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Surface membrane polarity of proximal tubular cells: Alterations as a basis for malfunction

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Surface membrane polarity of proximal tubular cells: Alterations as a basis for malfunction. The surface membrane of proximal tubular cells is organized into distinct apical and basolateral membrane domains. The establishment and maintenance of these biochemically, structurally and physiologically distinct domains involves a multi-stage process involving cell-cell, cell-ECM interactions, and polarized targeting mechanisms. Ischemia, via cellular ATP depletion, results in a series of structural, biochemical and functional alterations that lead to loss of proximal tubular cell surface membrane polarity. Of central importance is the rapidly-occurring, duration-dependent disruption and dissociation of the actin cytoskeleton and associated surface membrane structures. This results in numerous cellular alterations including loss of cell-cell contact, cell-extracellular matrix adhesion and surface membrane polarity. Redistribution of surface membrane proteins and lipids into the alternate domain results in the cells inability to function properly. Repair of these disorders involves re-establishment of the actin cytoskeleton and apical and basolateral surface membrane domains. Recent information indicates growth factors may play a role in hastening this repair process.

Epithelial cell surface membrane polarity is necessary for the vectorial movement of ions, water, and macromolecules between biological compartments. The surface membrane of epithelial cells is organized into distinct apical and basolateral domains. These domains are distinguishable biochemically, structurally, and physiologically, each containing specific membrane components including ion channels, transport proteins, enzymes, cytoskeletal associations, and lipids. These components determine what cellular processes including absorption, secretion and exchange the cell will perform. The establishment and maintenance of this specialized organization is a multistage process involving the formation of cell-cell and cell-substratum contacts, and the targeted delivery of plasma membrane components to the appropriate domains. In many cases these dynamic processes require the proper organization of the cytoskeletal elements of the cell. For instance, the increased reabsorptive surface area contributed by the microvilli is dependent upon the actin cytoskeleton; the stability of cell substratum junctions, such as desmosomes and focal adhesions, are dependent on actin and intermediate filaments (IF); and finally vesicular transport throughout the cell is in part dependent upon the microtubule and actin cytoskeletal elements.

This review will first describe the basic organization of polarized epithelial cells with an emphasis on the differences between apical and basolateral membrane domains, highlighting the functional significance of these differences. Finally, the role of ischemia-induced changes in surface membrane polarity and their role in altered cellular function will be examined.

Structural characteristics of proximal tubule cells

A primary function of the proximal tubule cell (PTC) is selective reabsorption of molecules from the glomerular filtrate. To accomplish this task PTC has adapted specialized structures for reabsorption and structural stability, and utilizes a variety of targeting mechanisms to place receptors and other membrane components at their appropriate site to enable directional transport [1]. Figure 1 shows the overall characteristics of a proximal tubule cell, some of the key structural elements and the importance of domain specific components for cellular function.

Proper functioning of epithelial cells requires not only structural polarization but also polar distribution of surface membrane components, including enzymes, receptors, ATPases, ion channels, and lipids [2]. For example, sodium reabsorption by proximal renal tubular cells is dependent on the polarized delivery of specific carrier proteins such as the Na^+/H^+ antiporter and the Na^+-dependent glucose, amino acid, and phosphate cotransporters to the apical membrane, combined with localization of the Na^+,K^+-ATPase to the basolateral membrane (BLM).

Extensive differences in membrane external leaflet lipid composition also exist between the apical and basolateral domains, and result in large physiochemical differences [3]. The apical membrane cholesterol:phospholipid (C:PL) and sphingomyelin:phosphatidylcholine (SPH:PC) ratios are high, resulting in high anisotropy and insulating capacity. In contrast, the BLM C:PL and SPH:PC ratios are low, with the phosphatidylcholine (PC) and phosphatidylinositol (PI) contents being relatively high. This results in a membrane that is more fluid (low anisotropy) and across which diffusion can occur more rapidly. Membrane lipid composition and anisotropy also influence the function of intrinsic membrane proteins, such as Na^+,K^+-ATPase and the Na^+-dependent glucose cotransporter.

