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Review

Intestinal tight junctions and their importance in health and disease: role of dietary lipids

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The function of intestinal tight junctions (TJ) can be measured experimentally, and is modified by influencing the intracellular signals responsible for directing the synthesis, assembly, and reformation of the components of TJ. Altering TJ composition modifies transepithelial electrical resistance, salt and fluid absorption, and possibly membrane composition and permeability. Food constituents modify the function of the intestine, including the TJ. The function of TJ changes in health and disease, and a future therapeutic objective may be to correct altered TJ function by pharmacological or nutritional means. © Elsevier Science Inc. 1997 (J. Nutr. Biochem. 8:2–12, 1997.)

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Introduction

The barrier function of the intestine is maintained by the tight junctions (TJ), also known as the zona occludens (ZO). The number and continuity of TJ increase as enterocytes differentiate.[1] TJ are important to intestinal barrier function, can be regulated, and thereby play a role in nutrient uptake.[2] In the absence of convective water flow, TJ forms a barrier to molecules 11.5Å and greater in Stokes radius.[3] TJ are specialized cell membrane domains at the contact region of neighboring epithelial cells, and can be seen only by electron microscopic examination. Latin terminology was introduced into the cell junction literature by Farquhar and Palade (1963)[4] to describe the shape and total area of membrane-membrane contact at junctions. The term "zonula" is used to described junctions that extend as a belt around the entire cell; "fascia" describes a sheet-like area; "macula" describes a spot or small localized area. ZO, fascia, and macula occludens (ZO, FO, MO) were observed in a variety of epithelial cells. FO and MO exhibit essentially the same substructure as ZO, except that the sealing elements are short segments forming a loose network over the lateral surface of the membrane (*Figure 1*).[5,6]

Whereas plasma membranes have pores with a radius of about 4 Å,[7] epithelia like the intestinal mucosa have larger pores with radii of 30–40 Å.[8] Water and small solutes may pass through an extracellular route between cells, i.e. the paracellular route. Epithelia like the small intestine have a comparatively low electrical resistance. Movement across TJ and through the paracellular route may account for up to 90% of the total movement of substances across some epithelia. For example, at least 85% of passive ion flow across mammalian small intestine occurs by this paracellular route.[9] The proportion of low molecular weight solute such as sugars transported by this pathway depends on its concentration in the intestinal lumen, the maximal transport rate (V_{max}) of its carrier, the function of the TJ, and the degree of associated solvent drag.[10,11] This function can be changed by altering TJ subunit number,[12] or state of openness.[13,14] Nutrients alter TJ permeability,[10,11,15] and this functional change modifies strand architecture and condensation of the perijunctional ring of actin and myosin. These

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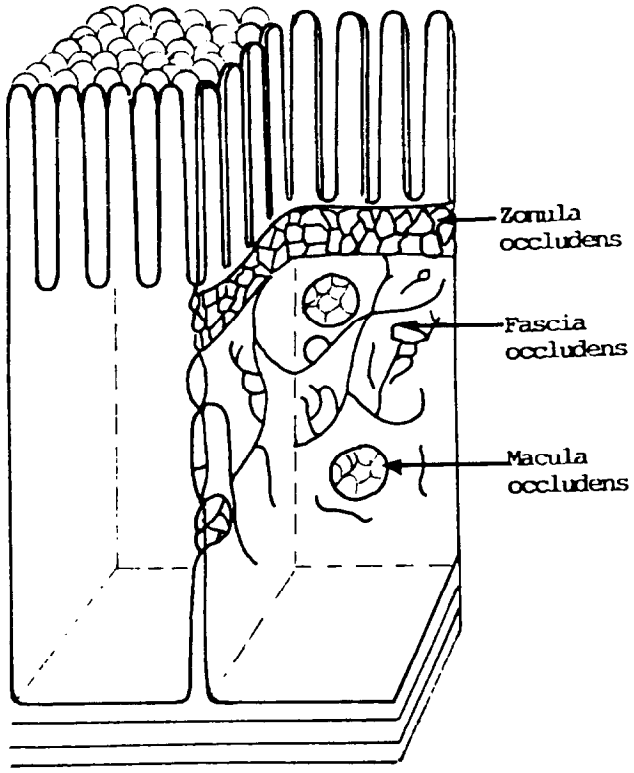

Figure 1 Schematic diagram showing the organization and localization of the three different types of occluding junctions. (Taken with permission from Polak-Charcon, 1991.)

nutrient effects are energy dependent, and may be triggered by turnover of the brush border membrane Na^+-glucose cotransporter, $SGLT_1$.[15]

Cingulin is a peripheral membrane phosphoprotein which links TJ to the cytoskeleton.[16] Actin microfilaments also attach to the TJ.[17,18] Inhibitors of protein synthesis, such as cycloheximide and puromycin, impair the development of TJ.[19–21] Each strand of the TJ may consist of a row of proteins. The degree of tightness is affected by the status of the cytoskeleton,[22] and varies in response to intracellular signals involving protein kinase C, phospholipase C, adenylate cyclase, calmodulin, and G-protein receptors.[23] Calcium plays a paramount role in the permeability of TJ.[24]

