in this protein that may only partially reduce transporter expression/activity. The clinical significance of this is as yet unknown, but there is ongoing research to suggest that such mutations may be involved in the pathogenesis of more common GI disorders, such as perhaps some cases of irritable bowel syndrome.

**Congenital Chloridorrhea.** This is a rare congenital diarrheal disease of infancy that manifests with severe, chloride-rich diarrhea. The disease can rapidly become fatal if left untreated. Genetic analyses of families with this disorder mapped the underlying defect to a gene previously studied for its role in the pathogenesis of colon cancer, known as down-regulated in adenoma (DRA) (16). When the gene was expressed in model systems, it was shown to be capable of exchanging chloride for bicarbonate (or hydroxyl) ions. This identified DRA as a critical anion exchanger responsible, in cooperation with a coupled NHE, for the electroneutral absorption of NaCl across the small and large intestines in the period between meals. Subsequent work has shown that an additional anion exchanger, putative anion exchanger 1 (PAT1), can also contribute to NaCl absorption by the intestine (38). However, in patients suffering from congenital chloridorrhea, presumably PAT1 activity is insufficient to compensate for the loss of DRA. This genetic disorder thus not only illuminates the function of an important intestinal transport protein but also illustrates for students how proteins characterized for their role in a given disease state may in fact have unsuspected and unrelated functions in another. Indeed, the precise role, if any, of the loss of DRA that is seen in adenomas for the subsequent development of colon cancer has yet to be elucidated.

**Diarrheal diseases caused by factors other than transporter mutations.** Of course, the vast majority of diarrheal diseases are unrelated to mutations in specific transporter molecules but rather are caused by infections and/or inflammation. The classic example of a diarrheal disease is cholera. While not a new concept, I find that I can always get the attention of students at any level by showing a picture of a victim who survived an attack of cholera, surrounded by the two nurses who supported him through his illness as well as the hundreds of intravenous saline bottles that were needed to maintain his hydration while he lost many liters of fluid to his stool over several days. While *Vibrio cholerae*, the causative agent of cholera, is a strictly luminal pathogen, it hijacks normal epithelial signaling pathways to irreversibly stimulate chloride secretion while simultaneously inhibiting sodium/hydrogen exchange, thus blocking electroneutral NaCl absorption. The mechanisms whereby cholera toxin dysregulates intestinal transport mechanisms are well known (11) and therefore will not be elaborated here. Suffice it to say that diarrheal illness is familiar to most students from personal experience, although the dramatic fluid losses that occur in cholera (up to 20 l/day), with accompanying loss of electrolytes, certainly illustrate the ability of the intestine to influence whole body fluid and electrolyte homeostasis.

More recent data, on the other hand, suggest that diarrheal symptoms may be beneficial under some circumstances. It has been known for many years that treatment of patients infected with invasive GI pathogens, such as *Salmonella typhimurium*, with antidiarrheal drugs may significantly increase the risk of a disseminated infection. Other enteric pathogens, on the other hand, such as *S. typhi*, cause disease predominantly by entering the bloodstream. We compared the effects of these two closely related bacteria on epithelial transport function and found that *S. typhimurium*, but not *S. typhi*, significantly increased the capacity of epithelial cells for chloride secretion (7). Likewise, if we induced chloride secretion across epithelial cells independently using cholera toxin, the ability of either bacteria to invade the cells was markedly compromised. We can speculate, therefore, that the diarrheal response may represent a primitive host defense mechanism that limits the invasion of pathogens, particularly beyond the gut. However, of course, this “beneficial” diarrhea may still come at the cost of significant dehydration and electrolyte derangements that can be fatal if left untreated in susceptible individuals, such as young children and the elderly.

