Directional Fluid Transport across Organ–Blood Barriers: Physiology and Cell Biology

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Directional fluid flow is an essential process for embryo development as well as for organ and organism homeostasis. Here, we review the diverse structure of various organ–blood barriers, the driving forces, transporters, and polarity mechanisms that regulate fluid transport across them, focusing on kidney–, eye–, and brain–blood barriers. We end by discussing how cross talk between barrier epithelial and endothelial cells, perivascular cells, and basement membrane signaling contribute to generate and maintain organ–blood barriers.

ORGAN–BLOOD BARRIERS

Accurate control of the composition of the internal medium is key to the survival of the organism. This depends on the directional absorptive and secretory activity of more than 150 different epithelia, mediated by ~2000 ion and nutrient transporters, channels, exchangers, and receptors, encoded by ~5% of the human genome (Rodriguez-Boulan and Macara 2014). Parenchymal epithelial cells of the various body organs face on their luminal side a broad range of environments (e.g., gastrointestinal [GI] tract, urinary space, biliary space, retinal photoreceptors, cerebrospinal fluid), whereas on the basal side they face an internal medium of constant ionic composition constituted by the interstitial spaces and the blood circulation. The exchange of nutrients and waste products between organs and blood circulation is mediated by highly selective organ–

blood barriers evolutionarily adapted to support organ function or/and organism homeostasis. Hence, the structure of these barriers and the driving forces that regulate fluid transport across them differ significantly (Table 1). Organ–blood barriers can display two layers (endothelial cells [ECs] and basement membrane) when ECs constitute the main component of the barrier (Fig. 1A,C,E), or three layers when parenchymal epithelial cells constitute a principal component of the barrier (Fig. 1B,D,F). Perivascular and local resident cells contribute in diverse ways to barrier assembly and maintenance. Some barriers (e.g., brain, inner retina, GI tract) are formed by monolayers of ECs sealed by tight junctions (TJs) that carry out blood–tissue exchange mostly via transcytosis across ECs. Other barriers (e.g., kidney glomerulus, kidney proximal tubule [KPT], choroid plexus [CP], endocrine glands, outer retina) display fenestrated ECs, which facilitate

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Table 1. Organization and functional characteristics of organ–blood barriers

Organ/Barrier	Organ side	Capillary side (endothelium)	Net fluid transport	Driving force
Kidney				
Glomerular filtration barrier	Podocytes	Continuous fenestrated	Blood→kidney	Balance between hydrostatic and oncotic pressure
Kidney proximal tubule (KPT) barrier	KPT epithelial cells	Continuous fenestrated	Kidney→blood	Active transport of NaCl
Loop of Henle barrier				
Descending limb	Tubule epithelial cells	Continuous fenestrated (ascending vasa recta)	Kidney→blood	Osmotic gradient favoring the medullary interstitium
Ascending limb	Tubule epithelial cells	Continuous (descending vasa recta)	Renal epithelium impermeable to water	Active transport of NaCl
Distal convoluted tubule (DCT) barrier	DCT epithelial cells	Continuous, non-fenestrated	Kidney→blood	Active transport of NaCl
Collecting duct barrier	Principal cells	Continuous, non-fenestrated	Kidney→blood	Osmotic gradient favoring the medullary interstitium
Eye				
Inner retinal–blood barrier	Muller cells	Continuous, non-fenestrated	Bidirectional	Balance between hydrostatic and oncotic pressure
Outer retinal–blood barrier	RPE	Continuous fenestrated (choroid endothelium)	Eye→blood	Active transport of KCl
Ciliary plexus barrier	Nonpigmented and pigmented ciliary epithelium	Continuous fenestrated	Blood→eye	Active transport of NaCl
Trabecular meshwork	Juxtacanalicular trabecular meshwork	Discontinuous (Schlemm's canal ECs)	Eye→blood	Hydrostatic pressure
Brain				
General brain–blood barrier	Astrocyte foot processes	Continuous, non-fenestrated	Blood→brain	Active transport of NaCl
Choroid plexus barrier	Choroid epithelial cells	Continuous fenestrated	Blood→Brain	Active transport of NaCl

RPE, Retinal pigment epithelium; ECs, endothelial cells.

Fluid Transport across Organ–Blood Barriers

Figure 1. Organ–blood barriers in kidney, brain, and eye. Organ–blood barriers consist of a layer of parenchymal cells (*top*), a basement membrane (*middle*), and a layer of endothelial cells (*bottom*). (A) Kidney glomerular barrier, (B) kidney proximal tubule barrier, (C) brain–blood barrier, (D) choroid plexus barrier, (E) inner retina–blood barrier, and (F) outer retina–blood barrier. Net fluid transport (represented by a red arrow) can follow a transcellular route (C, D, E, and F) a paracellular route (A) or both (B). RPE, Retinal pigment epithelium; TJs, tight junctions.

the transport of proteins from the circulation into the tissue and vice versa. Here, we will review physiological and cell biological mechanisms that regulate directional fluid flow, an es-

sential process for organ and body homeostasis, across kidney–, brain–, and eye–blood barriers. We will end by discussing emerging roles of ECs and stromal components in the regulation

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of structure and function of organ–blood barriers.

DIRECTIONAL FLUID TRANSPORT ACROSS ORGAN–BLOOD BARRIERS

Polarized fluid transport is an essential process for lumen formation during development (Lowery and Sive 2005; Bagnat et al. 2007; Khan et al. 2013; Kolotuev et al. 2013). Furthermore, various body organs transport large amounts of fluid for their own homeostasis (e.g., the eye, discussed below) or as a hallmark of their contribution to the homeostasis of the organism. For example, the kidneys filter 180 L of blood daily into the urinary space; most of this ultrafiltrate is recovered into the blood by the activity of the KPT (Weinstein 2013). The intestine also absorbs large amounts of fluid, whereas the CP secretes 500–600 ml of cerebrospinal fluid into the brain ventricles.

