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Cell biology and physiology of the uroepithelium

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¹Laboratory of Epithelial Cell Biology and Renal Electrolyte Division, Department of Medicine, and ³Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania; and ²Departments of Pathology, Molecular Genetics and Microbiology, and Immunology, Duke University, Durham, North Carolina

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Khandelwal P, Abraham SN, Apodaca G. Cell biology and physiology of the uroepithelium. *Am J Physiol Renal Physiol* 297: F1477–F1501, 2009. First published July 8, 2009; doi:10.1152/ajprenal.00327.2009.—The uroepithelium sits at the interface between the urinary space and underlying tissues, where it forms a high-resistance barrier to ion, solute, and water flux, as well as pathogens. However, the uroepithelium is not simply a passive barrier; it can modulate the composition of the urine, and it functions as an integral part of a sensory web in which it receives, amplifies, and transmits information about its external milieu to the underlying nervous and muscular systems. This review examines our understanding of uroepithelial regeneration and how specializations of the outermost umbrella cell layer, including tight junctions, surface uroplakins, and dynamic apical membrane exocytosis/endocytosis, contribute to barrier function and how they are co-opted by uropathogenic bacteria to infect the uroepithelium. Furthermore, we discuss the presence and possible functions of aquaporins, urea transporters, and multiple ion channels in the uroepithelium. Finally, we describe potential mechanisms by which the uroepithelium can transmit information about the urinary space to the other tissues in the bladder proper.

uroplakins; exocytosis; endocytosis; tight junctions; stretch; urothelium

THE UROEPITHELIUM IS AN EPITHELIAL tissue that lines the distal portion of the urinary tract, including the renal pelvis, ureters, bladder, upper urethra, and glandular ducts of the prostate. Functionally, it forms a distensible barrier that accommodates large changes in urine volume while preventing the unregulated exchange of substances between the urine and the blood supply. This task is accomplished by specializations of the outermost umbrella cell layer, including high-resistance tight junctions (2, 135, 210), an apical glycan layer (94), and an apical membrane with a distinctive lipid and protein composition (86, 92, 151), and the ability of the umbrella cells to alter their apical surface area by exocytosis and endocytosis (10, 117, 132, 205). In addition to its role as a barrier, the uroepithelium can modulate the movement of ions, solutes, and water across the mucosal surface of the bladder (133, 151, 175, 189, 191, 192, 234). Furthermore, the uroepithelium releases various mediators and neurotransmitters, including ATP, adenosine, and ACh, from its serosal surface, which may allow the epithelium to transmit information about the state of the mucosa and bladder lumen to the underlying tissues, including nerve processes, myofibroblasts, and musculature (11, 24). Thus the uroepithelium is a dynamic tissue that not only responds to changes in its local environment but can also relay this information to other tissues in the bladder. In this review, we summarize recent findings regarding the cell biology and function of the uroepithelium, with particular emphasis on the outermost umbrella cell layer.

CHARACTERISTICS OF THE UROEPITHELIUM, ITS STEM CELLS, AND ITS REGENERATION

The uroepithelium, or urothelium, is composed of umbrella, intermediate, and basal cell layers (Fig. 1). Early studies reported that thin cytoplasmic extensions connect the various cell layers to the basement membrane (163), suggesting that the uroepithelium is pseudostratified. However, subsequent studies used serial sectioning and electron microscopy to show that the uroepithelium is stratified and that cytoplasmic extensions are rarely observed in the intermediate cell layers, but never in the umbrella cell layer (102, 178). We concur with Wu et al. (230) that the term transitional epithelium, which is often used in histology textbooks and connotes a pseudostratified epithelium, be discarded and that urothelium/uroepithelium be used in its place.

Umbrella cell layer. Umbrella cells (also known as facet cells or superficial cells) are a single layer of highly differentiated and polarized cells that have distinct apical and basolateral membrane domains demarcated by tight junctions (2, 135, 210). These cells are mono- or multinucleate (depending on species), polyhedral (typically 5- or 6-sided), and between 25 and 250 μm in diameter (Fig. 2A). The morphology and size of these cells are dependent on the filling state of the bladder. In unfilled bladders, umbrella cells are roughly cuboidal; in filled bladders, these cells become highly stretched and are squamous in morphology (85, 205). The periphery of each cell is bordered by a 500-nm ridge that is apparent in scanning electron micrographs (Fig. 2A). High-resolution atomic force microscopy indicates that the ridge is formed by interdigitation of the apical membrane of adjacent cells, which may zipper the apical membrane just above the tight junction and may con-

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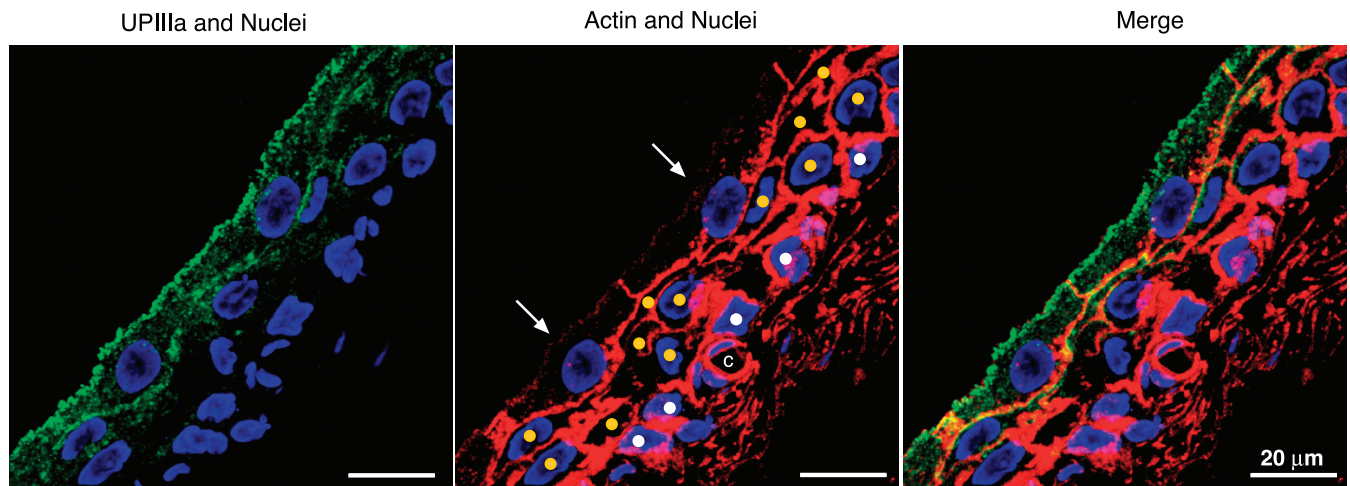


Fig. 1. Tissue architecture of the uroepithelium. Rat bladder uroepithelium was stained with FITC-phalloidin (green) to label the cortical actin cytoskeleton associated with plasma membranes of uroepithelial cells, TO-PRO3 (blue) to label nuclei, and an antibody that recognizes the cytoplasmic domain of uroplakin (UP) IIIa (red). *Left*: distribution of UPIIIa and nuclei. *Middle*: actin and nuclei. Umbrella cells are marked by arrows, intermediate cells by yellow circles, and basal cells by white circles. A capillary (c) below the basal cell layer is shown. *Right*: all 3 markers.

tribute to the high-resistance paracellular barrier formed by adjacent umbrella cells (120).

One of the mostly readily identifiable features of these cells is the scalloped nature of their apical plasma membrane, which comprises plaques and intervening hinge regions (Fig. 2*B*). The membrane associated with the hinge and plaque regions is highly detergent insoluble (138), likely reflecting the unusual lipid composition of this membrane, which is similar to myelin and rich in cholesterol, phosphatidylcholine, phosphatidylethanolamine, and cerebroside (86). Relative to the basolateral surface, only a small amount of actin is associated with the apical pole of the umbrella cell, and the apical plasma membrane may be devoid of actin (172, 226). However, other studies show that small amounts of filamentous actin are variably associated with the apical pole of the umbrella cell (cf. Fig. 5, *B* and *C*) (2, 15), possibly indicating that the amount of actin may change, depending on the physiological state of the uroepithelium before fixation, or that this pool of actin is difficult to fix. In the plaque regions, the outer leaflet of the plasma membrane appears to be twice as thick as the inner leaflet, forming an asymmetric unit membrane (AUM) (88, 164, 222) (Fig. 2*C*). The outer leaflet of the AUM appears thicker, because it contains a crystalline array of 16-nm-diameter AUM particles comprising six subunits that are arranged to form inner and outer rings (Fig. 3*A*) (146, 222). The major constituents of the AUM particles are a family of transmembrane proteins called uroplakins (UPs; Fig. 3*B*), which include UPIa, UPIb, UPII, UPIIIa, and UPIIIb (see below). A plaque contains ~1,000–3,000 AUM particles. The hinge areas contain at least one unique protein, which is called urohingin (233). Because the plaque regions are crystalline in nature, all other apically distributed nonplaque proteins, such as receptors and channels, may be localized to the hinge areas.

An additional feature of these cells is the presence of discoidal or fusiform-shaped vesicles (DFV) and associated cytokeratin filaments, which accumulate under and fuse with the apical surface of the cells in response to bladder filling (Fig. 2*B*) (85, 205). As described below, DFV are responsible for delivery of UPs and other proteins to the apical surface of the

cells. In humans and rabbits, the vesicles are primarily disc-shaped or ovoid (10, 102); in rats and mice, the vesicles are often long and spindle-shaped and have a fusiform appearance (Fig. 2*B*) (10). Similar to other regulated secretory granules, DFV have an acidified lumen (76); however, the functional significance of this reduced pH is unknown. DFV are intimately associated with cytokeratins, which form an elaborate subapical “trajectorial” network that may be unique to umbrella cells (Fig. 4*A*) (216). It is possible that the cytokeratins may directly bind to the cytoplasmic tail of UPIIIa (91), but this has not been addressed experimentally, and recent high-resolution imaging techniques show intermediate filaments in close association, but not in direct contact, with DFV (201). The constituents of the cytokeratin network include cytokeratins-7 and -20 (214, 216) but may also include other members of this family. The network forms a regular meshwork that begins ~150–300 nm below the apical plasma membrane; the walls of this meshwork run perpendicular to the apical plasma membrane, forming parallel tunnels (216).

DFV are found just below the apical membrane in the region that is devoid of the cytokeratin network and also within the tunnels of the cytokeratin mesh (Fig. 4*B*) (216). The walls of the network form smaller-diameter openings near the apical surface, which become larger at the opposite side of the network as some of the walls become shorter and individual tunnels fuse with one another, creating a larger entrance at the surface of the network than that which faces the basal surface of the cell. During bladder filling, the openings in the network become larger and the number of walls per unit area and their thickness decrease as the umbrella cells flatten and elongate (216). Presumably, the enlarged openings are conducive to trafficking of DFV during bladder filling. Along the apicolateral junction of the cell, the cytokeratins are arranged parallel to the apical plasma membrane and form a frame that may provide structural rigidity to the network and serve as an attachment zone for desmosomes that are concentrated in this subapical region (Fig. 4*A*) (215, 216).

Intermediate and basal cell layers. Deep to the umbrella cells is one to several strata of intermediate cells and a single

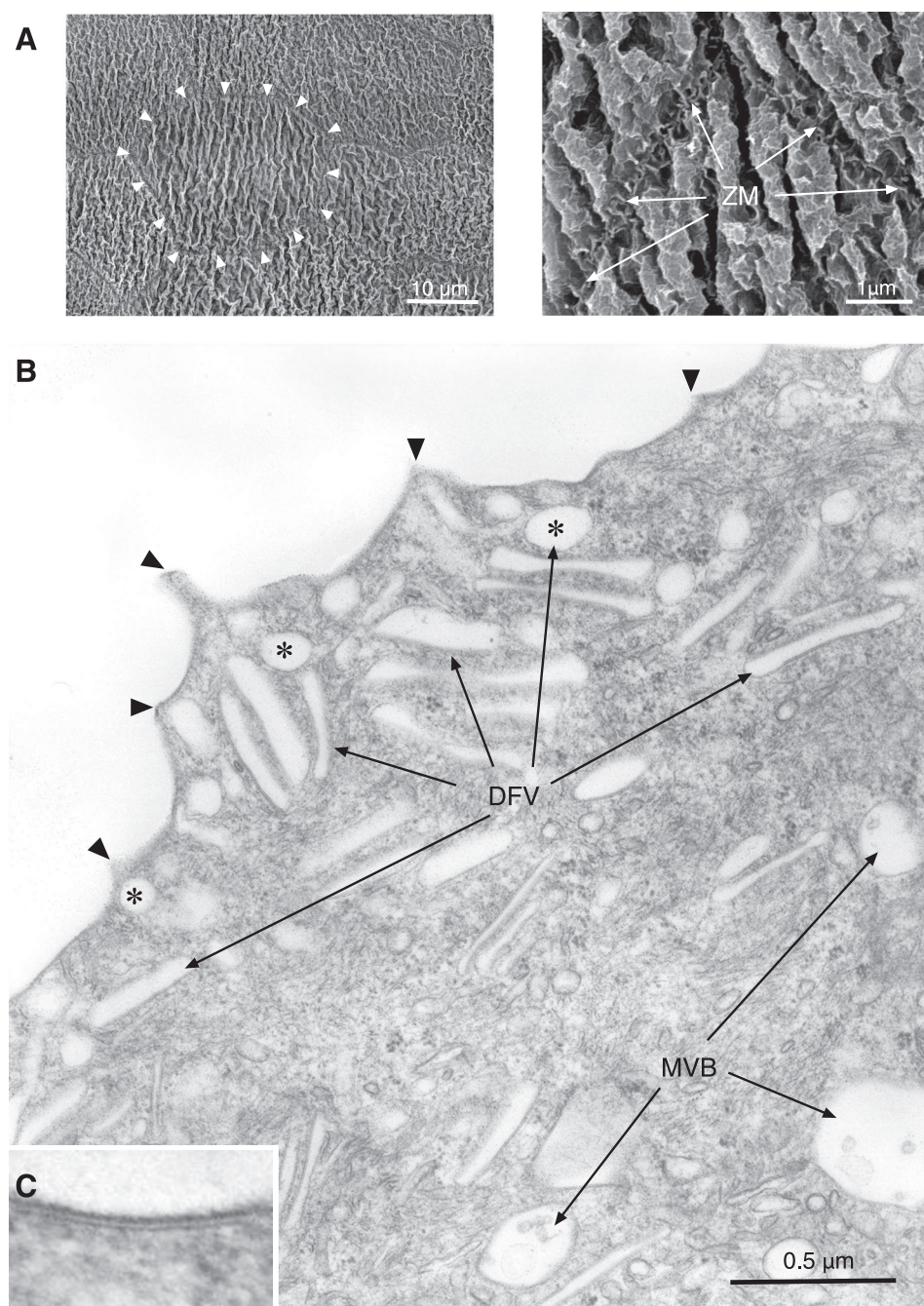


Fig. 2. Ultrastructure of bladder uroepithelium. *A*: scanning electron micrographs of mucosal surface of the bladder. *Left*: overview of the apical surface of rabbit umbrella cells. Borders of an individual umbrella cell are indicated by arrowheads. *Right*: ridge, consisting of zippered apical membrane (ZM), at the periphery of adjacent umbrella cells. *B*: transmission electron micrograph of the apical pole of a rat umbrella cell. Examples of discoidal/fusiform-shaped vesicles (DFV) are marked with arrows and hinges with arrowheads. Plaques are located in the intervening membrane between hinges. Apical cytoplasm of rat umbrella cells has disc-shaped (*) and fusi-form-shaped vesicles. Multivesicular bodies (MVB; i.e., late endosomes) are indicated by arrows. *C*: asymmetric unit membrane (AUM) at the apical surface of a rat umbrella cell. Contrast was adjusted using Photoshop.