Apical and BLM domains are separated from each other by the cellular junctional complex. This complex is also responsible for the epithelial cell barrier separating, for proximal tubule cells, the glomerular filtrate and blood compartments. In addition, the structural stability of both the epithelial sheet and individual cells is dependent upon junctional complexes to adhere cells to the extracellular matrix, (hemidesmosomes and focal adhesions), to stabilize cell-cell interactions (desmosomes, zonula adherens), to

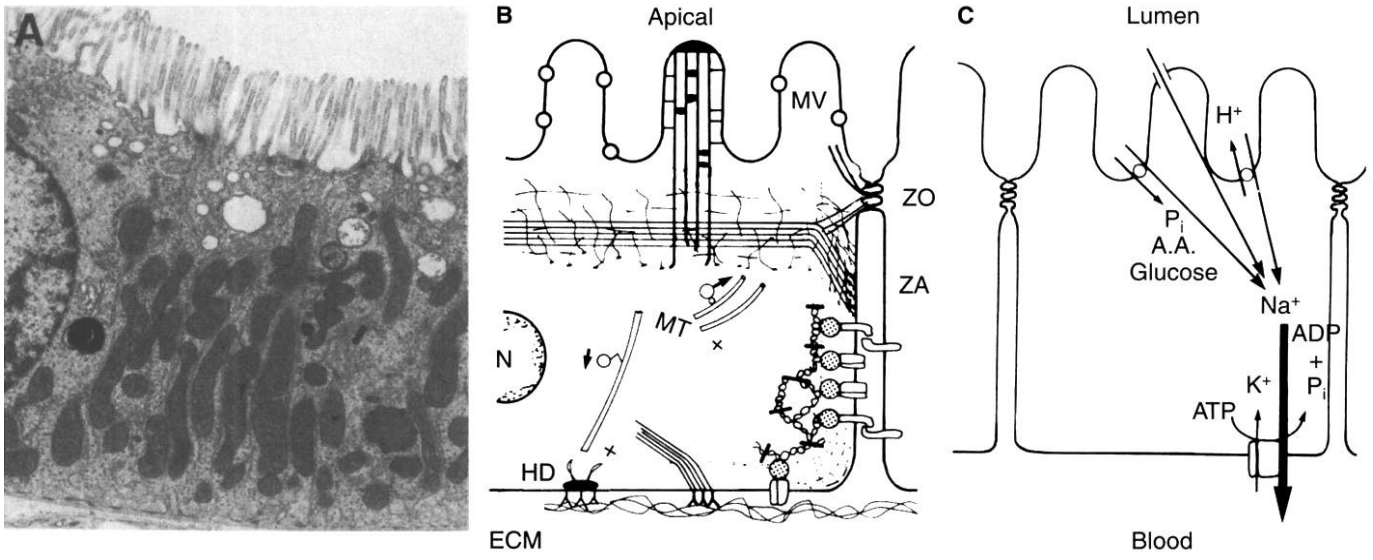

Fig. 1. *Proximal tubule cell cytoskeletal-surface membrane interactions.* **A.** Low power EM of proximal tubule cell. **B.** Cytoskeletal elements and their surface membrane interactions, cell-cell interactions and attachment to the ECM via integrins. **C.** Functional polarity of several apical and BLM transport proteins. Symbols are: (-o-) apical proteins; (–,=) myosin I; (▬) villin, fimbrin; (▩) ZO-1, cingulin; (≡) F-actin microfilaments; (—) fodrin; (◍) ankyrin; (⥽) cadherin; (⅄) integrin; (⊖) Na^+,K^+-ATPase.

permit communication between cells (gap junctions), and to form both a plasma membrane and extracellular barrier (tight junction).

The tight junction (zonula occludens) encircles the apex of the cell, fusing adjacent cells together and establishing the border between the apical and basolateral domains [4]. It forms a barrier to intradomain movement of intrinsic membrane proteins and outer leaflet phospholipids (the "fence" function), and to paracellular movement of solutes between biologic compartments ("gate function") [5].

The zonula adherens consists of a circumferential band of actin microfilaments of mixed polarity encircling the apex of the cell just basal to the zonula occludens. Myosin II, α-actin, and tropomyosin have all been identified, and are thought to both cross-link and provide contractile potential for F-actin. This complex is attached to the lateral membrane by an adhesion plaque containing α-actin, zyxin, vinculin, and radixin [6]. These, in turn, link to catenin/cadherin complexes, which mediate cell-cell adhesion. Cadherins are a superfamily of transmembrane glycoproteins that bind with each other in a homophilic manner to mediate Ca^{2+}-dependent cell-cell adhesion [7]. The family of "classical" cadherins includes at least 12 members, including uvomorulin (E- or epithelial-cadherin). Interactions with catenins appear important both for cell-cell binding and for interactions with the actin cytoskeleton [8]. Cadherins are also known to be phosphorylated, and tyrosine kinases may be involved in their regulation.

