Epithelial Organization: The Gut and Beyond

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ABSTRACT

Epithelial cells are essential to the survival and homeostasis of complex organisms. These cells cover the surfaces of all mucosae, the skin, and other compartmentalized structures essential to physiological function. In addition to maintenance of barriers that separate internal and external compartments, epithelia display a variety of organ-specific differentiated functions. Function is reflected in overall epithelial structure and organization, shape of individual cells, and proteins expressed by these cells. More than one epithelial cell type is often present within a single organ and, in many cases, individual cells differentiate to change their functional behaviors as part of normal development or in response to extracellular stimuli. This article discusses the diversity of epithelial structure and function in general terms and explores representative tissues in greater depth to highlight organ specific functions and their contributions to physiology and disease.
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Didactic Synopsis

Major teaching points

- Epithelial cells work in unison to form barriers that are necessary for separating self from non-self and preventing mixing of distinct compartments within complex organisms.
- Epithelial structure and organization varies widely and reflects the specialized functions of the sites at which epithelia are found.
- Core functions common to most epithelia include protection, sensation, transport, secretion, clearance, and repair.
- Polarization of epithelial cells, including development of distinct plasma membrane domains, targeted protein localization, and cytoskeletal organization is essential to epithelial function.
- Coordinated activity of transmembrane transport proteins as well as flux across the paracellular pathway, that is, the tight junction, mediates vectorial transport.
- Disorders of epithelial function can cause and contribute to disease.

Introduction

Epithelia cover the skin and line the cavities within internal organs. Here we will focus on organization and function of the epithelium. Distinctions among epithelia will be discussed along with commonalities, including fundamentals of organization, adhesion, polarity, and mechanical coordination.

A common, primary function of all epithelia is to form a barrier between different organs or compartments within organs to separate spaces with unique compositions. The epithelial structures that accomplish this and other tissue-specific functions vary widely and can be classified according to cell shape, orientation, and interactions with one another. Renal tubular, colon, bronchus, alveolar, and skin epithelia are examples of simple cuboidal, simple columnar, pseudostratified columnar, simple squamous, and stratified squamous epithelia, respectively (Fig. 1). While these diverse morphologies correspond to divergent specialized roles, most epithelia also share many of the following core functions.

Protection: Each organ resides in a unique environment. For example, the skin is regularly exposed to physical abrasion that can remove one or more epithelial layers. This would be an insurmountable challenge for the gut epithelium, which is composed of a single epithelial layer, but is easily managed by the multilayered stratified skin epithelium.

Diffusion barrier: Epithelial cells form barriers that separate distinct compartments, one of which is often the external environment. The permeability of these barriers varies, such that epithelia can be classified as either leaky or tight.

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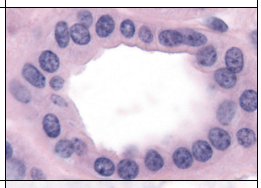
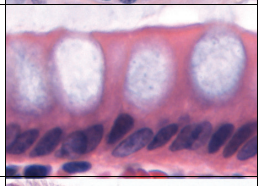
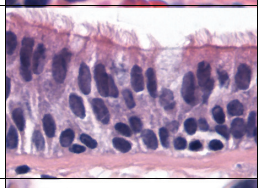
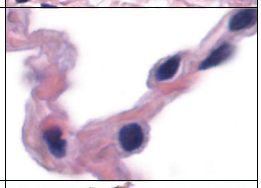
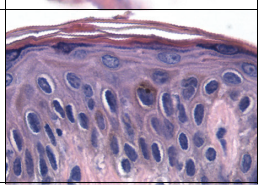
Epithelial type	Sites	Features	Representative image
Simple cuboidal	Renal tubule, thyroid follicle	Single layer of cuboidal cells; may have well-developed brush border	
Simple columnar	Intestines	Single layer of columnar cells; typically organized to allow rapid and continuous renewal and positioning of stem cells within a well-defined niche; well-developed brush border; nuclei are uniformly positioned close to the basement membrane	
Pseudostratified columnar	Trachea and upper airways	Single layer of columnar cells with striking variation in nuclear position, giving the appearance of stratification; reserve cells, which do not reach to mucosal surface, are capable of proliferation to replace damaged or senescent cells; may have cilia, which sweep mucus	
Simple squamous	Alveoli, glomeruli	Flattened, thin cells that form a barrier; shape allows rapid diffusion of gases and other materials across the cells	
Stratified squamous	Skin, oral cavity, esophagus	Multilayered epithelium with stem cells as the deepest layer. Can be keratinized or nonkeratinized.	

Figure 1 Diversity of epithelial cell shape and function.

The skin and urinary bladder, examples of tight epithelia, are nearly impermeant. In contrast, the small intestine is much leakier and allows paracellular flux of water, nutrients, and ions. The barrier and transport properties of the proximal renal tubule are similar to the small intestine, which is to be expected given the massive solute and fluid absorption that occurs at both of these sites. Within the nephron, the paracellular barrier becomes progressively tighter to generate and maintain the concentration gradients established by transcellular transport and countercurrent exchange.

Sensation: Epithelial cells can sense their environment and communicate with nerves. Examples include the cilia of inner ear epithelia that respond to sound waves, as well as other specialized cells that sense gustatory, visual, and olfactory stimuli. The tuft cell has recently gained attention as a sensory epithelial cell within the lungs and gastrointestinal tract (77, 100).

Transport: Many epithelia are involved in active and passive transcellular and passive paracellular transport. In many cases, this is facilitated by morphological specializations that markedly increase membrane surface area, such as intestinal microvilli (Fig. 2).

Clearing luminal materials: Many epithelial cells have cilia, which aid in moving substances within the lumen and may also sense fluid pressure and flow. Ciliated columnar epithelial cells are essential for transport of mucus containing entrapped bacteria and pollutants out of the airways. Ciliary failure occurs in Kartagener syndrome, which is characterized by chronic sinusitis, bronchiectasis, and situs inversus (reversal of the normal organ locations) (129). The latter emphasizes the role of cilia in defining left-right symmetry during embryogenesis. Defects in function of the primary cilium are also responsible for polycystic kidney disease (23, 60).

Secretion and lubrication: Epithelia transport ions, water, and other substances that hydrate the luminal surface. At many sites, the epithelial cells also elaborate mucins to aid in surface lubrication and support mucosal homeostasis. For example, mucins secreted by intestinal goblet cells contribute significantly to the mucosal barrier and, among other functions, limit contact between microbes and the epithelium (224). Defects in transcellular, aquaporin-mediated water transport are responsible for Sjogren's syndrome, in which lacrimal and salivary gland secretions that lubricate the eye and oral cavity, respectively, are insufficient (128, 242, 279).

