A COMPONENTS

8 Epithelia: biological principles of organization

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All cavities within the alimentary tract, from the small ducts and acini of the pancreas to the gastric lumen, are lined by sheets of polarized epithelial cells. Common to all of these epithelia is the ability to create selective barriers that separate lumenal and tissue spaces. Most epithelia are also able to direct vectorial transport of solutes and solvents. These essential functions are based on the structural polarity of individual cells, the complex organization of membrane domains, cell–cell and cell–substrate interactions, and integration with other cell types. This chapter reviews intestinal wall structure and examines how mucosal functions are supported by the organization of the gut and the biological properties of the epithelial barrier and epithelial transport. More detailed discussions of epithelial transport and nutrient processing can be found in Chapters 13–21.

Organization of the gut wall

The relation of the epithelial layer to other components of the gut wall is shown in Fig. 8.1. Four principal layers exist: mucosa, submucosa, muscularis propria, and serosa or adventitia. The mucosa consists of the epithelium, an underlying layer of loose connective tissue carrying nerves and vessels (i.e., lamina propria), and a thin layer of smooth muscle (i.e., muscularis mucosa). The mucosa also contains an array of lymphocytes, mast cells, macrophages, and, in disease states, polymorphonuclear leukocytes, all of which are capable of modulating epithelial function.

An underlying layer of fibroconnective tissue called the submucosa, which contains nerves, vessels, and lymphatics, supports the mucosa. The submucosa rests on the muscularis propria, which is composed of two or three layers of smooth muscle and is home to the myenteric plexus (see Chapters 2 and 6). In most instances, gastrointestinal organs are encased by an outermost delicate layer of fibrofatty tissue, the serosa, encircled by a continuous layer of mesothelial cells. In areas where no serosa exists, as in portions of the esophagus and in the distal colorectum, fibrofatty tissues interface with the external portion of the muscularis propria. These organs are said to have an adventitial, rather than a serosal, encasement.

Forceps biopsies retrieved endoscopically usually go no deeper than the muscularis mucosae, although thin wisps of submucosal tissue may occasionally be present. Suction biopsies more consistently penetrate the submucosa, although only the most superficial portion of the submucosa is obtained. Deeper portions of the wall appear in endoscopic samples by accident, such as in an aggressive snare of a sessile mucosal lesion, or by intention, as in endoscopic mucosal resection (see Chapter 137).

Similar to the gross anatomy, the microscopic anatomy of the gastrointestinal tract varies along its length. A simple columnar epithelium lines the stomach, small intestine, colon, pancreatobiliary ducts, and exocrine pancreas. In contrast, the oral cavity, esophagus, and anus are lined by a nonkeratinizing, stratified squamous epithelium that is capable of withstanding the mechanical stresses of swallowing and defecation but plays no role in transepithelial transport. The three-dimensional structure of epithelia also exhibits significant variation within the gastrointestinal tract, such as the prominent mucosal folds and villi in the small intestine (Fig. 8.1), the lobular organization of the exocrine pancreatic acini (Chapter 67), and the deep epithelial extensions into salivary and Brunner glands in the esophagus and duodenum, respectively. The liver, which lacks a single large lumen, is composed primarily of hepatocytes. These possess only a small apical (canalicular) surface and lack complex, deeper cell layers; capillaries and hepatocytes are separated by a thin basement membrane in the space of Dissé (see Chapter 79).

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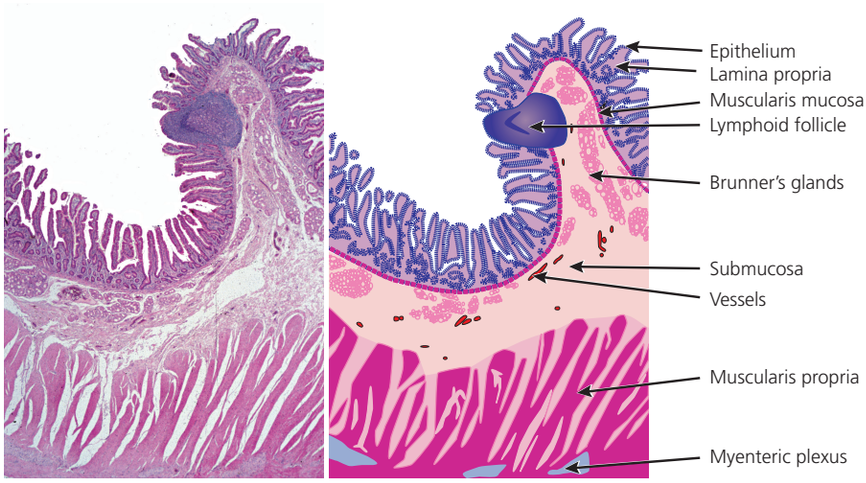

Figure 8.1 Organization of the intestinal wall. This transmural section of normal human duodenum exemplifies many of the structural features that are common throughout the gastrointestinal tract. The corresponding line diagram delineates specific structures.

Despite these complexities of regional specialization, central structural features critical to epithelial function are present throughout the gastrointestinal tract.

Organization of epithelial cells and sheets

To function properly as a barrier, epithelial cells must assemble into a multicellular sheet. This is a complex task that requires individual cells to establish a uniformly oriented polarity; to form intercellular junctions; and to develop stable interactions with the basement membrane (Fig. 8.2).

The need for cell polarity

A central function of many gastrointestinal epithelia is the vectorial transport of solutes and solvents. For example, parietal cells are polarized to effect acid secretion into the lumen (see Chapter 13). Imagine the ensuing havoc if acid were inadvertently secreted into the interstitium. Absorptive villous enterocytes of the small intestine are specialized to accomplish vectorial transport of ions, nutrients, and water from the lumen to the interstitium (Fig. 8.3) by expressing specific transporters within the apical (lumenal), but not basolateral, membrane domain [1–3]. These transporters often rely on a lumenal Na^+ concentration that is much higher than the intracellular Na^+ concentration required for cotransport of sugars, amino acids, ions, bile salt, and xenobiotics. In general, the absorbed solutes exit the cytosol by way of Na^+-independent facilitated transporters present in basolateral, but not apical, membranes, resulting in efficient transcellular transport. The high extracellular and low intracellular Na^+ concentrations that provide energy for this system are maintained by the exclusively basolateral Na^+,K^+-ATPase that pumps Na^+ out of the cell in exchange for K^+. Thus, the net result of apical Na^+-coupled transport is vectorial transport of both the specific solute and Na^+ from the lumen to the interstitium. The polarized distribution of these three classes of transport proteins – apical Na^+-coupled transporters, basolateral Na^+-independent transporters, and the basolateral Na^+,K^+-ATPase – is critical for active transepithelial transport. Note that the deposition of osmotically active molecules in the subepithelial interstitial space provides the driving force for passive water absorption. This integration of active transcellular and passive, primarily paracellular, transport explains the improved efficacy of oral rehydration solutions supplemented with Na^+ and carbohydrates [4,5]. Although details of these transport processes will be addressed in further detail, this outline of vectorial transport should emphasize that polarized distribution of surface membrane components within individual cells is essential to epithelial function. It should also be obvious that were the entire epithelial sheet not polarized uniformly, i.e., with all cells polarized in the same orientation, adjacent cells could

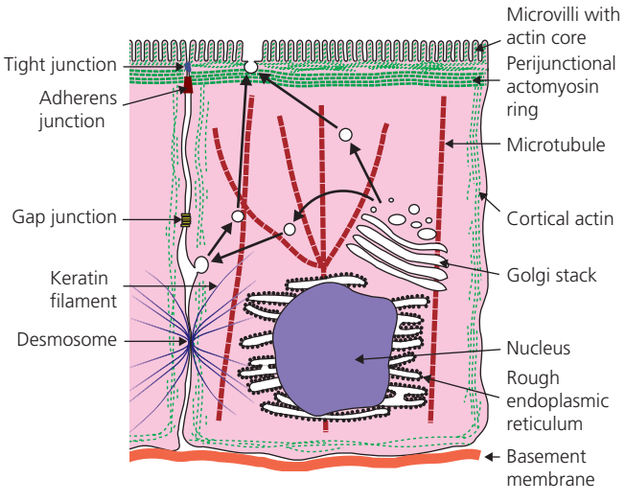

Figure 8.2 Organization of a typical gastrointestinal columnar epithelial cell. The precisely orchestrated architecture of polarized epithelia includes numerous specialized structures, such as cytoskeletal elements, transport vesicles, and intercellular junctions.