Desmosomes (macula adherens) lie in a loose band below the zonula adherens and are also distributed along the lateral membrane, forming cell-cell adhesions mediated by cadherins. Hemidesmosomes are distributed along the basal membrane, and form cell-substratum adhesions mediated by integrins [9].

Integrins are a family of heterodimeric transmembrane glycoproteins that mediate Ca^{2+}-dependent cell-substratum and heterophilic cell-cell binding [10]. There are at least 20 members, generated from permutations of pairings between at least 14 α- and 8 β-subunit isoforms. The pattern of binding to the various components of the extracellular matrix, such as fibronectin, laminin, and various collagens, varies with each integrin family member, but generally involves the Arg-Gly-Asp (R-G-D) tripeptide sequence. Integrins have also been implicated in heterophilic cell-cell adhesion [11]. Integrin function appears regulated by both extracellular and intracellular signals. Binding of an extracellular ligand apparently induces conformational changes in the intracellular portion of the β-subunit, which in turn regulate interactions with the actin cytoskeleton. Integrins are also known to be phosphorylated at serine and tyrosine sites.

Focal adhesions are distributed along the basal membrane, and anchor cells to the extracellular matrix [6] (Fig. 1B). Actin microfilaments are cross-linked by an α-actinin (in some *in vitro* cases this results in the formation of stress fibers) and linked to the membrane by a protein complex that includes talin, vinculin, and actin microfilament capping proteins. These, in turn, link to integrin heterodimers, which mediate cell-substratum adhesion.

Establishment and maintenance of epithelial cell polarity

Establishment of a polarized surface membrane begins with cell-cell recognition and contact mediated by cadherins [12, 13]. This allows for establishment of specific protein domains that serve as foci for assembly of junctional and non-junctional cytoskeletal structures, contributing to the formation of apical and basolateral domains. Other membrane proteins, such as Na^+,K^+-ATPase, are subsequently incorporated and organized within this cytoskeletal network.

Further genesis and maintenance of a polarized surface membrane depends upon the sorting and targeting of newly-synthesized proteins [1, 2]. A variety of pathways appear to be involved

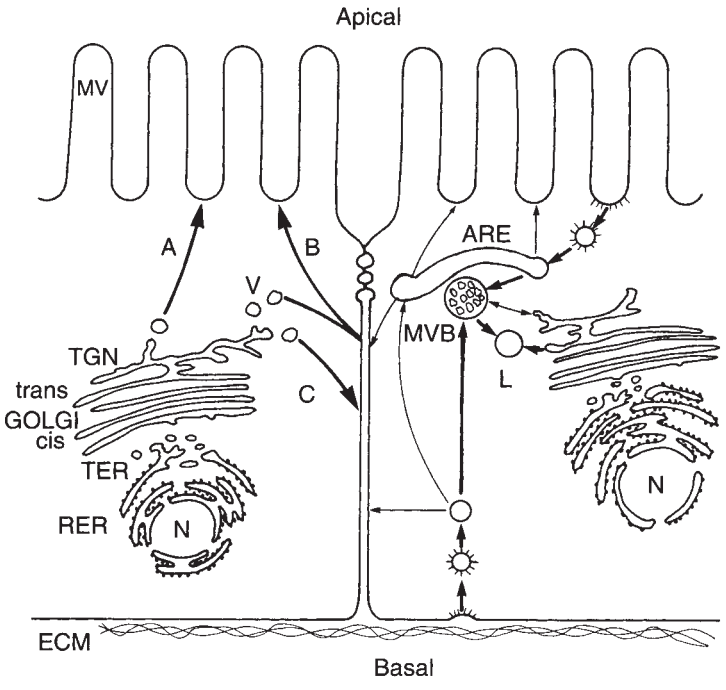

Fig. 2. *Establishment and maintenance of apical and BLM polarity via intracellular membrane trafficking.* **A.** Synthesis, sorting and targeting of newly synthesized surface membrane components. **B.** Endocytic, recycling and transcytotic routes for apical and BLM components. Thick arrows indicate degradative pathways. Thin arrows show recycling and transcytotic pathways. Abbreviations are: TGN, trans-Golgi network; TER, transitional endoplasmic reticulum; RER, rough endoplasmic reticulum; N, nucleus; ARE, apical recycling and sorting endosome; MVB, multivesicular body (prelysosomal degradative endosome); L, lysosome.