layer of basal cells (Fig. 1). The intermediate cells are often pear-shaped (pyriform), have a single nucleus, are ~ 10 – 15 μm diameter, and are connected to one another and the overlying umbrella cell layer by desmosomes (102) and, possibly, by gap junctions (78). The number of intermediate cell strata is species dependent: in rodents, the intermediate cells are one to two layers thick (Fig. 1); in humans, up to five strata are observed (102). The apparent thickness of the overall intermediate cell layer varies with the state of bladder filling. The strata of intermediate cells appear fewer in number in the distended than in the voided bladder (85). How this phenomenon is accomplished is unknown but could result from adjacent strata of intermediate cells sliding past one another during

filling. Whether this involves reversible breakage of cell-cell contacts is unknown. The layer of intermediate cells just beneath the umbrella cells is partially differentiated, and these cells can express UPs and have DFV (Fig. 1) (10, 230). As described below, this population of cells rapidly differentiates when the overlying umbrella cells are lost or removed. The basal cell layer comprises a single-cell stratum that forms intimate contacts with an underlying capillary bed (89) (Fig. 1). The basal cells are mononucleate, ~ 10 μm diameter, and adhere to the intermediate cells by desmosomes and to the basement membrane by hemidesmosomes (100, 102). The latter attachment is mediated by β_4 -integrin (100, 102). Although intermediate and basal cells express cytokeratin-13,

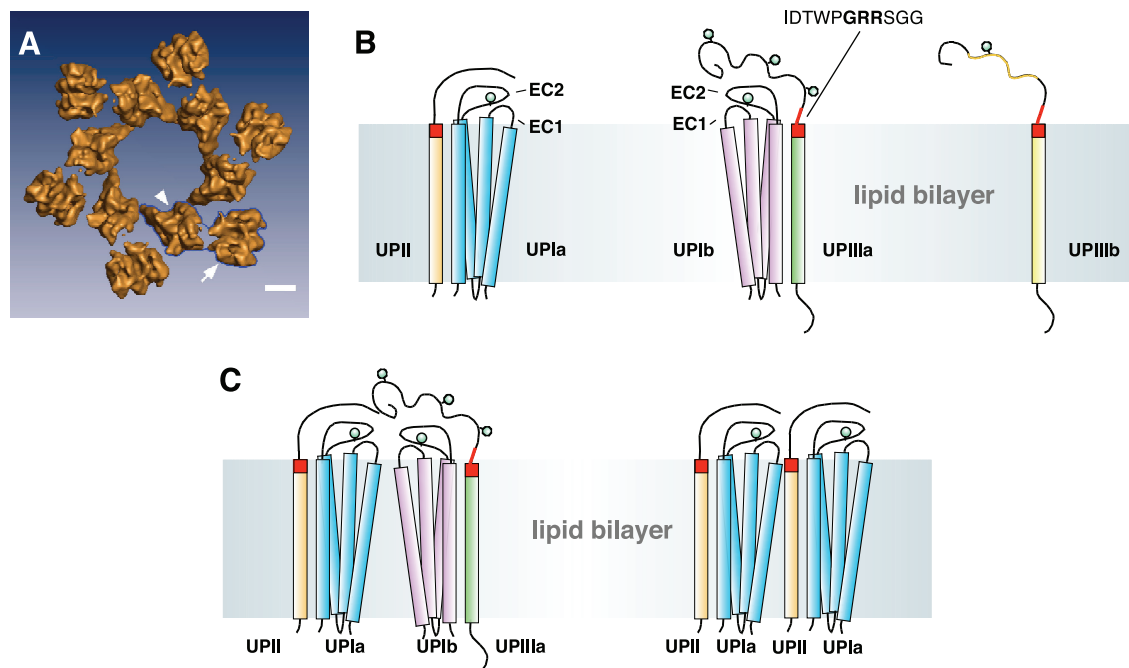


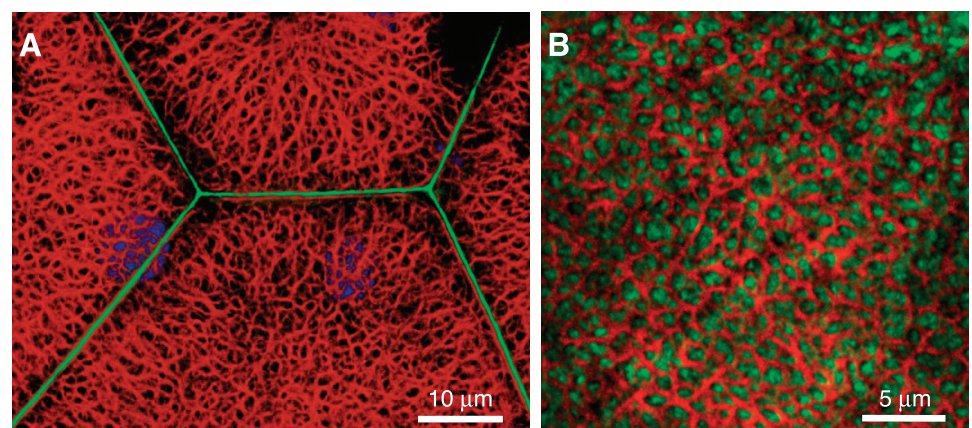
Fig. 3. Structure of AUM particles and UP subunits. **A:** 3-dimensional reconstruction of an AUM particle as determined by electron cryomicroscopy. Inner ring consists of UPIa/UII heterodimers (arrowhead), and outer ring consists of UPIb/UIIIa heterodimers (arrow). Scale bar, 2 nm. [From Min et al. (146).] **B:** schematic depiction of interactions between UPIa/UII and UPIb/UIIIa heterodimers. Extracellular loop (EC)-2 of UPIa or UPIb interacts with the extracellular domain of UII or UIIIa, respectively. Green circles show sites of N-linked glycosylation. Transmembrane domains of UPII and UIIIa/IIb contain a conserved region (red) that extends to the extracellular domain of UIIIa/UIIb. Sequence of the extracellular region of the conserved domain of UIIIa is shown in single amino acid code. Region of the extracellular domain of UIIb (yellow) is >90% identical to a portion of human DNA mismatch repair enzyme-related PMSR6 protein. [From Birder et al. (24).] **C:** interactions between heterodimers in inner and outer rings of the AUM particle (*left*) and between adjacent UPIa/UII heterodimers (*right*). [From Birder et al. (24).]

only basal cells express cytokeratin-5, -14, and -17 (214). All uroepithelial layers express cytokeratin-7, -8, -18, and -19 (214), and cytokeratin-20 is solely expressed in the umbrella cell layer (214, 216).

Uroepithelial stem cells and repair. In contrast to the epithelia that form the epidermis or line the gut and have relatively rapid turnover rates of 1.5–30 days, the turnover rate of the uroepithelium is estimated to be ~3–6 mo (85, 101). However, the uroepithelium shows enormous regenerative capability when it is damaged, and its patency can be restored rapidly, within days of significant damage (106, 119, 126, 212). Driven in part by investigations of uroepithelial regeneration, differentiation, and carcinogenesis and efforts to bioengineer bladders, there has been

significant interest in identifying uroepithelial stem cells. In other tissues, stem cells are thought to be clonal in origin, cycle slowly (but have a high regenerative potential), and give rise to other differentiated cell types. However, their identification in epithelial tissues is hampered by a lack of specific biochemical markers that can differentiate between persistent stem cells and transit amplifying cells, which arise from stem cells and are responsible for the normal turnover observed in epithelia, such as the epidermis (232). In this regard, a urological epithelial stem cell database that catalogs the expression of a large number of gene products, including cluster designation surface markers, has been described and may aid in identification of uroepithelial stem cells or markers for other cells in the uroepithelium (160).

Fig. 4. Trajectorial cyokeratin network of umbrella cells. **A:** 3-dimensional reconstruction of cyokeratin-20 network (red) at the apical pole of umbrella cells. Borders of adjacent umbrella cells are marked by the tight junction protein ZO-1 (green), and nuclei are shown in blue. [From Veranic et al. (212). Reprinted by permission of Springer Science+Business Media.] **B:** UP-positive DFV (green) are localized within tunnels formed by the cyokeratin-20-positive network (red). [From Veranic and Jezernik (216). Reprinted by permission of John Wiley & Sons, Inc.]



An additional feature of stem cells is that they are thought to reside in a protective environment, such as the limbus of the cornea or the crypts of the intestine. In the case of the uroepithelium, it is surmised that these pluripotent cells reside in the basal cell layer of the uroepithelium. Consistent with this possibility, there is a population of "label-retaining cells" in the basal cell layer that are positive for bromodeoxyuridine, even 1 yr after labeling (123). These cells are small, have low granularity, have high β_4 -integrin expression, and are clonogenic and proliferative when cultured (123). However, it remains to be established whether these label-retaining cells can differentiate into uroepithelium or whether there are any specific markers associated with these cells. Initial studies indicated that putative uroepithelial stem cells may reside in the ureter/trigone area of the bladder (153), but these regional differences were not observed in recent studies (123). An additional method to identify uroepithelial stem cells is implantation of a mixture of mouse embryonic stem cells and embryonic bladder mesenchyme under the kidney capsule (154). The optimal ratio of these two components allows for the generation of uroepithelium that, similar to native uroepithelial tissues, shows expression of UPs in the outermost umbrella cell layers, expression of p63 in the basal cells, and expression of the endodermal-associated transcription factor Foxa1 (154). By capturing cells at defined time points, it should eventually be possible to use these techniques to identify and characterize intermediates in the progression of embryonic stem cells as they develop into endoderm and then progenitor cells, uroepithelial stem cells, and, finally, uroepithelium.

An additional uroepithelial cell type that plays an important role in repair of this tissue is found in the outermost stratum of intermediate cells (those closest to the umbrella cell layer). These cells rapidly differentiate into umbrella cells when the cell barrier is disrupted as a result of senescence, bacterial infection, or experimental manipulation (12, 104, 125, 149). After a brief exposure to protamine sulfate, a polycationic peptide that forms pores in the apical membrane of the umbrella cells, there is a rapid, but selective, desquamation of the umbrella cell layer (126). Within 1 h of treatment, UPIIIa is seen to rapidly redistribute from an intracellular pool to the newly exposed surface of the intermediate cells, and formation of rudimentary (patchy) ZO-1-positive tight junctions is found along the borders of these cells. By 24 h, UPs are abundantly expressed at the surface of the exposed cells and ZO-1 is found to scribe the entire circumference of the cells. By 5 days, the entire uroepithelium has repaired itself, and it appears normal, except the umbrella cells are smaller in diameter (~ 15 – $25\ \mu\text{m}$) and do not reach their normal size until 10 days after exposure. Similar results are observed in bladders treated with chitosan, a positively charged polysaccharide that may act in a similar manner to protamine sulfate, which causes selective necrosis and desquamation of umbrella cells within 20 min of treatment (212). In this case, differentiation of newly exposed intermediate cells is even more rapid, with a scalloped apical membrane, a complete junctional ring, and a trajectorial network of cytokeratin-20 observed 1 h after chitosan treatment.

The cue(s) that triggers or promotes the rapid differentiation of intermediate cells is not known. Possible initiating events may include exposure of uncovered intermediate cells to growth factors or other mediators in urine [e.g., epidermal

growth factor (EGF)] or loss of cell-cell contacts between the intermediate cell and the overlying basolateral surface of the umbrella cells. Functional genomics may be a useful tool to explore differentiation in these model systems and has been used to examine changes in gene expression that accompany exposure of the uroepithelium to uropathogenic *Escherichia coli* (149). These bacterial cells express the FimH adhesin, which is found at the tip of slender pili, which extend from the surface of the bacterial cells, and specifically binds to UP1a (145, 148, 238). Binding induces an apoptotic response in the umbrella cell layer, causing this layer to slough off in ~ 6 h (148, 149). Within the next 12–48 h, intermediate cells rapidly differentiate into umbrella cells.

Expression of a number of gene products is affected in the basal and intermediate cell layers during bacterial infection, including downregulation of bone morphogenetic protein 4 and Wnt5a/ Ca^{2+} signaling, both of which are negative regulators of differentiation (149). In contrast, expression of a positive regulator of differentiation, E74-like factor, increases. Interestingly, changes in expression of these gene products are observed within 1.5–3.0 h of infection, indicating that the signals for differentiation may be initiated before loss of the umbrella cell layer and are apparently transmitted from the umbrella cells to the intermediate cells (149). How this information is transmitted between cell layers is unknown, but it may occur through gap junctions or through the release of mediators from the umbrella cells. It will be interesting to determine whether the same regulators of differentiation are induced by chemical mediators, such as protamine sulfate and chitosan, and to determine how changes in expression of bone morphogenetic protein 4, Wnt5a, and other proteins alter the differentiation program of the outermost intermediate cell layer.