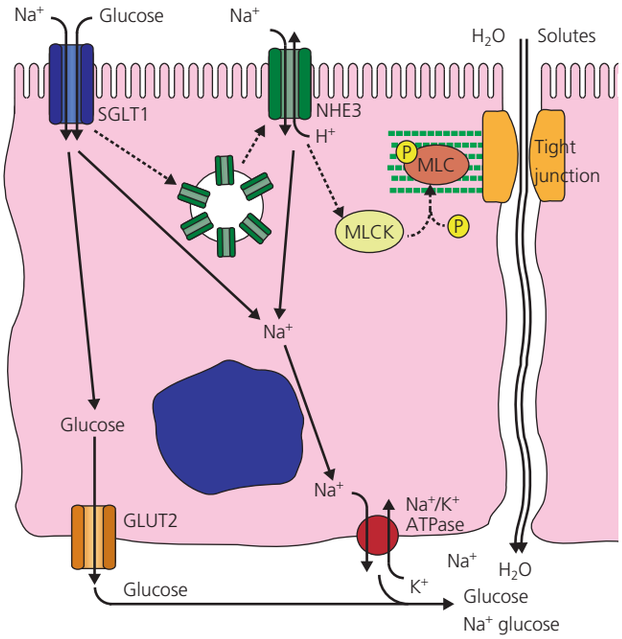

Figure 8.3 Coordination of transcellular and paracellular absorption of water, nutrients, and ions. The polarized delivery of SGLT1, GLUT2, and Na^+,K^+-ATPase to the appropriate apical (SGLT1) and basolateral (GLUT2 and Na^+,K^+-ATPase) membranes is essential for efficient vectorial glucose absorption. Research has shown that SGLT1 activation also stimulates apical NHE3-mediated Na^+ absorption, thereby linking these absorptive processes. Incompletely defined downstream signaling events subsequently activate myosin light chain kinase (MLCK). This, in turn, phosphorylates MLC and increases paracellular permeability, thereby linking transcellular and paracellular absorption.

negate one another's contributions, making net transport impossible. Thus, in addition to polarization of individual cells, it is critical that all cells within an epithelium respond to common cues to generate a unified, polarized epithelial sheet.

Cues to trigger polarization

The spatial cues that induce initial cell polarization and maintain it have generally been thought to require contact with the extracellular matrix and with other cells. For example, the interaction between the epithelial Ca^{2+}-dependent adherens junction protein, E-cadherin, on adjacent cells is a critical trigger for polarization. E-cadherin is concentrated at the adherens junction, the basal-most aspect of the apical junction complex (Fig. 8.2), where, through cytoplasmic linker proteins (e.g., α-actinin and α- and β-catenins), it is coupled to a perijunctional ring of actin and myosin filaments [6,7]. In addition to E-cadherin-mediated intercellular adhesion, interactions with matrix components through integrins, heparan sulfate proteoglycans, and other membrane proteins are also important in inducing polarization and maintaining differentiation [8–10].

The discovery of polarity genes encoding Par proteins in *Caenorhabditis elegans* and *Drosophila* has facilitated the identification of mammalian homologues, complexes of which define epithelial apical and basolateral plasma membrane domains [11,12]. Not surprisingly, Par protein mutations have been linked to disease. For example, many individuals with Peutz–Jeghers syndrome exhibit mutation of LKB1, the mammalian homologue of the *C. elegans* protein Par-4 [13]. LKB1 is a serine/threonine kinase that is activated by the STRAD adapter protein, which may also be mutated in Peutz–Jeghers syndrome [14]. STRAD-mediated LKB1 activation triggers early cell polarization in epithelial cells despite the absence of intercellular junctions [15]. One mediator of LKB1-initiated epithelial polarization may be AMP-activated protein kinase, which is activated by LKB1 and plays an important role in maintaining cellular energy balance [16].

The mutation of β-catenin that is often observed in human colorectal cancers and the increased risk of malignancy associated with Peutz–Jeghers syndrome suggest that disrupted cell polarization may contribute to neoplasia. The association of transcription factor-dependent E-cadherin repression with the epithelial–mesenchymal transition that is typical of invasive cancer [17] and the strong correlation between the loss of E-cadherin expression and the invasive phenotype of many human gastrointestinal neoplasms [18,19] further support this concept. Conversely, loss of E-cadherin allows unbound β-catenin to activate gene transcription. Cytosolic β-catenin activity is normally down-regulated by the adenomatous polyposis coli (APC) protein, explaining why mutations in APC lead to enhanced β-catenin activity and growth of adenomas in patients with familial adenomatous polyposis, as well as in those with spontaneous somatic APC mutations [20,21]. Clearly many functional connections exist between cell adhesion, polarity, growth, and tumor invasion (see detailed discussions in Chapter 24).

Structure of intercellular junctions

All polarized epithelia share a set of distinct intercellular junctions. These include, from the lumenal aspect, the tight and adherens junctions, which form continuous circumferential contacts, and, below these, desmosomes and gap junctions, which form macular or spot contacts. Together these junctions maintain polarity, seal the paracellular space, provide intercellular communication, and stabilize the monolayer.

A critical event in the generation and maintenance of cell polarity is the assembly of the apical junctional complex, which is composed of the adherens junction and the tight junction. The latter defines the boundary between apical and basolateral membrane domains; it prevents the mixing of transmembrane proteins and outer leaflet membrane

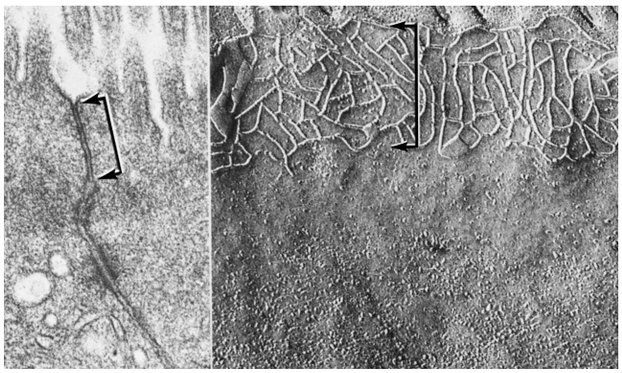

Figure 8.4 Tight junction ultrastructure. Electron microscopic appearance of the tight junction of a small intestinal absorptive epithelial cell. The transmission electron micrograph **(left)** shows that the tight junction is a zone of closely apposed cell membranes. Freeze-fracture electron microscopy **(right)** reveals the dense interconnecting network of strands that define the tight junction.

lipids between these domains. When examined by electron microscopy, tight junctions appear as 100- to 300-nm-deep zones where adjacent cells closely abut (Fig. 8.4). Series of punctate fusions or "kisses" between these plasma membranes form a seal between adjacent cells. These fusion sites are arrayed in a linear anastomosing fashion around the cell and correspond to the net-like series of grooves and strands seen in freeze-fracture replicas of epithelial cells (Fig. 8.4). The strands are formed by members of the claudin family of tetraspanning cell–cell adhesion proteins [22], which make contact in the intercellular space and define the ion selectivity of flow across the tight junction [23–25].