(Fig. 2). Apical and basolateral membrane proteins are synthesized in the endoplasmic reticulum, transported through the same Golgi complex and delivered to a trans-Golgi network where sorting occurs. Another method the cell uses to sort is observed with the Na^+,K^+-ATPase that is initially delivered equally to the apical and basolateral membranes of MDCK cells. However, the assembly, with cell-cell contact, of a basolateral actin cortical cytoskeleton allows for selective retention and stabilization of Na^+,K^+-ATPase. The end result is a polarized distribution, with higher levels in the basolateral domain [14]. A third method used by some cells, notably hepatocytes, to sort apical membrane proteins involves delivery of all membrane proteins to the basolateral surface and selective transcytosis of apical proteins to the apical surface. This transcytotic pathway is part of the continuous process of sorting and targeting that takes place in all epithelial cells, since they are continuously internalizing components from their surface.

Establishment of surface membrane lipid polarity is less well understood, in part because of the lack of immunologic techniques and domain-specific markers. Sphingolipids are preferentially transferred from the TGN to the apical domain, perhaps via vesicles containing co-clustered GPI-anchored proteins [15]. Possible mechanisms for the sorting of other lipid species are less clear. Proteins and lipids delivered to either the apical or basolateral surface are restrained by the zonula occludens "fence" function and by specific interactions with the actin cortical cytoskeleton. Selective retention in the membrane with exclusion from endocytosis may also play an important role.

Delivery of vesicles to either the apical or basolateral surface is an active process requiring ATP and MT- or actin-based motor molecules [16]. The MT motors kinesin and dynein are responsible for transport to the (+) or (−) end of MTs, respectively (Fig. 1B). The regulation of this motility and specificity of motor-membrane interactions are poorly understood but could be one mechanism by which targeting occurs. For example, a vesicle destined for the apical surface, toward the (−) end, may preferentially associate and activate dynein or release or inactivate kinesin. Accessory proteins and post-translational modifications are likely involved in these intricate motility pathways. In addition delivery to the plasma membrane requires movement through or around the cortical actin network. Direct vesicular movement along actin, possibly mediated by a myosin molecule, has been reported and may be necessary for fusion with the plasma membrane to occur [16]. Additional regulators of motility and fusion processes include GTP-binding proteins and calcium whose local concentration may be altered by extracellular signals emanating through cadherins or integrins.

The efficient sorting and recycling of endocytosed plasma membrane domain occurs with a high degree fidelity that is necessary to maintain surface membrane polarity [17]. During adsorptive endocytosis, plasma membrane containing receptor-ligand complexes are internalized via clatherin-coated pits (Fig. 2B). Subsequent processing involves sequential loss of clatherin from coated vesicles, fusion of these vesicles with an "early" endosomal compartment and progression to a lysosomal compartment via "late" multivesicular endosomal structures. Endosomal derived membrane components can also recycle back to the membrane of their origin [17] or undergo transcytosis to an alternate surface membrane domain. Distinct endosomal systems exist for each plasma membrane (PM) domain [17, 18]. An early or "sorting" set of endosomes lies close to either PM domain and receives cargo endocytosed exclusively from that domain [17]. In proximal tubule cells beta-2-microglobulin internalized into apical and basolateral early endosomes was shown to converge in a common, apically oriented, "late" endosomal compartment (multivesicular body) before delivery to lysosomes [18]. Translocation