Fluid transport was first observed in the gut (Reid 1902) and later confirmed by additional studies in intestine (Curran 1960; Parsons and Wingate 1961), gall bladder (Diamond 1962; Kaye et al. 1966), KPT (Windhager et al. 1959), retinal pigment epithelium (RPE) (Miller et al. 1982), and CP (de Rougemont et al. 1960). Although fluid absorption is known to occur mostly under isotonic conditions, various epithelia can transport fluid against an external osmotic gradient physiologically or under experimental conditions (Fig. 2A) (Parsons and Wingate 1961; Diamond 1962; Heisey et al. 1962). The Koefoed-Johnsen and Ussing model for active transport of NaCl across frog skin (Koefoed-Johnsen and Ussing 1958) framed the hypothesis of the coupled transepithelial NaCl/water transport (Curran and Solomon 1957). It was initially proposed that active water transport is an intrinsic property of certain epithelia accounted for by two membranes in series, one with low-solute permeability (high-reflection coefficient) and another one with high-water and solute permeability (low-reflection coefficient) (Curran 1960; Durbin 1960). This hypothesis evolved into the three-compartment model, in which compartments A, B, and

C are separated by membranes with high- and low-reflection coefficients (Fig. 2B) (Curran and Macintosh 1962). According to this model, active solute accumulation in B drives water by osmosis from A to B, whereas hydrostatic pressure drives water from B to C. Correlative morphological/physiological studies established the identity of these compartments: A is the lumen, B the lateral intercellular space (LIS), and C the interstitial space (Williams 1963; Whitlock and Wheeler 1964; Tormey and Diamond 1967; Bentzel et al. 1969; Maunsbach and Boulpaep 1980). The standing osmotic gradient model (Fig. 2C) (Diamond and Bossert 1967) proposed that directional water transport was achieved by active pumping of Na^+ into a region of the LIS proximal to the TJ, which was assumed nonpermeable to water and ions, and fluid exit at the other end of the LIS, which was open and did not consider the contribution of a basement membrane. In contrast with the postulates of this model, subsequent experiments showed a homogeneous distribution of the Na^+ , K^+ -ATPase in LIS (Farquhar and Palade 1964; Stirling 1972; Kyte 1976), and LIS swelling in actively transporting epithelia (Williams 1963; Kaye et al. 1966; Tisher and Kokko 1974), which is compatible with TJ with moderate osmotic water permeability at the proximal end and a basement membrane with high (but not infinite) hydrostatic permeability at the distal end (Fig. 2D) (Weinstein and Stephenson 1981). Although the LIS concept is essential to explain water transport against an osmotic gradient (physiologically relevant to water transport by gall bladder and intestine), modeling studies suggest that LIS is not an absolute requirement for vectorial water transport (Schafer et al. 1977). Indeed, net water transport across KPT (and RPE, and CP) may be the result of a small (2%–3%) favorable external osmotic gradient as observed across KPT epithelium (Andreoli and Schafer 1978; Andreoli et al. 1978), and interstitial oncotic pressure (Weinstein 1984). The osmotic model (Fig. 2E) (Spring 1998) relies on very high epithelial water permeability to transport water down a very small osmotic gradient in both directions, accounting for both absorption and secretion, depending

Figure 2. Directional fluid transport across epithelia. (A) Effect of osmotic gradients on the direction of fluid transport by gall bladder epithelium. (Panel is based on data in Diamond 1962.) Fluid flow was measured in gall bladder (y axis) to determine whether the epithelium was absorbing (red part of the trace) or secreting (blue part of the trace). The transepithelial osmotic gradient was manipulated to establish a driving force toward the lumen or the basolateral side (x axis). When the osmotic gradient was neutral (dashed line) isosmotic absorption was observed, and when the osmotic gradient reversed, the epithelium continued to absorb against the driving force until it eventually reversed the direction of the fluid toward secretion at higher osmotic gradients. (B) Three-compartment model (Curran and Macintosh 1962). The three compartments A, B, and C are separated by two membranes with a reflection coefficient $\sigma \approx 1$ (impermeable to solutes) and $\sigma \approx 0$ (permeable to solutes). Water moves from A to B by osmosis and from B to C because of hydrostatic pressure. (C) Standing osmotic gradient model (Diamond and Bossert 1967). Active solute pumping into the blind end of the lateral intercellular space (LIS) drives water flow into the compartment and exit from it through the open end. The model assumes an impermeable blind end of the LIS. (D) Three-compartment model compatible with epithelial architecture (Weinstein and Stephenson 1981). Active solute pumping by the Na^+ , K^+ -ATPase into the intermediate compartment (LIS) generates the osmotic force for water absorption from the lumen through the intracellular space. The increase in hydrostatic pressure inside the LIS drives fluid exit through the basement membrane (BM) into the interstitium. The model assumes a higher reflection coefficient of the tight junctions (TJs) relative to the BM. (E) Osmotic model (Spring 1998). A small transepithelial osmotic gradient is sufficient to drive fluid flow (secretion in the illustration) thanks to the very high water permeability of some epithelia. This model applies to absorptive and secretory epithelia depending on the direction of the osmotic gradient. (F) Water pump (Zeuthen and Stein 1994). Water is transported against an osmotic gradient coupled with one or more solutes (X) using the favorable electrochemical gradient for the cotransported solutes. In all figures, the osmotic gradient is represented by the red shading, in which lighter indicates lower and darker represents higher osmolality.

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Figure 3. Variable configuration of transporter polarity in secretory and absorptive epithelia. Different epithelial cells vary the localization of Na⁺, K⁺-ATPase, Na⁺, K⁺, Cl⁻, bicarbonate, and water transporters and channels to perform functions specific for each organ and tissue. (A) Kidney proximal tubule: The electrochemical gradient generated by the basolateral Na⁺, K⁺-ATPase is used by apical Na⁺/H⁺ exchanger NHE3 and Na⁺-coupled transporters (SGLTs) to absorb Na⁺ from the lumen. Apical anion exchangers (AEs) transport Cl⁻ inside the cell. Sodium and Cl⁻ exit through basolateral transporters and channels, producing net absorption of NaCl, which provides the driving force for water absorption via aquaporin-1. (B) Choroid plexus: Uses apical Na⁺, K⁺-ATPase and a different combination of Na⁺, Cl⁻, K⁺, and bicarbonate channels and transporters to secrete cerebrospinal fluid (CSF) into the brain ventricle. (Legend continues on following page.)

on the epithelium. An alternative model for uphill water movement involves water cotransport by solute transporters (Fig. 2F) (Zeuthen and Stein 1994). It has been questioned whether this mechanism would account quantitatively for the large amounts of transported water (Spring 1998; Zeuthen 2002). The Na^+ /glucose cotransporter SGLT1 has been proposed to transport water against an osmotic gradient (Loo et al. 2002), by incorporating water molecules within the transporter (Adelman et al. 2014). Other transporters that might mediate uphill water flux are NKCC1 in kidney (Hamann et al. 2005; Zeuthen and Macaulay 2012) and a K^+/Cl^- cotransporter (Zeuthen 1991a,b) in the CP. Furthermore, it has been speculated that Na^+ /glucose cotransport may contribute to paracellular fluid absorption (Turner 2000).

