# REVIEW ARTICLE

# ROLE OF CYTOSKELETAL STRUCTURE IN MODULATION OF INTESTINAL PERMEABILITY

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**The intestine contains the largest interface between man and his environment; thus, the intestinal barrier could be a key factor in health and disease states. This barrier is a highly selective gatekeeper that permits the passage of nutrients and prevents the penetration of harmful bacterial products and dietary antigens. The intestinal barrier is composed of immunological and nonimmunological compartments and the latter part is made up of multilayered structural and functional components. The intestinal epithelium and its paracellular tight junctions appear to be the key for integrity of this barrier. The cytoskeletal assembly is essential for maintaining epithelial structure, transport, and functional integrity, but is also pivotal for integrity of the paracellular pathway, especially the tight junction complex. Actin and microtubules are two cytoskeletal filaments that play key roles in regulation and maintenance of the intestinal barrier. Various noxious agents such as ethanol and/or oxidants can induce cytoskeletal damage and disruption of barrier integrity. The injurious effects of these compounds are mediated through upregulation and activation of inducible nitric oxide synthase (iNOS) and the resultant NO overproduction and nitration and oxidation of actin and tubulin. Oxidized cytoskeletal proteins result in depolymerization of cytoskeletal filaments, cytoskeletal disassembly and disarray and eventually disruption of barrier function. The disrupted barrier can initiate or perpetuate an inflammatory cascade that will result in intestinal mucosal injury and inflammatory bowel disease flare-up. There are several lines of repair/defense that help to brake this inflammatory cascade, reestablish barrier integrity and thus limit or terminate mucosal injury. One of these lines of defense is mediated through protecting factors such as epidermal growth factor, which prevents ethanol-induced and oxidative damage to the gastrointestinal epithelium.**

**Keywords** actin • cytoskeletal structure • intestinal permeability • tubulin

## Introduction

The intestinal tract acts as a filter—its selective permeability allows movement of nutrients from the intestinal lumen into the circulation—and as a barrier—it prevents the penetration of harmful compounds including microorganisms and their products, luminal dietary antigens, and other luminal proinflammatory factors.[1, 2] Intestinal permeability is thus a highly regulated dynamic process determined by interactions among several components including the unstirred water layer, mucosal surface hydrophobicity, the surface mucus coat, epithelial factors, and endothelial factors. Each of these structures has different permeability properties. However, the exact role of each component in modulation of intestinal permeability remains to be fully established. Transcellular and paracellular pathways are two routes for transepithelial permeation. Of these routes, the paracellular pathway, particularly the tight junction complex, has been most intensively studied and appears to be the most important in regulating the passage of proinflammatory and injurious luminal compounds.

### Epithelial cytoskeletal structure

The cytoskeleton is an essential structure for the integrity of all eukaryotic cells, including the gastrointestinal (GI) epithelium.[3 – 6] This intricate network of protein filaments extends throughout

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the cytosol[3, 4] and is not only essential for maintaining the normal structure, transport, and functional integrity of the cell, but is also pivotal for the integrity of the paracellular pathway. We have previously shown the importance of cytoskeletal stability in the GI mucosa *in vivo*[3, 4] and under *in vitro* conditions.[5 – 8] The cytoskeleton includes different types of protein filaments. Actin filaments and microtubules are two fractions of the cytoskeletal assembly that are involved in maintaining intestinal barrier function.

The actin component of the cytoskeleton is one of the most abundant proteins in the eukaryotic cytosol which can polymerize into filaments of highly dynamic alpha-double helices.[9, 10] In epithelial cells, such filaments make up a dense cross-linked actin cortex located on the inner side of the plasma membrane. Actin also makes up filamentous stress fibers, which traverse the cytosol and constitute short fibers extending into the lamellipodia in motile cells.[11] This structural element is essential in maintaining normal cellular physiology, structure, locomotion, and support functions.[7, 9 – 12] The actin-based skeleton also plays a critical role in regulating the permeability of epithelial monolayers.[12] These functions are based on the ability of monomeric G-actin to polymerize, and on the ability of F-actin polymers to resist disassembly. Despite the crucial role of actin in cell integrity, little is known of the role of F- and G-actin, especially in oxidant-induced loss of mucosal barrier function. It is speculated that oxidant-induced mucosal barrier dysfunction is caused by the oxidation, disassembly, and instability of the actin cytoskeleton, and that epidermal growth factor (EGF) and transforming growth factor-$\alpha$ (TGF-$\alpha$) prevent this by maintaining normal actin assembly and integrity. Though many studies have been performed to elucidate the interrelationships among actin stability, mucosal barrier function, and oxidant insult,[13] the role of the actin cytoskeleton in intestinal disease such as inflammatory bowel disease (IBD) remains elusive.