Another tetraspanning membrane protein, occludin, is present in tight junction strands, in intracellular vesicles, and, in some cell types, along the lateral membranes. Although occludin knockout mice are viable and appear to have intact tight junctions [26], abundant in vitro and in vivo data suggest that occludin plays a critical role in the organization and regulation of tight junctions [27–32] and, potentially, in the maintenance of the differentiated epithelial phenotype [33]. Like occludin, several members of the junctional adhesion molecule (JAM) family of proteins localize to tight junctions and along lateral membranes. JAM proteins appear to be necessary for the transepithelial migration of inflammatory cells [34–36]. A variety of other transmembrane proteins, including the coxsackievirus and adenovirus receptor [37,38], are also sequestered within tight junctions.

The cytoplasmic face of tight junctions contains a large number of peripheral membrane proteins that fall into different categories. Various peripheral membrane proteins link transmembrane proteins to the actin cytoskeleton, help to establish cell polarity, define specialized zones for vesicle targeting, or regulate gene transcription. An extensive review of these proteins is beyond the scope of this chapter.

Of special interest are the numerous proteins, such as ZO-1, with multiple protein interaction (PDZ) domains [39]. Although incompletely understood, ZO-1 appears to play a unique role in tight junction assembly and regulation. Initially recruited to nascent adherens junctions through interactions with α-catenin, ZO-1 is present from the earliest stages of tight junction assembly, prompting the suggestion that ZO-1 defines the site of tight junction assembly. A report that tight junction assembly is delayed in ZO-1 knockout epithelial cells gives credence to this idea [40]. ZO-1 may also serve as a scaffold for the assembly of multiprotein complexes. For example, ZO-1 interacts with claudin proteins, occludin, and actin filaments [41–43] and binds to other peripheral membrane proteins, such as the myosin-binding protein cingulin [44]. Most striking, ZO-1 and the related protein ZO-2 can each determine whether and where claudin polymerization occurs [45]. This multitude of protein–protein interactions has led to the hypothesis that the tight junction itself is composed of a stable multiprotein complex. However, measurement of unique rates of protein exchange within the tight junction, as well as between junctional and cytoplasmic pools, contradicts this appealing concept [46].

Directly below the tight junction lies the adherens (or intermediate) junction, in which the lateral membranes of adjacent cells lie parallel to each other and adhere by means of E-cadherin. At this site, the perijunctional ring of actin and myosin interacts with E-cadherin through α-actinin, vinculin, and α- and β-catenin. This perijunctional actomyosin ring is also essential to the maintenance of the tight junction [29,47]. Directly below the adherens junctions are desmosomes (Fig. 8.2). Distant relatives of the cadherin family, desmogelins and desmocollins, form cell contacts at desmosomes. These transmembrane glycoproteins associate with intermediate filaments rather than with actin filaments. Consequently, they anchor the cytokeratin-based cytoskeleton between neighboring cells and provide resistance to mechanical stress. Keratin gene mutations in some patients with inflammatory bowel disease and the spontaneous chronic colitis that develops in keratin-8 knockout mice emphasize the importance of keratin proteins in epithelial function [48,49].

To varying degrees, all epithelial cells of the gastrointestinal tract express gap junctions. At the site of the gap junction the cytoplasm of adjacent cells is in physical continuity through transmembrane channels formed by members of the connexin protein family [50]. Six connexin molecules assemble on each membrane to form a channel and, by adhering across the paracellular space, they create a lumen isolated from the extracellular space. Signaling molecules up to about 1500 Da (e.g., Ca^{2+}, inositol triphosphate) and small nucleotides can diffuse freely between cells and coordinate

physiological responses. There are numerous connexin genes in humans and each shows organ-specific diversity, which allows for organ-specific regulation of communication. Gap junctions coordinate epithelial function by allowing sheets of cells to behave as syncytia, for example coordinating exocytosis of zymogen granules from the pancreas (see Chapter 15). Gap junction communication in the liver, working through intracellular Ca^{2+} waves, modifies bile secretion in response to glucagon and vasopressin. Gap junctions are often mutated or down-regulated in gastrointestinal cancers [51], probably reflecting the aberrant intercellular communication that is characteristic of the neoplastic phenotype.

Polarized protein delivery

Plasma membrane proteins and secreted proteins pass through a series of distinct vesicular compartments as they are sorted to either the apical or basolateral surface. They share a common site of synthesis on ribosomes bound to the rough endoplasmic reticulum and undergo posttranslational modification (e.g., glycosylation) in the Golgi apparatus. Membrane proteins and secreted proteins are then sorted into distinct vesicles in the trans-Golgi network (Fig. 8.2). In most cells, proteins destined for the basolateral surface are delivered directly to that domain; studies using live cell imaging have suggested that this basolateral delivery may even be targeted to specific sites along the lateral membrane [52]. Detailed analysis of basolaterally targeted proteins has shown that specific amino acid sequences located within the cytoplasmic tail are sufficient to direct basolateral delivery [53]. Several of these sequences, including those with conserved tyrosine residues, are sorted by the epithelial adapter protein AP-1B [54]. This protein selects cargo destined for the basolateral membrane and also coordinates the assembly of the exocytic machinery necessary for fusion of transport vesicles with the plasma membrane. The exocytic machinery includes 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 [55,56]. Although not yet identified, other adapter proteins must also exist, because the sorting of E-cadherin, Na^+,K^+-ATPase, and proteins with dileucine-containing basolateral targeting motifs is AP-1B independent.

In contrast to basolateral proteins, apically targeted proteins are transported by both direct and indirect pathways [57,58]. Proteins that traffic directly to the apical membrane include those that associate with glycolipid- and cholesterol-rich membrane domains, such as the brush border hydrolase sucrose-isomaltase, as well as proteins that are targeted independently of these membrane domains, such as lactase–phlorizin hydrolase. Dependence on actin also differentiates these two direct transport pathways, as transport of sucrose-isomaltase occurs along actin tracks and is inhibited by actin depolymerization, whereas lactase–phlorizin hydrolase transport is actin independent. The targeting motifs that direct apical delivery have been more difficult to identify than their basolateral counterparts. Thus far, ectodomain glycosylation sites and transmembrane protein domains, including those that allow association with glycolipid- and cholesterol-rich membranes, have been implicated in apical targeting [59].

Direct trafficking to the apical membrane is not used by all proteins nor in all cell types. In hepatocytes, for example, all membrane proteins are first delivered to the basolateral surface. The apically destined proteins are then transcytosed to the apical, or canalicular, surface. A simple example of this type of sorting in hepatocytes and intestinal epithelia is provided by the polyimmunoglobulin (IgA) receptor, which binds IgA on the basal surface and is then transcytosed and released as secretory component into bile or the intestinal lumen [60]. In addition to specific targeting sequences that direct basolateral and then apical delivery, transcytosis also requires microtubules, which serve as tracks for the movement of transport vesicles from basolateral to apical surfaces. Why apical proteins without specific basolateral duties take this indirect pathway remains unclear. However, this mechanism is useful for the redistribution of apical proteins errantly targeted to the basolateral membrane, as well as for the sorting of membrane proteins during the initial stages of epithelial polarization.

Maintenance of membrane domains

Once delivered to the correct plasma membrane domain, proteins can be retained through 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. The complex functions of the apical membrane require intricate structures, such as parietal cell secretory canaliculi (see Chapter 13) and enterocyte microvilli. Assembly and maintenance of these membrane domains depends, in part, on ezrin–radixin–moesin (ERM) proteins, which play a critical role in the organization of apical, or free, membrane domains in species as diverse as *C. elegans*, *Drosophila*, and mammals, and in multiple cell types from epithelia to lymphocytes [61]. These highly conserved cytoskeletal proteins link membrane proteins to the actin cytoskeleton by way of an amino-terminal cargo-binding domain and a carboxy-terminal actin-binding domain. The cargo-binding domain can interact with transmembrane proteins either directly or through accessory proteins, such as NHERF-1, NHERF-2, and PDZK1 [62–67]. These accessory proteins can also anchor protein kinases (e.g., protein kinase A), thus serving as a scaffold for the organization of signaling complexes [66,68,69]. For example, the cystic fibrosis transmembrane regulator (CFTR) is bound to PDZ domains in NHERF-1 or NHERF-2. These interactions stabilize CFTR at the apical membrane and tether CFTR to protein kinase A, thereby enhancing the ability of protein kinase A to activate CFTR [68,70]. Ezrin-dependent mechanisms have also been implicated in

stimulated apical delivery of the apical Na^+/H^+ exchanger NHE3, after the initiation of Na^+/glucose cotransport in enterocytes, and in the acute surface delivery of the H^+,K^+-ATPase in histamine-stimulated parietal cells [71,72]. Studies in knockout mice lacking ezrin, the only ERM protein expressed in enterocytes, confirm the central role of this protein in organizing the apical membrane [73]. Enterocytes in these mice develop only primitive microvilli and fail to correctly target some proteins to the apical membrane. Moreover, the villi are irregularly shaped, with intravillous lumens and fused profiles. Not surprisingly, these mice fail to wean and die in the early postnatal period.