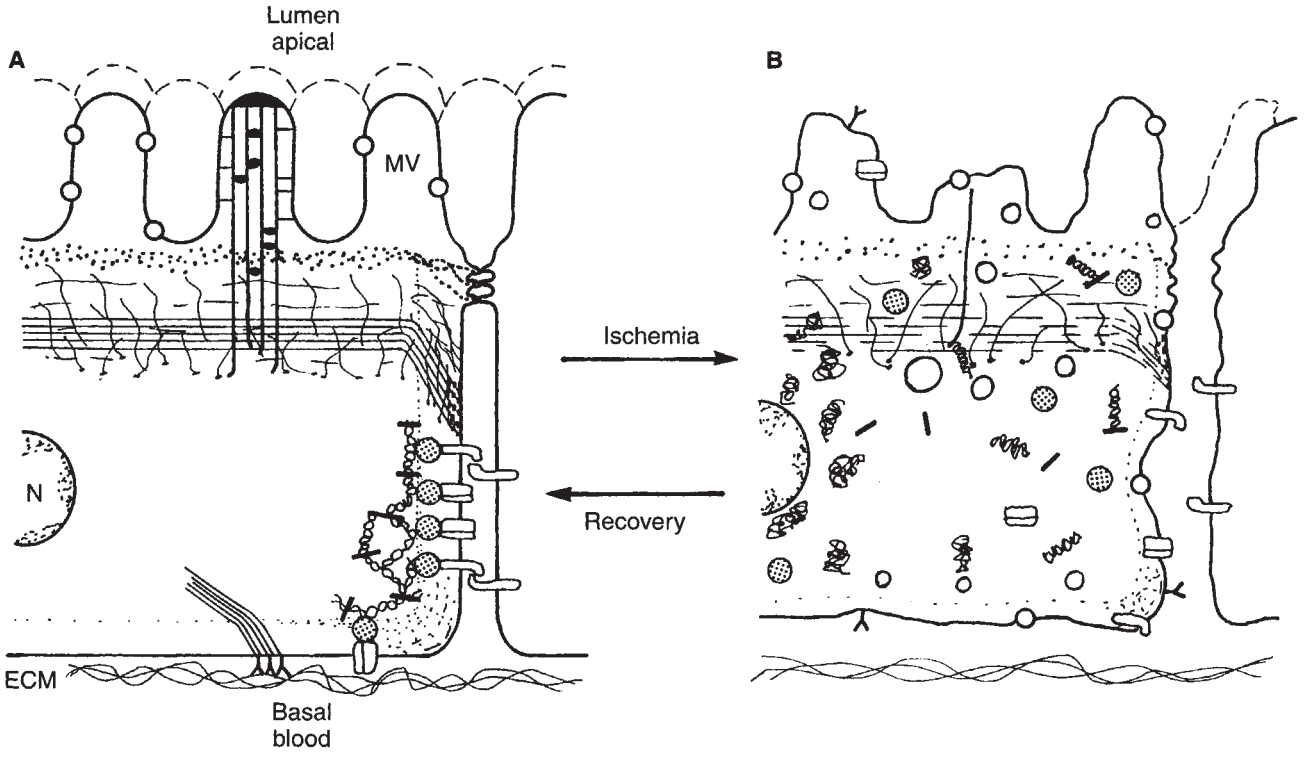

Fig. 3. *Effect of ischemia and reperfusion on the cytoskeleton, cytoskeletal-surface membrane interactions and surface membrane polarity in proximal tubule cells.* **A.** Cytoskeletal-surface membrane interactions as shown in Figure 1. **B.** Cytoskeletal and surface membrane alterations occurring during ischemia. Note loss of cytoskeletal-surface membrane interactions, cell-cell attachment, cell-ECM attachment and polarity of apical and BLM proteins. Cellular repair (recovery) is associated with correction of these alterations and reestablishment of a functionally polar cell.

of cargo from the early to the common late endosomal compartment appears to take place via a microtubule-dependent mechanism [17]. A different pathway has been demonstrated for transcytosis in MDCK cells when a specific marker of basolateral to apical transcytosis (the polymeric immunoglobulin receptor) was used [17, 19]. Material endocytosed through the basolateral endosomal system converged with apically endocytosed material at the level of the apical sorting endosome. The transcytosed (degradative) material to be did not converge with apically endocytosed material in the late apical endosomal compartment [17]. Microtubules were required for this process as well.

Altered epithelial cell polarity during ischemia: Role of the cytoskeleton and functional significance

Tissue ischemia occurs when blood flow is reduced to levels insufficient to maintain cellular energy levels. Depletion of ATP induces a series of structural, biochemical, and functional deviations. The kidney proximal tubule cell is particularly susceptible to ischemia, and serves as a useful model both *in vivo* and *in vitro*. Of central importance appears to be a rapidly-occurring, duration-dependent disruption and dissociation of the actin cytoskeleton and associated surface membrane structures.