VARIATIONS IN THE ORGANIZATION OF ORGAN–BLOOD BARRIERS

Different epithelial cells vary dramatically the localization of individual receptors and transporters to perform specific functions required by the host organ. In this section, we discuss organ blood barriers in kidney, brain, and eye and illustrate variations in the localization of transporters implicated in directional fluid transport in three epithelial barriers in these organs, KPT, CP, and RPE (Fig. 3).

Kidney

Kidney Glomerular Barrier

The glomerular filtration barrier filters the blood to remove waste products and excess solutes. From blood to tissue, it displays three components: fenestrated ECs, basement membrane, and podocytes, highly specialized epithe-

lial cells with foot processes that interdigitate, forming “filtration slits” (Haraldsson et al. 2008; Scott and Quaggin 2015). The EC fenestrations are ~ 60 nm in diameter; therefore, they exclude blood cells but allow the passage of proteins. However, the negatively charged glycocalyx that lines the capillary lumen, together with adsorbed plasma components is believed to be capable of restricting the passage of some molecules (Haraldsson et al. 2008; Scott and Quaggin 2015). The basement membrane is secreted by ECs and podocytes. The laminin matrix meshwork and proteoglycan negative charges limit filtration of intermediate-large molecules (i.e., proteins) (Jarad et al. 2006). Podocyte filtration slits display slit diaphragms, specialized junctional elements (5–15 nm diameter) composed of adhesion, and signaling molecules (nephrin, podocin, Neph1, Nph3, ZO-1, P-cadherin, CD2AP, Densin-180, FAT1), which contribute to regulate the filtration barrier (Haraldsson et al. 2008; Gagliardini et al. 2010). The blood is filtered through EC fenestrae and podocyte filtration slits into the Bowman’s (urinary) space, generating an ultrafiltrate with a composition similar to the blood except for the absence of proteins larger than 60 kDa.

Kidney Proximal Tubule Barrier

The KPT reabsorbs 2/3 of the 180 L of ultrafiltrate generated daily by both kidneys back into the blood circulation. To this end, the KPT’s absorptive epithelium organizes its polarity along the classical Koefoed-Johnsen and Ussing model (Fig. 3A) (Koefoed-Johnsen and Ussing 1958; Palmer and Andersen 2008). The Na^+ , K^+ -ATPase is localized basolaterally and provides the driving force for primary and secondary active transport. The electrochemical gradient generated by the Na^+ , K^+ -ATPase is used to

Figure 3. (Continued) NaCl secretion into the CSF provides the driving force for water exit through aquaporin-1. (C) Retinal pigment epithelium: The apical Na^+ , K^+ -ATPase pumps Na^+ into the cell and removes K^+ from the subretinal space, which is necessary for the dark current of the photoreceptors. However, net transport of fluid occurs in the apical to basal direction driven by KCl absorption via apical Na^+ , K^+ , 2Cl^- cotransport and basolateral exit via K^+ and Cl^- channels. Candidates are Maxi-K, CFTR, Bestrophin, and probably additional Cl^- channels.

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reabsorb Na^+ from the ultrafiltrate via a battery of Na^+ -coupled symporters and antiporters in the apical side and Cl^- /bicarbonate cotransporters in the apical and basal sides. Na^+ absorption is coupled to other processes such as extrusion of H^+ via the Na^+/H^+ exchanger NHE3, crucial for pH regulation, and uptake of glucose, amino acids, and phosphate into KPT cells (Weinstein 2013). Exit of these solutes via basolateral Na^+ -independent solute transporters completes their *trans*-epithelial transport from the ultrafiltrate into the blood.

Most of the net water absorption in the KPT occurs via a transcellular pathway mediated by aquaporin-1 (AQP1) (Schnermann et al. 1998) expressed both apically and basolaterally (Sabolic et al. 1992; Nielsen et al. 1993; Zhang et al. 1993). The remaining fluid flux occurs paracellularly via TJ expressing “leaky” claudins such as claudin-2 (Muto et al. 2010) and possibly claudin-10 and claudin-17 (Krug et al. 2012), which are also selective to cations (e.g., Na^+ , K^+ , Ca^{2+} , and Mg^{2+}) (Bomsztyk et al. 1984; Wilson et al. 1997, 1998; Muto et al. 2010).

Other Kidney–Blood Barriers

After the KPT reclaims most of the ultrafiltrate through the constitutive process described above, the distal nephron modifies the ultrafiltrate in a regulated fashion to precisely maintain water and electrolyte homeostasis of the internal medium (reviewed in Castaneda-Bueno et al. 2012; Knepper et al. 2015; McCormick and Ellison 2015; Pearce et al. 2015). All distal nephron segments use the same transporter configuration as the KPT, with the Na^+ , K^+ -ATPase at the basolateral membrane and a variable complement of apical ion transporters. Water permeability is also controlled by different aquaporin isoforms with distinct properties. The loop of Henle that descends into the medulla operates in tandem with the vasa recta as a countercurrent exchange mechanism that allows urine concentration. The thin descending portion is highly permeable to water because of apical and basolateral AQP1 (Nielsen et al. 1995b), whereas the thin ascending portion is impermeable to water and transports

Na^+ to the interstitium. The thick ascending portion mediates NaCl absorption through a basolateral Na^+ , K^+ -ATPase in coordination with apical NKCC2 and NHE3, and a lumen-positive transepithelial electrical gradient that drives cation absorption through the TJs. In the distal convoluted tubule and the collecting duct, Na^+ absorption is mediated by the apical cotransporter NCC and the Na^+ channel ENaC, respectively, which use the gradient created by the basolateral Na^+ , K^+ -ATPase. The collecting duct has principal cells, in which water permeability is regulated through translocation of AQP2 to the apical membrane in response to antidiuretic hormone (Nielsen et al. 1995a).

Brain–Blood Barriers

General Brain–Blood Barrier (BBB)

The BBB is constituted by a monolayer of continuous non-fenestrated ECs endowed with tight intercellular TJ, which restrict paracellular diffusion of ions and water. The tightness of TJ in BBB ECs is regulated by interactions with astrocytes, pericytes, and the extracellular matrix (ECM). Like epithelial cells, BBB ECs display a polarized distribution of receptors and transporters between the luminal and abluminal side that use energy to transport a net amount of fluid into the brain interstitium (Abbott 2004; Mokgokong et al. 2014; Zhao et al. 2015; Worzfeld and Schwaninger 2016); the mechanisms that control EC polarization remain largely unknown.