Organization of the cytoskeleton

The cytoskeleton is considered here in the context of the villous absorptive enterocyte, a cell type that has become an important model for studies of cytoskeletal structure and function in nonmuscle cells. The cytoskeletal organization of columnar epithelia throughout the gastrointestinal tract exhibits only relatively minor differences. The stabilization of epithelial cell structure first requires support for the tall columnar shape; in the absence of the cytoskeleton, a sphere would have the most thermodynamically favorable properties. Maintenance of cell shape is primarily a function of the actin microfilaments that form a network beneath the entire plasma membrane (Fig. 8.2). Bundles of 20–30 actin filaments also form the submembranous cores responsible for microvillous architecture. Within these cores, individual microfilaments are cross-linked to each other by actin-bundling proteins and to the microvillous membrane by a member of the myosin family, myosin IA. The microvillous actin bundles jut into the apical pole of the cell and associate with a terminal web composed of actin and type II myosin that interfaces with the apical junctional complex. The tension of this perijunctional actomyosin ring can be adjusted in response to physiological and pathophysiological stimuli, allowing modulation of epithelial barrier function (see Regulation of barrier function by physiological stimuli and Dysregulation of barrier function in intact epithelium).

Cables composed of intermediate filaments course through the cells and function as support cables for structural buttressing. Such tonofilament cables associate with plasma membranes and insert into the desmosomes. This network of intercellular junctions and intermediate filaments is required for the intestinal epithelia to interface with the turbulent environment of the gut lumen.

Microtubules also form a unique array in polarized epithelial cells. In contrast to nonpolarized cells in which microtubules radiate from a single microtubule organizing center adjacent to the nucleus, microtubules in polarized epithelia are aligned apicobasally. With the assistance of microtubule-dependent motor proteins, kinesins and dyneins, which can transport vesicles along microtubule arrays, membrane-bound structures are trafficked throughout the cell. This microtubule network is particularly important in transcytosis; microtubule disruption markedly slows this process.

Basement membrane

In addition to its important structural and supportive roles, the basement membrane serves as a source of signals for inducing epithelial cell polarity. All alimentary epithelia reside on a basement membrane that is 20–40 nm deep, consists of a fibrillar network, and rests on an underlying extracellular matrix (Fig. 8.1). The basement membrane in the alimentary tract, similar to basement membranes in other tissues, is composed primarily of laminin, heparan sulfate proteoglycans, and type IV collagen. Minor constituents that may be functionally important include thrombospondin and entactin/nidogen-1.

Laminin exhibits specific binding sites for type IV collagen, heparan sulfate proteoglycans, cell surface laminin receptors, and entactin. Other matrix components also possess binding sites for additional components, adding to the complexity of interactions between the epithelial cell and its surrounding environment. The major proteoglycans of the basement membrane, heparan sulfate proteoglycans, consist of long chains of glycosaminoglycans linked to a protein core. The structure of these massive molecules is often likened to a test-tube brush, with the bristles representing the glycosaminoglycan extensions. Proteoglycans probably organize water within the basement membrane, hydrating this environment through their capacity to bind water, and possibly imparting solute-sieving characteristics under conditions of bulk water flow. Although a controversial concept, impaired water and electrolyte absorption may cause the watery diarrhea seen in collagenous colitis, a disease that manifests thickening of the basement membrane [74,75].

Type IV collagen originates as a triple-stranded helical molecule, which, unlike other collagens, does not have its propeptides sheared from it after deposition in the extracellular space. Partially as a result of this, collagen IV does not cross-link into dense fibrils; instead it assumes a loose, net-like structure by associating with other collagen IV molecules. This mesh-like structure of collagen IV may provide the basic structure to the basement membrane.

Basement membrane components can exert significant effects on epithelia, including modulation of proliferation, adhesion, migration, differentiation, and even barrier function. In the intestine, type IV collagen is produced primarily by mesenchymal cells, heparan sulfate proteoglycans by epithelial cells, and laminin by both mesenchymal and epithelial cells. Many basement membrane components bind to a family of epithelial cell surface molecules, the integrins, which are connected to the actin cytoskeleton through linker proteins. Through such associations, structural elements within the cell are able to connect with, and potentially be influenced by, events occurring within the basement membrane and more deeply within the extracellular matrix.

Epithelial barriers

Various sites in the alimentary tract are threatened by the presence of acid, bile, undigested potentially antigenic proteins, bacterial proteins, and live bacteria. It is not surprising, therefore, that the epithelial barrier consists of numerous components that prevent injury from these varying insults. Some of these components are site specific and others are universally present. To discuss these barriers, we arbitrarily divide them into two major categories: those that are extrinsic to the epithelium (although in some instances produced by the epithelium) and those provided by the physical presence of the epithelium, which we describe as intrinsic barriers.

Extrinsic barriers

Extrinsic barriers confront the microenvironment overlying the epithelia.

Mucus

All alimentary epithelia are coated with a layer of mucus that protects against bacteria and surface shear forces (Fig. 8.5). Most surfaces, including those of the stomach, the intestine, the pancreatobiliary ducts, and the gallbladder, contain specialized cell types that synthesize, package, and secrete mucin. In the esophagus, mucin is derived from small glands that lie under the epithelium and connect to the lumen by way of delicate ducts. Although the precise chemical nature of mucus varies throughout the alimentary tract, the various mucin molecules share common features. They are viscous, polydispersed glycoproteins (250–20 000 kDa) of which about 80% of the mass is carbohydrate. At least eight human mucin-producing genes (*MUC*) have been identified. *MUC2* is the predominant form in intestinal and colonic surfaces. Esophageal *MUC2* expression can be a marker of Barrett esophagus: In inflammatory bowel disease and cancer, altered *MUC2* expression may occur.

Mucins act as a barrier by behaving as a viscous hydrated gel which undoubtedly attenuates shear forces that the epithelium would otherwise experience from lumenal particulates that are driven down the alimentary tract by peristaltic propulsion. In addition, carbohydrate groups on mucin molecules may bind to bacterial surfaces, thereby inhibiting surface adhesion and colonization. In some instances, mucin carbohydrates replicate epithelial carbohydrate binding sites to which bacteria can attach, presumably preventing colonization by acting as a molecular decoy. Given their extensive glycosylation, mucins can cross-link and aggregate bacteria. Such aggregation presumably aids in bacterial clearance. Exposure of epithelial surfaces to threats such as bacterial toxins and noxious chemicals often results in a reflexive secretory release of mucins, further augmenting their protective effects. Mucin depletion is a nonspecific histological indicator of ongoing injury often noted in biopsy specimens. The expression of mucin genes and the secretion of mucin by goblet cells respond to intestinal microbes and host-derived inflammatory mediators and are altered by infections, such as *Helicobacter pylori* in the stomach. Consistent with the essential protective role ascribed to mucins, mice deficient in Muc2, which is down-regulated in human inflammatory bowel disease, develop spontaneous colitis [76].