There is also an emerging, and evolving, appreciation of the mechanisms underlying diarrheal illness associated with intestinal inflammation, as seen in the IBDs of Crohn’s disease and ulcerative colitis. At one time it was assumed that diarrhea in these diseases, often the most distressing and disabling symptom, was brought about by excessive chloride secretion since a wide variety of cytokines and other inflammatory mediators can be shown to function as chloride secretagogues, at least acutely (3). However, in the setting of chronic inflammation, there have been some surprises as to mechanism in animal models of IBD as well as in human patients. Rather than an increase in chloride secretion, diarrhea appears to result instead from defective sodium absorption (14, 22). Thus, the colon may lose its normal ability to desiccate the stool. Coupled with alterations in motility, this leads clinically to diarrhea. Not only is there no evidence for upregulated chloride secretion in various models of IBD, but, in fact, chloride secretion appears to be profoundly suppressed (9, 22). It is tempting to speculate that this may contribute to the breakdown of epithelial barrier function that occurs in IBD, in the following way. Since chloride secretion arises predominantly from the crypts, it may be the case that the secretory process is expressed continuously, at least at a low level, to flush the crypt and maintain relative sterility compared with the large numbers of bacteria that are present in the bulk colonic lumen. When chloride secretion is absent, on the other hand, bacteria and their products can accumulate in the crypt lumen and may exert toxic effects on the epithelium. This putative mechanism seems deserving of additional study.

**Links to Therapeutics**

An understanding of transport regulation in the intestinal tract provides a solid basis for teaching about the mechanism of action of antidiarrheals and other drugs. Most available anti-diarrheal drugs act to slow intestinal motility rather than to influence transport processes per se, but this nevertheless allows the instructor to illustrate the interaction of “north-south” and “east-west” vectors for fluid movement. That is to say, if motility is slowed, absorptive processes, which normally predominate in the gut, have more time to remove fluid from the stool and thus lessen diarrhea. On the other hand, high...
throughput screening mechanisms have recently been applied to the discovery of potent blockers of CFTR, which show promise in preventing a variety of diarrheal syndromes, including diarrheal illness caused by cholera infection (19). Conversely, the screen has also identified CFTR activators, which may be useful in ameliorating the GI symptoms of cystic fibrosis (12).

Transport physiology has also come to the fore with the recent clinical introduction of the drug lubiprostone, which is an activator of CIC-2 chloride channels, an additional class of such channels present in the intestinal epithelium. This drug is marketed for the treatment of chronic constipation and constipation-predominant irritable bowel syndrome, which hitherto have been thought to represent disease states brought about by neuromuscular dysfunction (1). However, if chloride channels are opened and more water is thereby added to the intestinal contents, the symptoms of constipation appear to be reduced. The use of lubiprostone in additional GI conditions that relate to abnormal transport responses is currently being explored. This drug provides an excellent example of how knowledge of basic gastrointestinal physiology can lead to novel therapeutics for troublesome conditions.

Conclusions

It has been my goal in this article to convey an appreciation of the fact that our knowledge of intestinal epithelial transport function is advancing rapidly, particularly at the molecular level. Similarly, the epithelium is recognized as no longer simply a static barrier dividing the luminal contents from the body and permitting selective uptake of nutrients, but rather a dynamic structure whose permeability can selectively be regulated as needed for specific physiological settings. Barrier dysregulation may also occur inappropriately in the setting of disease. The molecular make up of the tight junctions that link adjacent epithelial cells permits, at least in part, this dynamic regulation, both via the intrinsic properties of the components of the tight junction and via the interaction of these molecules with the cellular cytoskeleton.

For both professional and graduate students, disease correlations, and in particular experiments of nature that lead to the disruption of individual transporters, allow the instructor to illuminate understanding. Even in introductory medical school lectures, I have been quick to incorporate information about the molecular character of GI transporter molecules as well as descriptions of how epithelial function is modulated by the intimate and lifelong relationship that the epithelium enjoys with commensal bacteria. Finally, the intestine is a vast portal intimately and lifelong relationship that the epithelium enjoys with commensal bacteria. Finally, the intestine is a vast portal

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REFERENCES