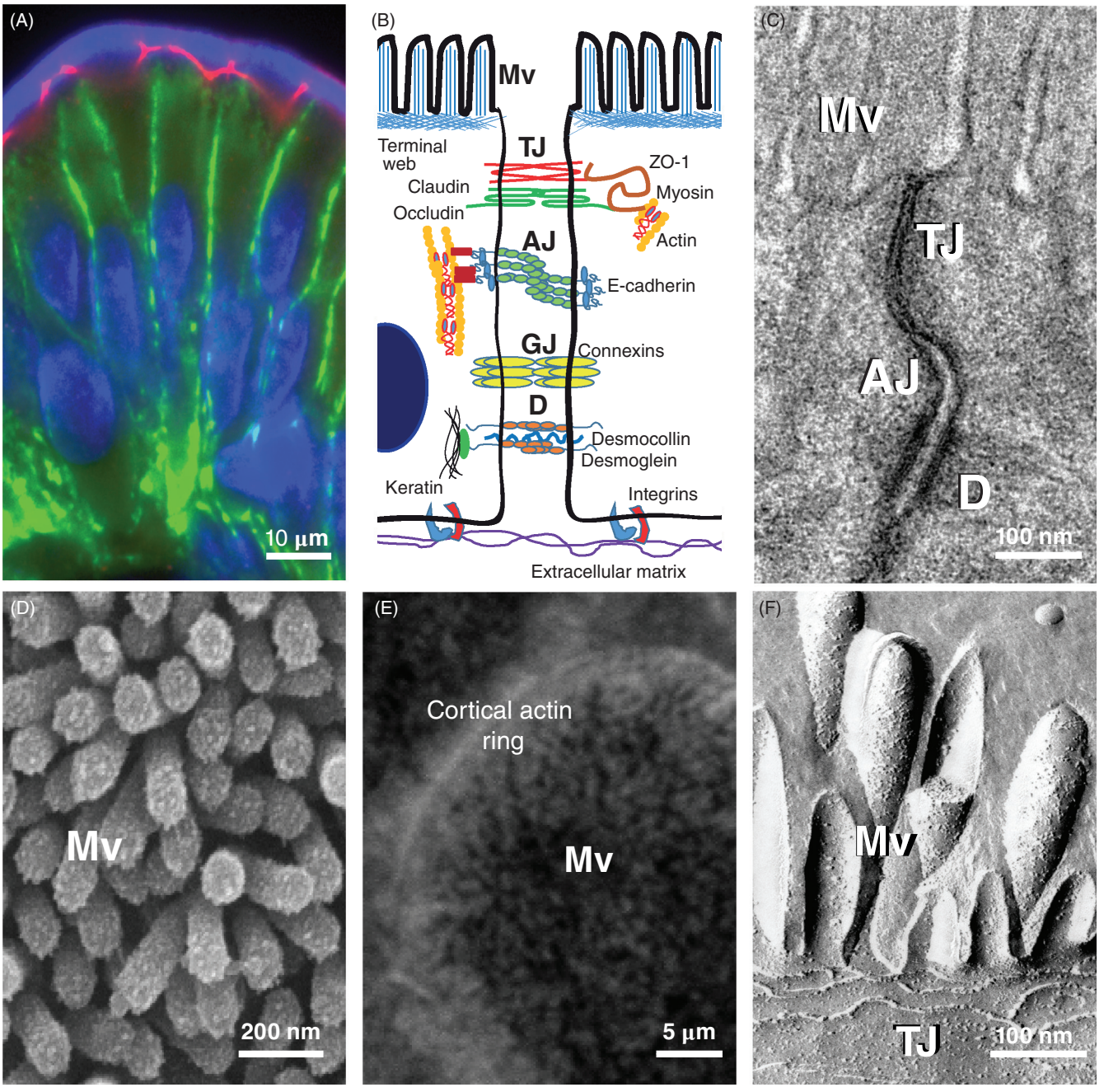


Figure 2 Epithelial cell architecture and organization. (A) Fluorescence micrograph of human small intestine labeled for E-cadherin (green), F-actin (purple), tight junction protein ZO-1 (red), and DNA (blue). Note the localization of each protein: actin is present within the cortical actomyosin ring and microvillus brush border, ZO-1 as bright puncta at tight junctions, and E-cadherin along basolateral membranes. (B) The apical junctional complex of an intestinal epithelial cell. Tight junction proteins include claudins, zonula occludens 1 (ZO-1), occludin, and F-actin, while E-cadherin, α-catenin 1, β-catenin, and F-actin interact to form the adherens junction. Myosin light chain kinase (MLCK) is associated with the perijunctional actomyosin ring. Desmosomes are formed by interactions between desmoglein and desmocollin, which are bound to keratin filaments. Integrins form focal adhesions with the extracellular matrix proteins. (C) Transmission electron micrograph showing junctional complexes between two enterocytes. The tight junction (TJ) is just below the microvilli (Mv), followed by the adherens junction (AJ). The desmosomes (D) are located basolaterally. (D) Scanning electron micrograph showing microvilli, as viewed from above. (E) Confocal micrograph of cultured small intestinal epithelial cells labeled for F-actin. In this apical view (from above the cell), microvilli (Mv) can be appreciated as small dot-like structures due to their F-actin core. The cortical actin ring that forms a belt around each epithelial cell can also be appreciated at the junction between the two cells shown. (F) Freeze-fracture electron micrograph showing apical microvilli (Mv) and tight junction strands (TJ) in a cultured intestinal epithelial cell. [Part C from Nature Reviews Immunology (261) and part F from Annual Reviews of Physiology (233) with permission.]

Regeneration and repair: Due to their inevitable interface with a hostile environment in many cases, epithelia must continuously regenerate. The location of the stem cell compartment varies among epithelia, but is most often at the base such that maturing cells migrate toward the lumen. Loss of normal stem cell growth control can result in cancer. Thus, while regeneration and the ability to repair after injury are essential, they must be precisely regulated to prevent disease.

Epithelial Development and Organization

As early as the morula stage, desmosomes and gap junctions are essential to maturation from the solid morula to the hollowed-out blastocyst. Eventually, three germ layers develop and each gives rise to unique epithelia: The skin, renal tubules, and gut are formed from ectoderm, mesoderm, and endoderm, respectively. Many of these epithelial cells retain the ability to form a central lumen that was demonstrated during blastogenesis. This process, lumenogenesis (160), is essential to development of hollow organs, including the gut, kidneys, lungs, and airways (79) and can be studied *in vitro* (28, 39, 205). Without a lumen, definition of apical and basal surfaces is difficult. Lumenogenesis can occur by a number of methods, including budding, wherein invagination occurs within an existing lumen or sheet of cells, as is seen in branching morphogenesis within the lungs and kidney. Apical constriction within a region can release these cells, as occurs during neural tube development (82). Cavitation results from apoptosis of centrally located cells and occurs in mammary ducts (22). Finally, hollowing occurs in luminal spaces created by cellular invagination or vesicular budding, which has described in Madin Darby Canine Kidney (MDCK) cells and possibly occurs in mammalian nephrons (170).

Epithelial polarization and adherens junction proteins

Epithelial cells must maintain contact with the basement membrane or, in the case of stratified epithelia, other cells that are in contact with the basement membrane (69). The side of the cell that binds the basement membrane is defined as basal, while the lumen-facing surface is termed apical, and the surface in contact with adjacent cells (in the case of simple epithelia) is the lateral membrane. These three surfaces differ in morphology, protein and lipid composition, and function. Generation and maintenance of polarity is, therefore, essential for specialized epithelial functions such as vectorial transport of ions and nutrients (6). This is so important that it extends to epithelial regeneration, and mitotic spindle orientation is precisely controlled during division of polarized epithelial cells (208).

Epithelial cell interactions with the basement membrane are, primarily, mediated by integrins, cell surface receptors that can bind to extracellular matrix proteins (147). These interactions are also essential for establishing and maintaining epithelial polarity on both the cellular and tissue level (28, 300). Different types of integrins attach to matrix proteins by recognizing typical amino-acid motifs, such as the RGD (Arg-Gly-Asp) motif in fibronectin (202). On lateral membranes, integrins mediate interactions between epithelial cells and adjacent cells, including immune cells and pathogens (15, 70, 257).

The first intercellular junctions that develop when epithelial cells make direct physical contact with one another are the adherens junctions (*zonula adhaerens*, Fig. 2) (59). These are not unique to epithelia; E-, N-, and VE-cadherins are found in, and can be used to define, epithelial, neural, and endothelial cells, respectively. Differential cadherin expression allows cells to "recognize" one another and bind to similar cells, which facilitates tissue organization (82). Other adhesion complexes include ephrin receptors and ligands, which are both expressed on cell surfaces; their interaction strengthens intercellular binding. Adherens junctions also contribute to contact inhibition, a form of growth control (127). This signaling is disrupted in many epithelial tumors, some of which transcriptionally repress E-cadherin, which is encoded by *CDH1*. Loss of E-cadherin-mediated intercellular adhesion explains the dyscohesive pattern of tumor infiltration that typifies lobular carcinomas of the breast and signet ring cell gastric cancers (20, 239). Germline mutations in *CDH1* are also responsible for some familial gastric cancers (81). In other cases, epithelial to mesenchymal transition (EMT) is accompanied by a shift from E-cadherin to N-cadherin expression.

E-cadherin recruits other proteins to intercellular junctions. These include the perijunctional ring of actin and myosin filaments to which E-cadherin is connected via cytoplasmic linker proteins α-actinin and α- and β-catenins (49, 130, 294). The cytoplasmic domain of E-cadherin binds to β-catenin, which is then linked to the F-actin meshwork by α-catenin. This latter protein recognizes the mechanical force developed due to cell-cell adhesion developed by cadherin, and responds to this force by unfurling, which exposes cryptic sites open to regulation by vinculin and other junction related proteins (13, 152, 297). These interactions between E-cadherin and the cytoskeleton are, in turn, essential for cell polarization and differentiation. Consistent with the idea that aberrant epithelial polarization may contribute to neoplasia, mutations in β-catenin and its regulator adenomatous polyposis coli (APC) protein are common early events in human colorectal cancer development (78, 132, 173, 181).

E-cadherin trafficking is associated with and dependent on (282) the cytoplasmic polarity protein PALS1 (Protein Associated with Lin Seven one). PALS1 binds to the apical transmembrane protein crumbs3 (Crb3) as well as a second cytosolic protein PATJ (PALS1-associated tight junction protein). This complex is essential for defining the apical membrane (288). Consistent with this, genetic deletion of

Crb3 results in neonatal death due to polarization and apical membrane function defects in intestines, kidneys, and lungs (288). Interestingly, the phenotype of mice lacking the apical actin-binding and linker protein ezrin is identical similar to that of Crb3 knockout mice (34, 225, 288), which may be due to an ezrin-binding domain within Crb3 (64).

Basolateral membrane domains are established, in part, by competition between the apical complex and a corresponding basolateral complex composed of Dlg (discs large), Scrib (scribble), and Lgl (lethal giant larvae) (295). These complexes actively repel one another; overexpression or loss of components of either complex increase or reduce, respectively, the size of the corresponding membrane domain (38, 254). Finally, a complex composed of cdc42, Par6, Par3, and atypical protein kinase C defines the position of the apical junctional complex (56, 115). This depends, at least in part, on binding of PALS1 to Par6 (partitioning defective 6) and the resulting linkage between the PALS1/PATJ/Crb3 and Cdc42/Par6/Par3/atypical protein kinase C complexes that maintain the apical membrane and apical junctional complex, respectively (64, 104, 220).

While specific roles in polarization of mammalian epithelia have not been defined for other members of the Par protein family, it is interesting to note that mutation of *LKB1* (Liver Kinase B1), the mammalian ortholog of the *C. elegans* protein Par4 is associated with Peutz-Jeghers syndrome (93,113,283). Peutz-Jeghers syndrome is characterized by gastrointestinal hamartomatous polyps (16). This can be understood when one recognizes that hamartomas represent disorganized masses composed of cell types that are otherwise appropriate for the site in which the lesion is found. Peutz-Jeghers syndrome patients also have increased susceptibility to gastrointestinal tract cancers, suggesting that the serine/threonine kinase LKB1 may be a tumor suppressor (93). Consistent with the links between cancer and loss of polarity, LKB1 activation by STRAD (STE20-related kinase adaptor) is sufficient to trigger epithelial polarization in the absence of the otherwise essential cues provided by E-cadherin engagement (10). Conversely, loss of E-cadherin signaling-dependent growth control, that is, contact inhibition, may occur in the absence of LKB1 due to upregulation of Wnt5a, which has been reported in both $Lkb1^{+/-}$ mice and polyps from Peutz-Jeghers patients (140).