It has been observed that the diffusion coefficients of hydrophilic molecules are substantially lower in mucin than in free solution. Some researchers have suggested that this alteration would diminish contact between the epithelial surface and lumenal threats such as acid. Given the depth of the mucin layer and the duration that lumenal contents are in contact with the epithelium, however, small molecules probably have sufficient time to equilibrate within the mucous gel.

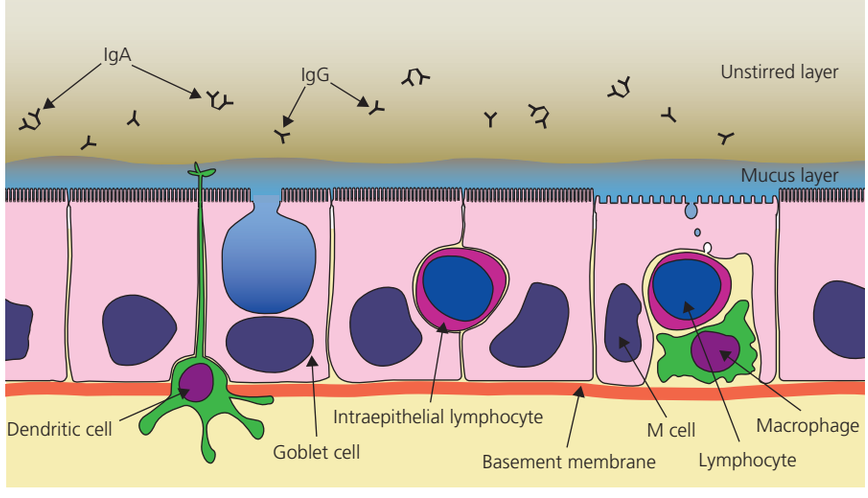

Figure 8.5 Epithelial barriers. The intestinal epithelium is the focal point around which the interaction of lumenal material and subepithelial cells, including those of the immune system, is organized. In addition to conducting vectorial transport and maintaining a cellular barrier, epithelia also contribute to host defense by elaborating mucus and transporting immunoglubulins.

Unstirred layer

Peristalsis creates a rather turbulent environment in the gastrointestinal lumen. This turbulence or convective force does not extend to the epithelial surface. The best estimate is that an aqueous layer with a thickness of 300–800 μm lies above the epithelia. This apical microenvironment is still, that is, it is unstirred. Convective forces then increase rapidly with increasing distance from the mucosal surface. Although not fully determined, the physiological impact of the unstirred layer on the immediate environment to which epithelial cells are exposed must be profound.

The presence and volume of the unstirred layer may significantly affect nutrient absorption. For example, if the epithelial transport system can transport a given nutrient more rapidly than the nutrient can diffuse into the unstirred layer, diffusion becomes the rate-limiting step in absorption. In contrast, polymeric nutrients that are broken down into monomers at the brush border (e.g., carbohydrates) may be formed at very high rates within the unstirred layer. Should local nutrient concentrations exceed transporter capacity, this in itself represents the rate-limiting step in absorption. Because the concentration of a molecule at the epithelial surface is unlikely to be equivalent to the concentration of that molecule in the center of the lumen, the challenge of measuring solute concentrations within the unstirred layer obscures the biophysics of many transport reactions within the intact intestine. Thus, the confounding issue of the unstirred layer must be confronted when analyzing molecular transport kinetics.

Secreted immunoglobulins

The epithelial surfaces in the alimentary tract are for the most part bathed by secretory IgA and IgG (Fig. 8.5). Secretory IgA is produced as a dimer by lamina propria plasma cells, transcytosed by the polyimmunoglublin receptor, and released into the lumen as a consequence of proteolytic clipping of the receptor. IgG is transcytosed by the neonatal Fc receptor [77]. By binding to lumenal threats such as pathogenic bacteria or toxins, secretory IgA and IgG act as barriers to antigenic material [78]. Although of extreme importance in host defense, this barrier is highly specific and dependent on prior antigenic sensitization [79]. Secretory IgA binding to the surfaces of pathogens may not only impede pathogen–epithelial interactions over most of the epithelial surface but also actually enhance pathogen–epithelial interactions at selected sites such as the M cells [80], a cell type responsible for the afferent limb of intestinal immunity.

Secreted bicarbonate

In contrast to the extrinsic barriers discussed previously, some extrinsic barriers have regional variation. One well-described example is the net bicarbonate (HCO_3^-) secretion by epithelia that interface with the acidic lumenal environment of the stomach. The proximal duodenum also must protect itself from gastric acid, as pancreatic bicarbonate secretions enter the gut lumen further downstream. The effects of epithelial bicarbonate secretion on the stomach and the duodenum are of central interest [81]. Clearly the first line of defense is the neutralization of intralumenal acid before it reaches the epithelium. As a result, the unstirred layer overlying the epithelium has a pH much closer to neutral than layers more distant from the mucosal surface [82–85]. In vivo confocal imaging of the gastric juxtamucosal alkaline layer in anesthetized mice shows that the pH set point of this layer is determined by the balance between epithelial H^+ and HCO_3^- secretion and not by the thickness of the unstirred layer [86]. Further, the presence or absence of the gastric mucus layer appears to have no effect on surface pH. Thus, apical HCO_3^- secretion by gastric surface foveolar cells and duodenal villous absorptive cells is an important example of a highly specific and regionally localized extrinsic epithelial barrier. Intracellular HCO_3^- may also play an important role in cytoprotection of the duodenal epithelium [87], providing further evidence of the complex interactions between multiple ion transporters [85].

Antimicrobial peptides

Gut epithelial cells produce and secrete peptides with antimicrobial functions. Several classes of peptides have been isolated from humans, including members of the defensin, cathelicidin, and histatin families. Paneth cells at the base of the crypts in the small intestine and ascending colon release certain enzymes with antimicrobial activity, including lysozyme and type II phospholipase A_2. Paneth cells also produce defensin peptides [88], of which some are released specifically in response to bacteria [89,90]. Some defensins not only exert direct antibacterial activity [91] but also orchestrate a protective host response by signaling to immune cells and by stimulating apical Cl^- and water secretion to flush the lumen [92,93]. The observations that the susceptibility gene *NOD2* for Crohn's disease is required for expression of some defensins [94] and that decreased defensin gene copy number may be related to colonic Crohn's disease [95] suggest that disruption of essential protective defensin functions may contribute to the pathogenesis of Crohn's disease [96].

Intrinsic barriers

The contribution and presence of specific extrinsic barriers vary in different regions of the gastrointestinal tract. In contrast, the intrinsic barrier is formed by the continuous sheet of epithelial cells that lines the entire gastrointestinal tract. This uninterrupted epithelial layer separates lumenal material from the subepithelial space.

Classically, discussions of epithelial barrier function consider two routes by which material may traverse the barrier;

the transcellular and paracellular pathways (Fig. 8.3). The exact physical site where solutes cross the epithelium was a topic of considerable controversy until it was recognized that proteins insert into and form channels across lipid bilayers. Similarly, the paracellular pathway was thought to be impermeable and unregulated, a misconception perpetuated well into the 20th century because of the static appearance of intercellular contacts seen in early electron micrographs. Indeed, even the term "tight" junction is a misnomer, as tight junctions form a transcellular barrier that selectively allows paracellular flux of ions and small molecules. Our present understanding of transcellular and paracellular transport emerged along with the recognition that both are physiologically regulated and vary widely in different tissues. Transepithelial transport of hydrophilic solutes along these two pathways will be discussed below. The movement of water and hydrophobic molecules across the epithelium poses unique challenges. Hydrophobic compounds can cross epithelial cells directly by virtue of their solubility in the lipid bilayer. For example, saturated fatty acids cross jejunal epithelial cell microvillous membranes at rates more than 10^{11}-fold faster than they diffuse in aqueous solution. Fat absorption, the most physiologically important transepithelial movement of hydrophobic compounds, is considered separately (see Chapter 18). Transmucosal water movement, although incompletely understood, is discussed later in this chapter (see Water movement across the epithelial barrier).