In functional epithelium, the polarized cells are organized into a continuous membrane by a variety of different junctional complexes including the TJ, gap junctions, zonula adherens (ZA), and desmosomes. The TJ are characteristic of the epithelial membranes.[25] The TJ form the intercellular seal that controls the diffusion of water and small solutes between the apical and basolateral compartments.[26] This has been described as a "gate function." Secondly, the TJ also have a "fence function," restricting the lateral diffusion of different components such as membrane lipids and proteins.[27]

The paracellular pathway of all epithelia studied so far behaves as an aqueous pathway restricting solute permeation on the basis of molecular size and charge. Ions can permeate through TJ and lateral intercellular spaces (shunt pathway) or through cells (cellular pathway), either across the apical and basal membranes or across the apical and lateral membranes, in series with the lateral space. Thus, TJ and cell membranes are parallel transepithelial permeation pathways. The number of junctional strands at focal points along the TJ decreases, with a consequent reduction of the electrical resistance of the paracellular route.[11,15] From a functional point of view, the correct parameter to assess epithelial leakiness is not the absolute value of the resistance of the shunt pathway (Rs), but the ratio Rs/Rc (Rc, resistance of the cell), that is, the paracellular resistance relative to the transcellular resistance.[28,29] The paracellular pathway is formed by two resistances in series, the resistance of the TJ itself and the resistance of the intercellular space. Part of the resistance of the paracellular pathway is due to the length, narrowness and tortuosity of the intercellular cleft.[30]

Composition

The major epithelial cell adhesion molecule is a transmembrane glycoprotein called E-cadherin.[31,32] E-cadherin interacts with actin-containing elements of the cytoskeleton. The polypeptides that link E-cadherin to the cytoskeleton structure have been called "catenins."[33] E-cadherin is the intercellular adhesive component of the ZA. ZA participate in morphogenetic events by mediating the specificity of intercellular recognition in different cell types.[32] Secondly, the switching of cadherin expression may be responsible for the segregation of cell types, and the formation of morphological boundaries between developing tissue.[34]

Epithelial cells position their apical surfaces away from collagenous matrices.[35] Recognition of the basement membrane protein laminin appears to play a critical role in this process.[36] Cytochalasins have many effects.[37] Whereas internalization of cytochalasin and binding of cytochalasin to the cytoskeleton is not energy dependent, the effects of cytochalasin on cytoskeletal condensation in TJ structure and permeability are energy dependent.[38]

All of the TJ components have been identified either by ultrastructural criteria alone (e.g. actin), or by producing monoclonal antibodies to relatively impure membrane fractions (e.g. ZO-1, cingulin, and BG9.1 antigen).[39,40] ZO-1,[41,42] and ZO-2[43] are bound to each other and to a 130 kDa (p130) and a 330 kDa protein.[44,45] In addition, ZO-1 binds directly to the cytoplasmic tail of occluden[40,46] and the cytoskeletal protein spectrin.[41] Occludin is a 65-kDa integral transmembrane protein that localizes to the TJ contact sites of both epithelial and endothelial cells. Occludin contains four transmembrane domains, as well as a COOH-terminal cytoplasmic domain necessary for the localization of occludin to the TJ.[46] The amino acid composition of the occludin suggests the existence of two extracellular loops that may act either to form tight paracellular seals through an interaction with loops on adjacent cells, or by interacting with the lipid bilayer of the adjacent cell membrane itself.

Other proteins that have been localized to TJ include cingulin (140 kDa),[16] 7H6 antigen (155 kDa),[47] the small GTP-binding proteins Rab 13[48] and Rab3B,[49] the proto on-

cogenes c-Src and C-yes,[50] and the Src substrate p120.[51] The role of these proteins in regulation of TJ structure and function remains to be determined, although recent evidence does suggest a role for the GTP-binding proteins in cytoskeletal organization.[52]