Desmosomes

As noted earlier, the adherens junction is the first intercellular junction formed when epithelial cells contact one another. In addition to supporting the development of separate apical and basolateral domains, the adherens junction and associated Cdc42/Par6/Par3/atypical protein kinase C complex position the apical junction complex (Fig. 2), which consists of the tight junction (*zonula occludens*), adherens junctions (*zonula adhaerens*), and desmosomes (*macula adhaerens*) (59). Together these junctions maintain polarity, seal the paracellular space, provide intercellular communication, and stabilize intercellular contacts to preserve overall epithelial integrity.

The adherens junction is located directly below the tight junction and followed closely by the desmosomes (59). The latter are composed of the desmosomal cadherin proteins desmoglein and desmocollin. In a manner that is analogous to catenin-dependent binding of E-cadherin to F-actin, desmosomal cadherins bind to keratin intermediate filaments via the cytoplasmic plaque proteins plakoglobin and desmoplakin (240, 291). This allows keratin filament networks within adjacent cells to be stably anchored to one another and provide tensile strength to epithelial structures. The importance of desmosomes is highlighted by the rare, blistering diseases caused by autoantibodies to desmosomal proteins (101, 219). In these diseases, defective desmosomal intercellular adherence allows adjacent cells within the skin and other stratified squamous epithelia to separate from one another. Fluid accumulates in the resulting spaces, causing severe blistering and, ultimately, sloughing of epithelial sheets.

Tight junctions and paracellular permeability

The epithelial cell membranes are sufficient to form a barrier to macromolecules, hydrophilic solutes, including ions, and water. However, all of these materials could, potentially, traverse the paracellular shunt pathway at sites of cell junctions. The adherens junctions and desmosomes provide strength that holds adjacent epithelial cells to one another (Fig. 2B), but these junctions do not seal the shunt pathway. That duty falls to the tight junction, the component of the apical junctional complex that is closest to the lumen (Fig. 2B). In simplest terms, tight junctions across various epithelia can be referred to as "tight" or "leaky" based on their permeability. For example, the urinary bladder is a tight epithelium with very little transepithelial ion conductance, i.e. high electrical resistance. In contrast, the small intestine is a leaky epithelium with substantial paracellular flux of ions, nutrients, and water. When examined by freeze fracture electron microscopy, tight junctions appear as an anastomosing series of strands that extends approximate 200 nm beneath the apical surface (Fig. 2C and F). In general, greater numbers of strands correlate with higher resistance (42). The specific protein composition of the tight junction also has a profound effect on paracellular permeability.

The tight junction also demarcates the boundary between apical and basolateral membranes; the adherens junction is located within the basolateral domain, where E-cadherin decorates the entire basolateral membrane surface in many epithelia. As a result, the tight junction has been suggested to have a fence function that prevents mixing of transmembrane proteins and lipids between apical and basolateral domains (168). However, perturbations that inhibit development of barrier function do not prevent polarized distribution of membrane proteins and specialized membrane structures, for example, microvilli (11, 194, 238, 268). Therefore, tight junctions,

which contribute to the organization of multicellular structures (194), are not essential for epithelial polarization.

Although the complete composition of tight junction strands remains to be defined, it is clear that their assembly is catalyzed by claudin proteins. The claudins are a family of 27 genes (178) that encode ~20 kD proteins with short intracellular N- and C-termini, 4 transmembrane domains, and 2 extracellular loops (ECLs) (135). Claudins can develop homotypic or heterotypic interactions in *cis* or *trans* orientations. This leads to development of claudin polymers, whose structure is just now being elucidated (250, 251). Whether these polymers comprise or merely direct assembly of the tight junction strands seen by freeze-fracture microscopy remains an open question (156).

The first ECL (ECL1) of channel-forming claudins forms the pore and at least part of the paracellular channel. These channels are exquisitely size selective, with a maximum diameter of ~6 Å, and are also charge-selective (274, 298). In the gastrointestinal tract, most channel-forming claudins, for example, claudin-2 and claudin-15, are cation-selective, meaning they preferentially allow paracellular flux of cations over that of anions. Other channel-forming claudins, for example, claudin-10a and claudin-17, preferentially accommodate Cl^-, $HCO3^-$, and NO_3^- anions (137, 138, 275). In contrast to the intestines, anion specific claudins are abundant in renal tubules and salivary glands (185, 186). While recent data show that claudin-based channels open and close in a manner similar to transmembrane ion channels (284), it should be recognized that the specificity of channels for different ions of similar size and charge is limited relative to that of transmembrane ion channels.

Some claudin proteins do not form channels, but their expression enhances the paracellular barrier by mechanisms that remain to be defined (272). The repertoire of claudin proteins expressed at any specific site is therefore a principal determinant of both barrier function and paracellular permeability to ions and water. Not surprisingly, expression of individual claudins varies along the length of the gastrointestinal tract, along the crypt villus axis, during development, and in the context of disease (55, 96, 209). Similar variability occurs along the length of the nephron (99).

While they are essential, claudins are not sufficient to form tight junctions (226, 276). A large number of other proteins are also present at tight junctions, including both transmembrane and peripheral membrane proteins. Among these, the most important appear to be the zonula occludens (ZO) family, composed of the scaffolding proteins ZO-1, ZO-2, and ZO-3, and the tight junction-associated Marvel proteins (TAMPs), occludin, tricellulin, and MarvelD3 (58, 71, 88, 107, 114, 211, 216, 241, 243). ZO family proteins contain PDZ domains that attach to a PDZ-binding motif at the C terminus of most claudins and contribute to claudin recruitment to the tight junction (14, 57, 210). Specific functions of the TAMPs have been more difficult to define, but it is becoming clear that they can regulate both claudins and other aspects of barrier function. Among these, mutations in tricellulin have been shown to be a cause of hereditary deafness (190, 216).

Ion and nutrient transport

Many epithelia transport nutrients, ions, and water. The spatial distribution of these transport proteins is essentially what makes a cell 'polar' in terms of both protein distribution and development of electrochemical gradients for the net movement of molecules across membranes. Common transporters include:

Cystic fibrosis transmembrane conductance regulator (CFTR): CFTR is an ATP-binding cassette (ABC) transporter found within apical membranes of gastrointestinal, pulmonary, hepatobiliary, and renal epithelia (180, 244). The channel is normally gated by ATP hydrolysis, but can be massively activated by cyclic AMP generated as a result of cholera toxin activity (80). The resulting intestinal chloride secretion creates the osmotic gradient that is responsible for the severe diarrhea that characterizes cholera. Conversely, CFTR defects result in failure to generate the osmotic gradient that is necessary for fluid secretion and mucin hydration, as exemplified by the use of mucoviscidosis to describe cystic fibrosis (136,157). In the absence of CFTR-dependent luminal hydration, pulmonary mucociliary clearance fails and mucus plugs obstruct pancreatic ducts leading to recurrent pneumonia and exocrine pancreatic insufficiency, respectively. Over 1,000 CFTR mutations have been described, most of which disrupt trafficking of the transporter to the apical membrane.

Na^+-glucose cotransporters (SGLT): The SLC5 family transporters SGLT1 and SGLT2 transport glucose across the apical membrane. SGLT1 is expressed in the intestines, while both SGLT1 and SGLT2 are expressed in the renal tubules (62, 85). The coupling of glucose transport with that of Na^+ allows glucose to be absorbed against a concentration gradient using the extracellular to intracellular Na^+ gradient as a driving force. Transport of each glucose molecule requires the energy provided by one or two Na^+ ions, for SGLT2 and SGLT1, respectively (120, 148).

Facilitated glucose transporters (GLUT): This large family of SLC2 nutrient transporters (32) is expressed throughout evolution (290). In mammalian epithelia, GLUT family transporters traffic primarily to the basolateral domain where GLUT2 ensures that glucose absorbed via SGLT1 and SGLT2 is able to exit the cell (149). Some GLUT transporters, for example, GLUT2 and GLUT5, traffic to the apical membrane where they mediate absorption of nonglucose hexoses, such as fructose (124, 166).

Na^+-H^+ exchangers (NHEs): Eight NHE isoforms exist, with NHE1 being expressed ubiquitously. NHE2 and NHE3 are expressed in lung, intestinal, and renal epithelia where

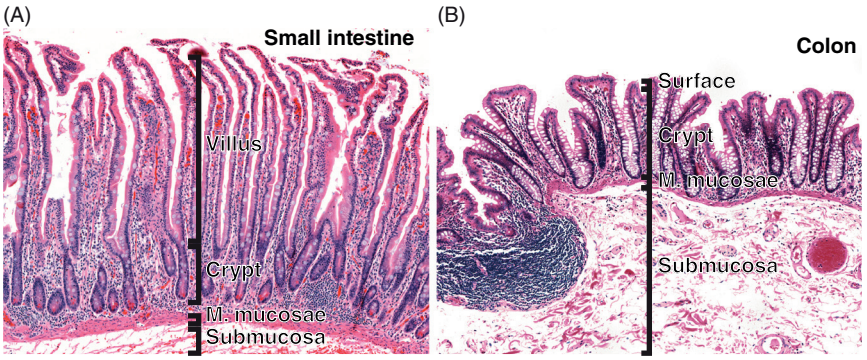


Figure 3 Comparison of small intestinal and colonic mucosal architecture. (A) Low-magnification image of hematoxylin and eosin-stained section of normal human duodenum. The mucosa can be separated into villus, crypt, and muscularis mucosae (m. mucosae) and sits atop the submucosa. The villi greatly expand mucosal thickness. (B) Low-magnification image of hematoxylin and eosin-stained section of normal human distal colon. The mucosa can be separated into surface, crypt, and muscularis mucosae (m. mucosae) and, like the small intestinal mucosa, rests on the submucosa.

they traffic to the apical membrane (97). The electrochemical gradients created by these SLC9 family exchangers are critical to water transport: *Nhe3* knockout in mice results in chronic malabsorptive diarrhea and defective water absorption in the proximal tubule (232). In humans, *NHE3* missense mutations result in congenital diarrhea (112). Reduced NHE3 activity also contributes to malabsorptive diarrhea in inflammatory bowel disease (144,248) and may promote disease-associated dysbiosis (143, 145).