Transcellular pathway

The transcellular pathway is highly restrictive to the passive flow of hydrophilic solutes. To traverse an epithelial cell, an ion or other hydrophilic solute must interact with three barriers in series: the apical membrane, the cytosol, and the basolateral membrane (Fig. 8.3). Although the cytosol has the potential to limit transcellular molecular flux, the two plasma membranes are the key barriers that restrict the passive movement of hydrophilic solutes across epithelial cells.

The lipid bilayers of the apical and basolateral membranes prevent massive flux of hydrophilic solutes and preserve transmembrane electrochemical gradients; the high resistance to passive ion flow across model lipid bilayers approaches impermeability. Biological membranes, which are composed of lipid bilayers and membrane proteins, are slightly less impermeable, but still capable of considerable resistances to passive ion flow that are several orders of magnitude greater than those of intact alimentary epithelial cell membranes. Integral membrane proteins such as transporters, pumps, and channels contribute to the relative permeability of alimentary epithelia. These proteins serve a critical need as the transmembrane movement of ions is essential for cellular homeostasis.

As discussed previously, vectorial transport relies on the polarized delivery of transporters, pumps, and channels to the apical and basolateral membranes. The specific example of glucose absorption in the small intestine will be considered here (Fig. 8.3). For detailed discussions of absorption and secretion see Chapters 13–20. Glucose is actively transported across the apical plasma membrane by the Na^+/glucose cotransporter SGLT1 [1]. The absence of this critical transporter results in glucose–galactose malabsorption, an autosomal recessive disease characterized by the failure to absorb these carbohydrates from the diet [97]. The energy source that allows efficient uptake of lumenal glucose by SGLT1 is the high extracellular, and low intracellular, Na^+ concentration; two Na^+ ions are absorbed along with each glucose molecule. The apical positioning of SGLT1 ensures that glucose is never secreted into the lumen, as the Na^+ gradient makes this thermodynamically unfavorable. The necessity for this apical SGLT1 targeting was clearly shown by defects in transepithelial Na^+ and glucose transport when an SGLT1 molecule engineered to include a basolateral targeting sequence was transgenically expressed in intestinal epithelial cells [2]. Once within the cytosol, Na^+ and glucose diffuse to the basolateral membrane. Here, Na^+ ions are pumped out of the cell and into the subepithelial and basolateral interstitium by the Na^+,K^+-ATPase, and glucose molecules diffuse across the membrane in a concentration-dependent manner facilitated by the glucose transporter GLUT2. The basolateral positioning of GLUT2 allows it to operate in the reverse direction, bringing glucose into the epithelial cell from the subepithelial interstitium, in the absence of lumenal nutrients. SGLT1 and GLUT2 are specific transporters for sugars; other transporters with similar properties manage the transport of amino acids and other nutrients. It should be apparent that the subepithelial deposition of Na^+ and glucose results in an osmotic gradient that drives water absorption (Fig. 8.3). This is exemplified by the severe diarrhea and dehydration that are usually fatal in patients with glucose–galactose malabsorption unless these sugars are eliminated from the diet (see Chapter 51).

Paracellular pathway

The paracellular pathway is a major pathway for passive solute permeation. Although plasma membranes tend toward high resistance, alimentary epithelia, with the exception of the esophageal epithelium, have low net resistance, meaning that they are relatively leaky. Detailed molecular, biophysical, and morphological analyses have shown that the paracellular pathway is largely responsible for the leakiness of these epithelia.

The paracellular pathway consists of the apical intercellular tight junction and the underlying paracellular space. Under most conditions the tight junctions are the rate-limiting barrier, restricting passive movement of hydrophilic solutes through the paracellular space. The permeability of tight junctions to ions and solutes varies between tissues and even between sites within tissues (e.g., crypt vs villus) and, in the resting physiological state, tight junctions may leak small

quantities of molecules the size of monosaccharides and disaccharides. The degree of this leakiness is regulated and, as will be described, greatly increased under some conditions [30,98,99].

The selective ionic permeability of the tight junction is largely defined by the expression of specific members of the claudin family of proteins [25,100,101]. Familial hypomagnesemia, a disease of deficient renal tubular Mg^{2+} reabsorption resulting from the loss of a single claudin isoform [23,102], is the best example of this selective mechanism. Although similar genetic losses of claudin family members have not been described in gastrointestinal disease, the unique claudin protein distributions along the length of the gastrointestinal tract, as well as along the crypt–villus axis, explain, at least in part, the variation in paracellular permeability at different sites [103]. Changes in the specific pattern of claudins expressed by intestinal epithelia may also contribute to disease. For example, inflammatory bowel disease is associated with increased claudin-2 expression and decreased claudin-5 and -8 expression [104–107]. In vitro studies suggest that claudin-2 expression increases tight junction permeability [101,108], which is consistent with the reported increased permeability in inflammatory bowel disease.

Water movement across the epithelial barrier

Despite the obvious importance of fluid transport across gastrointestinal epithelia, controversy remains about the relative importance of the transcellular vs paracellular routes. One route for transcellular water movement is through transmembrane channels created by members of the aquaporin protein family [109]. These small integral membrane proteins are well studied in tissues specialized for regulated water transport, such as the collecting duct of the kidney. Although aquaporins are expressed in gastrointestinal epithelia, and expression may be reduced in colitis [110], their contribution to water movement in the gastrointestinal tract remains unknown. Numerous mouse models deficient in specific aquaporins have shown defective water movement in salivary glands, pancreas, and liver, but only minor difficulty in handling water in the intestine [109,111,112]. Other models have suggested that the apical Na^+/glucose cotransporter SGLT1 serves as a molecular water pump, carrying a large number of water molecules with each glucose molecule transported [113]. However, the osmotic gradient discussed previously can completely explain the enhanced water absorption induced by Na^+/glucose cotransport, thereby raising some doubt as to the quantitative contribution of this pathway [114,115]. Transcellular water movement may also occur across lipid membranes; water movement is much less restricted than that of hydrophilic solutes. However, the mechanism by which water permeates biological membranes in the absence of specific channels is uncertain.

Although there is general agreement that water flux requires both transcellular and paracellular routes, the relative contribution of each is controversial. Some data suggest that about 50% of the water absorption stimulated by Na^+/glucose absorption is paracellular. Data from a variety of other epithelia show that interepithelial differences in hydraulic conductivity, a measure of force-induced water flux, correlate reasonably well with transepithelial electrical resistance. Finally, data suggest that a leaky paracellular pathway is necessary to support the massive water secretion that accompanies acute immune-mediated diarrhea [30]. These observations suggest that the paracellular pathway is a major route for water flow across intestinal epithelia whether such flow is driven by hydrostatic or osmotic pressures [116–118].

Transport of xenobiotics

The gastrointestinal tract, particularly the small intestine, is actively involved in the transport and metabolism of foreign chemical compounds, including environmental toxins and therapeutic agents. Many of these so-called xenobiotics are absorbed transcellularly; some are lipophilic and dissolve easily in lipid membranes. Others take advantage of apical uptake pathways that are normally expressed, such as the apical Na^+-dependent bile salt transporter or members of the organic anion transporting polypeptide family [3,119,120]. Basolateral transporters, such as multidrug resistance associated protein 3, ABCC3, may then allow xenobiotics to traverse the basolateral membrane [121]. A significant fraction of these compounds may never reach the basolateral membrane, as members of the cytochrome P450 system expressed in enterocytes may contribute a "first-pass" effect of their own [122]. Analogous to the effects of drugs metabolized by the hepatic cytochrome P450 system, enterocyte nuclear receptors can up-regulate expression of enterocyte drug transporters and cytochrome P450 enzymes [3,119]. These layers of regulation not only challenge the maintenance of steady-state drug levels but also increase the potential for drug interactions [123]. Dysregulation of xenobiotic metabolism and transporter activity may be a pathogenetic mechanism in inflammatory bowel disease [124]. Genetic polymorphisms in these transporter proteins may also lead to significant variation in clinical responses [125,126].

