Reassembly of the Permeability Barrier

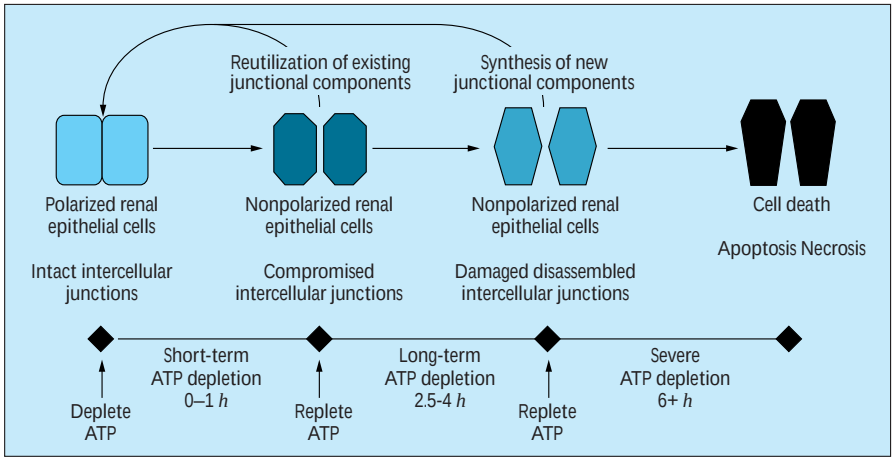

FIGURE 16-4

Cell culture models of tight junction disruption and reassembly. The disruption of the permeability barrier, mediated by the tight junction, is a key lesion in the pathogenesis of tubular dysfunction after ischemia and reperfusion. Cell culture models employing ATP depletion and repletion protocols are a commonly used approach for understanding the molecular mechanisms underlying tight junction dysfunction in ischemia and how tight junction integrity recovers after the insult [6, 12, 42]. After short-term ATP depletion (1 hour or less) in Madin-Darby canine kidney cells, although some new synthesis probably occurs, by and large it appears that reassembly of the tight junction can proceed with existing (disassembled) components after ATP repletion. This model of short-term ATP depletion-repletion is probably most relevant to transient sublethal ischemic injury of renal tubule cells. However, in a model of longterm ATP depletion (2.5 to 4 hours), that probably is most relevant to prolonged ischemic (though still sublethal) insult to the renal tubule, it is likely that reestablishment of the permeability barrier (and thus of tubule function) depends on the production (message and protein) and bioassembly of new tight junction components. Many of these components (membrane proteins) are assembled in the endoplasmic reticulum.

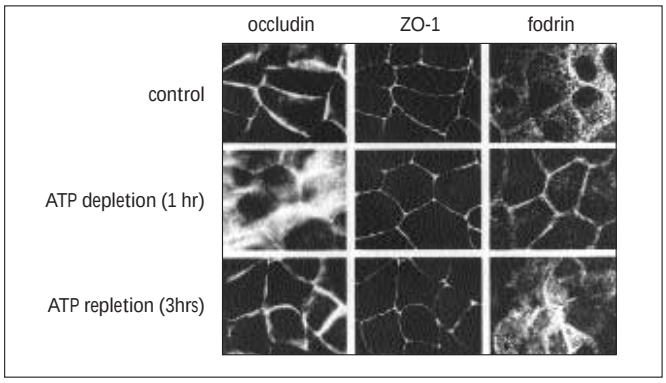

FIGURE 16-5

Immunofluorescent localization of proteins of the tight junction after ATP depletion and repletion. The cytosolic protein zonula occludens 1 (ZO-1), and the transmembrane protein occludin are integral components of the tight junction that are intimately associated at the apical border of epithelial cells. This is demonstrated here by indirect immunofluorescent localization of these two proteins in normal kidney epithelial cells. After 1 hour of ATP depletion this association appears to change, occludin can be found in the cell interior, whereas ZO-1 remains at the apical border of the plasma membrane. Interestingly, the intracellular distribution of the actin-cytoskeletal–associated protein fodrin also changes after ATP depletion. Fodrin moves from a random, intracellular distribution and appears to become co-localized with ZO-1 at the apical border of the plasma membrane. These changes are completely reversible after ATP repletion. These findings suggest that disruption of the permeability barrier could be due, at least in part, to altered association of ZO-1 with occludin. In addition, the apparent co-localization of ZO-1 and fodrin at the level of the tight junction suggests that ZO-1 is becoming intimately associated with the cytoskeleton.