Choroid Plexus

The CP is a secretory epithelium and produces cerebrospinal fluid (CSF), necessary for the normal function of the brain (Fig. 3B). The CSF's ion and nutrient composition is very similar to the blood. Brain ventricles contain ~50–70 ml of CSF; this volume is replaced 4–7 times per day through CP secretion of ~500–600 ml of CSF (Cserr 1971; Wright 1978). This high rate of fluid secretion occurs in the basal-to-apical direction and is achieved via the polarized distribution of apical and basolateral transporters. The Na^+ , K^+ -ATPase and

other transporters, including NKCC1, KCC4, and NHE1, which are normally basolateral in other epithelia, are instead localized apically in the CP. The mechanisms responsible for this atypical transporter localization are not yet fully understood. The Na^+ pumping activity of the Na^+ , K^+ -ATPase is coordinated with basolateral bicarbonate, Cl^- and Na^+ transporters and with the activity of carbonic anhydrases that promote the intracellular formation of bicarbonate (Davson and Luck 1957; Davson and Segal 1970; Jacobs et al. 2008).

What are the forces that drive fluid transport apically in CP? CSF may have an osmolality slightly higher than blood (Goldberg et al. 1965; Davson and Segal 1996), which has been proposed to drive osmotic movement of water toward the CSF (Damkier et al. 2013). The relative contribution of the paracellular versus transcellular pathways to CSF secretion is still undefined. Transcellular water transport is facilitated by AQP1 (Oshio et al. 2005) and paracellular transport may occur through TJ, which display claudin-2 as well as non-pore-forming claudins (Lippoldt et al. 2000; Wolburg et al. 2001; Kratzer et al. 2012), which classifies CP as an epithelium of “intermediate” TJ (i.e., midway between leaky and tight) based on a reported transepithelial electrical resistance of $\sim 170 \Omega \cdot \text{cm}^2$ (Saito and Wright 1983).

Eye–Blood Barriers

Several circulation systems irrigate different areas within the eye; their structural and functional features are designed to accomplish the particular needs of each region. Whereas, the inner retina is perfused by retinal blood vessels with continuous non-fenestrated capillaries similar to those of the brain, the outer retina is nourished by fenestrated choroidal capillaries, acting in concert with the RPE. In addition, there are other key eye–blood barriers in the eye. Two are involved in the formation (ciliary body) and reabsorption (trabecular meshwork) of the aqueous humor. A third one is the barrier between the cornea, an avascular tissue, and the peri-corneal blood vessels that contribute to corneal nutrition.

Fluid Transport across Organ–Blood Barriers

The Inner Retina–Blood Barrier (iRBB)

Because the neural retina is part of the central nervous system, it is not surprising that the iRBB and BBB share many structural and functional features. Inner retina capillaries are non-fenestrated, designed to tightly restrict the passage of solutes across the endothelial monolayer. As in the BBB, pericytes and glial cells also participate in the establishment and maintenance of the iRBB (reviewed in Klaassen et al. 2013; Arboleda-Velasquez et al. 2015). In addition, changes in the physical properties of retinal EC basement membrane associated to diabetic retinopathy are causative of EC alterations and barrier dysfunction (Chronopoulos et al. 2010; Yang et al. 2016).

The Outer Retina–Blood Barrier (oRBB)

The retina accumulates large amounts of water from the metabolic turnover of retinal neurons and photoreceptors and from pressure-induced movement of water from the vitreous body (Hamann 2002). Water is removed from the inner retina by Muller cells (Moseley et al. 1984; Marmor 1999; Nagelhus et al. 1999) and from the subretinal space by the RPE (Hamann 2002). The RPE, like CP, is a neuroepithelium. However, unlike most other epithelia, the apical plasma membrane (PM) of RPE cells is not free, but is in contact with 50–100 photoreceptors (rods and cones), separated by a virtual compartment, the subretinal space (Fig. 1F). A major task of the RPE is to generate the correct ionic environment for the function of the photoreceptors, controlling tightly the subretinal K^+ and Na^+ levels to allow photoreceptors to generate the “dark current,” essential for vision (Strauss 2005). A second major function of RPE is to transport a net amount of fluid in the apical to basal direction, creating a negative pressure in the subretinal space that helps keep the neural retina attached to the RPE (Adijanto et al. 2009). Hence, defective fluid transport by RPE is a cause of important human eye pathologies such as macular edema and retinal detachment (Hamann 2002).

Fluid transport in RPE is driven by the net transport of Cl^- and K^+ in the apical to basal

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direction and is facilitated by AQP1 (Strauss 2005). Lactate, produced in large amounts by the retina and a major food source for photoreceptors, also contributes to fluid transport (Philp et al. 1998; Hamann et al. 2003; Adijanto et al. 2009). Lactate levels in the subretinal space are 3–10 times higher than in the blood (Strauss 2005) and is transported efficiently to the choroid blood by proton-coupled transporters MCT1 and MCT3 at the apical and basolateral membrane of RPE, respectively (Philp et al. 1998).

As in both CP and PT, this task requires coordinating Na^+ , K^+ -ATPase activity with those of bicarbonate and Cl^- transporters. Diurnal variations in the activity of the retina result in changes in the levels of Na^+ , K^+ , and CO_2 in the subretinal space, which are balanced by changes in the activity of the various Na^+ , K^+ , Cl^- , and bicarbonate transporters (Fig. 2C).

RPE TJs constitute the main barrier between the subretinal space and the choriocapillaris; they generate a paracellular resistance, $\sim 10\times$ higher than the transcellular resistance, which defines the RPE as a “tight” epithelium (Strauss 2005). Most water moves through AQP1 in the apical and basolateral membranes of RPE. As with KPT and CP, net water transport from RPE to choroid occurs secondarily to ion transport processes (Strauss 2005). However, the organization of fluid transport differs markedly from that of KPT and CP. Like CP, RPE cells localize Na^+ , K^+ -ATPase to the apical PM; however, fluid transport in RPE is in the opposite direction (basal) of fluid transport in CP (apical). Unlike basal fluid transport in KPT, which depends on the net basal transport of NaCl, basal fluid transport in RPE depends on the net transport of KCl. Apical Na^+ , K^+ -ATPase pumps Na^+ toward the subretinal space generating a Na^+ gradient that facilitates the entrance of Cl^- and K^+ by a bumetanide-sensitive Na^+ , K^+ , 2Cl^- cotransporter in the apical PM (Kennedy 1990; Miller and Edelman 1990; Bialek et al. 1995; Hu et al. 1996). In mammals, two different genes code for two isoforms of NKCC cotransporters (NKCC1 and NKCC2) and they are both selectively inhibited by bumetanide. To our knowledge, the identity of the apical NKCC cotransporter in RPE has never been deter-