In vivo, ischemia induces disruption of microvillar actin cores and the apical circumferential actin microfilament network, with redistribution of actin from the apical pole to throughout the cytoplasm [20]. *In vitro*, using the proximal tubule-derived LLC-PK_1 cell line, antimycin A-induced ATP depletion resulted in disruption of the cortical cytoskeleton and redistribution of actin into large cytoplasmic aggregates primarily located in the perinuclear region [21]. Within the first five minutes of ATP depletion, there was a significant conversion of monomeric (G) actin to polymeric (F) actin [21], possibly involving effects on G-actin sequestering proteins and F-actin capping and nucleation-inhibiting proteins. These changes have also been confirmed in mouse proximal tubule cells grown in primary cultures [22]. Concurrent with this disruption of the actin microfilament network was a dissociation of the cortical cytoskeleton. *In vivo*, Na^+,K^+-ATPase, and spectrin became Triton X-100 soluble (that is, cytoskeleton-dissociated), with the Na^+,K^+-ATPase being free of actin, spectrin, and uvomorulin [23]. *In vitro*, ankyrin and spectrin distributed throughout the cytoplasm and were not associated with each other [24]. Concurrent with or following this disruption and dissociation of the cortical actin cytoskeleton, the surface membrane undergoes extensive changes (outlined in Fig. 3B). These include alterations in microvilli morphology, disruption of cellular junctions, and loss of surface membrane polarity. Apical microvilli are lost by fragmentation with shedding into the lumen and internalization into the cytoplasm. Disruption of the zonula occludens leads to increases in intercellular permeability (loss of "gate" function) [25], and membrane lipids and proteins are free to cross domains (loss of "fence" function). Apical domain sphingomyelin and cholesterol contents decrease while PC, PI, and total phospholipid contents increase, thus enhancing apical membrane fluidity [26]. Na^+,K^+-ATPase, released from the cortical cytoskeleton, redistributes into the apical domain, while the apical marker protein leucine aminopeptidase redistributes into the basolateral

domain [26]. Alterations in both intermediate filament protein expression and location have also been observed following ischemia. In addition, a recent study also documented the dissociation of ezrin from the cytoskeleton during anoxia [27]. Similar investigations using the BS-C-1 cell line and H_2O_2 oxidative stress have revealed disruption of focal adhesions with loss of talin from the basal cell surface [28]. The disruption detaches the associated integrins, allowing them to redistribute into the apical domain. Additional changes occurring to MTs and IFs throughout ischemia ATP depletion have not been analyzed extensively, but are suggested by some earlier studies. In addition, recent information shows microtubules in S_3 cells, although not grossly disrupted during ischemia *in vivo*, undergo fragmentation during the first hour of reperfusion. This defect was corrected during 24 hours of reperfusion [29].

The functional ramifications of these changes in cytoskeletal structure and surface membrane polarity are substantial. Loss of microvilli results in a markedly decreased apical membrane surface area. Increases in intercellular permeability allow for increased "backleak." Redistribution of membrane lipids change membrane physiochemical properties, which may affect integral membrane protein function. For example, reduced glucose reabsorption after ischemic injury appears related in part to decreases in the apical SPH:PC ratio. In brush border membrane vesicles (BBMV) isolated after 15 minutes of ischemia, the V_{max} for Na^+-dependent glucose transport and the number of phlorizin binding sites decreased dramatically compared to controls. Though this could represent a redistribution of Na^+-dependent glucose "carriers" to the alternate domain, carrier-mediated Na^+-dependent alanine transport was not similarly affected. Moreover, glucose transport in BBMV was highly correlated with the SPH:PC ratio and inversely correlated with membrane fluidity. In addition, recovery from ischemia returned glucose transport to control levels concurrent with the normalization of the apical SPH:PC ratio.

Finally, redistribution of membrane proteins to the alternate domain may result in substantial changes in domain-specific functions. Perhaps the best-studied example in this category involves Na^+,K^+-ATPase. Under physiologic circumstances, sodium enters the cell via a variety of apical transport proteins, providing the energy for uptake of a variety of solutes while moving down its electrochemical gradient. Sodium is then transported out of the cell up its electrochemical gradient via basolateral Na^+,K^+-ATPase (Fig. 1C). Under these circumstances vectorial transport of sodium and other solutes is coupled to ATP utilization. With ischemia-induced redistribution of Na^+,K^+-ATPase to the apical membrane, sodium that has entered the cell may be transported out of the cell across either the apical or the basolateral membrane. Apical transport via Na^+,K^+-ATPase results in a futile cycle, with transport of sodium now uncoupled from ATP utilization. *In vivo* evaluation of sodium reabsorption by micropuncture and by lithium and sodium reabsorption confirmed decreased proximal tubule sodium absorption after ischemia correlated with apical redistribution of Na^+,K^+-ATPase. Direct evidence for functioning of apical Na^+,K^+-ATPase came from *in vitro* cell culture studies utilizing LLC-PK_1 cells [30]. Basolateral redistribution of apical transport proteins could similarly affect cellular transport.