Synthesis, assembly and sealing—formation and reformation

The process of TJ formation can be followed through 1) the development of transepithelial electrical resistance (TER); 2) the decrease in permeability to extracellular markers such as insulin, polyethylene glycol or mannitol; 3) transmission and freeze-fracture electron microscopy using ruthenium red or horseradish peroxidase; and 4) immunofluorescent techniques to observe the distribution of actin, tubulin, and other junctional-associated proteins. The re-formation of TJ after trypsinization of cells in culture requires protein synthesis,[20,21] extracellular calcium,[53–55] and the presence of intercellular Ca2+-dependent adhesion molecules.[31,56] The synthesis and assembly of junctional components are temporarily separated by several hours.[20] After their initial synthesis (which may occur in the absence of calcium), ZO-1, cingulin, and a 192 kDa peptide accumulate in a vesicular compartment located beyond the Golgi apparatus.[13,16,39,57] The transport and incorporation into the surface membrane of these proteins is then achieved by a microfilament and calcium-dependent exocytotic fusion. Microfilaments also contribute to the subsequent assembly and sealing of the strands.[58–60] Glycosylation of TJ-associated proteins does not appear to be important, as tunicamycin (an inhibitor of the transfer of N-linked oligosaccharides to nascent protein) has no effect on the degree of sealing of TJ.[59–61] This indicates that either junctional peptides are not glycosylated, or that glycosylation is not essential for their sealing capacity.[59–61] Monensin markedly slows the transit of newly synthesized proteins from the mid to the trans Golgi,[62] and this decreases TER. In contrast, cytochalasin B does prevent the sealing of TJ, indicating that the assembly of already synthesized junctional components requires the participation of actin filaments. This may involve either a need for actin to position these components at the outermost end of the intercellular space, or else actin may be necessary for their anchorage to the plasma membrane. The synthesis of TJ components appears to be a multifactorial phenomenon. It may proceed in the absence of Ca2+, cell-cell contracts, and even in the absence of cell-substratum interactions.[59,60] Synthesis of TJ components occurs quickly, and insertion into the plasma membrane occurs by a process which requires Ca2+ in the extracellular medium. The rho family of GTP-binding proteins is implicated in the regulation of filamentous actin organization, as well as in the organization and permeability of the TJ itself.[52]

TJ formation requires protein synthesis[20] that may proceed in the absence of cell attachments.[63] Several different TJ-associated proteins have been reported, such as ZO-1, a 225-kDa phosphoprotein;[13] cingulin, a 140-kDa peptide,[16] and a 192-kDa peptide.[39,57] Once synthesized, these components accumulate in a vesicular compartment located beyond the Golgi apparatus. Their transport to and incorporation into the surface membrane is achieved by an exocytic fusion that requires calcium. TJ formation is under the control of the cytoskeleton.[22,38,64,65] Once assembled, the regulation of TJ permeability is in part dependent on intracellular cAMP levels: as intracellular cAMP is increased, TER rises.[66] Ca2+-stimulated development of TER by MDCK cell monolayers is delayed by exogenous dibutyryl cAMP (db-cAMP);[67] conversely, activation of protein kinase C by phorbol esters causes the decline of TER.[68]

Proteolytic enzymes affect the structure and permeability of TJ,[54] but also induce de novo formation of TJ in several epithelial tissues and cells in culture.[69] For example, when HT-29 human colonocytes in culture are treated briefly with protease, they rapidly assemble large amounts of TJ fibrils.[70,71] The addition of trypsin to polarized HT-29 cells induces the assembly and proliferation of TJ elements.[69] Protein synthesis is unnecessary for the induction or the assembly phases of these elements,[72] whereas the cytoskeleton is involved in TJ assembly in protease treated HT-29 cells.[73]

The cell possesses regulatory mechanisms that influence the structure and the functional permeability of the TJ. These regulatory signals include adenosine 3′-5′-cyclic monophosphate (cAMP), phospholipase C (PLC), tyrosine kinases, calcium and protein kinase C (PKC), calmodulin, and the heterotrimeric G proteins.[22] TJ resistance rises with increased intracellular cAMP[66] or with exposure to a calcium ionophore.[74] Activation of PKC with phorbol esters reduces TJ resistance.[75]

The energy requirement for protease-induced formation of TJ in HT-29 cells has been established by the use of metabolic inhibitors.[76] The proteolytic activity of the protease is essential for the induction phase, but not for the assembly phase of the FO.[72] The method of domain-selective biotinylation has been used to follow the kinetics of protein delivery to the surface of brush border and basolateral membranes in Caco-2 human colonocytes.[77] Some endogenous apical plasma membrane proteins follow a direct route from the Golgi apparatus to the cell surface, whereas others follow a transcytotic route. The direct route has been observed for basolateral membrane proteins, whereas apical plasma membrane proteins use both direct and transcytotic routes.

TJ demonstrate ion selectivity sequences and TJ sieving characteristics.[23] TJ provide a seal between epithelial cells, thereby producing a diffusion barrier to the intercellular movement of ions and molecules. TJ play no role in the polarized distribution of molecular species other than lipids.[54,78–80] Polarization proceeds with the insertion or removal of molecules after TJ are assembled.[55]

Calmodulin and calpain are also involved as intercellular signals in the assembly and sealing of TJ. Thus, the cell possesses mechanisms that determine the structure and permeability of its TJ and G-proteins: these include adenylate cyclase, PLC, PKC, calmodulin (CaM). As well, the cytoskeleton may participate in such mechanisms.[23] On activation, PLC converts PIP_2 into IP_3 (1,4,5-triphosphate) and diacylglycerol (DAG). IP_3 mobilizes Ca^{2+} from internal reservoirs, and DAG activates PKC. Activation of calmodulin by Ca^{2+} induces arrangement of actin microfilaments into a continuous ring that circles the cell. Once a ring of actin

filaments has been formed, and once cell-cell contacts at the adherens junction are established, the TJ develops and the monolayer acquires transepithelial electrical resistance.