PARACELLULAR AND TRANSCELLULAR ION, SOLUTE, AND WATER TRANSPORT BY THE UROEPITHELIUM

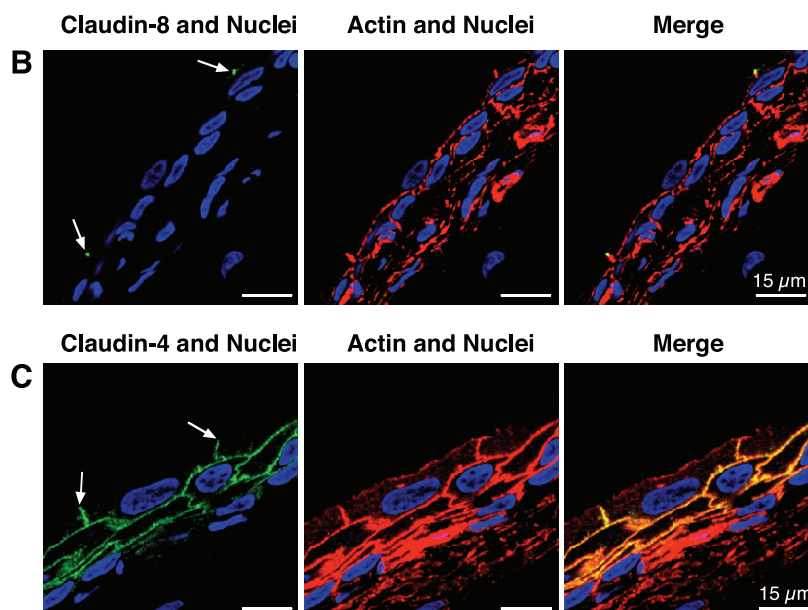
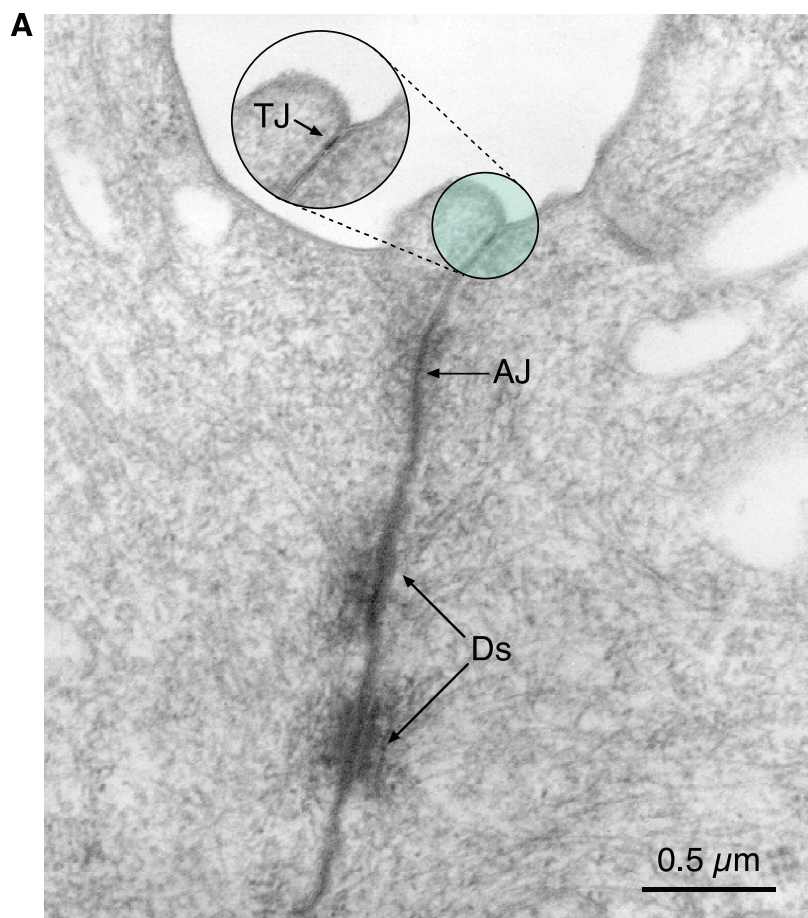
One of the most critical roles of the uroepithelium is formation of a regulated barrier to ion, solute, and water flow. This function is primarily associated with umbrella cells, which have an exceptionally high transepithelial resistance ($20,000$ to $>75,000\ \Omega\cdot\text{cm}^2$) that results from a high apical membrane resistance combined with a parallel junctional resistance that approaches infinity (134, 135). In addition, the apical membrane of these cells has a low, but finite, permeability to water, urea, and ions (36, 151). It is generally assumed that the urine held in the bladder is identical to that excreted by the kidneys. However, the composition of urine can change during its passage from the renal pelvis to the ureters/bladder (130, 181, 221), and the uroepithelium is exposed to very large and sometimes changing concentration gradients of ions, pH, and solutes, as well as mechanical stimuli, as the bladder fills and empties. Thus it should not be surprising that pathways for transport of Na^+ , K^+ , Cl^- , urea, creatinine, and water have been described in the uroepithelium (134, 135, 167, 175, 189–192, 223). These pathways, coupled with the large surface area of the uroepithelium and the long storage times of urine, indicate that, depending on the physiological status of the organism, the uroepithelium may play an unappreciated role in ion, solute, and water homeostasis.

Claudin expression in the uroepithelium. The junctional complex is a zone of attachment between adjacent epithelial

cells and is located at the intersection of their apical and lateral membranes. It includes the tight junction and adherens junction, both of which form continuous belts, and desmosomes (Fig. 5A). The latter form individual circular plaques that are arranged in a row just below the adherens junction and are also found along the basolateral cell surface. Although the adherens

junction and desmosomes have important roles in cell-cell adhesion, the tight junction modulates paracellular transport (gate function) and restricts the movement of lipids and membrane proteins in the exofacial leaflet of the apical and basolateral plasma membrane domains (fence function) (8, 9). Cytoplasmic and transmembrane proteins are associated with

Fig. 5. Claudin expression in rat bladder uroepithelium. *A*: junctional complex of the umbrella cell. Positions of tight junction (TJ), adherens junction (AJ), and desmosomes (Ds) are indicated with arrows. *Inset*: higher-magnification view of the tight junction. Contrast was adjusted with Photoshop. *B*: claudin-8 (green) is expressed at the apicolateral junction of the umbrella cell layer. Phalloidin staining (red) demarcates cell borders of the uroepithelium. *C*: claudin-4 (green) is associated with the basolateral surface of umbrella cells and plasma membranes of intermediate and basal cell layers. Arrows in *B* and *C* indicate location of the tight junction in the umbrella cell layer.



the tight junction (9). Examples of the former include ZO-1, as well as regulatory GTPases, phosphatases, and kinases. Transmembrane proteins associated with the tight junction include the claudins, occludin, the coxsackie/adenovirus-associated receptor, the junctional adhesion molecules, and tricellulin (9). The claudins are a family of 24 proteins that are localized to tight junctions in a tissue- and segment-specific manner (114, 165). Common features of claudins are their size (20–25 kDa), their domain structure, which includes four transmembrane segments and two extracellular loops and a COOH-terminal PDZ-binding motif that promotes interactions between the claudins and proteins, such as ZO-1, that contain PDZ motifs (73). Claudins play an important role in regulating paracellular transport, which is the subject of a recent review by Angelow et al. (9).

The specific claudins associated with the uroepithelium are now being enumerated and their localization is being described. Claudin-4, -8, -12, and, possibly, -13 are found in the uroepithelium of rat, mouse, and rabbit bladders (Fig. 5, B and C) (2, 149). In all three species, claudin-8 (Fig. 5B) and -12 are specifically localized to the apicolateral tight junctions of the umbrella cell layer. In contrast, claudin-4 is associated with the tight junctions, as well as the basolateral surface of the umbrella cells and the plasma membrane of the underlying cell layers (Fig. 5C). Nonjunctional claudin expression has been described in other tissues (66, 75, 137). Although its function is unknown, it may serve to promote cell-cell adhesion, act as a reserve pool of claudin molecules, or provide a mechanism to limit paracellular ion flow across the uroepithelial tissue when the umbrella cell layer is disrupted. Claudin expression has also been examined in human tissues and cultures of human uroepithelium. Using probes for claudin-1 to -10, Varley et al. (210) found that claudin-3, -4, -5, and -7 are expressed in human ureteric uroepithelium. Claudin-3 is localized at the umbrella cell tight junction, claudin-5 is expressed at the basolateral surface of the umbrella cell layer, claudin-4 is distributed at the intercellular borders of all uroepithelial cell layers, and claudin-7 is localized to the membranes of the intermediate and basal cell layers. Furthermore, RT-PCR data indicate that claudin-1, -2, -8, and -10 may also be expressed in the human uroepithelium. This list may not be exhaustive, as the immortalized TEU-2 cell line, which is derived from human ureter, expresses claudin-1, -4, -5, -7, -14, and -16 at the junction of the superficial cell layer (168). In contrast, claudin-1, -4, -5, and -7 are found along the lateral surfaces of these cells, and claudin-2, -8, and -12 are found in vesicular structures scattered throughout the cell cytoplasm. Because these TEU-2 cells are transformed, it will be important to determine whether claudin-12, -14, and -16 are expressed in native human uroepithelium.

What is striking is the large number of claudins that are associated with the tight junction of the umbrella cells. Some of the diversity may result from species differences or expression that reflects the growth and differentiation state of cultured uroepithelium. Claudin expression in culture epithelium can be modulated by the nuclear hormone receptor peroxisome proliferator-activated receptor- γ , which stimulates uroepithelial differentiation and promotes claudin expression and localization at the tight junctions and intercellular borders of the cultured cells (210). In addition, the uroepithelium that lines the ureters, bladder, and upper urethra may be distinct (169,

230), and these regional differences may be reflected in region-specific claudin expression.

An important next step is to understand which claudin(s) contributes to the high-resistance paracellular pathway that is associated with the umbrella cell layer. In essence, this pathway appears to be nonconductive (estimated to be $>300,000 \Omega \cdot \text{cm}^2$) (134, 135), at least in quiescent tissue, which indicates that the individual claudins or combination of claudins expressed by the umbrella cell may not form pores or may form pores with very high resistance. Future studies that examine the role of claudins will be aided by a growing list of knockout (KO) mice lacking expression of individual claudins and techniques that allow for the in situ transduction of rat bladder umbrella cells using adenoviruses (112, 166). For example, virus-encoded short hairpin RNAs could be used to downregulate expression of single or multiple claudins. Additional topics for exploration are as follows. 1) How is the paracellular barrier maintained or altered as the umbrella cells increase their apical surface area and outer diameter (i.e., junctional ring) as the bladder fills? 2) What happens during bladder voiding when the process reverses?

Study of claudins will also contribute to our understanding of bladder-associated disease processes. A growing literature indicates that changes in claudin expression may alter the metastatic potential of numerous tumor types (199), and alterations in expression of tight junction proteins may also play an important role in the genesis of bladder cancer (84). Furthermore, disruptions of tight junctions may contribute to bladder conditions such as outlet obstruction, bacterial cystitis, spinal cord injury, and interstitial cystitis (IC), all of which are characterized by alterations of the uroepithelium and umbrella cell junctional complex (12, 106, 119, 127, 128, 149, 213, 215, 237). In most cases, the cellular defects that lead to disruptions of tight junction function are not understood, but in IC it may be related to release of antiproliferative factor (APF) from the uroepithelium. APF is a nonapeptide (T-V-P-A-A-V-V-V-A) that is identical to residues 541–549 of the sixth transmembrane domain of the Wnt ligand receptor Frizzled 8 (109). As its name implies, APF inhibits proliferation of uroepithelial cells (108). The putative receptor for APF is the cytoskeleton-associated protein 4/p63, which is expressed by isolated uroepithelial cells (44). Cultured uroepithelium from IC patients shows increased paracellular flux of tracers and decreased expression of ZO-1 and occludin (237). It is striking that treatment of normal uroepithelial cells with APF results in an IC-like phenotype, characterized by increased paracellular flux and decreased expression of tight junction-associated proteins (237). Further study of these pathways should provide additional information about how tight junctions are modulated in bladder diseases such as IC.

Expression and function of aquaporin water channels in the uroepithelium. In mammals, aquaporins (AQPs) form a family of 13 integral membrane proteins, which form pores and principally transport water (AQP-1, -2, -4, -5, and -8) or glycerol and other small solutes (AQP-3, -7, and -9) (217). They are widely expressed by epithelial cells, including those lining the nephrons of the kidney, endothelial cells, and the epidermis, as well as nonepithelial cells, such as adipocytes. They play important roles in fluid homeostasis in the kidney, glandular fluid secretion, water flux in the brain, modulation of glycerol content in the skin and fat cells, and cell migration

(217). In the case of the bladder, there was little expectation that the uroepithelium would express AQPs, inasmuch as it was traditionally regarded as a passive barrier. However, there are reports that water transport may occur across the uroepithelium (130, 175, 189), the most striking example of which is the complete and daily resorption of urine from the bladders of hibernating bears (152).

Studies in rats and humans indicate that the uroepithelium expresses a number of AQPs (147, 175, 189). In rats, AQP-2 and -3 are found along the basolateral membrane of the umbrella cells and the plasma membranes of the intermediate and basal cells (Fig. 6) (189). No expression is observed at the apical membrane of the umbrella cells. It is intriguing that, in the bladders of dehydrated (vs. water-loaded) animals, expression of AQP-2 increases by ~50%, whereas expression of AQP-3 increases by ~200%, indicating regulation of AQP expression by water load (189). In human tissues, expression of AQP-3, -4, -7, -9, and -11 is detected by RT-PCR (175). The distribution of AQP-3 is similar to that observed in rats, whereas the distribution of AQP-4 and -7 is reported to be cytoplasmic. However, in the images of tissue published by Rubenwolf et al. (175), AQP-4 appears to associate in part with the membranes at cell-cell contacts, and a fraction of AQP-4 and -7 appears to be at the apical surface of the umbrella cell layer.

The function of AQPs in the uroepithelium is an open question; however, several roles have been proposed (189). One possibility is that the AQPs allow the uroepithelial cells to regulate cell tonicity or volume. This may be important during conditions in which solutes or ions are absorbed by the uroepithelium or when the cells lose water to a hypertonic urine. Alternatively, bulk water flow may occur across the uroepithelium. Although the nature of this bulk flow pathway is unclear (and may be species specific), it may involve initial passage

through an apical AQP, possibly AQP-4 or -7, and then egress through a basolateral AQP such as AQP-2 or -3. Because AQP-3 can also transport urea, it may also be important for dispersion of this or other solutes from the uroepithelial cells. The availability of KO mice lacking AQP expression (217), as well as uroepithelial cell cultures that can be engineered to not express individual AQPs, should allow these functions to be tested experimentally.