Na^+-K^+-Cl^- cotransporters (NKCC): Like apical SGLT transporters, these SLC12 family transporters use the Na^+ gradient to drive electroneutral absorption of one Na^+, one K^+, and two Cl^- ions into the cell. Basolateral NKCC1 provides the Cl^- ions that are secreted via CFTR in some forms of diarrhea (215). In the kidney, NKCC2 mediates absorption of these ions across the apical membrane in the thick ascending limb (TAL) of the nephron (7, 74, 304).

Epithelial Na^+ channel (ENaC): These channels are expressed on the apical membrane of highly resistive epithelia, including the distal colon, bronchi, distal tubule, and collecting duct of the kidneys (50,54,68,196). ENaC is upregulated by aldosterone and glucocorticoids and is important for Na^+ absorption. Interactions between ENaC and CFTR are of pathophysiological importance in cystic fibrosis, where ENaC is upregulated in the absence of a functional CFTR (214). ENaC is a target of diuretics, for example, amiloride, that inhibit ENaC-dependent Na^+ absorption and, by osmotic means, increase urinary volume (196).

Compartmentalization of epithelial functions

Compartmentalization may be most obvious within the small intestine, where distinct functions are distributed along a longitudinal axis, from duodenum to terminal ileum, as well as a vertical axis, from crypt to villus (Fig. 3). At the base of each villus, the epithelium invaginates into the lamina propria and forms the crypts of Lieberkühn (131). Each crypt contains stem cells that are responsible for renewal of crypt and villous epithelium (269). As a result of the patterned organization of crypts and villi within the small intestine, each villus is populated by cells derived from multiple crypts. The primary proliferative compartment is within the crypt base, where the rapidly cycling, LGR5-expressing stem cell pool is present (12, 111, 296). These cells are susceptible to chemotherapeutic agents and radiation, both of which interfere with DNA replication. Other cells repopulate the LGR5-expressing stem cell pool under these conditions (206, 234, 259, 292). Within the small intestine and proximal colon, the crypts also contain Paneth cells (167, 203, 301), which contain large cytoplasmic granules within the apical cytoplasm. Among other substances, these granules, which can be secreted into the lumen, contain antimicrobial peptides that help to maintain the relatively sterile composition of the crypt (40, 53, 286). Paneth cells also synthesize factors that promote development and maintenance of the stem cell niche (227, 271). As newly created cells exit the crypt base, they enter the transit-amplifying zone, a region at the top of the crypt where mitosis continues and increases the number of daughter cells produced exponentially (218). The undifferentiated transit-amplifying cells have secretory capacity and are the primary epithelial cells that secrete Cl^- in response to pathogens and toxins, e.g. cholera toxin (41). As they migrate toward the villus mitosis ends and the cells differentiate into absorptive enterocytes, goblet cells, and enteroendocrine cells (159). As a reflection of these disparate functions, epithelial transport proteins are differentially expressed along the crypt:villus axis. For example, the apical and basolateral Cl^- transporters (CFTR) (Fig. 4A) and the Na^+-K^+-Cl^-cotransporter NKCC1 (Fig. 4B) are primarily expressed in the crypt base (244) and transit-amplifying zone (215). Conversely, the Na^+-H^+ exchanger NHE3 (Fig. 4C)

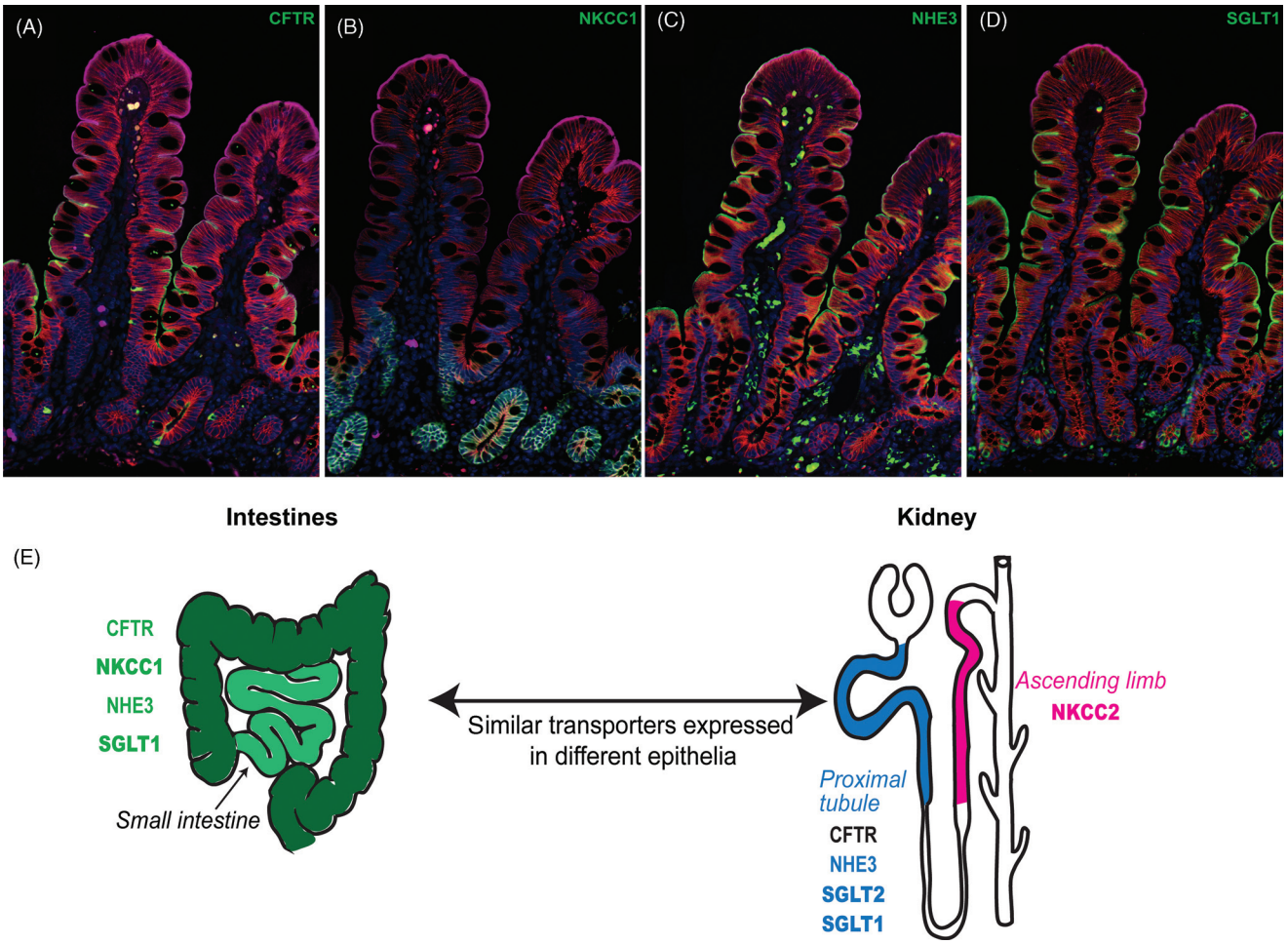


Figure 4 Segregation of epithelial transport proteins along the crypt:villus axis. Fluorescence micrographs of human small intestine labeled for E-cadherin (red), F-actin (purple), and DNA (blue). (A) Note the localization of CFTR (green) to the apical membrane of epithelial cells within the lower villus and crypt, the principal site of chloride secretion. (B) Note that NKCC1 (green) is expressed in the same population of cells that expressed CFTR but is localized to the basolateral membrane, where colocalizes with E-cadherin. (C) NHE3 (green) is localized to the apical membrane and predominantly expressed on cells within the villus, where the bulk of absorption takes place. (D) Similar to NHE3, SGLT1 (green) is localized to the apical membrane of villous absorptive enterocytes. Bright autofluorescence (green) of red blood cells highlights villous capillaries that run just beneath the basement membrane is present in some images (particularly panel C). (E) These transporters are expressed throughout small intestine and colon; similar transporters are expressed within the nephron in a site-specific manner.

and Na^+-glucose cotransporter SGLT1 (Fig. 4D) are primarily expressed in the villus at the brush border (apical) membrane (106). Although the renal tubules are only organized along a single axis, from proximal tubule to collecting duct, a similar functionally compartmentalized organization is present (Fig. 4E).

Mucosal secretion

The efficacy of mucosal barriers can be measured by multiple parameters including the immune barrier, the physical barrier to microorganisms, and the physical barrier to solutes, ions, and water. Each of these is defined by distinct components of the mucosa. The immunological barrier includes both innate and adaptive arms of the immune response and is essential to prevent infection and sepsis. Particularly in the case of the gastrointestinal tract, the mucosa is also the primary site at which the immune system encounters antigens and, therefore, plays an important role in immune education.

Despite intestinal colonization by diverse microorganisms, most intestinal epithelial cells do not come into direct contact with the microbiota. This is largely due to mucin, which in the case of the intestines is secreted by goblet cells (116). Mucins are organized into a single loose mucus layer within the small intestine. In the colon, where bacterial colonization is far higher, an inner mucus layer that is physically attached to the epithelial cells by means of transmembrane mucin proteins is present beneath the loose outer layer (117). Bacteria can infiltrate the outer mucus layer but are excluded from the denser inner mucus layer.