The circumferential ring of actin and myosin is wrapped around each absorptive cell, and contracts in response to ATP and divalent cations. This alters TJ structure and possibly permeability function.[81,82] The effect of cytochalasin D (an actin-binding agent) is energy-dependent, and alters TJ form and function.[83] TJ resistance rises with increased intracellular cAMP,[66] or exposure to a calcium ionophore;[74] activation of PKC with phorbol esters reduces TJ resistance.[75] The cytoskeleton may be involved in transducing these intracellular events to the TJ to alter their function.[12]

TJ are constantly undergoing assembly/disassembly by means of a vesicle shuttle mechanism from the cytoplasm below the junction. One type of signal consists of Ca^{2+}, microfilaments, cAMP, and perhaps other intracellular processes. A second type of signal derives from a mechanism that senses pressure/volume changes in the intercellular space (ICS). This latter signal can be either inhibitory (ICS pressure/volume high) or stimulatory (ICS pressure/volume low) (*Figure 2*).[84] As these authors note: "The tight junction is made more permeable (conductive) if ICS pressure and volume is increased. This would tend to increase junctional backflux (relative to junctional forward flux) of salt and water if transjunctional driving forces are favorable. Net NaCl and water transport would thus decrease across the epithelium, provided that the active transcellular transport remains unchanged. A decrease in ICS volume/pressure would make the junction less conductive, thereby diminishing backflux and enhancing net transport efficiency. The model thus ties modulation of TJ permeability to changes in ICS pressure/volume which, in turn, depends on the activity of active transcellular transport." In the case of the jejunum, glucose-dependent Na^+ transport might be expected to increase volume/pressure in ICS, and thus raise TJ conductance with enhanced forward flux.[65]

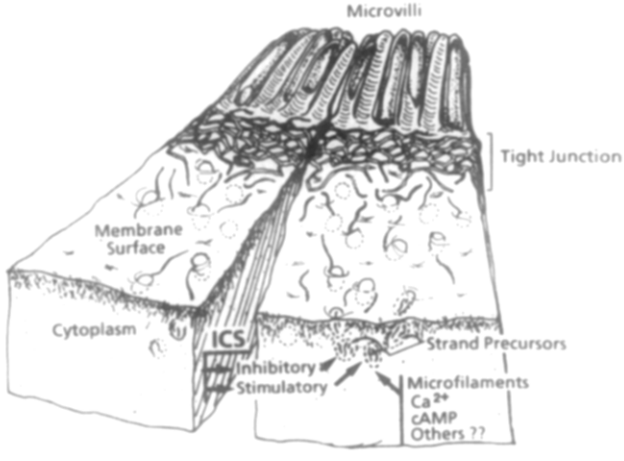

Figure 2 Model for tight junctional assembly/disassembly involving a shuttle mechanism via submembranous vesicle containing strand (fibril) precursors. Strand precursors would assemble into mature strands and fuse into the membrane below the main meshwork. Eventually strands are integrated into the main meshwork. Microfilaments aided by Ca^{2+}, cAMP, and perhaps unknown kinases regulate shuttle movement and/or vesicle-membrane fusion. Disassembly is the same process in reverse. Signals emanating from ICS pressure/volume changes can either enhance or retard the process, but may even constitute the primary implementing signal. (Taken with permission from Bentzel et al., 1992.)

Microtubules and microfilaments

Microtubules are important in the targeting of proteins to membranes; colchicine and nocodazole inhibit but do not block the transport to the apical surface of aminopeptidase N and sucrase-isomaltase.[85,86] Microtubule inhibitors have little effect on the delivery of basolateral proteins to the cell surface.[87] Thus, microtubules act as facilitators of vesicular transport to the apical surface, and of basal-to-apical transcytosis.[88] This facilitation appears to be essential for the final polarity of some apical proteins, but only an enhancer for others.

Microfilaments link to or anchor intramembranous TJ elements. This, in turn, alters TJ strand processing in an unknown manner.[89] Alternatively, microfilaments may constitute a continuous subjunctional web of filaments that attaches to the TJ from below. As microfilaments contract or relax, junctional permeability is altered mechanically.[37] There is a relationship between intercellular space volume/pressure and TJ permeability, but the resistance response is not simply the result of collapse of ICS. Instead, the resistance response is a complex interplay of cytoplasmic signals, which eventually result in the induction of new TJ elements into the junctional membrane domain.[84] In the small intestine, increments in TER after exposure of the mucosa to hypertonic solutions is associated with proliferation of TJ strands and an increase in TJ depth, as shown on analysis of freeze fracture electron micrographs.[90] In many leaky epithelia mucosal hypertonicity leads to the collapse of intercellular spaces.[91,92] Phalloidin, a drug known to induce microfilament hyperplasia, produces an extensive proliferation of TJ elements in rat hepatocytes.[93] Thus, microfilaments are involved in TJ assembly (as suggested by the effect of cytochalasin B), whereas microtubules are not important in this process.