Solute transport in the uroepithelium. In addition to water, solutes such as urea and creatinine can be reabsorbed by the ureter and bladder or may enter the lumen of the bladder during states of low urine osmolarity (192, 221). Although the net transport of water and solutes is usually in the direction of the concentration gradient, the magnitude of the change (up to 20%) and its rapidity indicate that transport may be facilitated or active in nature (130, 221). There are two subfamilies of urea transporters, UT-A and UT-B (183). UT-A includes six isoforms that are encoded by a single gene, are found along the nephron (and liver, heart, and testis), and are important in the formation of concentrated urine. UT-B exists as a single isoform and is found on red blood cells and other tissues, including the vasa recta, where it functions to recycle urea and concentrate urine. UT-B is also expressed in the uroepithelium, where it is localized to the basolateral surface of the umbrella cells and the plasma membrane of the underlying intermediate and basal cells (Fig. 6) (140, 191, 192). Some UT-B is also found within the cytoplasm of the uroepithelial cells, presumably in endocytic or biosynthetic vesicles. In one study, UT-B expression was increased in the ureters and bladder in water-restricted animals (140); in another study, UT-B protein expression in the ureter, but not the bladder, was significantly increased in water-restricted compared with water-loaded animals (191). Creatinine also increases in the bladder tissue but is not modulated by water restriction or loading (192).

The function of the urea transporters is most likely to dissipate urea that enters the uroepithelium from the urine and, in doing so, modulates cell volume and osmolality (191). Consistent with this possibility, urea nitrogen concentration in the ureter and bladder is three to five times greater than in plasma and comparable to that in the renal cortex (192). The concentrations of urea nitrogen are highest in water-restricted animals and lowest in water-loaded animals. Presumably, urea crosses the apical membrane of the umbrella cell passively or through an apical transporter such as UT-A and then transits through the basolateral UT-B urea transporters, through the interstitial space (and likely other cell layers), until it encounters the subepithelial capillary bed and is absorbed into the bloodstream (191).

Ion transport in the uroepithelium. Several pathways for ion transport have been described in the uroepithelium (133, 223). The best understood is the pathway mediated by the amiloride-sensitive epithelial Na^+ channel (ENaC) (134, 135), which is expressed at the apical surface of the umbrella cell layer (184). Na^+ absorption by ENaC is driven by the basolaterally localized Na^+/K^+ -ATPase, which generates the net electrochemical gradient that promotes Na^+ entry into the cells (135, 223). Under basal conditions (when uroepithelial tissue is not stretched), Na^+ absorption is the primary contributor to the measured short-circuit current (a measure of active ion transport) of ~1–2 $\mu\text{A}/\text{cm}^2$ and the transepithelial potential difference of nearly –30 mV (135, 223). When isolated uroepithe-

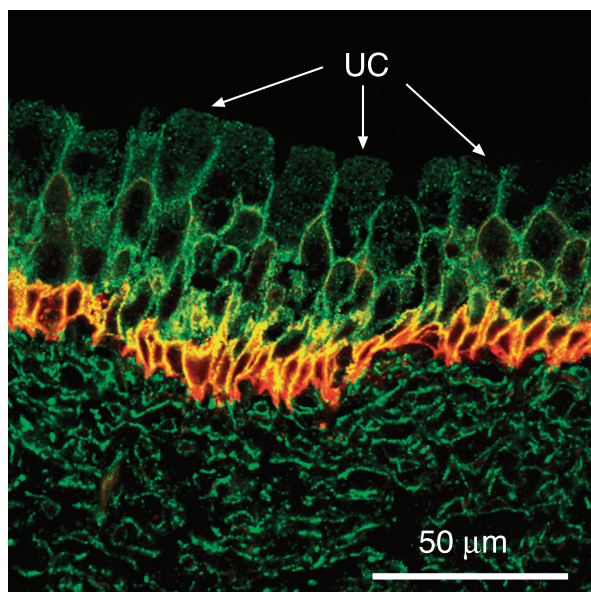


Fig. 6. Aquaporin (AQP)-3 and urea transporter (UT)-B distribution in uroepithelium. UT-B (green) is distributed on the basolateral surface of dog bladder umbrella cells and is present on plasma membranes of intermediate and basal cell layers. AQP-3 (red) shares a similar distribution but is heavily expressed in the basal cell layer. Arrows, apical surface of representative umbrella cells (UC). [From Spector et al. (192).]

lium is mounted in Ussing chambers and bowed outward by an increase in the fluid level in the mucosal hemichamber (which mimics bladder filling), amiloride-sensitive current significantly increases (132, 223), likely the result of increased apical membrane exocytosis of ENaC-containing DFV. Beyond its well-described function in mediating Na^+ transport, ENaC is also proposed to play a role in modulating ATP release from the uroepithelium (61, 62), and recent studies indicate that ENaC may have a mechanosensory role in modulating stretch-induced exocytosis at the apical membrane of the umbrella cell (234). These studies are described below.

In addition to increasing Na^+ transport, stretch also stimulates electroneutral Cl^- and K^+ transport (223). The channel(s) responsible for Cl^- transport is unknown; the pathway for K^+ transport includes an apically expressed nonselective cation channel (NSCC) that conducts Cs^+ (a hallmark of NSCCs) and is sensitive to tetraethylammonium, Gd^{3+} , La^{3+} , and high concentrations of amiloride (100 μM) (223, 234). An important mechanosensory role for this channel was recently proposed (234). In response to stretch, this channel may conduct Ca^{2+} across the apical membrane of the umbrella cell, which stimulates Ca^{2+} -dependent Ca^{2+} release and apical membrane exocytosis. The identity of the NSCC is unknown but may be one of several transient receptor potential (TRP) family members that are expressed in the uroepithelium, including polycystin-1 and -2 (96; unpublished observations).

Renal outer medullary K^+ channel [inwardly rectifying K^+ ($\text{K}_{\text{ir}}1.1$) channel] expression was recently described in the uroepithelium (190). In the kidney, this channel is important for K^+ secretion in the collecting duct and salt transport and K^+ and Na^+ resorption in the thick ascending limb. It is expressed at the apical membrane of the umbrella cells and, to a lesser extent, in the cell cytoplasm. Its expression levels are unaffected when animals are fed a diet depleted of K^+ , and its function in the uroepithelium is unknown. However, it may act to regulate cell volume or transmembrane electrical potential, which may be important for uroepithelial sensory transduction, and it may also act to modify the K^+ content of the urine.

Additional K^+ channels have been identified in the uroepithelium. These include the heparin-binding EGF (HB-EGF)-modulated inwardly rectifying channel $\text{K}_{\text{ir}}2.1$ and the large-conductance (maxi-K) channel (198). Wang et al. (223) showed that pressure-stimulated K^+ secretion was augmented by treatment of the serosal surface with charybdotoxin (an inhibitor of Ca^{2+} -activated K^+ channels), but not the maxi-K channel-specific inhibitor iberiotoxin. Apamin [an inhibitor of the small-conductance K^+ (SK) channel] and glibenclamide [which inhibits ATP-sensitive K^+ (K_{ATP}) channels] also altered K^+ secretion. Most recently, Yu et al. (234) used RT-PCR to identify stretch-modulated K^+ channels that are expressed in the uroepithelium. Message for the following channels was observed: the Ca^{2+} -activated K^+ channels KCNMA1 [large-conductance K^+ (BK) channel] and KCNN1-4 [small- or intermediate-conductance K^+ (SK/IK) channel], the two-pore K^+ channels KCNK2 (TREK-1) and KCNK4 (TRAAK), and the inwardly rectifying ATP-modulated K^+ channels KCNJ8 ($\text{K}_{\text{ir}}6.1$) and KCNJ11 ($\text{K}_{\text{ir}}6.2$) (234). Immunofluorescence was used to confirm that $\text{K}_{\text{ir}}6.1$ was expressed at the basolateral surface of the umbrella cells and plasma membrane of the underlying cell layers. Most intriguingly, treatment with the channel openers NS309 (which targets SK/IK) and chro-

makalim (which targets K_{ATP}) causes a net increase in apical membrane exocytosis (234), possibly by decreasing compensatory endocytosis at this membrane.

Other ion channels with a potential sensory role have been described in the uroepithelium (14). These include TRPV1 and TRPV4, both of which are important in modulating bladder function (25, 69, 122, 193), TRPV2 (33), and TRPM8 (54, 122, 193). The latter may be important in a neonatal response to instillation of cold fluids into the bladder (193). Additional channels include the P2X family of ATP receptors, which act as nonselective cation channels. All seven P2X receptors (P2X_1 , P2X_2 , P2X_3 , P2X_4 , P2X_5 , P2X_6 , and P2X_7) have been identified in the uroepithelium (27, 58, 129, 197, 200, 218, 225). KO mice lacking expression of P2X_2 or P2X_3 subunits show altered bladder function and decreased stretch-induced apical exocytosis when their bladders are filled (42, 43, 219, 225). Other studies have shown nicotinic receptor expression in the uroepithelium (19) and, possibly, acid-sensing ion channels (122). The potential functions of these channels in sensory transduction are described below.

FUNCTION OF UPs

The UPs are a family of five proteins that assemble into AUM particles and are the major constituents of the apical membrane plaques of the umbrella cell. They have a variety of functions, including formation of the barrier to solute and water flow across the apical membrane (91, 92), modulation of the bladder function (1), promotion of sperm/egg fusion in lower vertebrates (81, 82, 177), and, possibly, regulation of urogenital development (99). Furthermore, they are co-opted by uropathogenic bacteria, which use UPIa as a receptor for bacterial adherence (238). The UPs include UPIa and UPIb, which share 40% homology, have four transmembrane domains and two extracellular loops, and are members of the tetraspanin family of proteins (Fig. 3B) (228). The other UPs are single-span membrane proteins and include UPII, UPIIIa, and UPIIIb (52, 228). The latter is a minor constituent of plaques. In mammalian tissues, UPIa, UPII, UPIIIa, and UPIIIb are only expressed in the uroepithelium and are concentrated in the umbrella cell layer; however, as noted above, some UP expression is also seen in the upper intermediate cell layers. In addition to the uroepithelium, UPIb is expressed in the cornea and conjunctiva and, possibly, the lung (3). UPs are likely to have formed by gene duplication and are absent in some vertebrates but are found in others, including *Xenopus laevis* (frog), *Gallus gallus* (chicken), and *Danio rerio* (zebrafish) (68). Additional information about UPs and their function can be found in an excellent review by Wu et al. (230).

Assembly of UPs into AUM particles. The following scenario of AUM assembly is proposed (230). The initial site of AUM formation is in the endoplasmic reticulum (ER), where UPIa forms heterodimers with pro-UII, and UPIb pairs with UPIIIa or UPIIIb (Fig. 3B) (52, 229). UPIb can exit the ER when expressed alone, whereas the other UPs can only exit as heterodimers (207). Exit of UPIb from the ER likely depends on specific residues within its transmembrane domains, inasmuch as swapping these domains with those in UPIa results in defective exit from the ER (206). In the Golgi apparatus, N-linked glycans on pro-UII are converted from the high-

mannose to the complex type, which is thought to trigger a change in the folding of the UPIa/pro-UPII dimer that is permissive for heterotetramer formation. In the *trans*-Golgi network (TGN), the proteinase furin cleaves the UPII propeptide, initiating oligomerization of the UPs into 16-nm AUM particles (90). Within the TGN, AUM particles are packaged into DFV before their eventual delivery to the apical plasma membrane of the umbrella cells (5, 87, 93). It is unknown whether UPs contain apical targeting signals and, if so, the nature of these signals. In other apical proteins, targeting information can reside in their cytoplasmic, transmembrane, or extracellular domains and can depend on residues within the peptide backbone of these proteins or posttranslational modifications such as N-linked glycosylation (63). It is also possible that only one member of the UP heterodimer (or heterotetramer) may contain sorting information. CD63, a tetraspanin family protein similar to UPIa/UIPb, may be important for apical delivery of proteins in the principal cells of the cortical collecting duct (180), and UPIa/UIPb may play a similar role in targeting AUM particles to the apical surface of umbrella cells.

Recently, electron cryomicroscopy was used to give important structural insight into the organization of the AUM particles (146). UPIa/UIPb form rod-shaped structures that associate with UPII/UIPIIIa through interactions between their transmembrane domains. In addition, the extracellular "head" domain of UPII or UIPIIIa extends over the second extracellular domain of the cognate UPIa/UIPb protein (Fig. 3B). The UPIa/UIPII heterodimers form the inner ring of the AUM particle; the UIPb/UIPIIIa heterodimers form the outer ring. Interactions between adjacent UPIa/UIPII heterodimers in the inner ring are mediated through association of UPIa with the neighboring UPII of the adjacent UPIa/UIPII pair, whereas interactions between UPIa/UIPII in the inner ring and UIPb/UIPIIIa in the outer ring occur through attachments between the extracellular head regions of UPII and UIPIIIa (Fig. 3C). As finer-resolution tools are employed, it should be possible to better model the AUM particle and identify the specific residues that are involved in promoting interactions within the AUM particle and define which of these interactions are necessary to form apical membrane plaques.

Contribution of UPs to bladder function. The generation of KO mice lacking expression of UPII or UIPIIIa is providing insight into the functional role of UPs in bladder function. As expected, UIPIIIa KO mice have few plaques, and those plaques that form are likely the result of UIPIIIb forming heterodimers with UIPb (91). UPII KO mice form no plaques, which is consistent with a single isoform of this UP (116). Among the defects described in the UIPIIIa KO mice is an increase in methylene blue permeability across the apical surface of the umbrella cells (91), indicating a disruption of the apical membrane function of these cells. This observation was subsequently corroborated in studies that reported an increase in water and urea permeability across the umbrella cell layer of the UIPIIIa KO animals but no change in junctional permeability (92). The underlying mechanism by which UIPIIIa deficiency leads to increased permeability is unknown. One possibility is that plaques help organize or rigidify lipids in the outer leaflet of the plasma membrane and, in doing so, decrease water permeability across the apical surface of the umbrella cells (230). However, it is possible that the lipid composition or associated cytoskeleton may be altered in these KO animals, as

the umbrella cells display abnormal apical membranes studded with microvilli and the cells are smaller and are hyperplastic compared with normal cells (91). It has not been reported whether UPIIIa KO mice share a similar phenotype, but it is likely that they do.