mined directly. Based on their affinities for the transported ions, the ionic environment of the subretinal space would allow the proper functioning of NKCC1, but not of NKCC2. The latter is evolutionarily adapted for its function in the extreme ionic environment of the renal medulla, in which NaCl concentrations can be up to 3–4 times higher than blood. Importantly, NKCC1 is expressed basolaterally in almost all epithelia studied to date (Ginns et al. 1996; He et al. 1997; Delpire et al. 1999; Evans et al. 2000; Shillingford et al. 2002; Del Castillo et al. 2005; Pena-Munzenmayer et al. 2005; Carmosino et al. 2008); hence, if NKCC1 turns out to be the apical bumetanide-sensitive transporter in RPE, this would be another example of inverted polarity in the RPE, similar to the Na^+ , K^+ -ATPase. Part of the K^+ internalized by the Na^+ , K^+ -ATPase is transported back to the subretinal space through the potassium channel Kir7.1, which is tightly coexpressed with Na^+ , K^+ -ATPase in various body epithelia. In addition to NKCC, intracellular Cl^- and Na^+ concentrations are also regulated by bicarbonate exchangers, which, together with lactate transporters MCT1 and MCT3, regulate the subretinal and intracellular pH. Accumulation of Cl^- results in a high intracellular Cl^- concentration (~ 60 mM) that, coupled to the favorable transmembrane electric potential across the basolateral PM, promotes exit of Cl^- toward the choroidal space, with K^+ as a counterion. The transporters mediating K^+ and Cl^- exit toward the choroid are poorly characterized. Candidates for this role are a Maxi-K channel, ClC-2, cystic fibrosis transmembrane regulator (CFTR) and Bestrophin-1 (Strauss 2005). ClC-2 is a ubiquitous voltage-gated chloride channel, particularly abundant in epithelial tissues (Grunder et al. 1992; Thiemann et al. 1992; Jentsch 2008), believed to play key roles in transepithelial Cl^- transport processes in response to cell swelling and extracellular acidification (Jentsch 2005). Its knockout in mice causes blindness by retinal degeneration and male sterility (Bosl et al. 2001; Edwards et al. 2010) consistent with its presumed localization in blood–tissue epithelial barriers such as the RPE in the outer retina and Sertoli cells in

the testis (Jentsch 2005; Strauss 2005). Bestrophin-1, encoded by the gene BEST1 (formerly VMD2) causes a group of eye diseases collectively called “bestrophinopathies” (Marmorstein et al. 2009). The best understood is Best vitelliform macular dystrophy (BVMD), a dominant trait characterized electrophysiologically by a normal clinical electroretinogram (ERG) and a diminished light peak. Bestrophin-1 is a Ca^{2+} -activated Cl^{-} channel that is expressed in the basolateral PM of RPE and Madin–Darby canine kidney (MDCK) cells (Marmorstein et al. 2000). Many of the mutations that cause human disease cause intracellular retention of Bestrophin-1 (Milenkovic et al. 2011; Johnson et al. 2013, 2014), likely a result of disruption of its tight pentameric structure, recently reported by Long and coworkers (Kane Dickson et al. 2014); however, the signals and mechanisms that mediate its basolateral distribution in both MDCK and RPE cells remain unknown.

EPITHELIAL POLARITY ORGANIZES ORGAN–BLOOD BARRIERS

As discussed above, the variable organization of ion transporters in different epithelia is key to explain the extent and directionality of fluid transport across epithelia. However, the mechanisms used by the cell to localize these transporters at the correct PM domain and to vary their localization in different epithelial cells remain largely unknown. Here, we will briefly review apical and basolateral sorting mechanisms and discuss some mechanisms that may contribute to the variable localization of ion transporters in different epithelia.

Compartments Involved in Apical–Basolateral Protein Sorting

The study of the intracellular routes and sorting compartments involved in apical–basolateral PM protein sorting in MDCK cells was initially made possible by the introduction of the prototype polarized epithelial cell line MDCK (Cereijido et al. 1978) and the demonstration that enveloped RNA viruses, for example, influenza and vesicular stomatitis virus, bud from opposite domains of the PM (Rodriguez-Bou-

lan and Sabatini 1978; Rodriguez-Boulan and Pendergast 1980). Early studies in the 1980s showed that the envelope proteins of these viruses, for example, influenza hemagglutinin (HA) and VSVG protein are appropriate model apical and basolateral PM proteins and are sorted by epithelial cells using the same compartments and mechanisms used for their own PM proteins. These studies showed that apical and basolateral PM proteins are synthesized in the endoplasmic reticulum (ER) and sorted in the *trans*-Golgi network (TGN) into different carrier vesicles for delivery to their corresponding PM domains (Fig. 4). Starting in the 1980s, studies by several laboratories have defined the endocytic and recycling routes of MDCK cells, highlighting the presence of separate apical sorting endosomes (ASEs) and basolateral sorting endosomes (BSEs), and two sets of recycling endosomes, the common recycling endosome (CRE) and the apical recycling endosome (ARE), which participate in the sorting of apical and basolateral PM proteins in the recycling route (Fig. 4) (Mostov et al. 2003; Rodriguez-Boulan and Musch 2005; Rodriguez-Boulan et al. 2005; Mellman and Nelson 2008; Folsch et al. 2009; Apodaca et al. 2012; Rodriguez-Boulan and Macara 2014). Finally, studies in the last decade have shown that TGN and endosomes do not work totally independently but, rather, cooperate in the sorting of PM proteins. Indeed, many newly synthesized PM proteins follow a transendosomal route from the TGN to the PM, with the final sorting decision made at the level of different endosomal compartments (Mellman and Nelson 2008; Folsch et al. 2009; Gonzalez and Rodriguez-Boulan 2009; Weisz and Rodriguez-Boulan 2009). Preliminary data from our laboratory (R Schreiner, S Salvezza, and EJ Rodriguez-Boulan, unpubl.) suggest that TGN and CRE are intimately associated (Fig. 4), reflecting a possible coordinated role in protein sorting.