Role of TJ in membrane composition and polarity

The functional polarity of epithelial cells depends on the asymmetric distribution of enzymes and nutrient transport systems between the apical and the basolateral regions of the plasmalemma, separated by the TJ fence.[88,94] Glycoproteins that are bound to the cell surface via a glycolipid (glycosylphosphatidyllinositol, GPI), are also highly polarized in the apical surface of several epithelial cell lines. GPI is used as an anchor by a group of plasma membrane proteins, and may also act as an apical targeting signal.[88,95,96] GPI does not account for the distribution of the large majority of apical plasma membrane proteins.

The apical or luminal plasma membrane of epithelial cells has a different lipid composition than the basolateral membrane.[97] The lipids of the basolateral membrane are similar to those of the plasma membrane of non-epithelial cells, being enriched over the total cellular lipids in cholesterol, sphingomyelin, and phosphatidylserine, at the expense of phosphatidylcholine and phosphatidylinositol. The brush border membrane (BBM) is a specialized plasma

membrane and has a different lipid composition as compared with the basolateral membrane (BLM); for example, the BBM is enriched in glycosphingolipids,[98] and the molar ratio of glycosphingolipids to phospholipids and cholesterol is 1:1:1.[98–102] Lipids diffuse rapidly in membranes,[103] but the maintenance of the difference between the BBM and BLM is attributed in part by the TJ, which restrict random diffusion between the two membrane domains.

There are also differences in lipid composition between the inner and outer leaflet of membranes. For example, glycosphingolipids are localized exclusively to the exoplasmic bilayer leaflet of mammalian cell membranes.[104] The monolayer of glycosphingolipids may fulfill a general role of membrane stabilization.[105] Cells require a stable and uniform lipid composition of the cytoplasmic leaflet, and the lipid composition of the exoplasmic leaflets is adapted to the needs of the particular external environment.[97] A key role of the expolasmic lipids is the sorting of intracellular proteins and lipids.[106–109] Lipid polarity is achieved by lipid sorting along the biosynthetic pathways, or by the involvement of microdomains in lipid sorting. Glycosphingolipid PC reaches the outer leaflet of the plasma membrane by vesicular transport. With a vesicular mechanism for distributing the correct lipids to the apical and the basolateral membranes, it might be expected that sorting would occur in the membrane compartment from which vesicles exit for the cell surface.

Transport from the Golgi to these two distinct membranes has been characterized in Caco-2 cells, using fluorescent and radioactive sphingolipid analogs. The cell surface polarity of lipids is likely generated by lipid sorting in the trans Golgi.[110] This sorting may occur by an interaction of the glycophospholipid-anchor with the putative glycosphingolipid microdomain.[111] Membrane-spanning proteins are required in the apical microdomain to transmit the information concerning its apical identity toward the cytoplasmic surface, where it is needed for apical targeting of the budding membrane vesicles.[106] There are protein microdomains in the trans Golgi network/reticulum has been demonstrated.[112] It is possible that the glycosphingolipids in the exoplasmic bilayer leaflet may associate with apical proteins, either directly or via a putative sorting protein.[106]

The molecular arrangement at the membrane contact sites of the TJ is not understood, but is unlikely to be due to a simple continuity between the external leaflets of the apical plasma membrane in adjacent epithelial cells.[113] The TJ act as a diffusion barrier for probes incorporated in the exoplasmic leaflet, but not in the cytoplasmic leaflet of the plasma membrane.[108,109]

Mechanisms that may be involved in determining the spatial polarity of a protein in the BBM or in the BLM, such as Na^+K^+-ATPase, include: 1) restriction of the lateral mobility of the enzyme in regions of cell-cell contact by the TJ; 2) developing specific linkages between the Na^+K^+-ATPase and cytoskeletal proteins; and 3) direct targeting of newly synthesized enzymes from the Golgi complex to the BLM domain (*Figure 3*).[114] However, evidence from various tissues demonstrates that the development of the Na^+K^+-ATPase polarity is not dependent directly on TJ formation, but rather the TJ may be involved in maintaining an asymmetric distribution of the enzyme in polarized epithelial