In addition to metabolic clearance, active secretion rapidly clears many xenobiotics from the intestine. This secretory activity, which is primarily mediated by MDR1, an apical multidrug resistance transporter family member [124,127], exhibits significant interindividual variability, similar to absorption and metabolism. Interestingly, *MDR1* mutations have been associated with inflammatory bowel disease in some patient populations [128], and a knockout mouse lacking *mdr1* spontaneously develops colitis [129,130]. *MDR1* polymorphisms may also be related to disease behavior in ulcerative colitis [127,131], suggesting that defective export of an unidentified xenobiotic contributes to the pathophysiology of intestinal disease [132].

Epithelial homeostasis and responses to disease and injury

Commensal bacteria

Bacteria normally colonize the entire human gastrointestinal tract, with the highest concentration and number of species in the colon. Although bacterial cells outnumber the cells of the human body by about 10-fold, most of these organisms belong to only three evolutionary divisions that are, at least in part, modified in a host-specific manner [133–135] (see Chapter 25). These nonpathogenic bacteria are termed commensal, to distinguish them from well-characterized pathogenic species such as *Salmonella*, *Shigella*, and *Clostridium difficile*. It appears that the commensal host–bacteria relationship is beneficial [136], as normal gut function is highly dependent on resident bacteria. Such probiotic effects take several forms, including competing with pathogens for attachment to the epithelial surface, triggering intracellular signal transduction events that limit disease, and inciting the epithelium to release antimicrobial compounds [137–139]. Studies in germ-free mice show that the normal development of immune cell lineages in the bone marrow and the lamina propria and local humoral defense depends on the presence of the commensal bacteria in the gut [140,141]. Inductive effects on epithelial cell gene transcription have also been observed [142]. For example, introducing commensal *Bacteroides* species into germ-free mice extensively alters the bacterial and epithelial transcription profiles toward gene products that enhance nutrient uptake and metabolism [143–145], revealing a possible association with obesity [146,147].

Although the molecular mechanisms remain poorly understood, published reports of the probiotic effects of commensal bacteria are increasing. For example, sterilization of the gut greatly enhances disease severity in some murine models of colitis [138,139,148]. This effect appears to be at least partially mediated by toll-like receptors (TLRs) that specifically recognize bacterial products [138,139,149–152]. Beneficial effects of probiotics have been reported in experimental disease as well as in ulcerative colitis and pouchitis [153–155]. The effects of bacteria on intestinal function are discussed in detail in Chapters 25, 48, 49, and 52.

Physiological epithelial injury

Epithelial injury is most readily apparent when gaps within the epithelium such as erosions or ulcerations are present. However, because the gut has a remarkable ability for repair, many forms of focal acute injury do not result in functionally significant defects. One example is the rapid sealing of wounds that must occur during the physiologically normal turnover of gastrointestinal epithelia. Gut epithelial cells turn over, on average, once each week through coordinated proliferation, migration, apoptosis, and sloughing. For example, small intestinal enterocytes arise from the stem cell compartment, i.e., the crypt, migrate upward through the proliferative zone, and undergo an ordered process of differentiation as their phenotype is modified from undifferentiated secretory cell to fully differentiated villous absorptive cell. They are then sloughed from the villous surface. Membrane proteins, with half-lives considerably shorter than that of the epithelial cell, also turn over. The composition of these proteins changes remarkably during the process of differentiation. Lipid turnover undoubtedly occurs as well but technical challenges hinder its documentation. Perhaps most remarkable in this continual process of renewal is that, at sites of epithelial cell detachment, the barrier remains intact [156,157]. In vivo imaging studies suggest that a presently undefined substance fills the "gap" left as the epithelial cell exits the villus, preventing diffusion of lumenal material into the subepithelial villous core [156,158]. The renewal process must then be completed by some form of wound closure, possibly involving cytoskeletal contraction [158–161].

Regulation of barrier function by physiological stimuli

Intestinal permeability can be regulated by physiological processes. For example, it is well documented that Na^+/nutrient cotransport enhances the permeability of absorptive tight junctions to molecules the size of amino acids and glucose [99,162,163]. Although the physiological significance of these Na^+/nutrient cotransport-induced increases in tight junction permeability remains controversial, the process probably underlies the observations that, at high lumenal glucose concentrations, both glucose and amino acid absorption exceed the capacity of their respective transcellular transport systems [164–166]. The concept of "solvent drag" offers an explanation [167]. For example, as described previously, active transcellular Na^+ and nutrient absorption result in the development of a transepithelial osmotic gradient that drives water absorption. Solvent drag is the mechanism by which water absorption across the tight junctions with increased permeability allows the solvent (i.e., water) to drag nutrient-sized molecules (i.e., free glucose within the unstirred layer) across the tight junction. In this manner, transcellular absorption is amplified by paracellular absorption [167]. The latter depends on generation of a suitable transepithelial osmotic gradient and increased permeability of tight junctions to small molecules. This mechanism can also enhance paracellular absorption of undegradable compounds, such as D-amino acid-substituted short peptides and creatinine [168,169]. Oral pharmaceutical delivery may benefit from use of this pathway.

Physiological tight junction regulation is initially triggered by the apical Na^+/glucose cotransporter SGLT1 (Fig. 8.3). This initiation of Na^+/glucose cotransport activates a signal transduction pathway that induces increased NHE3-mediated apical Na^+/H^+ exchange, resulting in mild cytoplasmic

alkalinization and enhance Na^+ absorption [170,171]. These events are accompanied by the activation of myosin light chain kinase, which phosphorylates the myosin II regulatory light chain and triggers contraction of the perijunctional actomyosin ring [172]. Although the molecular details of the subsequent tight junction remodeling are not yet defined, it is clear that this myosin II regulatory light chain phosphorylation is required for Na^+/glucose cotransport-induced increases in tight junction permeability.

Dysregulation of barrier function in intact epithelium

Despite an intact epithelium, tight junction permeation to inert solutes is enhanced in many inflammatory, infectious, ischemic, and immune-mediated intestinal diseases [173,174]. For example, permeability defects in celiac sprue can be reversed by a gluten-free diet [175]. Similarly, in both graft-versus-host disease and HIV infection, increased serum lipopolysaccharide levels (reflecting leakage of lumenal contents) correlate with disease severity [176,177].

Permeability defects are also seen in Crohn's disease; increased permeability in patients with inactive disease can predict disease reactivation [178,179]. In addition, permeability defects are present in a subset of healthy first-degree relatives of patients with Crohn's disease [180,181]. This has led to speculation that a primary defect in tight junction barrier function may cause Crohn's disease [181–183]. Despite this, it is clear that the inflammation of Crohn's disease can also cause increased permeability, as barrier function can be restored by treatment with antibodies that neutralize tumor necrosis factor [184]. Conversely, tumor necrosis factor acutely reduces barrier function in cultured intestinal epithelial monolayers and jejunal epithelia of intact mice [116,185–187].

In a remarkable demonstration of how pathophysiological events can hijack physiological regulatory mechanisms, investigators have shown that tumor necrosis factor disrupts the intestinal epithelial tight junction by way of myosin II regulatory light chain phosphorylation [188]. In vitro studies have shown that this is due to both transcriptional and enzymatic activation of myosin light chain kinase [185,186,189,190], and that similar increases in myosin light chain kinase expression and enzymatic activity correlate with disease activity in inflammatory bowel disease [191]. Moreover, in vivo work has shown that myosin light chain kinase-driven loss of barrier function is required for the development of acute tumor necrosis factor-mediated diarrhea [30]. These data have led to the proposal of a disease model in which impaired mucosal barrier function leads to increased leakage of lumenal contents and inappropriate immune stimulation, subsequent interferon-γ and tumor necrosis factor release, and further loss of barrier function. The result is a self-amplifying cycle of barrier dysfunction and inappropriate immune activation [173]. Thus, compromised barrier function may be a critical event in the initial pathogenesis and subsequent exacerbation of inflammatory bowel disease and other intestinal diseases.