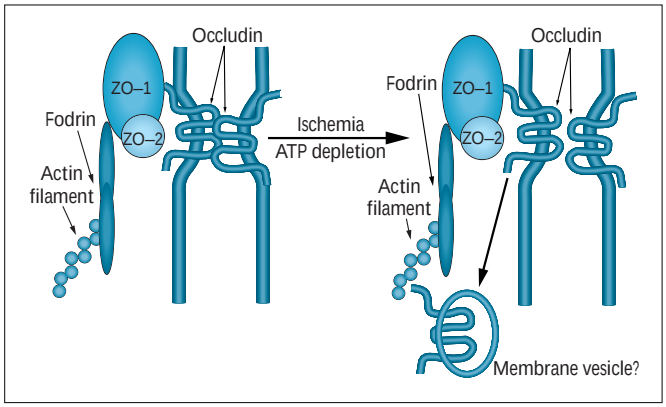

FIGURE 16-6

ATP depletion causes disruption of tight junctions. Diagram of the changes induced in tight junction structure by ATP depletion. ATP depletion causes the cytoplasmic tight junction proteins zonula occludens 1 (ZO-1) and ZO-2 to form large insoluble complexes, probably in association with the cytoskeletal protein fodrin [12], though aggregation may also be significant. Furthermore, occludin, the transmembrane protein of the tight junction, becomes localized to the cell interior, probably in membrane vesicles. These kinds of studies have begun to provide insight into the biochemical basis of tight junction disruption after ATP depletion, although how the tight junction reassembles during recovery of epithelial cells from ischemic injury remains unclear.

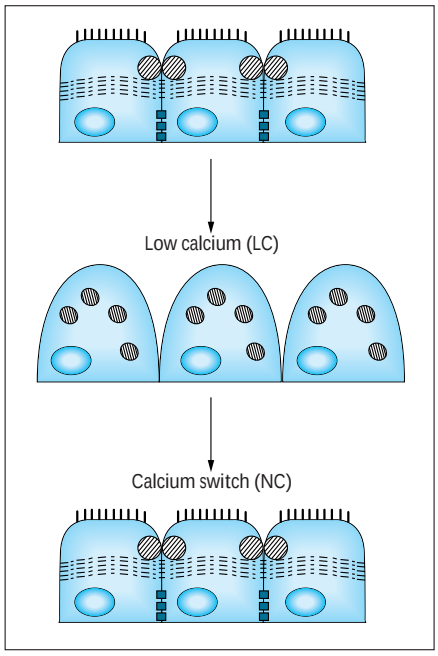

FIGURE 16-7

Madin-Darby canine kidney (MDCK) cell calcium switch. Insight into the molecular mechanisms involved in the assembly of tight junctions (that may be at least partly applicable to the ischemia-reperfusion setting) has been gained from the MDCK cell calcium switch model [43]. MDCK cells plated on a permeable support form a monolayer with all the characteristics of a tight, polarized transporting epithelium. Exposing such cell monolayers to conditions of low extracellular calcium (less than 5μM) causes the cells to lose cell-cell contact and to "round up." Upon switching back to normal calcium media (1.8 mM), the cells reestablish cell-cell contact, intercellular junctions, and apical-basolateral polarity. These events are accompanied by profound changes in cell shape and reorganization of the actin cytoskeleton. (*From* Denker and Nigam [19]; with permission)

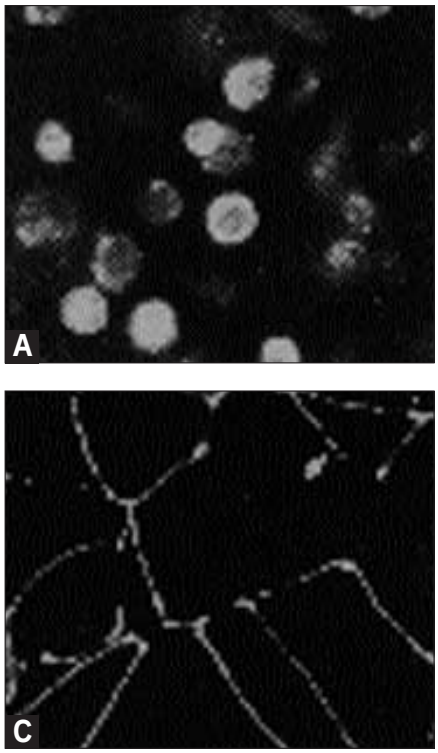

FIGURE 16-8

Protein kinase C (PKC) is important for tight junction assembly. Immunofluorescent localization of the tight junction protein zonula occludens 1 (ZO-1) during the Madin-Darby canine kidney (MDCK) cell calcium switch. In low-calcium media MDCK cells are round and have little cell-cell contact. Under these conditions, ZO-1 is found in the cell interior and has little, if any, membrane staining, **A**. After 2 hours incubation in normal calcium media, MDCK cells undergo significant changes in cell shape and make extensive cell-cell contact along the lateral portions of the plasma membrane. **B**, Here, ZO-1 has redistributed to areas of cell-cell contact with little apparent intracellular staining. This process is blocked by treatment with either 500 nM calphostin C, **C**, or 25μM H7, **D**, inhibitors of PKC. These results suggest that PKC plays a role in regulating tight junction assembly. Similar studies have demonstrated roles for a number of other signaling molecules, including calcium and G proteins, in the assembly of tight junctions [12, 13, 16–19, 37, 44–46]. An analogous set of signaling events is likely responsible for tight junction reassembly after ischemia. (*From* Stuart and Nigam [16]; with permission.)