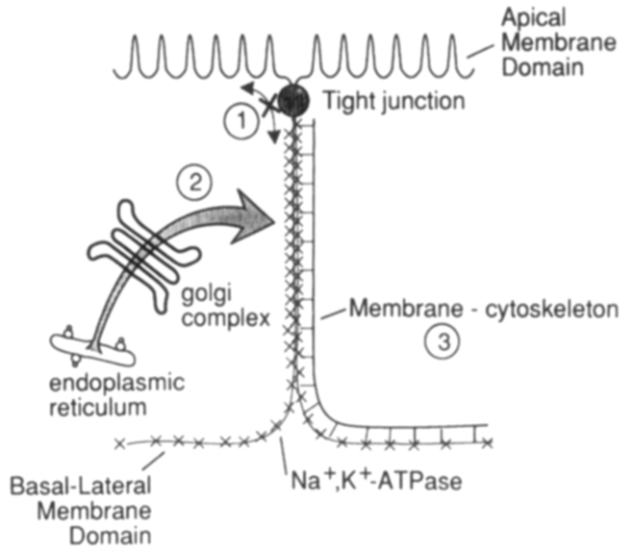

Figure 3 Mechanisms involved in the localization of Na^+K^+-ATPase in epithelial cells. 1. The tight junction may restrict diffusion of Na^+K^+-ATPase from the lateral to the apical membrane domain. 2. Newly synthesized Na^+K^+-ATPase is targeted to the basal-lateral membrane domain. 3. Accumulation of Na^+K^+-ATPase on the basal-lateral membrane domain may occur as a result of direct intermolecular interaction with the membrane-cytoskeleton. (Taken with permission from Hammerton & Nelson, 1991.)

cells by prohibiting the diffusion of the Na^+K^+-ATPase to the apical membrane.[114]

Membrane fluidity may play an important role in regulating enzyme activity. In liver cells, membrane fractions enriched with the basolateral (sinusoidal) and apical (canalicular) domains are differentially affected by fluidizing agents, which increase activity in the apical membrane without affecting activity in the basolateral membrane. There is also an interaction between Na^+K^+-ATPase and the cytoskeleton: the spectrin(fodrin)-based membrane skeleton is co-distributed with Na^+K^+-ATPase, although fodrin is synthesized in developing rat intestine several days before the Na^+K^+-ATPase is detected.[115]

Cell proliferation and polarization

Cell proliferation and polarization has a spatial component in the crypt-villus axis. Most of the changes in the morphological, biochemical, and functional characteristics of cells observed during differentiation occur in the apical membrane of the cells.[116] The functional development of the apical membrane may occur even before the complete sealing of the ICS: the polarized development of the sodium-dependent glucose transporter ($SGLT_1$), and of the sodium/hydrogen antiport (NHE) systems, precede for a few hours the development of the TJ.[117] When senescent epithelial cells slough, the extrusions are quickly covered as processes from neighboring cells migrate under the sloughing cell and established new TJ contact. In this way the junctional "zipper" closes the space underlying the sloughing cells, so that a macromolecular barrier can be retained.[118]

Signal transduction

The final structure of the TJ may exhibit from one to ten strands. G-proteins are a subset of a large family of guanine

nucleotide peptide proteins that participate in the transduction of extracellular stimuli (Figure 4). These heterotrimers are composed of α, β, and γ subunits. The β and γ subunits appear to be essential for the interaction of the G-proteins with receptors.[119] Some bacterial toxins may ADP-ribosylate the α-subunit. The signal pathway consists of a receptor, a G-protein transducer, and an amplifier protein. Binding of a ligand to its receptor in the plasma membrane promotes the activation of the G-protein, allowing GTP to replace GDP in the α-subunit. The activated α-GTP dissociates from the β and γ subunits, and one or both of these interact with the amplifier. The intrinsic GTPase activity of the α subunit hydrolyses GTP to GDP, and α-GDP recombines with the βγ complex.[23] The amplifier produces an intracellular messenger, which, in turn, activates a specific kinase system to phosphorylate the target protein, and thereby to achieve a biological response.

The TER is not influenced by activation of adenylate cyclase, but a profound inhibition of the level of TER is achieved with db-cAMP (a permeable analog of cAMP) with forskolin (a stimulator of adenylate cyclase), and with IBMX (an inhibitor of phosphodiesterase).[23] Thus, adenylate cyclase does not appear to be a protein involved during the Ca^{2+}-switch. Phospholipase C participates in the assembly and sealing of TJ, because when it is inhibited by neomycin, TER remains low.[120] Despite the effect of the stimulation that TPA elicits on PKC,[121,122] it decreases the value of TER in MDCK cells.[23]

Adaptation of function by nutritional means

The TJ may be important to influence: 1) membrane composition, and therefore the transport properties of the intestine; 2) cell proliferation and differentiation of the cells along the crypt-villus axis, and therefore the digestive and transport maturity of the intestine; and 3) electrical resistance, and therefore the absorption/secretion of salt and water. The synthesis, assembly, and function of TJ may be influenced by a variety of intracellular signals. Thus, TJ may be subject to regulation and to adaptation. Does food influence the function of TJ? Yes: exposure of the mucosal surface of the mammalian small intestinal epithelium to glucose or amino acids, such as would occur during a meal, elicits focal dilatations in absorptive cell TJ. These are not due simply to hydrostatic pressures associated with transjunctional fluid movement.[83,118] Paralleling this response, once the electrical resistance of diverse epithelia is corrected for length/cm^2 of epithelium, resistance increases with the number of strands in an exponential manner, and not in a direct fashion as would be expected from the sum of resistors in series. This has been ascribed to the presence of channels that fluctuate between open and closed states.[30] There is insulation of neighboring channels in each strand provided by anastomoses between strands, so that the channels are compartmentalized in the transepithelial direction. In this compartmentalized flickering channel model, junctional resistance increases exponentially with the number of strands.[80] Because the TER of an epithelium does not bear a simple relationship to the number and distribution of TJ strands, other elements need to be considered.[59] With the compartmentalized flickering channel model, current flowing through one segment can only cross the next strand if it also has an open channel, so that conductance will be markedly restricted. Thus, because of branching and anastomosing of its ridges, TJ exhibit an exponential relationship between the number of strands and TER.[80]