Trafficking Mechanisms Involved in Apical–Basolateral Sorting

Studies, largely in MDCK cells, have revealed that epithelial cells sort their PM proteins using a dizzying repertoire of apical and basolateral

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Figure 4. Contributions of protein trafficking to establishing epithelial polarity. Epithelial cells are polarized with apical and basolateral membrane domains separated by tight junctions (TJs). Selective trafficking of domain-specific proteins in and out of these domains allows epithelia to perform their many functions. Apically and basolaterally targeted proteins use various sorting signals (*right* panel). Apical sorting signals can be associated with lipid rafts and basolateral sorting signals are often dependent on clathrin and the clathrin adaptors AP-1 and AP-2. Different routes are indicated in colored arrows and can traverse several endosomal compartments. Biosynthetic route (black): It originates in the endoplasmic reticulum (ER), then proteins traffic to the Golgi and they are sorted in the *trans*-Golgi network (TGN). Then proteins are delivered directly to the basolateral or apical membranes, they can traverse apical sorting endosomes (ASEs) and apical recycling endosomes (AREs) en route to the apical membrane or they can reach the basolateral membrane via common recycling endosomes (CREs). Apical recycling route (blue): Proteins are endocytosed from the apical membrane via clathrin, AP-2, and the small GTPases Rab4 and Rab5. They transit to the ASE, CRE, and ARE before reaching the apical membrane again via Rab11a. Basolateral recycling route (red): It originates in the basolateral membrane via endocytosis mediated by clathrin, AP-2, and Rab4 and 5. Proteins transit through the CRE before reaching the basolateral membrane again. Transcytotic route (green): Similar to the basolateral recycling route, it originates in the basolateral membrane, but from the CRE proteins are directed to the ARE and reach the apical membrane. BB, Basal body.

targeting mechanisms, rather than a single binary mechanism. The multiplicity of sorting mechanisms is ontologically explained by the requirement of different epithelia for maximal flexibility in localizing individual transporters apically or basolaterally to perform their tissue- or organ-specific functions (Mostov et al. 1992;

Rodriguez-Boulán et al. 2005). Typically, apical sorting signals are complex and may be found in either the luminal, transmembrane, or cytoplasmic domains of the PM protein (Weisz and Rodriguez-Boulán 2009). The first apical sorting signal identified was glycosyl-phosphatidylinositol (GPI), which mediates attachment of

GPI-anchored proteins to the PM (Chan et al. 1988; Brown et al. 1989; Lisanti et al. 1989). Because GPI has affinity for PM microdomains enriched in glycosphingolipids and cholesterol, termed “lipid rafts,” experiments showing that GPI-anchoring promoted apical targeting of chimeric proteins (Brown et al. 1989; Lisanti et al. 1988, 1989) provided the first experimental support for the lipid raft hypothesis for apical sorting (Simons and van Meer 1988). Subsequent experiments showed that other apical proteins, for example, influenza HA, interacted with lipid rafts (Skibbens et al. 1989). The lipid raft concept is still evolving: current ideas indicate that they are formed in the Golgi complex as very dynamic structures that require aggregation into larger rafts via a variety of proteins (e.g., the tetraspanin MAL1 and certain lectins) to act as sorting platforms (Puertollano et al. 1999; Fullekrug and Simons 2004; Weisz and Rodriguez-Boulán 2009). Lipid raft association is not a universal mechanism for apical trafficking, as many apical proteins are not associated with lipid rafts; some apical proteins are sorted through direct interaction with microtubule motors, for example, rhodopsin (Sung and Tai 2000).

In contrast, sorting of basolateral proteins is mediated by basolateral sorting signals (BLSS), that is, simple peptide motifs in the protein's cytoplasmic domain, often resembling tyrosine (YXX Φ , NPXY) and dileucine [D/E]XXXL[L/I] motifs used for clathrin-mediated endocytosis (CME) (Bonifacino and Traub 2003; Gonzalez and Rodriguez-Boulán 2009; Bonifacino 2014; Rodriguez-Boulán and Macara 2014). Other basolateral signals are unrelated to endocytic signals, for example, the tyrosine motifs in LDLR (Matter et al. 1994) and VSVG protein (Thomas et al. 1993), the GDNS motif of transferrin receptor (TfR) (Odorizzi and Trowbridge 1997), the multicomponent basolateral signal of polymeric Ig receptor (Reich et al. 1996), the monoleucine motifs found in CD147 (Deora et al. 2004) and stem cell factor (Wehrle-Haller and Imhof 2001), the EXEX $\Phi\Phi$ motif found in the M₃ muscarinic receptor (Iverson et al. 2005), the PXXP motif in the epidermal growth factor receptor (He et al. 2002), and the PDZ-

binding domains in syndecan-1 (Maday et al. 2008).

During endocytosis, clathrin generates endocytic vesicles via the heterotetrameric clathrin adaptor AP-2, which interacts with endocytic signals via specific pockets characterized in detail by X-ray crystallography (Owen and Evans 1998; Kelly et al. 2008). Likewise, clathrin plays a key role in basolateral traffic at both biosynthetic and recycling routes (Deborde et al. 2008), albeit using a different clathrin adaptor, AP-1. AP-1 exists as 10 different combinatorial heterotetramers of the medium (μ 1A, μ 1B), γ (γ 1, γ 2), σ (σ 1A, σ 1B, σ 1C), and β subunits (Bonifacino and Traub 2003; Bonifacino 2014). Two of these variants have been implicated in basolateral sorting. One of them, AP-1B, is epithelial-specific and appears to regulate basolateral trafficking in both biosynthetic and recycling routes (Folsch et al. 1999; Ohno et al. 1999; Gan et al. 2002; Cancino et al. 2007; Gravotta et al. 2007). The ubiquitous, highly related AP-1A adaptor, which differs from AP-1B in the medium subunit (μ 1A instead of μ 1B), mediates basolateral PM protein sorting proximally to AP-1B, probably at the level of the TGN (Carvajal-Gonzalez et al. 2012; Gravotta et al. 2012; Rodriguez-Boulán et al. 2013). Two other clathrin adaptors, AP-3 and AP-4, have been postulated to participate in basolateral sorting (Nishimura et al. 2002; Simmen et al. 2002), although the original observations supporting this claim have not been followed up. It appears that, although clathrin and clathrin adaptors regulate the localization of many basolateral proteins, there are alternative sorting adaptors involved in basolateral PM localization. To date, these include Naked, involved in basolateral sorting of some epidermal growth factor receptors (Li et al. 2004) and ankyrin G, which participates in cooperation with β 2 spectrin in basolateral sorting of E-cadherin (Kizhatil et al. 2007).

Domain-Specific Cytoskeletons in Apical–Basolateral Segregation

PM proteins may be segregated through interactions with asymmetrically distributed cytoskeletal elements. Interactions with apical inter-

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mediate filaments may contribute to the apical localization of PM proteins in epithelia (Rodriguez et al. 1994; Salas et al. 1997). The actin cytoskeleton displays different apical and basolateral organizations (Drenckhahn et al. 1983, 1985; Nelson and Veshnock 1986, 1987b; Rodman et al. 1986; Drenckhahn and Dermietzel 1988). At the apical PM, the actin cytoskeleton contributes to the formation of microvilli through proteins such as Villin (Friederich et al. 1989; Costa de Beauregard et al. 1995) and Ezrin (Takeuchi et al. 1994; Saotome et al. 2004; Fehon et al. 2010). In RPE, Ezrin is localized at both apical and basolateral membranes (Bonilha et al. 1999), interacting with different membrane proteins through different Ezrin-binding adaptors, apical EBP50 and basolateral SAP97 (Bonilha and Rodriguez-Boulant 2001). For example, Ezrin contributes to the apical localization of transporters like NHE1 (Denker et al. 2000) directly, or indirectly, via EBP50, in the case of CFTR (Short et al. 1998) and NHE3 (Reczek et al. 1997; Murthy et al. 1998).