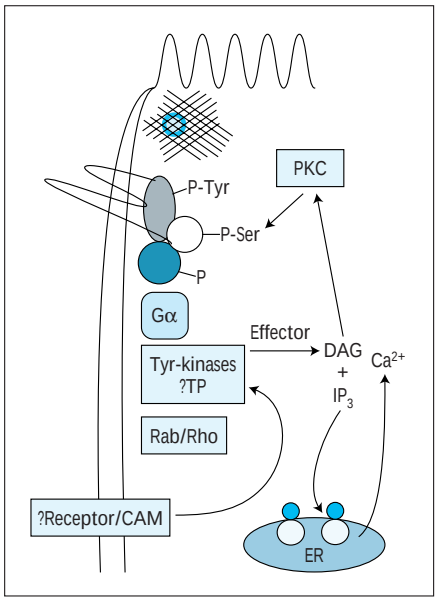

FIGURE 16-9

Signalling molecules that may be involved in tight junction assembly. Model of the potential signaling events involved in tight junction assembly. Tight junction assembly probably depends on a complex interplay of several signaling molecules, including protein kinase C (PKC), calcium (Ca^{2+}), heterotrimeric G proteins, small guanodine triphosphatases (Rab/Rho), and tyrosine kinases [13–16, 18, 37, 44–53]. Although it is not clear how this process is initiated, it depends on cell-cell contact and involves wide-scale changes in levels of intracellular free calcium. Receptor/CAM—cell adhesion molecule; DAG—diacylglycerol; ER—endoplasmic reticulum; Gα—alpha subunit of GTP-binding protein; IP3—inositol trisphosphate. (*From* Denker and Nigam [19]; with permission.)

The Endoplasmic Reticulum Stress Response in Ischemia

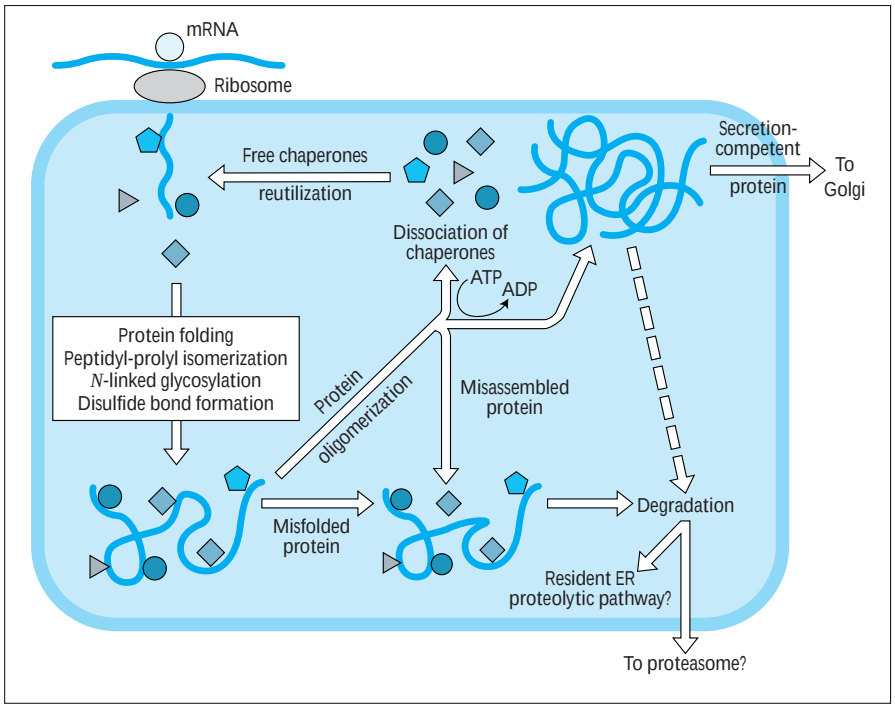

FIGURE 16-10

Protein processing in the endoplasmic reticulum (ER). To recover from serious injury, cells must synthesize and assemble new membrane (tight junction proteins) and secreted (growth factors) proteins. The ER is the initial site of synthesis of all membrane and secreted proteins. As a protein is translocated into the lumen of the ER it begins to interact with a group of resident ER proteins called molecular chaperones [20, 54–57]. Molecular chaperones bind transiently to and interact with these nascent polypeptides as they fold, assemble, and oligomerize [20, 54, 58]. Upon successful completion of folding or assembly, the molecular chaperones and the secretion-competent protein part company via a reaction that requires ATP hydrolysis, and the chaperones are ready for another round of protein folding [20, 59–61]. If a protein is recognized as being misfolded or misassembled it is retained within the ER via stable association with the molecular chaperones and is ultimately targeted for degradation [62]. Interestingly, some of the more characteristic features of epithelial ischemia include loss of cellular functions mediated by proteins that are folded and assembled in the ER (*ie*, cell adhesion molecules, integrins, tight junctional proteins, transporters). This suggests that proper functioning of the protein-folding machinery of the ER could be critically important to the ability of epithelial cells to withstand and recover from ischemic insult. ADP—adenosine diphosphate.