A glucose-induced decrease in TER is associated with an increase in junctional Na^+ and mannitol permeability, and "activation" of the Na^+ nutrient co-transporters present on the BBM of small intestinal absorptive cells represents the initial trigger that elicits these changes in cytoskeletal structure and TJ structure and permeability. As a result of altered junctional sieving of nutrient-sized molecules, substantial paracellular uptake of nutrients occurs via solvent drag as water moves across the TJ.[10] For example, at concentrations of 125 mM glucose, at least one-third of glucose absorption in rat intestine in vitro occurs via this paracellular solvent drag mechanism. However, the relative importance of paracellular transport of nutrients in vivo is debated.[123,124]

Models

Protamine induces the formation of TJ in Necturus gallbladder, and reversibly decreases the conductance of the cation selective pathway through the TJ.[125] More strands appear in the main meshwork, and TJ depth increase.[126] The effects of protamine on TJ formation in the intestine is unknown. Protamine does not increase epithelial cell cAMP, nor does it induce collapse of intercellular spaces, because solute-coupled water flux increases.[125]

The function of the TJ is influenced by the presence of glucose, amino acids, and excessive amounts of bacteria in the small intestine. Is there any influence of dietary lipids? There is indirect evidence that this may be the case, since pre-exposing cultured cells with long-chain fatty acids alters the development of electrical resistance. The development of TER in fatty acid-supplemented and control clone 4 MDCK cell monolayers was examined after trypsinization.[127] The phospholipid acyl group composition of these cells was altered by culturing them in serum-free medium supplemented with different fatty acids. The unsaturation index of the phospholipid of the cell was more than doubled in cells receiving either of the two 18:3 isomers (18:3 n-3 or 18:3 n-9), as compared to control and to 18:1 (n-9) supplemented cells. The rate at which steady state TER was achieved slowest in cells supplemented with 18:3 (n-6), and this was accompanied by a 20 fold decrease in PGE_2 synthesis. Modulation of membrane cholesterol has an even greater effect than alterations in phospholipid acyl group composition on the formation of TJ.[128] Whereas steady state TER and ion cell activity is not affected, the change in the rate of TER development in the 18:3 (n-6) supplement cells and the change in membrane lipids and PGE_2 synthesis raises the possibility that this method may be used to examine the mechanisms of the effect of enrichment of BBM lipids on TJ function.

Role of tight junctions and intestinal permeability in inflammatory bowel disease

The continuous lining of epithelial cells along the gastrointestinal tract mediates absorption of dietary nutrients as well

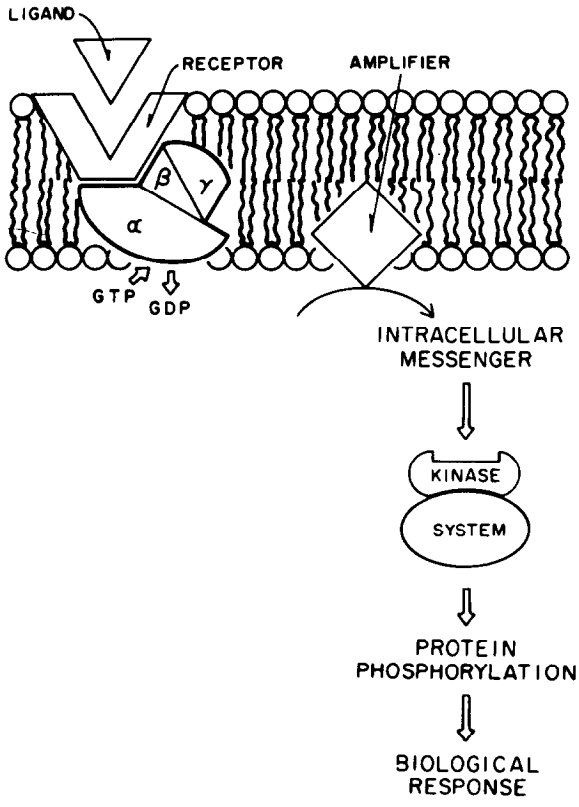

Figure 4 Schematic representation of signal transduction. The basic signal pathway consists of three proteins: a receptor, a transducer (G-protein, a-b-g subunits) and an amplifier protein. Binding of a ligand to its receptor in the plasma membrane promotes the activation of a G-protein. The receptor-G-protein interaction allows GTP to replace GDP in the a subunit. The activated a-GTP and the b and g subunits dissociate, and one or both of these subunits may, thus, interact with the amplifier. The intrinsic GTPase activity of the a subunit hydrolyses GTP to GDP, and a-GDP recombines with the bag complex, thus ending the activation cycle. The amplifier produces an intracellular messenger, which in turn, activates a specific kinase system to phosphorylate the target protein. (Taken with permission from Balda, 1992.)