Microtubules are one of the principal protein structures in the eukaryotic cytosol; they are distributed in a radial array and represent the major structural element of the cytoskeleton. As such, they play a central role in maintaining cellular integrity, structure, and transport function.[14–16] Microtubules provide a system for directing intracellular transport and secretion, as well as coordinating cytosolic organelle movement. They also regulate cell morphology, cell migration, and cell polarity, and maintain the plane of cell division.[16–18] Disruption of the microtubule cytoskeleton can severely limit cell function, and if not reversed, can adversely affect its integrity and viability.[8, 19] Microtubules maintain the overall shape and stability of the plasma membrane. These functions are based on the ability of the tubulin subunits to polymerize and on the ability of microtubules to resist depolymerization. We recently showed the crucial role of this structural component in the maintenance of mucosal barrier function.[20]

## Tight junction complex

Macromolecular permeation across the epithelial layer takes place either through transepithelial or paracellular routes. Studies in animal models show that increased transcellular intestinal permeation is associated with an increase in the number and size of epithelial endosomes.[21] The paracellular pathway, however, seems to be the major route of transepithelial macromolecular permeation. This pathway is a complex array of structures that are mainly controlled by tight junctions between epithelial cells. Tight junctions appear to be key regulators of intestinal permeability to macromolecules such as endotoxin and other bacterial byproducts.[2] The physiology of this tightly regulated conduit is not fully known. However, this dynamic gateway is able to change its size under various physiological and pathological conditions.[1] Madara showed that transepithelial resistance and tight junction structure can be altered rapidly by osmotic load.[22] Luminal osmotic load, especially when activated by sodium co-transport, can increase paracellular permeability to large molecules in rat jejunum.[23] In addition, intestinal permeability increases following ingestion of hypertonic solution[24] and meal-related solutes such as glucose. This change in intestinal permeability after meal ingestion should enhance the ability of the small intestine to harvest the maximal amount of nutrients,[25] but it can also increase the risk of exposure to luminal proinflammatory compounds.

One of the main structures essential for the integrity of this conduit is the intricate cytoskeletal protein filament.[26, 27] Actin plays a major role in gut barrier integrity in general and in regulation of intestinal tight junction rings in particular.[28] Barrier dysfunction has been linked to actin disruption induced by oxidants.

Tight junctions are also composed of other structural proteins including occludin and actin anchoring protein (ZO-1), which could be the target of oxidative or other toxin injury and result in disruption of intestinal barrier integrity.[29, 30]

### Role of the cytoskeleton in maintaining intestinal barrier integrity

As mentioned above, the critical characteristic of the epithelium of the GI mucosa is its ability to maintain a highly selective permeability barrier. The rules that govern this selection process are not fully understood. However, molecular size and physiochemical properties of the substances might play a major role in the selection process. Hence, the transepithelial and paracellular pathways are two major routes for epithelial permeation; the integrity of epithelial barrier function depends on the presence of both healthy epithelial cells and a functionally normal paracellular pathway.

It is not surprising that cytoskeletal damage induced by various agents results in disruption of epithelial barrier function and hyperpermeability. For example, oxidant-induced mucosal barrier dysfunction is caused by the oxidation, disassembly, and instability of the actin cytoskeleton. In addition, disruption of microtubules by either oxidants,[31] ethanol,[6, 32] colchicine or antimitotic agents can severely limit cell function and the structural integrity of the intestinal barrier.[8,17, 26]

The mechanism responsible for cytoskeletal damage in various settings is not fully understood. We have shown that ethanol-induced cytoskeletal damage causes upregulation and activation of iNOS, NO overproduction, nitration and oxidation of tubulin, decreased levels of stable polymerized tubulin, and increased levels of disassembled tubulin. Eventually, extensive damage to the cytoskeletal assembly results in disruption of the intestinal monolayer barrier function.[32] On the other hand, oxidative stress results in oxidation of F- and G-actin (carbonylation), decreases the stable F-actin fraction (index of stability), increases the monomeric G-actin fraction (index of disassembly), and eventually results in loss of monolayer barrier integrity.[13] Thus, alcohol- and oxidant-induced oxidation, disassembly, and instability of the cytoskeletal protein appear to play a key role in loss of intestinal barrier integrity.

### Protecting mechanisms modulate cytoskeletal stability

EGF and TGF-α play a major role in regulating proliferation, differentiation, barrier function, and repair processes throughout the GI mucosa.[33, 34] Numerous studies, including our own, have shown that EGF and TGF-α can effectively protect GI barrier integrity against injurious agents, including oxidants.[5, 6, 35] The major source of luminal EGF is the salivary gland, as shown by increased intestinal permeability after sialoadenectomy in a rat model.[36] The specific mechanisms underlying this protective phenomenon are being elucidated. We have shown that growth factors prevent oxidant- and alcohol-induced disassembly and instability of actin and microtubules and, thus, mucosal barrier dysfunction. We have also shown that EGF prevents ethanol-induced and oxidative damage to GI epithelium through both protein kinase C (PKC) and calcium-dependent pathways.[6, 20, 37 – 38] This is not surprising since PKC signaling and cytosolic calcium concentration play a key role in epithelial cell health and function.[37]

### Significance and future perspective

The intestinal barrier is the most important interface connecting man to his surrounding environment. It is now postulated that a breach in this barrier might be the primary event in the pathogenesis of several systemic and intestinal disorders including IBD. Indeed, it is now believed that IBD is a result of an abnormally exaggerated immune response to normal intraluminal proinflammatory factors such as bacterial byproducts in susceptible individuals with a dysregulated mucosal immune system. In this scenario, intestinal barrier integrity can play a key role since it can prevent exposure of the mucosal immune system to intralumuinal factors and thus prevent initiation and/or perpetuation of the inflammatory cascade in the presence of hostile luminal factors or in subjects with dysregulated immune systems. Thus, better understanding of barrier function can provide an opportunity for development of new therapeutic options for many systemic and GI inflammatory disorders. Potential agents are PKC mimetics, cytoskeletal stabilizers, and growth factor mimetics. Further studies are needed to explore these very promising therapeutic avenues.

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