The apical redistribution of integrins may also have important functional ramifications, as adherence of unattached cells to cell monolayers is increased in cells previously exposed to ATP depletion [22], oxidant stress [28] and this interaction appears dependent on the RGD sequence of integrins. Such cell-cell interactions within a renal tubule lumen may well lead to cell clumping and lumenal obstruction [31].

Partial confirmation of a central role for actin cytoskeletal changes in surface membrane alterations comes from studies using cytochalasin D, which disrupts actin microfilaments by binding to their "plus" end. Perfusion of kidneys with cytochalasin D, under conditions in which disruption was selective for the actin microfilaments, resulted in loss of microvilli with membrane fusion and fragmentation, and well-correlated time-dependent decreases in sodium and lithium reabsorption [20]. Morphologically and physiologically, these changes were similar to changes seen during ischemia. Cytochalasin D has also been shown to disrupt the zonula occludens "gate" function in enterocytes.

An intriguing and potentially important problem is the increased sensitivity to ischemia of proximal compared to distal tubule cells with respect to a variety of morphologic, biochemical, and functional derangements. For example, *in vivo* renal ischemia for up to 50 minutes induces extensive apical actin microfilament disruption in proximal tubule cells, but essentially no alterations in distal cells. Similarly, ischemia for 15 minutes induces extensive disruption of tight junctions and redistribution of Na^+,K^+-ATPase to the apical domain in proximal cells, but no such changes in distal cell [23] (Molitoris BA, Dahl R, unpublished data). Finally, ischemia-induced impairment of sodium reabsorption is also largely due to proximal tubule dysfunction, with distal tubule function little-affected, as evidenced by good correlations between the fractional excretion of sodium and lithium [32]. The observation that cytochalasin D infusion also affects proximal but not distal tubular sodium reabsorption [20] raises the possibility that intrinsic differences in actin cytoskeletal susceptibilities may play an important role in this differential sensitivity.

Re-establishment of apical and basolateral membrane polarity occurs during the recovery phase of ischemic acute renal failure and has been demonstrated for leucine aminopeptidase, Na^+,K^+-ATPase, and apical and BLM lipids (Fig. 4) [26, 32]. The mechanisms responsible for restitution of surface membrane polarity have not been determined, but primarily involve actual remodeling of surface membrane domains in previously damaged cells as opposed to extensive cellular proliferation. The rate at which repolarization occurs is dependent on the severity of the injury. Fifteen minutes of ischemia (mild injury) required only 24 to 48 hours of reperfusion, while 50 minutes of ischemia (moderate to severe injury) required several days for re-establishment of apical and basolateral membrane polarity *in vivo* [32]. In both cases, return of structural polarity (cytoskeleton) is a prerequisite for re-establishment of surface membrane polarity.

The study of factors involved in and responsible for cellular recovery from ischemia is now being pursued with vigor, but remains in its infancy. Epidermal growth factor, insulin-like growth factor, and hepatocyte growth factor [33], have all been shown to accelerate recovery. Heat shock proteins have been shown to accumulate following ischemia [34]. Recovery from ischemia may be aided by an acidic environment which was shown to stabilize the actin cytoskeleton during *in vitro* ATP depletion [35]. Finally, the addition of glycine which acts as a cytoprotectant during ischemia may facilitate a more rapid recovery following ischemia [36]. However, the underlying cellular mechanisms by

which these agents provide for enhanced recovery remain to be determined. For instance, such basic questions as whether the disrupted and dissociated actin cytoskeletal components are re-utilized or if synthesis of new proteins is required remain unanswered.

In summary, ischemia in kidney proximal tubule cells induces a rapidly-occurring, duration-dependent disruption of the actin cytoskeleton. This, in turn, leads to disruption of associated surface membrane structures and untethering of integral membrane proteins. Lipids and proteins move to alternate domains, and cell-cell and cell-substrate contacts are lost. The cell is no longer able to perform vectorial transport, and cellular and organ-level dysfunction ensues. We hypothesize re-establishment of cytoskeletal integrity occurs early on during the recovery phase (reperfusion) and mediates re-establishment of surface membrane polarity and the return of cellular function (Fig. 3).

Acknowledgments

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