The primary functions of the gastrointestinal (GI) tract have traditionally been perceived to be limited to the digestion and absorption of nutrients and electrolytes, and to water homeostasis. A more attentive analysis of the anatomic and functional arrangement of the GI tract however suggests that another extremely important function of this organ is its ability to regulate the trafficking of macromolecules between the environment and the host through a barrier mechanism. The intestinal epithelial barrier controls the equilibrium between tolerance and immunity to non-self-antigens by regulating antigen trafficking both through the transcellular and paracellular pathways. When the finely tuned trafficking of macromolecules is deregulated in genetically susceptible individuals, autoimmune disorders can occur. Celiac disease is characterized by loss of intestinal barrier functions, and evidence is now accumulating suggesting a role of increased intestinal permeability in the early steps of the disease pathogenesis. This new paradigm subverts traditional theories underlying the development of autoimmunity, which are based on molecular mimicry and/or the bystander effect, and suggests that the celiac disease autoimmune process can be arrested if the interplay between genes and environmental triggers is prevented by reestablishing intestinal barrier function. Understanding the role of the intestinal barrier in the pathogenesis of celiac disease is an area of translational research that encompasses many fields and is currently receiving a great deal of attention. This chapter reviews the recent advance in intestinal mucosal biology involved in gliadin trafficking and possible alternative therapeutic approaches to correct the barrier defect typical of celiac disease.
entry of luminal noxious antigens and to prevent the loss of ions, water and other serum components from the circulation to the intestinal lumen. The main structure of this barrier function is the apical membrane of enterocytes connected to each other by a continuous network of TJs which tightens the lateral intercellular space between neighboring cells. Thus, the epithelial barrier can be differentiated into a trans- and paracellular pathway component. While in tighter epithelia like the large intestine (passive) transcellular transport accounts for almost the overall permeability of ions, leaky epithelia as the small intestine are characterized by a highly conductive paracellular pathway with a conductance contribution being higher than that of the transcellular pathway [1]. In addition, several other epithelial properties like mucus and glycocalyx covering the apical surface contribute to barrier function.

Depending on the specific intestinal segment under consideration, antigen uptake may depend upon other specialized structures/cells, such as M cells above Peyer’s patches in the ileum. Whether or not epithelial apoptosis significantly contributes to the overall paracellular permeability is still a matter of debate. At least in semitight epithelia like the colon, a significant contribution has been directly shown by conductance scanning measurements [2]. In the colon, upregulation of the apoptotic rate to 12% changes the epithelium from a semitight to a leaky one [3].

Another barrier property which has only recently gained more attention is the transcellular uptake of antigens by endocytosis and subsequent transcytotic transport through the enterocytes to the basolateral compartment of the epithelium. This process is electrically silent and may be more relevant for the uptake of antigens than for the entry of ions (see detailed description below).

As far as the paracellular pathway is concerned, its permeability is mainly dependent on TJ competency, while the lateral intercellular space has only a limited contribution to the paracellular route [4].

![Fig. 1. Composition of intercellular TJs. The 3 key elements of intercellular TJs include: (1) the structural TJ proteins occludin and claudins, (2) the scaffold proteins ZO-1, ZO-2, fodrin, cingulin, symplekin, 7H6 and p130, and (3) the actin cytoskeleton.](image-url)

**Structure of Tight Junctions**

**Transmembrane TJ Proteins**

To date, multiple proteins that make up the TJ strands have been identified (fig. 1) and include occludin [5] and members of the claudin family [6], a group of at least 20 tissue-specific proteins. The junctional adhesion molecule, a protein belonging to the immunoglobulin superfamily, has been described as an additional component of the TJ fibrils [7]. Junctional adhesion molecule reportedly binds to zonula occludin (ZO)-1, so aiding ZO-1 localization to the junctional complex [8]. Occludin cDNA analysis revealed that the predicted 504-amino-acid polypeptide (65 kDa) contains 4 transmembrane spanning domains with 2 extracellular loops and internal NH₂- and COOH- termini. Occludin expression levels and its distribution correlate with the number of TJ strands in a variety of epithelia [9]. The claudins are 20- to 27-kDa proteins that each contain 2 extracellular loops with variably charged
amino acid residues among family members and short intracellular tails [9].