Mucus is composed of proteins that are glycosylated such that about 80% of their mass is carbohydrate. The core mucin

proteins are encoded by at least 20 different genes in humans. MUC2 is the predominant mucin expressed in the small intestine and colon (117, 118). In mice, deletion of the *Muc2* gene leads to spontaneous intestinal disease, demonstrating the importance of mucins to mucosal protection (19, 270).

In addition to preventing large objects, including undigested food and microbes, from directly contacting the epithelial surface, mucus creates a zone in which luminal fluids are not mixed uniformly. This unstirred layer allows brush border digestive enzymes to break down complex proteins, fats, and carbohydrates and generate high local concentrations of the resulting smaller molecules that are amenable to transport across the epithelium (197). Beneath the unstirred layer lies the epithelium, which forms the barrier to macromolecules, ions, and water. Similarly, goblet cells within airway epithelium secrete mucins that lubricate the surface and capture particulates and microbes; thereby limiting entry of these materials into the alveolar airspaces (89).

Polarized protein delivery

Polarized delivery of proteins and lipids to the plasma membrane is essential for epithelial cell organization and function. In most polarized cells, basolateral surface proteins are delivered directly to that domain, possibly to specific sites within lateral membranes (121). The targeting information is encoded by amino acid sequences within the cytoplasmic tail, or sometimes, the N-terminal domain, as with E-cadherin (33). In some cases, these tyrosine-containing sequences are sorted by the epithelial adapter protein AP-1B (87, 252). The vesicular transport process, which is also involved in endocytic recycling of apical and basolateral membrane proteins, involves members of the Rab family of small guanosine triphosphate (GTP)-binding proteins and SNARE proteins that target delivery of transport vesicles to specific membrane domains (65).

Apically targeted proteins can be transported by both direct and indirect pathways (204, 231). Proteins that traffic directly to the apical membrane include those that associate with glycolipid- and cholesterol-rich membrane domains, such as the apical hydrolase sucrose-isomaltase, and may also be transported along actin microfilaments (37, 142, 223, 287). Ectodomain glycosylation sites and transmembrane protein domains have been implicated in apical trafficking (26), but have been more difficult to identify than their basolateral counterparts.

Some proteins have special functions that require them to traverse a more complicated route. One example is the polyimmunoglobulin receptor (pIgR), which initially traffics to the basolateral membrane where it binds to IgA released by lamina propria plasma cells. Upon IgA binding, the ligated pIgR is endocytosed and directed to fuse with the apical plasma membrane (247). Here, proteases cleave pIgR, part of which is released as the secretory component protein that cross-links IgA molecules (35, 163). In addition to specific targeting sequences within pIgR, transcytosis also relies on tracks defined by microtubules. It remains unclear as to why some apical proteins, without any specific basolateral functions also take this indirect pathway. Nevertheless, this mechanism is useful to correct errors in initial trafficking of apical proteins and for membrane protein sorting during the early stages of epithelial polarization.

Maintenance of membrane domains

Once delivered to the correct plasma membrane domain, it is important that proteins be retained. This frequently depends on interactions with actin-based cytoskeletal proteins. For example, the Na^+,K^+-ATPase is stabilized on the basolateral membrane domain by attachment to the cytoskeleton through the linker proteins ankyrin and spectrin (192). E-cadherin bound to p120 catenin is stable at the membrane, but is targeted for endosomal recycling once detached, which can be regulated by RhoGEFs and GAPs (29, 146). Some differentiated epithelial cells have special functions that require intricate structures, such as the apical secretory canaliculi and microvilli of parietal cells and enterocytes, respectively. Organization and maintenance of these domains is guided by ezrin–radixin-moesin (ERM) proteins, which are evolutionarily conserved in organisms including *C. elegans*, *Drosophila*, and mammals, and are found in many cell types, including epithelia and lymphocytes (191). ERM proteins have separate cargo-binding and actin-binding domains. The cargo-binding domain can interact with transmembrane and peripheral membrane proteins either directly or through accessory proteins, such as NHERF-1, NHERF-2, and PDZK1 (141, 228). As a result, ERM proteins can organize signaling complexes. For example, the cystic fibrosis transmembrane regulator (CFTR) binds to NHERF-2 PDZ domains, which stabilize CFTR at the apical membrane (Fig. 4A) and tether it to protein kinase A. This enhances the ability of protein kinase A to activate CFTR (17, 189). Ezrin is also involved in trafficking of the apical Na^+/H^+ exchanger NHE3 to the enterocyte brush border (Fig. 4C) following initiation of Na^+-glucose cotransport and of the gastric H^+K^+-ATPase to parietal cell canalicular membranes after histamine stimulation (154, 230, 302).

Maintenance of the polarized distribution of plasma membrane transport proteins is essential to nutrient transport (108, 264). In addition to allowing hydrophilic solutes to cross the apical membrane, transmembrane transporters create the ionic and electrochemical gradients that are essential to movement of water and passively transported solutes. They help in maintaining the pH of the gut lumen and allow symbiotic microbes to thrive. This is especially true for the ileum and the colon, where CFTR mediated transport of Cl^- and short-chain fatty acid dependent secretion of HCO_3^- regulates luminal pH. Defects in bicarbonate secretion or pH neutralization leads to inflammation and altered microbiome composition in CFTR knockout mice (21, 236).

At the microvillus brush border, the Na^+-glucose cotransporter SGLT1 (Fig. 6D) uses the extracellular to intracellular Na^+ gradient to transport one glucose and two Na^+ ions

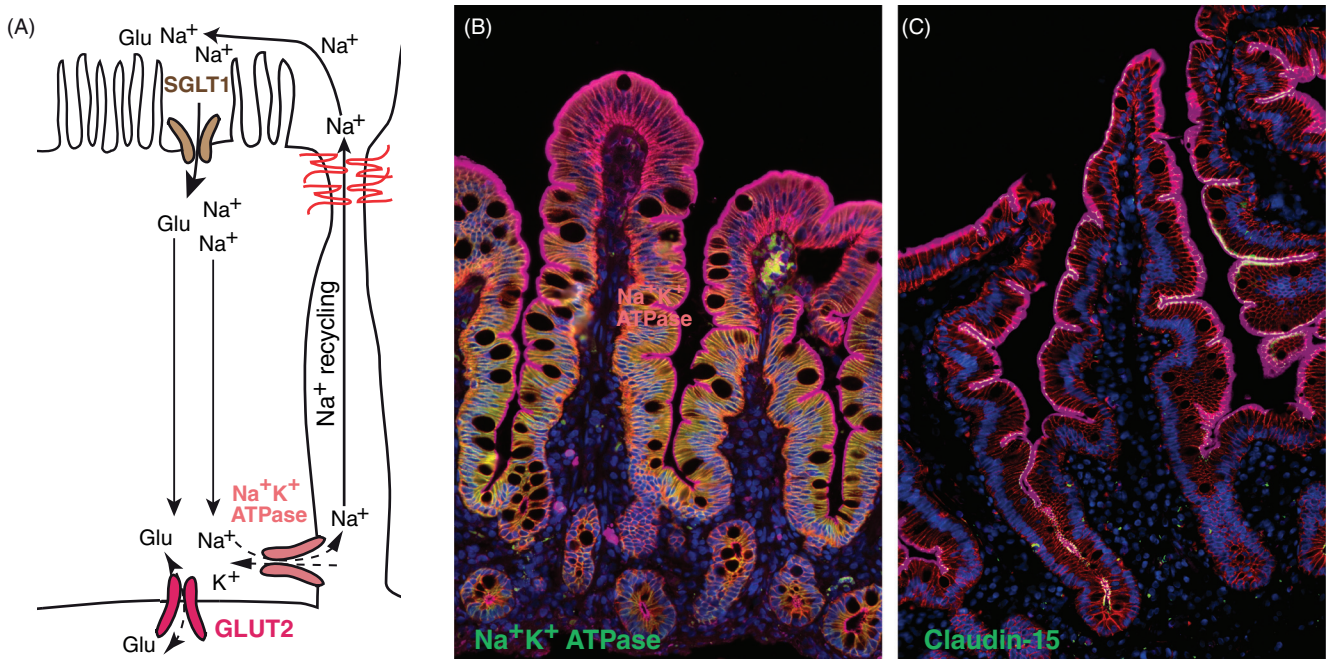


Figure 5 Epithelial glucose transport as a model of Na^+-coupled nutrient absorption. (A) Glucose (Glu) absorption begins with transport across the apical, brush border membrane via SGLT1-mediated Na^+ cotransport. Both glucose and Na^+ diffuse to the basolateral membranes where they exit the cell by way of GLUT2 and Na^+K^+ATPase, respectively. As described in the text, this and the many other Na^+ absorptive pathways present can deplete Na^+ from the lumen and thereby inhibit further absorption. Tight junction proteins claudin-2 and claudin-15 allow Na^+ to diffuse, passively, across the tight junction according to the concentration gradient to replenish luminal stores and allow continued nutrient absorption. (B) Fluorescence micrograph of human small intestine labeled for Na^+K^+ATPase (green), E-cadherin (red), F-actin (purple), and DNA (blue). Note the localization of the Na^+K^+ATPase to the basolateral membranes of villus and crypt epithelial cells. (C) Claudin-15 (green) is distributed in a dot-like pattern at epithelial cell junctions. By light microscopy, claudin-15 appears to colocalize with the very apical extent of E-cadherin, which is otherwise restricted to the basolateral membrane as well as the dense cortical (perijunctional) F-actin ring.