as regulating fluid and electrolyte balance across this mucosal lining. An equally important function of the epithelium is to serve as a selective barrier and to prevent access of the luminal contents to the interior of the body. The barrier function of the intestine consists of several components, including the BBM of absorptive cells and the TJ of the paracellular space.[129] The ability of the intestinal mucosa to act as a barrier is commonly referred to as "permeability." Current research has focused on examining alterations in TJ function within the paracellular pathway as the main determinant in altered intestinal permeability.

In the intestine, disruption of the mucosal barrier and subsequent increases in intestinal permeability may play an important role in the etiology and pathogenesis of various intestinal and systemic diseases. Increased intestinal permeability has been described in such diverse small bowel conditions as gluten enteropathy,[130] acute alcoholism,[131] burn injury,[51] and inflammatory bowel disease (IBD).[132]

In the surgically created self-filling blind loop of rat jejunum,[133] the total junctional depth is greater in treated than in control rats. This structural change is paralleled by a 3 fold increase in TER, and a decrease in short circuit current in the blind loop epithelia.[134] These alterations may be an example of "crosstalk" between TJ conductance and intrinsic transport properties in an epithelium. When the rat ileum is studied 8 weeks after 70% proximal resection, the transport of sodium, chloride, and bicarbonate is unchanged, but epithelial resistance falls.[135] Morphometric analysis at four levels of the crypt/villus axis yields no demonstrable change of the number of horizontal strands in the short bowel as compared to the control ileum, whereas the depth of the total TJ increase only slightly. The decrease in epithelia resistance may be due to mucosal hyperplasia, leading to an increase in TJ area per cm^2 serosal area exposed in the Ussing changer.

Patients suffering from active IBD exhibit an increased intestinal permeability,[136,137] and increased permeability after treatment of Crohn's disease is a good predictor of subsequent symptomatic disease relapse.[138] However, a primary question relating to the etiology of IBD is whether the inflammation occurs as an appropriate response to the entry of pathogens across a defective tight junction in the intestine (suggesting an intrinsic permeability defect), or whether the inflammation occurs as an aberrant immune response to a normal stimulus (suggesting an acquired permeability defect).[139] An acquired permeability defect can result from a direct action of the proinflammatory cytokines IFNδ and TNFα on epithelial barrier function.[140] Thus, during an inflammatory response, the increased concentration of IFNδ and TNFα in the lamina propria may cause an enhanced permeation of luminal antigens, resulting in the initiation of a vicious cycle, where inflammation prompts increased epithelial permeability, which then worsens inflammation.

Further evidence to support a role for an intrinsic permeability defect in patients with IBD comes from a study showing that mice develop a condition resembling Crohn's disease when intestinal cell-cell interactions are interrupted by disrupting cadherin function.[141] These mice were immunocompetent, so perhaps IBD can arise in the absence of any immune dysfunction if the crypt epithelium becomes disrupted.

Individuals with the highest risk of developing Crohn's disease are those with a first-degree relative who already has Crohn's disease. Approximately 10% of this group ultimately develop disease. A working hypothesis is that people destined to develop Crohn's disease may have increased intestinal permeability before the development of the disease, and that it is this intrinsic permeability defect that leads to the condition. This hypothesis predicts that this patient population would have increased intestinal permeability even in the absence of clinical evidence for Crohn's disease. This was the scenario proposed by Dan Hollander in 1986 when he reported that the relatives of patients with Crohn's disease have increased intestinal permeability.[142] Although this hypothesis made sense and the initial data was encouraging, some subsequent studies failed to confirm these findings.[143] However, what all these studies did not take into account was that only a small subgroup of the relative population (10%) could reasonably be expected to be at risk. With such a small subpopulation in a larger group of "normal" relatives, significant differences between the

total group and normal controls could be easily overlooked. Taking these considerations into account, Meddings and colleagues have reexamined Hollanders' hypothesis and found that a subgroup of relatives clearly have increased intestinal permeability in the absence of clinical disease.[144,145] In the latter study, these observations were extended to demonstrate that this group of patients with increased permeability also have immunological evidence of increased antigen exposure in circulating B-lymphocytes. Therefore, increased intestinal permeability appears to be important in the initiation of perpetuation of Crohn's disease, and a subgroup of individuals at risk of developing this disease can be identified by determining that they have increased intestinal permeability. With active disease, intestinal permeability is again increased and the degree of abnormality correlates with other indices of disease activity.[146,147] It remains unknown whether attempting to normalize the altered intestinal permeability will prevent the onset of clinical disease in relatives, or the recurrence of symptomatic disease in persons with established disease.

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