Deficiencies in UP expression are also manifested in altered bladder function as measured using cystometry (1). This technique measures the changes in intraluminal pressure that occur as the bladder is filled with buffer solutions through a catheter implanted in the dome of the bladder. In a normal mouse bladder, the baseline pressure remains fairly constant for the majority of the extended filling phase and then rapidly rises as the detrusor muscle contracts, signaling the onset of micturition. The pressure then falls during voiding and returns to baseline. In UPII and UPIII KO animals, there is a significant increase in nonvoiding contractions, which are seen as small, recurring peaks in the cystometrogram that are not accompanied by release of buffer (1). The UPII KO mice have the most severe phenotype, with males affected more than females, and show elevated intermicturition pressure, spontaneous activity, bladder capacity, and residual volume (indicating that the animals do not void completely). The observed detrusor overactivity is not obviously a result of changes in detrusor sensitivity, as isolated muscle strips show similar sensitivities to the ACh receptor agonist carbachol (and slightly lower sensitivities in female mice) (1). It is therefore possible that the uroepithelium in these KO animals may have defects in expression of signaling receptors and/or release of mediators that stimulate increased afferent nerve activity (see below), thus contributing to the detrusor overactivity.

Relationship of UP expression, vesicoureteral reflux, and other defects in kidney development. One of the most striking defects in the UPII and UIPIIIa KO animals is the presence of enlarged, obstructed ureters (91, 116), which leads to the flow of urine from the bladder back into the kidney (vesicoureteral reflux), resulting in hydronephrosis and death in some of the animals (91). Vesicoureteral reflux is a hereditary disease that affects ~0.5–1.0% of humans, but its mode of inheritance is not well understood (53). Although the KO mice indicate that defects in UPs or plaque assembly contribute to vesicoureteral reflux, two groups have examined patients with primary disease and observed no genetic linkage between the disease and defects in the UIPIIIa locus (71, 110). It is possible that the enlarged ureters observed in the UP KO mice reflect enhanced uroepithelial proliferation, which secondarily leads to urine reflux. Intriguingly, mutations in the UIPIIIa gene may be a rare cause of renal hypodysplasia (98, 179), a disease characterized by developmental defects that include small kidneys with reduced numbers of nephrons. The underlying basis for these kidney defects is unknown but may be related to changes in signaling pathways that are associated with UIPIIIa.

UPs and signaling. In addition to their role in forming plaques and modulating permeability across the apical membrane of the umbrella cell, work in *Xenopus* indicates that UPs may also play a role in cell-surface signaling events. *Xenopus* UIPIIIa and UIPb (xUPIIIa and xUIPb) are expressed in the kidney, urinary tract, ovary, and eggs of the frog (81, 177), and message for xUPIa, xUPII, and xUIPIIIb mRNA is also detected in frog tissues (68). Unexpectedly, antibodies to the extracellular domain of xUPIIIa block egg fertilization (177). Further work has shown that xUPIIIa and xUIPb form a

complex at the plasma membrane that includes the surface ganglioside GM-1 (81) and the cytoplasmic nonreceptor tyrosine kinase *src* (81). Upon binding the oocyte, sperm cells release a proteinase(s) that likely cleaves a conserved G-R-R188 motif in the membrane proximal region of xUPIIIa (82). The proteinase is probably related to cathepsin-B, which also recognizes peptides containing G-R-R residues and can cleave and activate xUPIIIa (82). Proteolysis of xUPIIIa activates *src*, which phosphorylates Tyr²⁴⁹ in the cytoplasmic domain of xUPIIIa, and stimulates Ca²⁺ release downstream of *Xenopus* phospholipase C γ (82), which signals resumption of meiosis II. Although it remains to be shown whether the UPs play a role in regulating signaling during urinary tract development in mammals, such a requirement could explain the significant abnormalities associated with loss of UPs in mammalian systems (99). It is also worth noting that G186 in xUPIIIa is equivalent to G202 in the conserved domain of human UPIIIa (Fig. 3B), the mutation of which results in renal hypodysplasia (179). Thus cleavage of human UPIIIa may play an important, but unknown, role in kidney development. Although these studies are suggestive, a recent analysis of bacteria-induced apoptosis provides striking evidence that UPIIIa-dependent signaling events may occur in mammalian uroepithelial cells and may play an important role in the host response to infections by uropathogenic *E. coli* (203).

Role of UPs in the pathogenesis of infections by uropathogenic *E. coli*. UPs not only play important roles in normal bladder function, they can also be co-opted by bacteria to promote bacterial adhesion to and invasion of the uroepithelium (Fig. 7). Uropathogenic *E. coli* account for >80% of urinary tract infections and interact with the surface of the uroepithelial cells by way of the FimH adhesin protein. The receptor for FimH is UPIa, which is glycosylated at Asn¹⁶⁹ with a modified glycan that contains Man(6)GlcNAc(2) (231). However, FimH can bind other mannosylated proteins, including the $\alpha_3\beta_1$ -integrin (60). As described above, association of the bacteria with the umbrella cell can lead to apoptosis and shedding of the umbrella cell layer, which may be a protective mechanism to remove infected cells (148, 149). In the bladder, apoptosis is induced upon binding of bacterial lipopolysaccharide to the Toll-like receptor 4 (185, 186). However, *E. coli*-induced apoptosis of uroepithelial cells may also depend on UPIIIa expression (202, 203). It is intriguing that binding of the FimH adhesin to cultured uroepithelial cells results in casein kinase II-dependent phosphorylation of Thr²⁴⁴ in the cytoplasmic domain of UPIIIa (203). In turn, this leads to a rise in intracellular Ca²⁺ that may stimulate apoptosis. Inhibition of casein kinase II or the rise in intracellular Ca²⁺ blocks bacterial invasion, as well as uroepithelial apoptosis, *in vitro* and *in vivo*. Thus treatments that target this signaling pathway may provide new modalities of treatment for recurrent bladder infections. Important unknowns include the following. 1) How does FimH binding to UPIa induce phosphorylation of UPIIIa? 2) How does phosphorylation of Thr²⁴⁴ lead to increased intracellular Ca²⁺? 3) What are the relative contributions of Toll-like receptor 4 and UPIIIa signaling to the bacteria-induced apoptosis observed *in vivo*?

Upon binding to the umbrella cell apical surface, a significant number of UPIa-tethered uropathogenic bacteria are internalized by these cells. These intracellular bacteria can propagate the infection as well as serve as a reservoir for recurrent

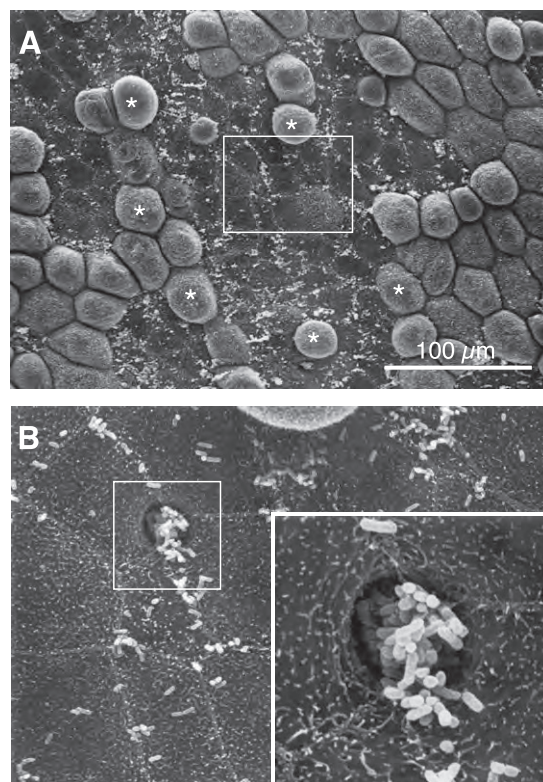


Fig. 7. Association of uropathogenic *Escherichia coli* with bladder uroepithelium. A: *E. coli* adherent to the surface of the uroepithelium. Apical surfaces of umbrella cells flatten in response to bacterial adhesion, whereas adjacent umbrella cells with few adherent bacteria (*) maintain a dome-shaped morphology. B: higher-magnification view of bacterial adherence to umbrella cells and invasion between cells shown in boxed region in A. Higher-magnification view of boxed region in B shows site of bacterial invasion (inset).

infections. Bacterial internalization is reported to be dependent on the Rho family GTPases Rac1 and Cdc42 (142), caveolae/lipid rafts (56), or clathrin, AP-2 adaptor, and alternate clathrin adaptors (59). A caveat associated with these studies is that they were performed in cell lines that bear little resemblance to native uroepithelium and, as described below, there are no clathrin-coated pits or caveolae at the apical surface of the native umbrella cell (10, 29). However, it is possible that the machineries associated with these forms of endocytosis may be induced and then recruited to the sites of bacterial invasion, or may be expressed in the underlying cell layers. The GTPase dynamin, which is involved in the scission of many types of endocytic vesicles (143), was recently implicated in *E. coli* invasion of umbrella cells (201).

In situ studies of bacterial internalization in mouse bladders indicate that *E. coli* stimulates DFV exocytosis near the site of bacterial attachment (28). This would recruit extra UPIa receptors at the bacterial-host cell interface and provide additional membrane to surround the bacterium, in what is likely to be an actin-dependent process (226). As the membrane coalesces around the bacteria, there is formation of tubular invaginations and then a vacuole-like compartment with attached membranous tethers, which may result from collapse of the tubular membrane around the bacteria (28). The vacuole generated is apparently a modified DFV and is positive for Rab27b (a regulatory GTPase associated with DFV; see below) (28). It is

intriguing that these bacteria-laden vacuoles can be stimulated to undergo exocytosis when treated with the secretagogue forskolin, and such treatment can significantly enhance bacterial clearance from infected bladders (28). Thus agents that stimulate exocytosis may have therapeutic potential in the treatment of recurring bacterial infections. Bacteria not only survive in umbrella cells, but they can also form intracellular bacterial communities (7, 174), which may further serve as reservoirs for recurrent infections.

MEMBRANE TRAFFIC AT THE APICAL POLE OF THE UMBRELLA CELL

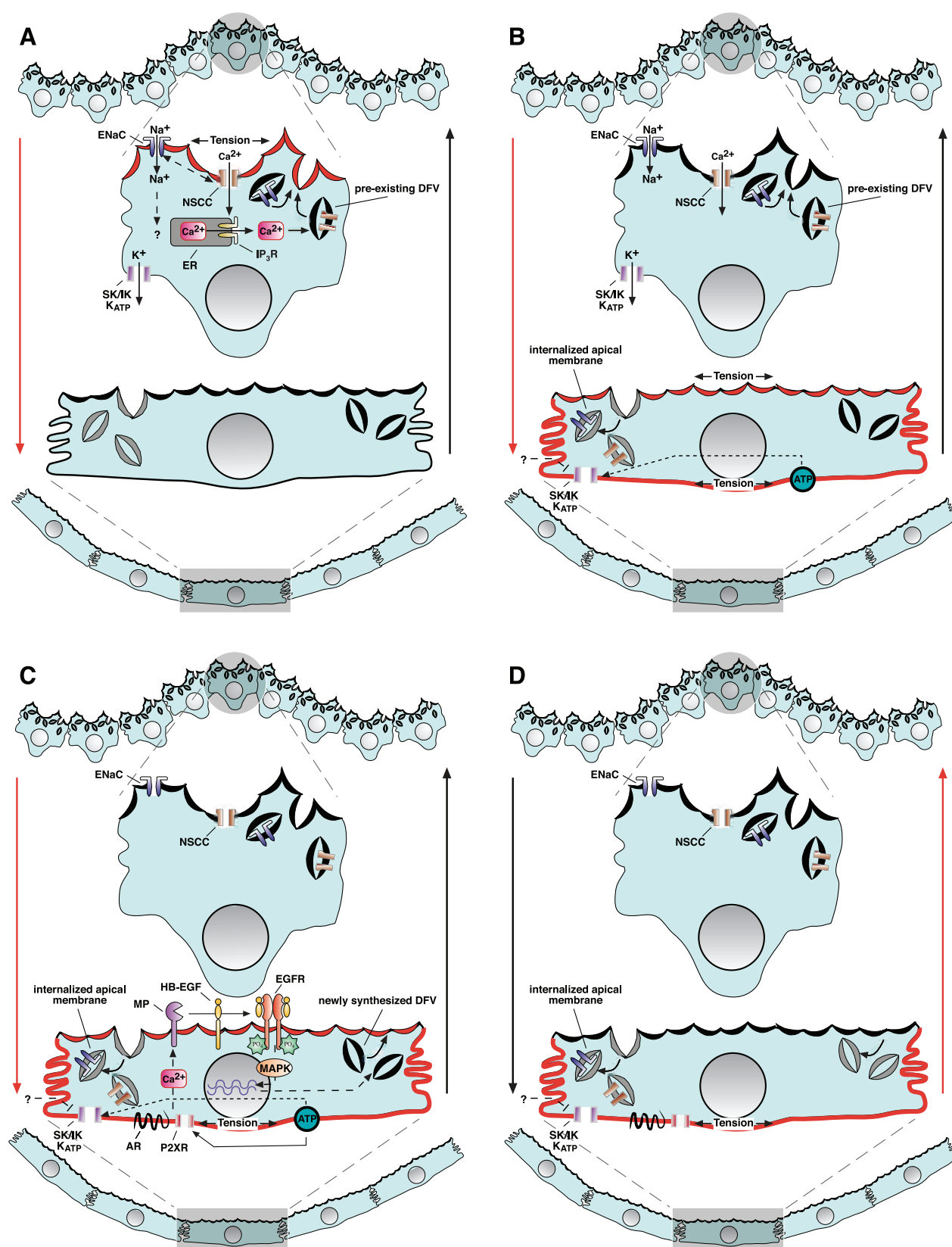
A crucial role for the uroepithelium is maintenance of barrier function in the face of large changes in urine volume as the bladder fills and empties. During bladder filling, this is accomplished by several mechanisms. At the tissue level, the folded surface of the bladder mucosa unfurls; at the cellular level, the apical membrane of the umbrella cell unfolds as well as expands as a result of exocytosis of subapical DFV (111, 112, 131, 132, 205, 224, 225, 234). During voiding, the process is thought to work in reverse as added apical membrane is recovered by endocytosis (10, 85, 164) and the apical membrane of the umbrella cells and the mucosal surface of the bladder refold. Beyond their role in allowing membrane expansion and recovery, exocytosis and endocytosis are likely to play crucial roles in the pathogenesis of urinary tract infections and in modulating the barrier function of the epithelium by ensuring delivery and turnover of UPs and lipids to the apical surface of the umbrella cells. Furthermore, these pathways may also regulate the sensory function of the uroepithelium, in part, by governing the surface content of receptors and channels at the apical surface of the umbrella cells. These receptors/channels allow the uroepithelium to sense conditions in the extracellular milieu and then communicate changes to the underlying tissues. The mechanisms that drive the surface expansion of umbrella cells have been actively studied in the last few years (see below). However, few studies have directly addressed events that occur during bladder voiding, and this is an open area of inquiry.