At the lateral PM, the actin cytoskeleton is organized through association with specific proteins such as catenins (Drees et al. 2005; Yamada et al. 2005), IQGAP1 (Watanabe et al. 2004; Tanos et al. 2015), and Ankyrin (Nelson and Veshnock 1986; Kizhatil and Bennett 2004; Kizhatil et al. 2007). In turn, the basolateral actin cytoskeleton may stabilize certain PM proteins (Drenckhahn et al. 1985, 1993). Such mechanism was initially proposed for Na⁺, K⁺-ATPase in MDCK cells, in which a direct interaction of the pump's α subunit with ankyrin promotes its association with the spectrin cytoskeleton, which is concentrated laterally in MDCK cells (Morrow et al. 1989; Nelson and Hammerton 1989; Nelson et al. 1990). In epithelia where the Na⁺, K⁺-ATPase is apical, like the RPE and CP, this association with spectrin and ankyrin may contribute to the inverted polarized distribution of the pump (Gundersen et al. 1991; Marrs et al. 1993; Alper et al. 1994). However, whether these proteins form a complex has not been determined directly and the role played in determining polarized distribution is not clear because spectrin and Ankyrin

may be also present at the basolateral membrane in CP (Marrs et al. 1993; Alper et al. 1994).

Mechanisms Controlling Variable Localization of Ion Transporters in Different Epithelia

Differential Expression of Sorting Signals or Mechanisms

The various strategies used by epithelial cells to localize proteins to apical or basolateral domains may contribute to the variable localization of transporters involved in directional fluid transport. As different sets of signals mediate apical and basolateral trafficking, differential splicing may determine the presence or absence of a specific sorting signal and result in changes in the polarity of a given PM protein. This strategy may be used to change the localization of NKCC1 and aquaporins (Brown and Nielsen 2008; Carmosino et al. 2008). NKCC1 is expressed in the basolateral membrane of most epithelia, directed by a di-leucine motif found in the carboxy-terminal cytoplasmic tail. The dileucine motif is encoded by optionally spliced exon 21. In most epithelia, NKCC1 is expressed with exon 21 but not in neuroectoderm-derived epithelia. This could explain apical targeting of NKCC1 in CP and RPE. However, a report in human cultured RPE cells shows that the messenger for the splice variant that contains the dileucine motif is slightly more abundant than the other splice variant in this epithelium (Vibat et al. 2001).

Not surprisingly, because BLSS are usually dominant over apical sorting signals and many basolateral proteins contain cryptical apical signals, variable expression of a component of the basolateral sorting machinery contributes to the most striking variations in epithelial polarity observed to date. RPE and KPT do not express the clathrin adaptor AP-1B, leading to apical or depolarized localization of a plethora of cognate basolateral proteins in these epithelia (Diaz et al. 2009; Schreiner et al. 2010). These proteins include adhesive PM proteins such as coxsackie adenovirus receptor (CAR), junctional adhesion molecule C (JAMC), and neural cell adhesion molecule (NCAM), hormone re-

ceptors such as parathyroid hormone receptor (PTHr), and nutrient receptors such as low-density lipoprotein receptor (LDLR) and TfR. Clearly, lack of expression of AP-1B must contribute in a significant way to the normal physiology of these epithelia.

Expression of Different Combinations of Transporter Subunits

Most transporters are either homo- or hetero-oligomers. For example, the family of P-type ATPases, to which Na^+ , K^+ -ATPase belongs, as well as many amino acid, sugar, and lactate transporters, are heterodimers of a single pass highly glycosylated subunit that acts as a chaperone for intracellular transport, and a multi-span transmembrane subunit that constitutes the functional transporter. Studies with monocarboxylate (lactate) transporters, which display variable localization in different epithelia, indicate that sorting signals may be found in the glycosylated subunit (CD147) or in the multi-span subunit, according to the particular MCT (Deora et al. 2005; Castorino et al. 2011). Although the basolateral sorting of Na^+ , K^+ -ATPase has been attributed to signals in the α (Muth et al. 1998) or β (Vagin et al. 2005) subunits, to domain-specific restriction by the ankyrin–spectrin cytoskeleton (Nelson and Veshnock 1987a) or to intercellular adhesion between β subunits (Padilla-Benavides et al. 2010), the mechanism that contributes to its reversed apical distribution in RPE or CP remains unknown.

Differential Localization of Domain-Selective Cytoskeleton

In contrast with MDCK and other kidney cells, which concentrate the ankyrin–spectrin cytoskeleton basolaterally (Drenckhahn et al. 1985; Drenckhahn and Bennett 1987), RPE and CP concentrate this cytoskeleton apically (Gundersen et al. 1991; Alper et al. 1994), which could contribute to the apical localization of Na^+ , K^+ -ATPase in these epithelia. However, this mechanism, although suggested, has not been proven.

CROSS-SIGNALING BETWEEN EXTRACELLULAR MATRIX, PERIVASCULAR, AND ENDOTHELIAL CELLS ORGANIZE ORGAN–BLOOD BARRIERS

Seminal work by several groups has established a key role of ECM in the development and maintenance of tubular epithelial tissues (Hall et al. 1982; Burute and Thery 2012; Roignot et al. 2013; Overeem et al. 2015). Growing evidence suggests that signals from the basement membrane and perivascular cells may regulate the functional properties of endothelial and epithelial cells in organ–blood barriers. In this section, we review several examples that illustrate such cross-signaling events.

In the brain, the general BBB is formed by ECs, which form part of a “neurovascular unit” that includes vascular smooth muscle cells, pericytes, glial cells (including astrocytes), and neurons (Arboleda-Velasquez et al. 2015; Sweeney et al. 2016). ECs and pericytes share a common basement membrane and the communication between both cell types, as well as their interaction with the ECM and surrounding glial cells, is essential for the integrity of the blood–tissue barrier. Pericytes maintain the BBB by both decreasing endothelial transcellular transport and maintaining proper EC intercellular junctions (Armulik et al. 2010; Bell et al. 2010; Daneman et al. 2010). Furthermore, pericytes are key for the polarization of astrocyte endfeet (Armulik et al. 2010) and, in turn, astrocyte-secreted laminin maintains the integrity of BBB through, at least in part, regulation of pericyte differentiation (Yao et al. 2014). Moreover, astrocytes control the production of matrix metalloproteinase 9 by pericytes, which can degrade the basement membrane and promote the degradation of TJ proteins in ECs (Bell et al. 2012). Consistent with a key role of ECM in the maintenance of BBB integrity, EC-specific ablation of $\beta 1$ integrin results in the alteration of EC intercellular junctions and BBB disruption (Yamamoto et al. 2015). Thus, it is clear that the cross talk between vascular cells and the stroma in the brain is a complex network whose imbalance disrupts the BBB.