across the apical membrane (260). GLUT2, a facilitated transporter located on the basolateral surface, then allows glucose to diffuse across the membrane to the interstitium (Fig. 5A) (36, 45). This arrangement allows GLUT2 to operate in the reverse direction to provide glucose to intestinal epithelial cell from the blood stream when luminal nutrients are not present (2, 123). Finally, Na^+ is transported across the basolateral membrane via the Na^+K^+-ATPase (Fig. 5B). The initiation of apical Na^+-glucose cotransport triggers a MAP kinase cascade that induces trafficking of Na^+/H^+ exchanger 3 (NHE3) from intracellular storage pools to the apical membrane (83, 102, 154, 302). NHE3 translocation further enhances transcellular Na^+ absorption. At the same time, myosin light chain kinase (MLCK) activation increases tight junction permeability to facilitate passive, paracellular water and nutrient absorption (177, 262, 263). In this manner, SGLT1-mediated Na^+-glucose cotransport initiates a sequence of events that activate multiple modes of nutrient absorption. The driving force for all of these is the Na^+ concentration gradient across the apical membrane that is, ultimately, maintained by the activity of the basolateral Na^+K^+-ATPase (Fig. 5B). This process cannot continue if the luminal-cytoplasmic Na^+ gradient is not maintained or if luminal Na^+ is depleted. The former is avoided by providing metabolites that allow intracellular ATP generation. The latter depends on recycling of absorbed Na^+ from the basolateral interstitium back into the lumen. Recent data indicate that this Na^+ recycling requires either claudin-2 or claudin-15 (Fig. 5C), which are tight junction proteins that form paracellular Na^+ channels. This process exemplifies many of the vectorial transport systems that are essential functions of polarized epithelia.

Random distribution of transporters to both apical and basolateral domains would markedly disrupt glucose absorption and could even lead to net glucose secretion into the lumen. Similar problems can be envisioned for just about any of the transmembrane and transport proteins. Thus, particularly in transporting epithelia, including the gut and renal tubules, maintenance of polarity is absolutely required for continued physiological function.

Organization of the cytoskeleton

The villus absorptive enterocyte has been a useful model for studies of cytoskeletal structure and function in polarized epithelia. Columnar epithelial cells (Figs. 1 and 2A) require support to maintain their shape; without a cytoskeleton, they would collapse into a more thermodynamically favorable sphere. Cell shape depends on networks of actin microfilaments that lie beneath the apical and basolateral membranes and are cross-linked with spectrin and other proteins.

Intermediate filaments and microtubules also support the basolateral compartments and intercellular junctions (195).

Microfilament (actin) bundles also form the microvillus cores. Assembly of microvilli also depends on intracellular myosins and ERM proteins as well as extracellular mucin-like protocadherin and protocadherin-24 that provide inter-microvillar linkage (46, 228). Myosin-1a is particularly important in stabilizing microvilli (278), and, although mice lacking myosin-1a are viable and grow normally, the morphology of the brush border is disturbed (175), levels of the enzyme sucrose isomaltase are reduced, and myosin-1c is aberrantly localized to microvilli (176,265). The microvillous actin bundles integrate with the terminal web composed of actin and type II myosin that interfaces with the apical junctional complex (122, 162) (Fig. 2A and E).

Microtubule organization in polarized epithelial cells is distinct from that of non-polarized cells, where microtubules radiate from a single microtubule-organizing center adjacent to the nucleus. In polarized epithelia, microtubules are aligned apicobasally (76) where they support trafficking of kinesins and dyneins. These motors transport vesicles along microtubule arrays, and are particularly important in transcytosis, as occurs during IgA secretion (73).

The role of the cytoskeleton can be modified and manipulated by certain pathogenic and nonpathogenic microorganisms in the gut. *Listeria monocytogenes*, which can cause often gastroenteritis, interacts with E-cadherin (exposed during cell extrusion on the villus tip) via bacterial internalin proteins (24, 201), and then uses phospholipases to slip into the cytoplasm. Here, *Listeria* replicate and express the actA protein (47), which polymerizes actin to form 'comet tails' that propel the bacterium and allows penetration across membranes into neighboring cells. Cytoskeletal elements are therefore not only important to enterocyte structure, but also for physiological function and disease propagation.

Organ Specific Epithelial Organization and Function

Renal tubular epithelium

The nephron is the basic functional unit of the kidney, which allows re-absorption of most ions and nutrients from the glomerular ultrafiltrate. Similar to the intestinal epithelium, nephrons are lined by epithelia expressing transport proteins that are similar, and sometimes identical, to those of the intestines (Fig. 6). In the kidneys, SGLT2, which cotransports one Na^+ ion to provide the driving force each glucose molecule, mediates the bulk ofglucose reabsorption in the proximal tubule S1 segment. SGLT1, which, uses the energy provided by cotransport of two Na^+ ions to transport each glucose molecule and can function in the absence of a glucose concentration gradient, and absorbs remaining glucose in S2 and S3 segments (171). Familial renal glucosuria is due to defects in SGLT2 (SLC5A2) (4, 171), while SGLT1 and SGLT2 inhibition are used to induce renal glucose wasting in some diabetic patients. Disruptions of Na^+-Cl^- transport

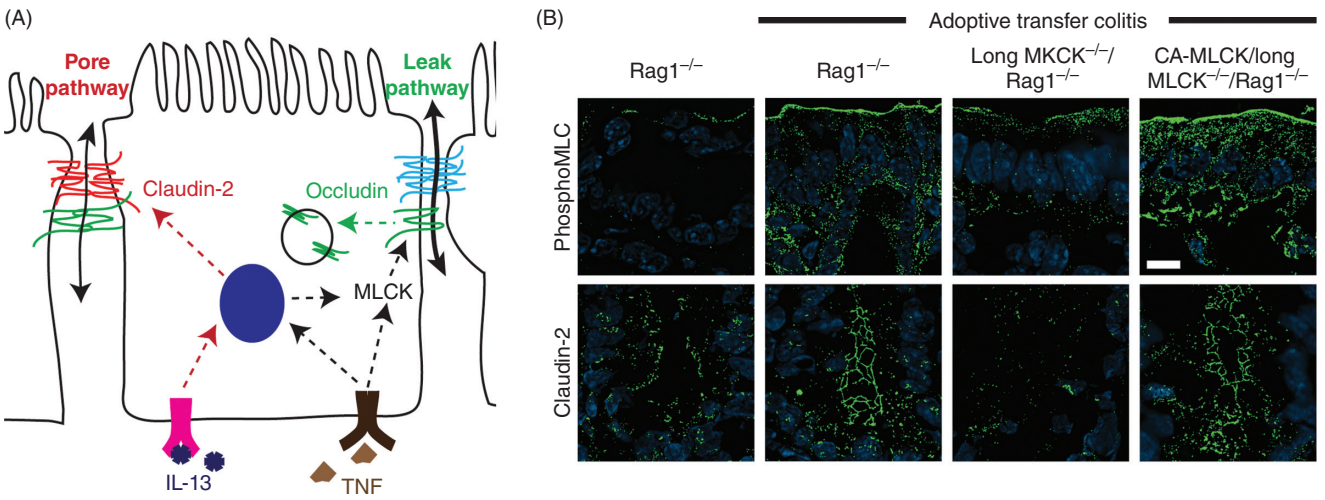


Figure 6 Molecular mechanisms of pore and leak pathway regulation by the immune system during disease. (A) IL-13 results in transcriptional activation of claudin-2 expression and resulting increases in pore pathway permeability. In contrast, TNF activates myosin light chain kinase transcription and enzymatic activity. These lead to endocytic removal of occludin from the tight junction and increased leak pathway permeability. (B) Transgenic, intestinal epithelial-restricted expression of constitutively active-MLCK restores sensitivity of long MLCK$^{-/-}$ mice to adoptive transfer colitis, an immune mediated experimental IBD induced by transfer of CD4$^+$CD45RB^{hi} T cells into Rag1$^{-/-}$ immunodeficient recipients. Long MLCK$^{-/-}$ mice are protected from disease-associated MLC phosphorylation (green, upper panels) and claudin-2 upregulation (green, lower panels). Tissue specific, intestinal epithelial expression of a constitutively active MLCK (CA-MLCK) catalytic domain restores disease-associated MLC phosphorylation and claudin-2 upregulation. Thus, although inflammatory cytokines can specifically and differentially activate pore and leak pathways, the regulation of these paracellular flux routes is linked in disease. Bar = 10 um. [Part B adapted from Gastroenterology (245) with permission.]

due to mutations in the thiazide-sensitive Na^+-Cl^- symporter (NCC, SLC12A3) or other transporters, including NKCC2 (SLC12A2) and the Cl^- channel (CLCNKB), result in Gitelman syndrome and Bartter syndrome, respectively (134). These disorders are both characterized by metabolic alkalosis, hypokalemia, and Mg^{2+} deficiency; Bartter syndrome patients also display Ca^{2+} deficiency while, for reasons that are not yet clear, Gitelman syndrome actually enhances renal Ca^{2+} absorption (133, 235). Because the diuretic furosemide targets NKCC2, patients taking this drug can occasionally develop symptoms similar to Gitelman syndrome. Although renal claudin-2 expression is not essential, it does increase the efficiency of Na^+ recovery from the tubule lumen by coordinating with transcellular Na^+ absorption (200). Other claudins expressed within the nephron include the anion selective claudins 10a and 17 (186). Unlike the intestine, which is discussed below, aquaporins play a major role in water transport. In the collecting duct, vasopressin dependent AQP2 function is an essential determinant of urinary water volume (105, 179, 193, 279).