Exocytic and endocytic responses at the apical surface of umbrella cells and their modulation by apical and basolateral surface tension. Exocytosis in umbrella cells has been studied in cell culture (51, 204), in situ (111, 112, 225), and in isolated

uroepithelial tissue mounted in modified Ussing chambers (112, 131, 132, 205, 224, 225, 234). In the absence of mechanical stimulus, the umbrella cells in tissue appear to be quiescent, and there is little evidence of endocytosis or exocytosis (205). This is consistent with recent observations that constitutive apical endocytosis is significantly diminished in highly differentiated cultures of the uroepithelium (118). However, umbrella cells in situ exist in a dynamic mechanical environment, and the epithelium is experiencing outward directed forces during bladder filling or inward forces as the detrusor muscle contracts, actively pushing the mucosa inward as it refolds (Fig. 8). Outward bowing of the uroepithelium mounted in Ussing chambers (which simulates bladder filling) increases exocytosis as measured by surface biotinylation (205, 234), stereology (205), release of secreted proteins (111, 112, 205), and transepithelial capacitance (C_T ; where $1 \mu\text{F} \approx 1 \text{ cm}^2$ surface area) (112, 131, 132, 205, 224, 225, 234). C_T primarily reflects changes in apical surface area and correlates well with other measures of exocytosis (112, 205, 224). There is ample evidence that the increase in surface area is mediated by exocytosis of DFV (10, 111, 112, 205, 225). Stretch also stimulates endocytosis (205), but how it does so and the spatiotemporal regulation of the exocytic and endocytic response were only revealed recently.

Studies by Yu et al. (234) indicate that the exocytic and endocytic responses in the umbrella cell are governed by mechanical forces that affect tension at the apical and basolateral poles of the cell as the mucosal tissue unfolds during filling and refolds after voiding. When uroepithelium mounted in Ussing chambers is bowed outward, the exocytic response (monitored by changes in C_T) is biphasic and includes an initial rapid increase followed by an extended response that lasts for hours (15, 205, 234). The initial increase is referred to as the early-stage response and is not sensitive to inhibitors of protein synthesis, secretion, or reduced temperatures (15, 205, 234). It apparently reflects exocytosis of a preexisting population of DFV. The early-stage response is initiated by increased tension (i.e., stretch) at the apical pole of the umbrella cell as the tissue is bowed outward and is not dependent on pressure per se (234). The rate of the response is dependent on how fast the tissue bows outward, with faster rates of bowing resulting in faster rates of exocytosis and vice versa (234). The early-stage response is likely important during bladder filling, when the

Fig. 8. Model for exocytic and endocytic responses to bladder filling and voiding. *A*: as the bladder fills, the mucosal surface unfurls and increased tension at the apical pole of the umbrella cell triggers opening of a nonselective cation channel (NSCC), stimulating influx of extracellular Ca^{2+} . Increased intracellular Ca^{2+} concentration triggers Ca^{2+} -dependent Ca^{2+} release from inositol trisphosphate (IP_3)-dependent stores, which triggers the early-stage exocytic response. This response is likely driven by fusion of a preexisting pool of DFV with the apical plasma membrane of the umbrella cell. Exocytosis may amplify the initial response by stimulating delivery of additional stretch-sensing channels to the apical surface of the cell. Epithelial Na^+ channel (ENaC), perhaps acting upstream of the NSCC, is also opened and may regulate the exocytic response by changing the membrane potential or the driving force for the entry of other ions. IP_3R , IP_3 receptor; SK/IK, small/intermediate-conductance K^+ channels; K_{ATP} , ATP-sensitive K^+ channel. *B*: as the epithelium bows further, outward tension in the basolateral membrane increases, stimulating stretch-induced endocytosis. Enhanced endocytosis would modulate the exocytic response by stimulating internalization of apical membrane channels and other sensory molecules (not shown). The mechanosensor at the basolateral membrane is unknown but may include integrins, the cytoskeleton, or stretch-modulated K^+ channel(s), which may close in response to the increased tension or other intracellular mediators such as ATP. *C*: as the bladder fills and tension is present at mucosal and serosal surfaces of the umbrella cell, heparin-binding epidermal growth factor (HB-EGF) is cleaved at the apical surface of the umbrella cell by an unknown metalloproteinase (MP) and then binds to and activates apical EGF receptors (EGFR). In turn, EGFR activates ERK and p38 MAPK, which trigger the late-stage exocytic response by promoting new protein synthesis and, possibly, biosynthesis of new DFV. Transactivation of the EGFR may occur in response to ATP that is released upon stretch and binds to purinergic receptors (P2XR), stimulating an increase in the intracellular Ca^{2+} concentration. Adenosine, acting through adenosine receptors (AR), may also act by stimulating transactivation of the EGFR. *D*: during voiding, tension would be released from the apical surface of umbrella cells but would increase in the basolateral membrane as the detrusor contracts and actively pushes the mucosa toward the lumen of the bladder. Increased tension would further stimulate endocytosis of the apical membrane and its constituents, readying the umbrella cell for the next cycle of filling. Red arrows at periphery indicate bladder filling (*A–C*) or voiding (*D*). For clarity, we do not include underlying cell layers or connective tissue; however, umbrella cells likely transmit tension to the interconnected matrix, intermediate cells, and basal cells, and they, in turn, may impact or promote events in the overlying umbrella cell layer. [Adapted from Yu et al. (234).]



uroepithelium is in a dynamic mechanical state and the mucosal surface is unfolding and subjected to tension in response to the increased volume of urine (Fig. 8A) (234).

As the epithelium continues to bow outward, the initial increase in apical tension is followed by a rise in basolateral membrane tension (Fig. 8B), which stimulates the previously described stretch-induced endocytosis (205, 234). This response may be similar to the compensatory endocytosis that inevitably follows an exocytic burst in neurons/neuroendocrine cells (16). In both cases, endocytosis modulates the increase in surface area, regulates signaling pathways/responses, and ensures recovery of the protein machinery and membrane needed for additional rounds of exocytosis. Although endocytosis and exocytosis are stimulated by stretch, the net effect is to increase apical surface area (205, 234).

The late-stage response is characterized by a gradual increase in exocytosis, and it is regulated differently from the early-stage exocytic response (see below). The late-stage requirement for protein synthesis and secretion may indicate that it involves exocytosis of a newly synthesized pool of DFV or synthesis of proteins required to maintain the exocytic response (15, 205, 234) (Fig. 8C). However, it is also possible that there is more than one population of DFV or a non-DFV population of exocytic vesicles. The late stage is initiated after the tissue starts to bow outward, occurs downstream of autocrine EGF receptor (EGFR) activation, and predominates as the bladder reaches capacity and the uroepithelium approaches a mechanical equilibrium (Fig. 8C). Under these conditions, the rate of exocytosis is presumably greater than the role of endocytosis, thereby causing a slow, but steady, increase in surface area.

Upon voiding, the membrane added during filling must be rapidly recovered. The mechanisms that govern this process are not well understood, but preliminary analysis indicates that it occurs in response to increased tension at the serosal surface of the tissue (Fig. 8D) and can be mimicked *in vitro* by an increase in the pressure head in the serosal hemichamber of the Ussing chamber (15, 234), which bows the tissue inward. Such inward bowing is likely important during voiding, when the detrusor contracts and pushes the uroepithelium inward, refolding the uroepithelium and readying it for the next round of filling (Fig. 8D). Some recent insights into the voiding pathway are described below.

Modulation of early-stage exocytic events by ion channels, Ca^{2+} , and the cytoskeleton. The mechanosensors involved in sensing and transducing membrane stretch during bladder filling (i.e., during the early-stage response) may include ENaC and an apical NSCC (234). Both are mechanosensitive, and, in other tissues, NSCCs often conduct Ca^{2+} into the cell, stimulating Ca^{2+} -dependent Ca^{2+} release (79). Consistent with this possibility, removal of Ca^{2+} from the buffer bathing the apical surface of the umbrella cells inhibits the early-stage response (234). Furthermore, early-stage exocytic events are sensitive to inhibitors of the inositol trisphosphate receptor, but not ryanodine, indicating that extracellular Ca^{2+} is stimulating the aforementioned Ca^{2+} -dependent Ca^{2+} release from inositol trisphosphate-dependent stores (Fig. 8A) (234). Ca^{2+} may directly (or indirectly) stimulate the early-stage exocytic response by promoting Ca^{2+} -dependent fusion. The function of ENaC in this response is unclear, but it may act to depolarize the apical membrane in response to stretch, thus driving Ca^{2+} into the cell. There are many examples of NSCCs that are

voltage sensitive (95, 103, 187), and activation of ENaC could drive the opening of the NSCC. Alternatively, increased Na^{+} absorption through ENaC may modulate cross talk between ion channels at the apical and basolateral domains of the umbrella cells, changing the driving force for apical Ca^{2+} entry.

It is unknown whether there are mechanosensors that act upstream of the NSCC or ENaC. For example, increased apical stretch is likely to increase tension/compression in the underlying cytoskeleton, which may activate integrins that not only modulate cytoskeletal tension but may also play important roles in initiating and/or propagating signaling responses at the apical surface of epithelial cells (4, 97). The α_2 -, α_3 -, α_5 -, α_6 -, α_v -, β_1 -, and β_4 -integrins are reported to be expressed in the uroepithelium (188, 227). Early-stage events are dependent on an intact actin cytoskeleton and, possibly, intermediate filaments, but not microtubules (234). However, no published studies have explored a role for integrins in umbrella cell exocytic traffic.

In contrast to the early-stage response, the late-stage response shows little dependence on ENaC, the NSCC, or the cytoskeleton (234). If it is assumed that there is a single population of DFV, then it is unlikely that stretch-sensitive channels or the cytoskeleton are general requirements for DFV exocytosis. Instead, they may act by specifically initiating the early-stage exocytic response. However, as noted above, there may be more than one population of DFV or exocytic vesicle, and this could explain the different requirements for their exocytosis. Further exploration is needed to understand the differences in the membrane pools involved in the early- and late-stage exocytic events and how they are regulated.

Late-stage exocytic events: role of autocrine activation of the EGFR, stimulation of MAPK cascades, and new protein synthesis. The late-stage (but not the early-stage) response depends on autocrine activation of EGFRs localized on the apical surface of the umbrella cell (Fig. 8C) (15). This activation is a downstream consequence of metalloproteinase-dependent HB-EGF cleavage and autocrine binding to the EGFR and is impaired by inhibitors of metalloproteinases, diphtheria toxin (which specifically binds to HB-EGF), and inhibitors such as function-blocking antibodies that prevent EGFR activation (15). Downstream of EGFR signaling, activation of ERK and, possibly, p38 MAPK signaling cascades can regulate changes in gene expression. The latter may explain the late-stage requirement for protein synthesis. Although EGFR activation is known to be stretch sensitive in umbrella cells, the metalloproteinase has yet to be characterized, and the upstream pathways that sense the stretch and promote HB-EGF cleavage are unknown. Transactivation is a process whereby an upstream stimulus such as elevated intracellular Ca^{2+} , exposure to radiation, or activation of G protein-coupled receptors promotes proteolytic processing and release of ErbB family ligands, typically HB-EGF, that rapidly bind to and activate the EGFR (48). It is intriguing that raising intracellular Ca^{2+} in umbrella cells by treatment with thapsigargin (an inhibitor of Ca^{2+} uptake by the ER-localized Ca^{2+} -ATPase) or the Ca^{2+} ionophore A23187 stimulates a slow, steady increase in C_T (characteristic of the late-stage response) (224), and preliminary data indicate that purinergic signaling pathways may stimulate the late-stage response by transactivating the EGFR.

Purinergic signaling pathways as modulators of umbrella cell membrane traffic. Ferguson et al. (62) were the first to report that the uroepithelium releases ATP from its serosal surface in response to stretch. Subsequent studies found that ATP is released from both surfaces of the uroepithelium (136, 225), and this release is blocked by inhibitors of vesicular transport, connexin hemichannel activity, ABC protein family members, and nucleoside transporters (115, 225). Because many of the drugs employed in these studies are not specific, the exact mechanism(s) of ATP release remains an open question.

The ATP released by the uroepithelium may act in an autocrine manner by binding to uroepithelium-associated P2X receptors, which then alter uroepithelial functions. For example, stretch-induced exocytosis is inhibited by addition of apyrase (a membrane-impermeant ATPase) or the purinergic receptor antagonist pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid to the serosal, but not mucosal, surface (225). Increased exocytosis is observed on addition of ATP or purinergic receptor agonists to the serosal or mucosal surface of the uroepithelium, even in the absence of pressure (225). However, Lewis and Lewis (136) only observed small changes in exocytosis in response to exogenous ATP. Consistent with a role for P2X receptors in uroepithelial membrane traffic, bladders of KO mice lacking expression of P2X₂ and/or P2X₃ receptors fail to show increases in apical surface area when stretched (225). Treatments that prevent release of Ca²⁺ from intracellular stores or activation of protein kinase A also block ATP γ S-stimulated changes in exocytosis (225). These results indicate that the ATP released from the uroepithelium may bind to P2X, and possibly P2Y, receptors on the umbrella cell. In turn, downstream activation of Ca²⁺ and protein kinase A second messenger cascades may act to stimulate membrane insertion at the apical pole of umbrella cells. As described above, stimulation of exocytosis by ATP may require transactivation of EGFRs. In fact, ATP-induced increases in exocytosis depend on EGFR activation (unpublished observations); however, additional studies are needed to define how the signaling cascades downstream of purinergic receptor activation lead to HB-EGF cleavage.