Healing of epithelial wounds

The destruction of gastrointestinal epithelial cells, as occurs in erosions and ulcers, leads to the loss of epithelial barrier function. Both the magnitude and duration of injury determine the epithelial response. Wounds representing loss of approximately 1–10 epithelial cells close extremely rapidly, within 30 min or less, by a purse-string closure mechanism [159–161,192]. This response depends on some of the same cytoskeletal mechanisms discussed previously, with small GTPases (e.g., Rho) directing the assembly of a ring of actin cables at the edge of the wound (Fig. 8.6) [159,193]. These cables, which are connected across adjacent cells by intercellular junctions, assemble within minutes of wounding and then begin to contract by a mechanism that requires myosin light chain kinase activity [159]. As noted, some data suggest that a similar purse-string mechanism maintains the epithelium after the extrusion of single apoptotic cells, which is a normal physiological process.

Larger epithelial wounds must also be sealed quickly. The initial rapid cell migration, termed restitution, involves a dramatic cytoskeletally directed modification of cell shape (Fig. 8.6) [194]. The columnar cells bordering a wound, normally tall, spread to become flattened, taking on an almost squamoid appearance and maximizing the basement membrane surface area covered by each cell [194]. Often seen in intestinal endoscopic biopsies, this flattened appearance is an easily recognized marker of ongoing epithelial restitution. Persistent injury also stimulates cell growth. The same stimuli often evoke both motogenic (migration-promoting) and mitogenic (proliferation-promoting) effects [195–199]. Thus, inflammatory mediators and growth factors may promote initial reepithelialization of wounds by initiating restitution, and support this process over extended periods by enhancing cell proliferation.

Interactions of epithelia with subepithelial cells

Gut epithelial function may be modulated by a host of local factors derived from nonepithelial sources, such as growth factors, cytokines, and chemokines, which are discussed in detail in Chapters 4 and 7. Direct interactions between epithelial cells and the immune system also occur; an obvious example involves the M, or microfold, cell [200]. This specialized epithelial cell resides in the convex dome epithelium that overlies mucosal lymphoid follicles. Although indistinguishable from adjacent enterocytes by light microscopy, electron microscopic evaluation shows that the basal membrane of M cells is retracted from the basement membrane, forming a cleft into which lymphocytes and macrophages migrate (Fig. 8.5). M cells actively sample lumenal material by bulk endocytosis; the transport vesicles are then released into the cleft, permitting extremely rapid delivery of

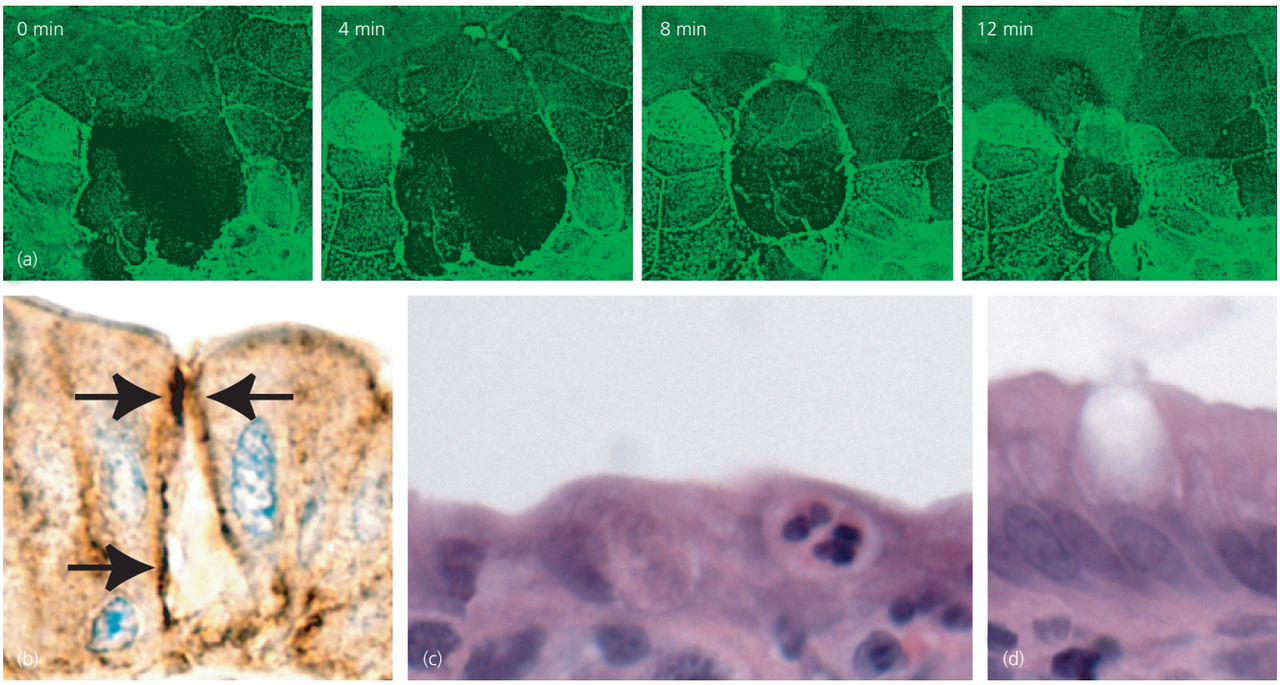

Figure 8.6 Mechanisms of epithelial wound closure. **(a)** A time-lapse series of cultured intestinal epithelial cells expressing fluorescent actin shows that a ring of actin forms to surround a wound within minutes after injury. This ring then contracts in a purse-string manner, rapidly closing the wound and restoring barrier function. **(b)** Immunohistochemical analysis of a small wound present in a patient with Crohn's disease shows that, similar to in vitro experimental wounds, phosphorylated myosin II regulatory light chain is concentrated at the closing edge of the injury site. **(c)** Larger wounds cannot heal by simple purse-string wound closure. This specimen from a patient with ulcerative colitis shows that intestinal epithelia flatten to spread and rapidly reseal the surface, a process called restitution. **(d)** Contrast the flattened shape of epithelia during restitution with the tall columnar shape of epithelial cells in an area of intact mucosa. Panels **(a)** and **(b)** are from Russo et al [159], with permission from Elsevier.

lumenal material to immune cells. Infectious organisms may exploit this pathway as a route of invasion. Other epithelial cells not confined to follicle-associated epithelium may also be capable of transporting antigens to mucosal immune cells [201], and a specialized population of dendritic cells within the ileal lamina propria actually extend slender processes across the tight junction to directly sample lumenal antigens and bacteria (Fig. 8.5) [202–204]. Finally, although not yet well described, additional cell types, beyond epithelial and immune cells, clearly regulate epithelial function [205–207].

Integration of mucosal function

The gastrointestinal mucosa is a complex structure that coordinates a variety of critical functions. These include balancing barrier function with the need for transepithelial movement of ions, nutrients, and antigens; rapid repair of mucosal injuries; and beneficial interactions with the array of mucosal immune cells. The precise integration of these functions centers on the epithelium, which is continuously repaired by rapid wound closure, restitution, and other mechanisms. The gut epithelial barrier, which restricts passive movement of molecules, is complex and dynamic. Maintenance of this barrier depends on the integrity of cellular plasma membranes and intercellular tight junctions. The mucosal barrier also benefits from the contributions of mucus, epithelial secretory products, such as HCO_3^-, and secreted immunoglobulins. Potential threats within the lumen are continuously surveyed and managed by M cells, intraepithelial and lamina propria lymphocytes, dendritic cells, and macrophages. From the examples provided in this chapter, and other chapters in this textbook, it is evident that dysregulation of any of these functions can result in diseases with overlapping clinical presentations. Thus, future studies should aim to better understand the interplay between the intricate systems that comprise the gastrointestinal mucosa.

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