**Cytoplasmic TJ Plaque Proteins**

The cytoplasmic plaque of TJs includes multiple proteins that have been characterized at the molecular level, and several others that await further characterization. By interacting with each other and with cytoskeletal proteins, these scaffold elements (ZO-1, ZO-2, ZO-3, ZO-1-associated protein kinase) functionally couple integral membrane TJ proteins to actin microfilaments [10]. TJ plaque proteins also appear to be direct targets and effectors of different signaling pathways. The first described and best-characterized TJ plaque component is ZO-1, a 225-kDa multidomain protein. ZO-1 and ZO-2 associate with each other in heterodimers [6] in a detergent-stable complex with an uncharacterized 130-kDa protein (ZO-3). Most immunoelectron-microscopic studies have localized ZO-1 precisely beneath membrane contacts [11]. A number of TJ plaque proteins, including ZO-1 and ZO-2, possess one or more approximately 90-amino-acid PDZ domains that mediate protein-protein interactions with other PDZ-containing proteins (see below). These PDZ-containing proteins belong to the membrane-associated guanylate kinase family of proteins [12]. Several other peripheral membrane proteins have been localized to the TJ, including 7H6 [9], Rab 13 and 3b [13], Go_1,2 [13, 14], protein kinase C [15], symplekin [16] and cingulin [17].

**The Actin Cytoskeleton**

To meet the many diverse physiological and pathologic challenges to which epithelia are subjected, the TJ must be capable of rapid and coordinate responses that require the presence of a complex regulatory system. There is now a large body of evidence suggesting that structural and functional linkage exists between the actin cytoskeleton and the TJ complex of absorptive cells [18–20]. The actin cytoskeleton is composed of a complicated meshwork of microfilaments whose precise geometry is regulated by a large cadre of actin-binding proteins. The architecture of the peripheral actin cytoskeleton strategically localized to regulate the paracellular pathway appears to be critical for TJ function. Most of the peripheral actin is positioned under the apical junctional complex where myosin II and several actin-binding proteins, including α-catenin, vinculin, radixin and cingulin, have been identified [21].

**The Transcellular Pathway**

Another important route for antigens to cross the epithelium is transcellular uptake by transcytosis [22, 23]. Costaining experiments of endocytotic vesicle compartments have shown evidence for apical endocytosis being an initial step in transcytosis (fig. 2) [24]. However, little is known about the postendocytosis antigen modifications and release into the basolateral compartment. Finally, limited information is available on the regulation of transcytosis by proinflammatory signals; however, TNF-α and IFN-γ have been reported to have a stimulatory influence [25, 26].

**Intestinal Permeability and Its Regulation**

Specific regulation of the epithelial barrier function has been described via changes in epithelial TJ structure and function which has been shown to be relevant in intestinal inflammation and celiac disease [27–29]. Much work has been generated during the last decade to explain the mechanisms contributing to TJ disruption in response to proinflammatory cytokines like TNF-α and IFN-γ [30, 31] which are elevated in celiac disease. In acute sprue stages as Marsh IIIc, TJ strand architecture is altered in freeze fracture electron microscopy as a direct correlate of the barrier impairment [32]. A similar pattern and extent of TJ changes is also observed in epithelial...
cell models like HT-29/B6 after exposure to TNF-α and/or IFN-γ [33]. This is the complex result of several regulatory influences on the cellular TJ domain which comprises transcriptional regulation as well as cleavage and redistribution of TJ proteins off TJ strands.

In T84 cells, IFN-γ has been shown to cause redistribution of occludin, claudin-1, claudin-4 and junctional adhesion molecule A from the TJ [34]. This redistribution depends on endocytosis of TJ proteins. In contrast to calcium removal inducing clathrin-dependent endocytosis of tight and adherens junction proteins into a subapical cytoplasmic compartment [35], TJ protein internalization in response to IFN-γ is due to macropinocytosis [36] and leads to the formation of a vacuolar apical compartment, which is the result of a myosin-II-mediated cytoskeleton contraction, since it can be prevented by inhibition of rho-associated protein kinase but not by myosin light chain kinase. This indicates that rho/rho-associated protein kinase signaling is the underlying mechanism [37]. This is corroborated by experimental evidence from Crohn's disease, where pharmacological inhibition of rho kinase is able to prevent inflammation via nuclear factor κB inhibition [38]. The TNF-α-induced increase in epithelial permeability was associated with a nuclear-factor-κB-dependent increase in both transcription and activation of myosin light chain kinase in Caco-2 monolayers [39, 40]. In addition, there is also experimental evidence in epithelial cell models for a NFκB-independent barrier effect of TNFα by transcriptional activation of myosin light chain kinase via TNF receptor II leading to cytoskeletal tight junction dysregulation [41].

In addition, also the expression of TJ proteins is affected by proinflammatory cytokines as expected from the findings in celiac disease patients described above. The reduction in electrical resistance as a measure of barrier function in intestinal epithelial HT-29/B6 cell monolayers was accompanied by a decrease in occludin-specific mRNA after TNF-α treatment. Reporter gene analysis of the human occludin promoter showed its downregulation by TNF-α and IFN-γ, suggesting transcriptional regulation [42]. Furthermore, the pore-forming TJ protein claudin-2 is upregulated by TNF-α in HT-29/B6 monolayers, which is consistent with elevated claudin-2 levels in patients with active celiac disease [unpubl. data]. In T84 cells, TNF-α leads to the appearance of a 10-kDa claudin-2-specific fragment.