P1 (adenosine) receptors may also be modulators of umbrella cell exocytosis. All four known adenosine receptors (A₁, A_{2a}, A_{2b}, and A₃) are expressed in the uroepithelium (235). A₁ receptors are found on the apical surface of the umbrella cells; the other adenosine receptors are expressed along the basolateral surfaces of the umbrella cell layer. When adenosine is added to the serosal or mucosal surface of the uroepithelium, exocytosis increases; however, this response is abolished by addition of adenosine deaminase, which converts adenosine to inosine (235). Agonists of the A₁, A_{2a}, and A₃ receptors increase exocytosis after serosal administration, but only A₁-selective agonists significantly stimulate exocytosis after mucosal administration. Antagonists of adenosine receptors, as well as deaminase, have no effect on stretch-induced capacitance increases. However, adenosine potentiates the effects of stretch when added to the mucosal surface of stretch-stimulated epithelium (235). Similar to ATP, the adenosine-induced changes in exocytosis are inhibited when EGFR activation is prevented (unpublished observations). This indicates that transactivation may be a common pathway for agents to stimulate late-stage exocytic events.

Regulation of DFV exocytosis by Rab GTPases. Vesicular traffic is regulated by small Rab family GTPases, which modulate cargo selection, vesicular transport, and vesicle fusion (141). The uroepithelium expresses multiple Ras family GTPases, including Rab4, Rab5, Rab8, Rab11, Rab13, Rab15, Rab25, Rab27b, Rab28, Rab32, RhoA, RhoC, and Ras1 (38, 112). Rab27b is intriguing, because it is expressed by umbrella cells and is localized to DFV (38); however, its functional role in the uroepithelium has not been described. In other cell types, Rab27 modulates the biogenesis and exocytosis of regulated secretory granules (65); in the bladder, it likely plays a similar role in the biogenesis of DFV and/or the exocytosis of these organelles. More recent studies have examined the role of Rab11a (112), which is known to regulate exocytosis in the biosynthetic and endocytic transport pathways of other cell types (34, 37, 72, 144, 208). Similar to Rab27b, Rab11a is localized to DFV, where it colocalizes with UPIIIa. However, it is unknown whether Rab27b and Rab11a are associated with identical or distinct populations of DFV. Stretch-regulated DFV exocytosis is significantly impaired in animals transduced with adenoviruses expressing a dominant-interfering mutant of Rab11a (112). Furthermore, by coexpressing mutant Rab11a and human growth hormone (hGH), a secretory protein that is sorted into DFV and secreted into the urinary space (111, 112), it is possible to confirm in situ that the Rab11a GTPase modulates DFV exocytosis. A next step is to identify Rab11a effectors that promote these events. Possible effectors include Sec15A, FIP1-5, and myosin Vb (55, 57, 124, 155, 182).

Why DFVs associate with multiple Rabs is unknown; however, such redundancy is common in other cells with regulated secretory vesicles/organelles (65). There are several possible scenarios. 1) Rab27b and Rab11a act in series. Rab27b (or Rab11a) may associate with the vesicles emerging from the TGN, and then Rab11a (or vice versa) may be recruited as the vesicles mature, facilitating late exocytic events (e.g., docking and fusion of mature DFVs with the plasma membrane). 2) Rab11a- and Rab27b-positive DFVs generate a hybrid organelle in a manner analogous to the fusion of Rab11a-positive recycling endosomes and Rab27a-positive late endosomes in cytotoxic T lymphocytes (144). Such fusion would result in recruitment of Rab11a and Rab27b onto the same vesicles, an event that may be a prerequisite for stretch-induced exocytosis. 3) The two Rab GTPases may associate with distinct populations of DFVs and, in a parallel fashion, act to independently regulate DFV exocytosis. For example, one GTPase may modulate the early-stage exocytic events, and the other could modulate the late-stage events. 4) Both GTPases are found on all DFVs and act sequentially to promote stretch-induced exocytosis. This mechanism is analogous to the Rab conversion during endosome maturation, in which Rab5 on early endosomes recruits numerous effectors, including the HOPs complex, which serves as a guanine nucleotide exchange factor to activate Rab7 on the maturing endosomes before their fusion with late endosomes (170). These scenarios are not mutually exclusive, and sequential activation may occur in the other models as well.

Fusion of DFV with the apical plasma membrane. The exocytosis of secretory granules culminates in the fusion of vesicles with their target membranes and has recently been reviewed by Sudhof and Rothman (194). Briefly, fusion is dependent on the formation of a *trans*-complex of target

membrane-soluble *N*-ethylmaleimide-sensitive factor attachment protein (SNAP) receptors (t-SNAREs) on a target membrane with a cognate v-SNARE found on the vesicle, which assemble into a four-helix bundle that is promoted/stabilized by Sec1/Munc18-like proteins. As the helical bundle zippers toward the fusion pore, it forces membranes close enough to fuse. In cells that undergo regulated exocytosis, such as umbrella cells, complete zippering is prevented by interactions of the four-helix bundle of SNAREs with complexin and synaptotagmin. Complexin interacts with the v- and t-SNAREs and acts as a clamp, whereas synaptotagmin likely acts to sense the influx of Ca^{2+} (the stimulus for regulated exocytosis), reversing the action of complexin and promoting fusion of the membranes. After fusion, the proteins that make up the SNARE complexes, now present in *cis*-complexes on the target membrane, are dissociated from one another by the action of the ATPase *N*-ethylmaleimide-sensitive factor, relieving them for additional rounds of fusion or recovery by endocytosis.

The SNAREs associated with the umbrella cell apical membrane and DFV have been described (29). They include the t-SNAREs syntaxin-1 and SNAP-23 (but not SNAP-25) and the v-SNARE synaptobrevin (vesicle-associated membrane protein 2). The identity of the synaptotagmin and Sec-1/Munc18-like proteins in umbrella cells is unknown. It is intriguing that all three SNARE components appear to be uniformly localized to the DFV and the apical plasma membrane. This may indicate that DFV can undergo homotypic fusion during exocytosis (so-called compound exocytosis); however, no evidence of DFV-DFV fusion is apparent in serial-sectioned tissue (205). An alternative possibility is that v-SNAREs at the apical membrane are present as inactive *cis*-SNARE complexes, which may be in the process of being cleared from the surface by endocytosis. An additional possibility is that the t-SNAREs use the DFV as apical carriers and, while in transit, are inactive until they arrive at the apical plasma membrane. For example, Sec-1/Munc18-like proteins, which interact with the NH_2 terminus of syntaxins and maintain them in a closed inactive conformation (194), may be associated with syntaxin-1 during transit. An intriguing question is whether fusion occurs randomly or at distinct sites along the apical cell surface. Although there is little information in this regard, it has been proposed that DFV may fuse at the hinge regions of the apical plasma membrane (85).

Biogenesis of DFV. In the classical model, DFVs are proposed to be a pool of recycling vesicles that are consumed by exocytosis during bladder filling but renewed during voiding, when the apical membrane is recovered by endocytosis (85, 164). However, endocytosed marker proteins (including fluid-phase and membrane-bound lectins) only label a small fraction of the total DFV pool (6, 87, 164), indicating that the majority of DFV may be formed *de novo* from newly synthesized proteins. Most recently, it was shown that when bladders are filled *in situ* with membrane or fluid markers and then allowed to void (a treatment that stimulates stretch- and voiding-induced membrane trafficking events), there is little colocalization between the internalized markers and Rab11a-positive DFV (112). In addition, there is little colocalization between internalized markers and a dominant interfering version of Rab11a that is associated with DFV but prevents exit of endocytic tracers from the endosomes of other cell types (112). Furthermore, the secretory protein hGH is found in DFV and is

released in response to bladder filling (112). Importantly, in contrast to membrane proteins, hGH does not undergo cycles of endocytic recycling and is significantly diluted once it is released into the urinary space (111, 112). These studies indicate that DFV are not recycling vesicles but are most likely formed *de novo* along the biosynthetic pathway. However, additional studies are needed that follow the fate of internalized UPs and define whether endocytosed UPs are recycled or have some other fate, such as degradation.

Apical endocytosis in umbrella cells. Several studies have reported apical endocytosis in umbrella cells (6, 36, 87, 118, 142, 164, 171, 205, 225). Similar to the exocytic pathway, apical endocytosis in umbrella cells is also regulated, and little endocytosis is measurable in quiescent tissue (205). This is consistent with studies in cultured uroepithelium that show constitutive endocytosis in developing cultures, but not in differentiated cells (118). Apical endocytosis is stimulated during filling (i.e., stretch-induced endocytosis) and upon voiding (131, 205, 234), but the relationship between these pathways is unknown, and it is unclear whether they share similar modes of regulation. The physiological stimulus in either case appears to be increased tension at the basolateral surfaces of the umbrella cells (15, 234), but how basolateral tension is sensed and transmitted remains to be established. One possibility is that it involves integrins, which are known to sense stretch and are localized to the basolateral surface of the umbrella cells (107, 188, 227). In fact, agents that block integrins and their downstream signaling pathways impair voiding-induced endocytosis (unpublished observations). Furthermore, agents that disrupt the actin cytoskeleton block voiding-induced endocytosis (unpublished results), and the apical endocytosis that follows a reversal of osmotic stretch is dependent on the actin and microtubule cytoskeleton (132). Other studies implicate the K^+ channels SK/IK and K_{ATP} as possible regulators of stretch-induced apical endocytosis (234), but how they modulate the endocytic response is unknown.

Endocytosis can proceed by clathrin- or caveolin-dependent mechanisms or through pathways that involve neither clathrin nor caveolin (143). Clathrin, caveolin, and some forms of nonclathrin, noncaveolar endocytosis require the activity of the large dynamin GTPase (143), which promotes vesicle scission. The apical surface of umbrella cells lacks clathrin-coated pits, and clathrin is not associated with the apical membrane of the umbrella cell (10, 29). Furthermore, we have observed that the AP-2 adaptor complex, which is required for many forms of clathrin-dependent endocytosis, is expressed along the basolateral surface of the umbrella cell, but not at its apical surface (unpublished observations). Caveolin-1 is expressed in the uroepithelium early in development but is not expressed in the mature tissue (17). These data appear to rule out a role for clathrin- or caveolin-mediated endocytosis in the recovery of apical membrane. Interestingly, the lack of clathrin and the presence of long membrane protrusions led Born et al. (29) to suggest that there is no apical endocytosis and, instead, to propose that extra-apical membrane is pinched off and released into the urinary space. However, we have observed significant apical endocytosis by a nonclathrin, noncaveolar pathway (unpublished results), and recent studies by Terada et al. (201) indicate that dynamin-2 is associated with DFV and is required for apical endocytosis of *E. coli*.

An unresolved question is the fate of internalized apical membrane. As described above, the classical model proposes that it reestablishes the population of DFV (85, 164). However, previous studies showed that internalized membrane and fluid are found in multivesicular endosomes/late endosomes and lysosomes (6, 36, 87, 164, 171), and not in DFV. These studies indicate that, in umbrella cells, internalized apical membrane proteins are delivered to lysosomes, where they are degraded. Consistent with this possibility, the fate of biotinylated apical membrane proteins internalized in response to stretch is degradation (205). Furthermore, recent studies in the Buff mouse, which has a defect in the Sec-1-related protein VPS33a, show decreased numbers of DFV and a dramatic accumulation of UP- and AUM-positive multivesicular endosomes in the apical cytoplasm of their umbrella cells. VPS33a is one subunit of the HOPS complex that is important in trafficking steps that lead to protein degradation in the endosomal system, including multivesicular endosome-lysosome fusion (162). Thus accumulation of UPs/AUM in the multivesicular endosomes of Buff mice could result from defects in endosome/lysosome fusion (76) and is consistent with the notion that endocytosed apical membrane is delivered to multivesicular endosomes before degradation in lysosomes. The reason for the decreased number of DFV in Buff mice is not known but could result from increases in DFV exocytosis and/or apical membrane endocytosis, or direct fusion of DFV with lysosomes in a process known as crinophagy (76). Similar to Buff mice the umbrella cells of KO mice lacking expression of lysosomal integral membrane protein (LIMP-2) exhibit a paucity of DFV and an accumulation of large numbers of multivesicular endosomes (67). In addition, the apical plasma membrane of LIMP-2 KO mice loses its scalloped appearance and lacks AUM (67). The underlying cause of these defects is not well understood, but the failure to degrade proteins (evidenced by the large accumulation of multivesicular endosomes) may result in cellular toxicity similar to that observed in lysosomal storage diseases (211). Finally, we have followed the fate of membrane and fluid-phase markers internalized from the apical surface of the umbrella cells after voiding and observed that these markers are first delivered to apical endosomes and then to lysosomes, where they are degraded (unpublished observations). Although our studies do not rule out a role for recycling from apical endosomes, they indicate that, upon voiding, a significant fraction of endocytosed marker is delivered to lysosomes, and not to DFV.

SENSORY AND EFFECTOR FUNCTIONS OF THE UROEPITHELIUM: UROEPITHELIUM-ASSOCIATED SENSORY WEB

Although the barrier function of the uroepithelium has been appreciated for decades, only recently has it become apparent that the uroepithelium also plays an important role in transmitting information about the bladder mucosa and its milieu to the nerve and muscle tissues of the bladder. A more extensive discussion of these issues can be found in a number of recent reviews (22–24, 50, 80), including one describing the uroepithelial associated “sensory web” (11). This web includes the uroepithelium, sensory afferent and efferent nerve processes that innervate the uroepithelium, and underlying tissues that include a subepithelial layer of myofibroblasts and the detrusor

muscle. Myofibroblasts arise from fibroblasts that undergo differentiation into smooth muscle-like cells (30). They are extensively linked by gap junctions, have close contact with nerves, and may serve as signaling intermediaries between the uroepithelium and nerves (30). Communication between the tissues that comprise the bladder ensures the proper function of the organ and explains why instillation of mediators such as ATP, carbachol, nicotine, vanilloid compounds, and the nitric oxide (NO) scavenger oxyhemoglobin into the lumen of the bladder alters neurotransmission and bladder function (13, 19, 46, 50, 64, 113, 150, 156, 157, 161, 173, 219), why the uroepithelium can modulate the spontaneous activity of the smooth muscle or muscle contraction (35, 83, 105), and why truncation of the spinal cord disrupts the uroepithelial barrier (12). The following is an abbreviated description of a proposed mechanism to explain how bidirectional communication between the uroepithelium and other tissues in the bladder ensures coordinated bladder function (11).