Lung epithelium

From the trachea to the airspaces, epithelial cells form the lining of the respiratory tract. The trachea and airways are lined by ciliated, undifferentiated columnar, secretory, and basal cells arranged as a pseudostratified epithelium (289). Airway epithelial cells maintain the surface liquid balance by the concerted actions of plasma membrane channels and tight junctions (229). Airspaces are lined by type I alveolar epithelial cells, which are large, flat cells that allow diffusion of gases between airspaces and capillaries (Fig. 1). Type I cell junctions are mainly responsible for alveolar epithelial barrier function (229). Type II alveolar cells are smaller, cuboidal, and have a granular cytoplasm. These cells secrete surfactant and are able to proliferate and differentiate to replace damaged type I pneumocytes.

Skin

The integument, or skin, is the largest organ within the human body and consists of the epidermis and subjacent dermis. The epidermis is composed of stratified squamous epithelium, which allows superficial layers to be eroded by surface trauma without substantial barrier loss. In skin, the basal layer of the epidermis consists of keratinocytes that are capable of division. Their progeny move upward to form the polyhedral spinocytes (27, 72, 249). Ultimately, the most superficial cells flatten, become anucleate, and compress into dense keratin bundles (Fig. 1). In addition to the interfollicular stem cells, damaged epidermis can also be replaced by stem cells within hair follicles, sebaceous glands (61, 153, 212). In some species, the esophagus, which is lined by a squamous stratified epithelium, is keratinized as well (98). As might be expected, these are species whose diet includes dry, abrasive materials, such as rodents (213). In contrast, keratinization of the esophageal epithelium only occurs in humans as a reactive process following chronic damage.

Urinary bladder

The urinary bladder presents a special problem for epithelia. The mucosal surface must expand markedly as urine accumulates. This could, potentially, be accomplished by thick mucosal folds, such as the gastric rugae. Instead, the bladder is lined by a stratified epithelium composed of a proliferative basal layer, an overlying intermediate layer, and, finally, a superficial cell layer (1, 150, 151). The latter, referred to as umbrella cells, are normally cuboidal. As the bladder fills and the mucosa is stretched, umbrella cells become large flattened cells that are able to maintain the barrier to water and ions. This near absolute barrier function is essential to maintain the concentrated or dilute urine generated by the kidneys.

Gastrointestinal Tract

The small intestine and colon, or large intestine, comprise the lower gastrointestinal tract. These organs are both essentially hollow tubes, with average lengths of 6 m and 1.2 m, for small intestine and large intestine respectively, in humans (91). Anatomically, the small intestine is broken into three sections; duodenum, jejunum, and ileum, from proximal to distal. Each of these regions has distinct functions, as discussed below. Functions as well as the composition of luminal contents differ significantly in terms of water, ion, and microbial content throughout the length of the small intestine and colon in a manner that corresponds to regional changes in epithelial function. Specialized functions of the small intestine and colon include:

Secretion: The proximal duodenum contains the mucosal Brunner glands, which secrete bicarbonate to neutralize the acidic contents received from the stomach. In general, large amounts of fluid secreted by the salivary glands, stomach, and small intestine mix with ingested liquids to hydrate the contents of the intestinal lumen.

Digestion: The "ampulla of Vater," located in the duodenum, acts as a conduit for biliary and pancreatic secretions, which aid in digestion and are also a source of water entering the intestinal lumen. Bile helps to emulsify dietary fats and form micelles that can be absorbed. Pancreatic secretions are rich in a variety of enzymes that break larger molecules into smaller units that can be absorbed by apical membrane transport proteins.

Absorption: The majority of absorption occurs within the duodenum and jejunum. In particular, the duodenum is responsible for the bulk of fatty acid and water-soluble vitamin absorption. In contrast, the greatest proportion of amino acids, carbohydrates, nucleotides, and ions are absorbed in the jejunum. Although calcium, iron and many vitamins

are absorbed in the duodenum and jejunum, vitamin B_{12} is a notable exception and is absorbed in the ileum (139). This is important to recognize, as ileal disease or surgical resection of the ileum can have profound effects on vitamin B_{12} despite having little impact on most other absorptive processes.

Fluid transport: The jejunum, ileum, and colon absorb the majority of fluid presented to the gastrointestinal tract. Overall, ingestion and secretion cause approximately 9 L to enter the gastrointestinal lumen each day, but only 200 mL is typically lost in the stools of humans.

In addition to functions found to varying degrees in many epithelia, gastrointestinal epithelia face unique challenges. One adaptation that is essential to meet the demands placed on gut epithelia is immanence of a surface area far greater than that of any other organ, including the skin. The massive transport occurring in the small intestine is facilitated by three levels of structural modifications that increase mucosal surface area. First, the mucosa of the small intestine and, to a lesser extent, the colon, is pulled into folds (Fig. 3). These folds increase mucosal surface area three-fold. Small intestinal surface area is further increased by the presence of villi, which create an additional 10-fold amplification (Figs. 3B and D). Finally, microvilli increase apical surface area 20-fold (Fig. 2C-F) (183). As a result, the absorptive area of the small intestine is 600-fold greater than if it were a simple smooth surface. While villi are not present, microvilli and mucosal folds also augment surface are of the colon (Fig. 3B) (183). To perform such specific and intricate functions, gut mucosae are supported by underlying tissues that provide structural integrity, vascularization, innervation, and drive peristalsis. While there are some regional specializations, the small intestine displays most of the features present in other areas of the gut.

The small intestine is divided into four concentric layers—the mucosa, the submucosa, the muscularis propria, and the serosa. The mucosa includes, at its luminal surface, the epithelium, which rests on the basement membrane. The lamina propria, which is composed of connective tissue, capillaries, lymphatics, and some nerve fibers, lies directly beneath the basement membrane (Fig. 3A). Notably, lymphatic vessels are not prominent in the colonic lamina propria, which explains why metastasis from colon cancers limited to the mucosa is rare. The lamina propria is also home to the mucosal immune system and contains neutrophils, T lymphocytes, B lymphocytes, plasma cells, macrophages, and other immune cells. In general, immune cells are not present within the epithelial compartment, i.e. across the basement membrane, where they can directly contact epithelial cells. Under normal conditions, the exception to this is T lymphocytes, a small number of which actively migrate within the epithelium and are thought to provide immune surveillance (51, 52). The presence of other immune cell types, for example neutrophils, within the epithelial compartment is generally associated with inflammatory pathologies. Lamina propria plasma cells secrete immunoglobulins that are transported into the lumen by epithelial cells (119,207,266) via the polyimmunoglobulin receptor (pIgR) and the neonatal Fc receptor (FcRn). These receptors bind immunoglobulins at the basolateral surface, initiate endocytosis, and then traffic across the epithelium to release their cargo into the lumen. Beneath the lamina propria lies the muscularis mucosa. This thin layer of muscle is innervated by Meissner's submucosal plexus. Together, the epithelium, lamina propria, and muscularis mucosae form the mucosa (Fig. 3A).

Water movement across the intestinal epithelial barrier

In the intestine, transepithelial water transport is largely paracellular and depends on the osmotic gradient developed by transcellular ion and solute transport (95). The cation selective claudin-2 pores can also act as paracellular water channels; their upregulation in infection is critical to infectious diarrhea and enteric pathogen clearance (221, 222, 258).While aquaporins are present, and their expression is altered in disease (86, 217, 256), the lack of gut phenotypes in aquaporin knockout mice suggests that (303) these proteins are more important for cellular homeostasis and transport of small signaling molecules (255). In contrast, aquaporins are essential for water transport and overall secretion in other tissues, including kidneys and salivary glands (242). Potential roles for other transmembrane channels, including the urea transporter-B (SLC14A1) and CFTR, for water transport across the apical membrane have been proposed, but it is not clear how water would then exit the cell (103).

Although the reliance of intestinal water transport on osmotic gradients is absolute, the rate of water flux can be slowed or accelerated by decreased or increased, respectively, tight junction permeability. One particular example of this is the increased tight junction permeability that occurs after epithelial exposure to tumor necrosis factor-α (TNF) (84). Under normal conditions, TNF reverses the normal direction of water flux such that there is net fluid secretion into the gut lumen, that is, diarrhea (184). However, if this increase in tight junction permeability is prevented, net water secretion does not occur (44). The water secretion under these conditions also depends on loss of the transepithelial Na^+ gradient, normally generated by the apical Na^+/H^+ exchanger NHE3, as a result of TNF-induced downregulation. Nature has provided striking evidence of the importance of NHE downregulation to this process via the TNF-related cytokine LIGHT, which increases tight junction permeability and but does not inhibit NHE3 (44). In contrast to the diarrhea induced by TNF, LIGHT modestly increases water absorption due to the increases in tight junction permeability and continued transcellular sodium transport driven by NHE3. Thus, although water transport in the intestine is largely paracellular, gradients generated by transcellular ion transport provide the necessary driving force.

Mechanisms of intestinal barrier regulation

As noted above, tight junction permeability is increased following activation of Na^+-nutrient cotransport (9, 18, 63, 164, 165). This is size-selective and limited to small, nutrient-sized molecules, for example, mannitol (63). In the small intestine, such transport occurs primarily in the villus, where Na^+-nutrient cotransporters are expressed. Previous studies suggested that this might reflect an increase in the number of paracellular channels (63), an idea that is consistent with the recent observation that trans-tight junction channels open and close dynamically (284) and the hypothesis that the open probability of these channels is increased by Na^+-nutrient cotransport. The physiological significance of this phenomenon with respect to paracellular amplification of transcellular nutrient absorption has been discussed above. Hints as to the mechanism of this regulation initially came from electron micrographs showing condensation of the perijunctional actin cytoskeleton following activation of Na^+-nutrient cotransport. Based on this observation, phosphorylation of the myosin II regulatory light chain (MLC), an essential intermediate in actomyosin contraction, was explored and found to be closely linked to Na^+-nutrient cotransport-induced increases in tight junction permeability. Further, inhibition of myosin light chain kinase (MLCK), the principal kinase that phosphorylates MLC, prevented increases in both MLC phosphorylation and tight junction permeability (263, 305). Thus, while it is theoretically possible that MLCK could phosphorylate other targets, and, conversely that other kinases, for example, rho kinase, could phosphorylate MLC, MLCK-mediated phosphorylation of MLC is central to physiological regulation of tight junctions. This regulation requires ZO-1 expression and depends on interactions mediated by the ZO-1 actin-binding region (ABR) (299).