A regulatory role of ECM, ECs, and perivascular cells is emerging for organ–blood barriers

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constituted by epithelial cells. (1) In the kidney, alterations in the composition and structure of the glomerular basement membrane, as well as loss of podocyte-expressed genes involved in ECM attachment, induce the disassembly of podocyte intercellular junctions and failure of the filtration barrier (reviewed in Scott and Quaggin 2015). There is evidence that the glomerular filtration barrier is regulated by cross talk between different glomerular cell types (Bartlett et al. 2016). Furthermore, loss of proteins that control epithelial polarization such as aPKC and the zebrafish Crumbs family member Crb2b results in aberrant podocyte cell–cell junctions and proteinuria (Scott and Quaggin 2015), underscoring the importance of epithelial polarity for proper glomerular barrier function. (2) In the testis, ECM proteins, that is, collagens and laminins, regulate the dynamics of intercellular junctions between Sertoli cells, a key process for spermatogenesis (reviewed in Siu and Cheng 2008). (3) In the gut, there is evidence that intestinal barrier function is controlled by the enteric glia (Aube et al. 2006; Savidge et al. 2007). (4) In the CP, deficit of collagen XVIII (Utriainen et al. 2004) or matrix metalloproteinase 8 (Vandenbroucke et al. 2012) was reported to cause disruptions of CP TJ and the CP barrier. (5) In the outer retina, it has been proposed that neural retina-secreted factors regulate the molecular composition and barrier function of chick RPE TJ (Rahner et al. 2004; Sun et al. 2008). Furthermore, it is well established that aging changes in the RPE basement membrane (named Bruch's membrane, which separates RPE from the fenestrated chorioid capillaries) (Fig. 1) that include thickening and lipid accumulation (Booij et al. 2010) impacts retinal function by impairing the transport of nutrients, oxygen, and waste products between the blood circulation and the outer retina. In addition, these changes may disrupt RPE–ECM interactions leading to functional alterations of the oRBB (Sorkio et al. 2014; I Benedicto, GL Lehmann, EJ Rodriguez-Boulan et al., in prep.). Future work should establish how these changes affect the onset and progression of retinal degenerative diseases, such as age-related macular degeneration.

ECs constitute instructive niches that control organogenesis during development (Lammert et al. 2001; Matsumoto et al. 2001). Exciting recent studies have uncovered key roles of ECs in stem cell renewal (reviewed in Rafii et al. 2016), tissue regeneration (Ding et al. 2010, 2011), and even the acquisition of very specific epithelial features such as apicobasal hepatocyte polarization (Sakaguchi et al. 2008). Recent data in our laboratory suggest that ECs secrete factors that regulate RPE basement membrane and decrease RPE paracellular permeability by modulating the composition and function of RPE TJ, the key constituent of the oRBB (I Benedicto, GL Lehmann, EJ Rodriguez-Boulan et al., in prep.). Interestingly, a similar reduction of paracellular permeability was shown for both KPT and airway epithelial cells when cocultured with ECs (Aydin et al. 2008; Chowdhury et al. 2010). Of note, the absence of ECs in zebrafish embryos results in an abnormal glomerular basement membrane and its defective interaction with podocyte foot processes (Majumdar and Drummond 1999). Thus, it is tempting to speculate that a common mechanism may regulate epithelial-mediated blood barriers throughout the organism, that is, basement membrane remodeling by EC-secreted factors, which in turn modulates epithelial intercellular junctions and barrier function. In addition to the regulation of the paracellular route, there is some evidence suggesting that ECs may also modulate epithelial transcellular transport. Endothelium-released nitric oxide regulates KPT lumen acidification in a Na^+ -dependent manner (Amorena and Castro 1997), and modulates Na^+ transport across KPT epithelial cells in vitro by controlling Na^+ , K^+ -ATPase activity (Linaz and Repine 1999). Moreover, ECs regulate the expression of several transporters in the KPT epithelial cell line HK-2 (Aydin et al. 2008). In summary, ECs seem to play a key role in the regulation of epithelium-based blood–tissue barriers. Because tissue-specific ECs establish highly specialized vascular niches (Nolan et al. 2013), it is plausible that the microvasculature of different blood–tissue barriers is equipped with specific angiocrine factors needed for the proper maintenance of each particular barrier. Future

studies are warranted to test this hypothesis, as well as the role of tissue-specific basement membranes and non-EC types in the regulation of epithelium-based blood–tissue barriers.

FUTURE PERSPECTIVES

More than 30 years of research on epithelial cell polarity have provided invaluable insights into the mechanisms that allow epithelia to perform crucial functions including water and solute transport. As constituents of different organ–blood barriers, epithelial cells are critical in physiological processes; therefore, disruptions in their biology lead to diseases like Fanconi's syndrome, diabetic nephropathy and hypertension (KPT), hydrocephalus (CP), and macular degeneration (RPE). Similarly, polarization of ECs is key for regulating the transcellular and paracellular routes between blood and tissue, and its imbalance may have profound implications in the onset and progression of pathologies associated to defective fluid transport such as brain and retinal edema.

It is increasingly clear that organ–blood barriers constitute functional units beyond the individual properties of the epithelial and endothelial components. More specifically, ECs along with ECM and other stromal components, play key roles in the regulation of epithelium-based barriers. They supply tissue-specific angiocrine factors and trigger outside-in signaling pathways that may contribute to the maintenance of epithelial polarity and the proper transport of solutes and water across the organ–blood barrier. This is an exciting emerging field with potential clinical applications that deserves further studies.

Despite intensive research, many open questions remain. The mechanisms that regulate epithelial and endothelial polarization are still incompletely understood. Uncovering such mechanisms will contribute to a better understanding of fundamental processes in epithelial physiology like regulation of water transport, or the nature and consequences of the cross talk between the different components of the barrier. Emerging technologies will be instrumental in elucidating these processes. In par-

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ticular, optical nanoimaging techniques with increasingly higher spatial and temporal resolution, high-throughput approaches to elucidate the properties of such complex system, and powerful gene manipulation technologies will test the translational relevance of the mechanisms discovered. All this considered, advancement in the field will aid, not only in the prevention and treatment of diseases, but also in the development of novel strategies for drug delivery across various organ–blood barriers.

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