Sensory input pathways. Within the sensory web, the uroepithelium acts as a sentinel that receives a broad array of “sensory inputs” in the form of mechanical stimuli, such as stretch, mediators present in the urine (e.g., ATP and growth factors), mediators released from nerve processes (e.g., ATP, substance P, and ACh) or other tissues in the bladder, and mediators released from the uroepithelium (e.g., ATP and adenosine) (Fig. 9). The uroepithelium detects these sensory stimuli by expressing a large number of surface receptors and ion channels, including EGF family ErbB1–3 receptors (15, 209), A₁, A_{2a}, A_{2b}, and A₃ receptors (235), α - and β -adrenergic receptors (20, 26), bradykinin receptors (41), cannabinoid receptors (74, 220), fractalkine receptor (236), neurokinin receptor (49), nicotinic and muscarinic receptors (M₁–M₅) (18, 22, 40, 77, 121), purinergic P2X₁, P2X₂, P2X₃, P2X₄, P2X₅, P2X₆, P2X₇, P2Y₁, P2Y₂, and P2Y₄ receptors (27, 58, 129, 197, 200, 218, 225), protease-activated receptors (47), VEGF receptor/neuropilins (39, 176), acid-sensitive ion channels (14, 122), ENaC (61, 134, 224), the NSCC (223, 234), TRAAK (234), TREK-1 (234), and the TRP family channels TRPV1, TRPV2, TRPV4, and TRPM8 (21, 22, 25, 33, 193) (Fig. 9).

Stimulation of these receptors/ion channels may have a number of consequences. They could alter the solute, water, or ion flow across the uroepithelium. Alternatively, they may increase or otherwise modulate membrane turnover at the apical surface of the umbrella cell. As described above, stimulation of adenosine receptors, P2X receptors, EGFRs, the NSCC, and ENaC is known to increase membrane turnover (15, 225, 234, 235). However, it is unknown whether all sensory input pathways have this effect. Membrane turnover could serve the following functions. 1) It could modulate the input response by increasing or decreasing the number of receptors/channels at the surface of the cell. 2) It could potentiate or maintain responses by providing a constant supply of nascent receptors, which would be cleared from the cell surface after binding their cognate ligands. This may be crucial for receptors that are constantly exposed to ligands present in urine and/or rapidly desensitize after binding ligand.

Sensory output pathways. An additional consequence of activating sensory input pathways is the release of mediators from the uroepithelium. These act as “sensory outputs,” which transmit information to the underlying tissues (Fig. 9). Sensory outputs that are likely to be physiologically relevant include

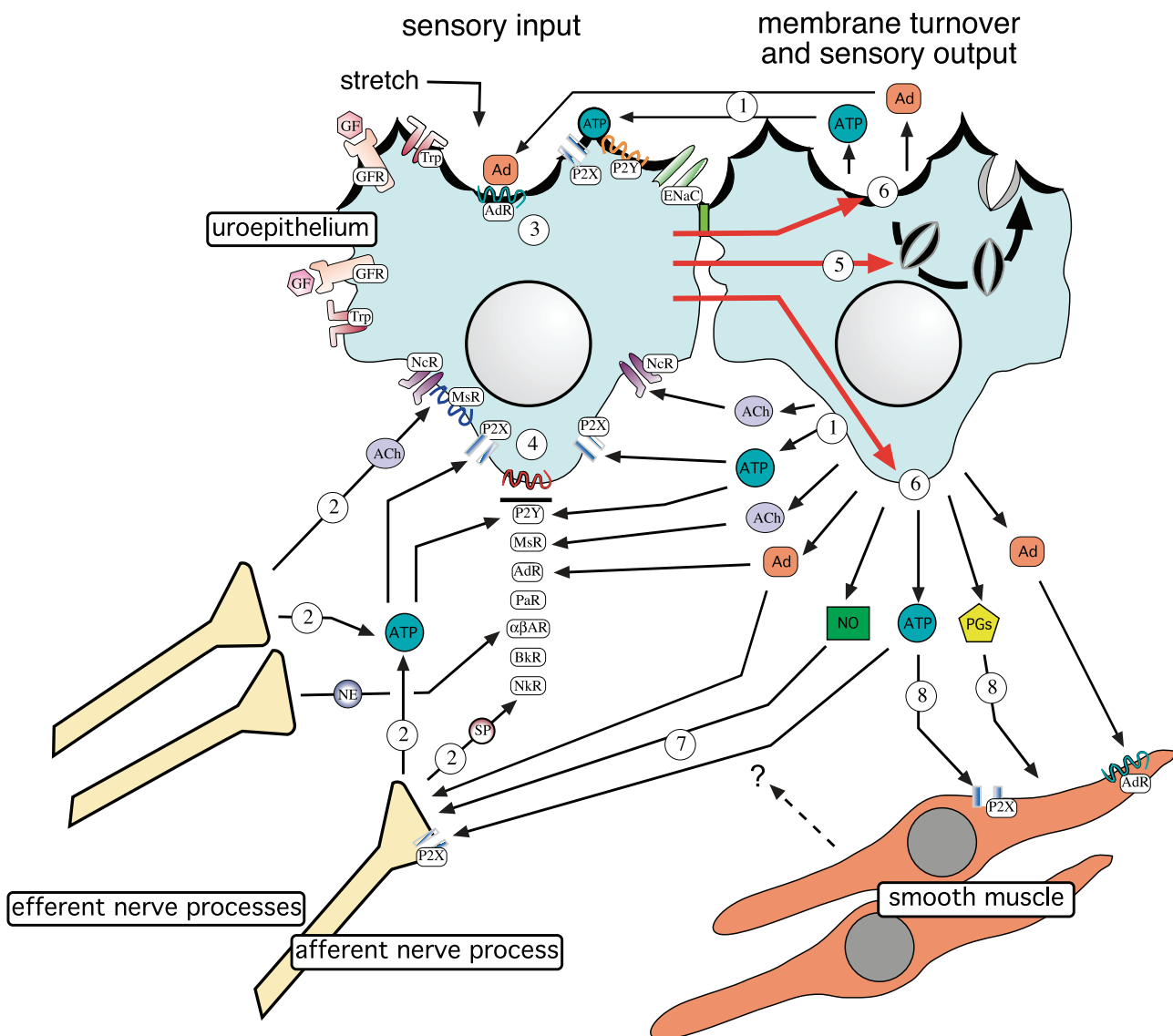


Fig. 9. Bidirectional communication between the uroepithelium and other tissues in the uroepithelium-associated sensory web. The uroepithelium receives “sensory input” in a number of different forms, including mechanical stretch as the bladder fills and the release of soluble mediators such as ACh, adenosine, and ATP from the uroepithelium (step 1), adjacent afferent/efferent nerve processes (step 2), and, possibly, the smooth muscle or myofibroblasts (not shown). In turn, soluble mediators bind to cell surface receptors on the apical surface of umbrella cells (step 3), basolateral surfaces of umbrella cells (step 4), and plasma membranes of underlying intermediate and basal cells (not shown). Receptor binding or channel activation results in changes in the uroepithelium, including membrane turnover at the apical plasma membrane of the umbrella cell (and, possibly, at other plasma membrane domains of the uroepithelium; step 5) and release of mediators such as ACh, adenosine, ATP, nitric oxide (NO), and prostaglandins (PGs; step 6), which act as “sensory outputs.” These outputs can act in an autocrine manner to further alter uroepithelial function (step 1), or they can bind to receptors on sensory afferent nerve processes (step 7) and/or the detrusor muscle (step 8) to regulate nerve and muscle function. α , β -AR, α , β -adrenergic receptor; Ad, adenosine; AdR, adenosine receptor; BkR, bradykinin receptor; GF, growth factor; GFR, growth factor receptor; MsR, muscarinic receptor; NcR, nicotinic receptor; NkR, neurokinin receptor; PaR, proteinase-activated receptor; Pr, proteinase; Trp, transient receptor potential channel family member. [Adapted from Apodaca et al. (11).]

ACh, adenosine, ATP, NO, and prostaglandins (20, 22, 43, 62, 70, 136, 225, 235). These outputs can act in a paracrine manner to modulate the function of other tissues in the bladder or in an autocrine manner to alter uroepithelial responses. Uroepithelial cells express all the machinery necessary to synthesize, transport, and metabolize ACh and release this transmitter in response to mechanical and chemical stimuli (80, 139). ACh may act in a paracrine manner to stimulate muscles and nerves or in an autocrine manner to stimulate uroepithelium-associated nicotinic and/or muscarinic receptors, including M_1 , M_2 , M_3 , M_4 , and M_5 . Activation of M_1 , M_2 , and M_3 receptors causes an

increase in intracellular Ca^{2+} , which is primarily driven by entrance of extracellular Ca^{2+} into the cells (77, 121). The increase in intracellular Ca^{2+} elicits the release of ATP (121), which may trigger purinergic stimulation of nearby afferent nerves. Adenosine is released from both surfaces of the uroepithelium (235) and may modulate sensory afferent function and the contraction of smooth muscle cells. ATP is also released from both surfaces of the uroepithelium in response to stretch (62, 136, 225), and, as described above, the serosal release of ATP stimulates the late-stage exocytic response. Serosal release of ATP may also alter the function of myofi-

broblasts and may stimulate nerve fibers, both of which lie in close proximity to the uroepithelium and express P2X and P2Y purinergic receptor subtypes (23, 30, 32, 195). NO is released from the uroepithelium in response to numerous stimuli and may relax smooth muscle and modulate afferent and efferent nerve functions (22, 26). Prostaglandins are also released from the uroepithelium in response to stretch and may play roles in modulation of nerve and detrusor functions (22). The flow of information between the uroepithelium and the other tissues is not unidirectional, inasmuch as the nerve fibers, myofibroblasts, or detrusor may release mediators that modulate the function of the uroepithelium by stimulating its sensory input pathways (Fig. 9).

The sensory web in action. The potential role of the uroepithelium in signaling bladder fullness illustrates how the sensory web may work (32, 225). Filling stretches the uroepithelium, activating mechanotransduction pathways, which are likely initiated by increased tension at the apical surface of the umbrella cells. The identity of the mechanotransducer(s) is unknown, but ENaC (61, 62), other mechanosensitive ion channels (14), or apical integrins could be involved; these channels or apical integrins would then trigger the uroepithelial release of ATP from both surfaces of the epithelium. The release of serosal ATP has at least two consequences. 1) It binds to P2X₂- and P2X₃-containing receptors on the uroepithelium to stimulate stretch-induced exocytosis at the apical surface of the cell (225), which would increase the volume capacity of the bladder. 2) It has been proposed that the serosally released ATP binds to receptors containing P2X₃ subunits on the sensory afferent nerve processes (43). The degree of afferent stimulation may signal the degree of bladder filling to the central nervous system (32, 219). Consistent with this hypothesis, KO mice lacking P2X₂, P2X₃, or P2X₂/P2X₃ receptor subunits can release ATP, but activation of bladder afferents is significantly decreased and KO mice show reduced micturition frequencies and increased bladder capacities (42, 43, 219). ATP released from the uroepithelium may also bind to myofibroblasts or smooth muscle cells and directly alter their function. The negative regulation of this purinergic pathway is likely mediated by ectonucleotidases that are present at the serosal surface of the uroepithelium and could rapidly decrease the pool of serosal ATP during bladder contraction (136, 225), presumably when the stimulus for ATP release is decreased.

The function of the ATP released from the apical surface of the umbrella cells is not known, but exposure of the mucosal surface of the epithelium to exogenous ATP or its analogs can trigger increased detrusor activity and can also stimulate increased membrane turnover in the umbrella cell layer (31, 45, 173, 225). The mucosa-released ATP could increase detrusor activity by binding to purinergic receptors at the apical surface of the umbrella cell, which would induce ATP release from the serosal surface of the uroepithelium. ATP-induced ATP release has been described in cultured uroepithelium (196), and such a mechanism would act as a positive-feedback loop to further amplify the original signal and stimulate detrusor activity through the purinergic mechanism described above. The ATP-induced apical membrane turnover may exert an additional positive effect on this pathway by ensuring the constant activation of newly inserted P2X receptors and the removal of ligand-bound receptors from the cell surface.

CONCLUSIONS

The uroepithelium forms an effective barrier to urine, toxic metabolites, and pathogens. Work in the past decade demonstrates that this barrier is multifactorial and includes surface glycans (158, 159), membrane lipids (86, 151), tight junction proteins such as claudins (2, 168, 210), and UPs (92, 203). UPs not only contribute to the permeability barrier at the apical membrane of the umbrella cells (92), but they also function as an integral part of the innate immune system, which stimulates apoptosis when these cells are infected with bacteria (148, 203). Barrier function also depends on membrane turnover at the apical surface of the umbrella cell, and recent studies are beginning to provide a general outline of how bladder filling and voiding lead to changes in exocytosis and endocytosis (15, 38, 112, 205, 224, 225, 234, 235). These pathways allow the barrier to accommodate changes in urine volume, and may also be important during bladder infections and for communication between the uroepithelium and the other tissues in the bladder. Beyond the role of the uroepithelium in forming a barrier, the presence of AQP, urea transporters, and ion channels indicates that the uroepithelium has all the machinery necessary to actively alter the composition of the urine (134, 175, 189–192, 223, 234). Therefore, this tissue may play an unappreciated but important role in water, salt, and solute homeostasis.

One of the most exciting functions of the uroepithelium is its potential role as a sensory transducer (11, 22). By receiving, amplifying, and transmitting information, the uroepithelium can convey information about the mucosa and urinary space to the nervous and muscular systems and help coordinate bladder function during filling and voiding. Furthermore, treatments that target the sensory input/output pathways of the uroepithelium can have clinical benefit (23, 49). For example, intravesical infusion of antimuscarinics ameliorates bladder overactivity (19, 50, 113), and administration of vanilloid compounds produces beneficial effects in patients with bladder disorders such as neurogenic detrusor overactivity or IC (13, 46, 64, 161). As a better understanding of the input/output pathways is gained, drugs that target these pathways may provide additional novel therapies for bladder-associated diseases.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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