The tight junction leak pathway

Building on the observation that MLCK is central to physiological regulation of tight junctions, a potential role for MLCK in TNF-induced tight junction regulation was explored. TNF caused marked increases in MLC phosphorylation within the perijunctional actomyosin ring, and either genetic or pharmacological inhibition of MLCK prevented this phosphorylation (43, 281, 305). Further, MLCK inhibition by either mechanism prevented TNF-induced barrier loss and diarrhea. Thus, similar to its role in physiological tight junction regulation, MLCK is a central mediator of pathological tight junction regulation. However, there is an important difference between these forms of tight junction regulation. Physiological Na^+-nutrient cotransport increases MLCK enzymatic activity and tight junction permeability to small molecules without marked effects on steady-state distribution of tight junction proteins. In contrast, TNF-induced barrier loss is associated with increased MLCK expression, enzymatic activation of MLCK, MLCK-dependent occludin endocytosis, and, perhaps most significantly, increased paracellular permeability to much larger molecules, including albumin, which has a hydrodynamic diameter of ~70 Å (43, 169).

While the mechanisms of occludin function remain enigmatic, several observations indicate that occludin removal from the tight junction is central to TNF-induced barrier loss. First, blockade of caveolar endocytosis, either pharmacologically or genetically, prevents TNF-induced occludin internalization and tight junction barrier loss. Further, occludin overexpression in vivo markedly attenuates TNF-induced barrier loss (169). Finally, in cultured monolayers, occludin overexpression or knockdown has been shown to reduce or increase, respectively, paracellular permeability to macromolecules (31, 273). This low-capacity paracellular route, which is not charge-selective and, according to best available estimates, allows flux of probes with diameters in the range of 100 Å has been defined as the tight junction leak pathway (Fig. 6A) (3, 261).

The tight junction pore pathway

Investigation of the ability of other cytokines to regulate tight junctions led to the discovery that IL-13 increases both paracellular permeability and claudin-2 upregulation (92). Experiments using siRNA to prevent increased claudin-2 expression demonstrated that this upregulation is required for IL-13-induced barrier loss (285). These studies also showed, however, that IL-13-induced claudin-2 expression increased paracellular permeability of Na^+ ions (non-hydrated ion diameter of 1.9 Å), but not of Cl^- ions (non-hydrated ion diameter of 3.6 Å) or 4 kD dextran (diameter of 28 Å). This is consistent with the known physiochemical properties of the claudin-2 pore, which can also accommodate water (diameter of 2.7 Å) as well as methylamine (non-hydrated ion diameter of 3.8 Å) and, to a lesser degree ethylamine (nonhydrated ion diameter of 4.6 Å) (221, 222, 274, 298). Importantly, this flux route, which is referred to as the pore pathway (Fig. 6A), is far more selective than the leak pathway in terms of both size and charge. The number of molecules transported, that is the carrying capacity, of the pore pathway is however far greater than that of the leak pathway. Notably, while the pore pathway across intestinal tight junction is cation selective, the pore pathway in many other epithelia is anion selective as a result of differential claudin isoform expression (75). Size-selectivity is confined to a narrow range in all claudins studied to date, regardless of charge selectivity.

The distinct effects of IL-13 and TNF on tight junctions indicate that the immune system can differentially regulate epithelial barrier properties by selectively upregulating pore or leak pathways (Fig. 6A). Further, these flux routes are differentially expressed along the crypt-villus axis, where the leak pathway dominates within the crypt while the pore pathway accounts for the bulk of paracellular flux in the villus (63). Moreover, as noted above, pores can be opened in response to physiological stimuli, such as Na^+-glucose transport (63, 263). Finally, the pore pathway is regulated developmentally. One striking example of this is the marked downregulation of

intestinal claudin-2 expression after weaning (96). Claudin-15 (Fig. 5C), which also forms a cation-selective channel, is coordinately upregulated. This is critical, since lack of either claudin-2 or claudin-15 is compatible with life, but mice lacking both claudin-2 and claudin-15 die within the neonatal period (253, 280). These mice die of malnutrition, due to inadequate paracellular recycling of transcellularly absorbed Na^+ (280). As noted earlier, this recycling is essential for ongoing Na^+-nutrient cotransport across the apical membrane (Fig. 5A). Given their similar functions in this context, it is therefore not clear why claudin-2 is replaced by claudin-15. This could, potentially, be explained by unidentified functional differences between these claudins. One hypothesis could be that claudin-2 has a greater carrying capacity than claudin-15. This would be consistent with the massive Na^+-nutrient cotransport that is needed prior to weaning, and the somewhat reduced Na^+-nutrient cotransport as the diet changes from maternal milk to solid foods. While no data are available to support this idea, the concept is consistent with the observed downregulation of the apical Na^+-glucose cotransporter SGLT1 that occurs in ruminants as the glucose content of the diet falls with the transition from milk to grass (66,277).

Contributions of intestinal barrier loss to immune-mediated disease

A potential role for increased paracellular permeability in intestinal disease was first proposed in studies of celiac and inflammatory bowel disease reported 35 years ago (199). This was followed by an innovative study that took advantage of the substantial contribution of genetics to development of inflammatory bowel disease. Analyses of Crohn's disease patients, their healthy first-degree relatives, and unrelated healthy controls revealed that a subset of healthy relatives had increased intestinal permeability probes (94, 174). Many have suggested that these relatives, that is those with increased permeability, might be at greater risk of subsequently developing Crohn's disease. However, this hypothesis has never been tested. Although a single case report does document development of disease in a previously healthy relative with increased permeability, this does not really address the issue, as the subject was at increased risk of developing disease regardless of intestinal permeability (109). The topic has remained controversial, as some studies have shown that healthy relatives with increased permeability tend to carry specific mutations in an IBD risk allele of *NOD2* (30), while other studies have failed to identify a specific genetic linkage (125). Nevertheless, several studies have shown that increased intestinal permeability in patients with inactive Crohn's disease is a marker of impending relapse (8, 48, 293). Whether this indicates the presence of subclinical inflammation and may be an opportunity for therapeutic restoration of barrier function has not been assessed in patients.

Although there are no perfect models of human inflammatory bowel disease in mice (126), immune-mediated models offer the greatest opportunity to understand potential contributions of epithelial dysfunction, including barrier loss, to disease pathogenesis. In contrast, many of the chemical colitis models, including DSS colitis, damage the epithelium so enormously that little epithelial function remains to be assessed. Using an adoptive transfer model of colitis in which naïve T effector cells are transferred to immunodeficient recipients (Fig. 6B), it has been shown that genetic inhibition of intestinal epithelial myosin light chain kinase (MLCK) attenuates disease (245). Conversely, expression of a constitutively active MLCK within the intestinal epithelium accelerates disease progression (245, 246). Interestingly, MLCK inhibition also prevented claudin-2 upregulation during disease (Fig. 6B). Thus, it is possible that both pore and leak pathways both contribute to disease pathogenesis. Many have proposed that the underlying mechanisms involve activation of the mucosal immune system by luminal materials that are able to traverse the pore or leak pathway. While this is an attractive hypothesis, it remains to be tested. Further, it will be important to consider the physiology that drives increased pore and leak pathway permeability in disease. Is this a compensatory mechanism that leads to some benefit? Alternatively, this could be a maladaptive response that ultimately promotes disease. The latter is the thesis on which the model of "leaky gut syndrome" has developed. While there are certainly many people with intestinal permeability beyond what would be considered the normal range, and some of these patients do have intestinal symptoms, a direct link is far from certain. Nevertheless, the topic remains of great interest, as increased intestinal permeability has been implicated in a plethora of extraintestinal diseases including diabetes mellitus, multiple sclerosis, graft vs. host disease, and even autism. The data supporting these links are, at present, weak. However, as our understanding of the dynamics of the gut lumen, including the contributions of the microbiota (5, 67, 90, 110, 158, 161, 187, 237), grow, the relationships between intestinal permeability and disease may become clearer.

It is unclear how the gut microbiome can directly influence barrier function during homeostasis. Changes to microbiota populations or a reduction in microbial diversity in the gut is linked to inflammation, IBD, obesity, and cancer, which suggests that metabolites elaborated by the microbiome might have a direct role in regulating intestinal permeability (25, 172, 182, 198, 267). However, studies to date have been limited to crude measures of intestinal barrier function and correlative analyses of tight junction proteins that do not demonstrate a direct connection between the microbiome and intestinal permeability and cannot distinguish barrier loss due to epithelial damage from that due to enhanced paracellular, that is, tight junction, permeability (188). Some studies have been performed in germ-free rodents, but it is notable that these animals have underdeveloped immune systems and exhibit excessive mucus production, both of which complicate functional analyses (155). Thus, while the microbiome is integrally linked to mucosal health, the effects of microbes on the barrier and, conversely, of the barrier on microbial populations, remain to be defined.

Conclusion

Many functions are conserved across diverse epithelial surfaces. Nevertheless, the specific functional requirements of each organ and anatomical site are reflected in the distinct organizations and activities of epithelia at those sites. We have sought to present common features while offering examples where properties diverge as epithelia adapt to local needs. In specific cases, we have also presented examples of epithelial dysfunction, and how this can result in aberrant physiology and disease.

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