Caring about the other 47% of the water channels. Focus on “Basolateral targeting and microtubule-dependent transcytosis of the aquaporin-2 water channel”

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It is said that when an experiment gives an expected result, nothing is really learned; rather, it is more often from the experiment that gives an unexpected result that something new is learned. In work reported in this issue, Yui and colleagues decided to care about, rather than ignore, their unexpected observations (14).

The water channel aquaporin-2 (AQP2) has been a seminal member of the family of membrane channels and transporters whose activity depends on regulated recycling from an intracellular membrane storage compartment to the plasma membrane, typically in a hormone-, secretagogue-, or neurotransmitter-dependent fashion (4). It is widely accepted that the mechanism of regulated water reabsorption from the lumen of the distal tubule and collecting duct of the nephron is due to the vasopressin-dependent recycling of AQP2 at the apical membrane of these epithelial cells (9). In addition, until now, it was thought to be equally likely that, once AQP2 left the biosynthetic pathway, it was targeted directly to the subapical membrane compartment or to the apical membrane. However, despite the predominant steady-state (sub)apical localization of AQP2 observed in an overwhelming number of in vivo and cell culture systems, Yui and colleagues provide experimental evidence, cobbled together with other data in the literature, for a more complex picture of AQP2 trafficking in polarized epithelial cells, one which surprisingly may include its targeting to the basolateral membrane and subsequent transcytosis to the apical membrane (Fig. 1).

In AQP2-transfected Madin-Darby canine kidney (MDCK) cells, Yui and colleagues describe conditions in cell-surface biotinylation and immunofluorescence experiments in which

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Fig. 1. Proposed basol-to-apical transcytotic trafficking of aquaporin-2 (AQP2). The proposed trafficking pathway of AQP2 through the transcytotic pathway [adapted from Yui et al. (14)] is indicated by green arrows, with the primary organelles involved in this pathway highlighted in blue. After its synthesis in the endoplasmic reticulum (ER) and arrival at the trans-Golgi network (TGN), AQP2 is rapidly delivered to the basolateral membrane and rapidly internalized by clathrin-dependent endocytosis, resulting in very little AQP2 expressed at the basolateral membrane at steady state. Inhibition of endocytosis by the 4°C block “exposes” this pool of AQP2 at the basolateral membrane (14). Hypertonicity, vasopressin (VP), and aldosterone also enhance basolateral expression of AQP2 in vitro (13) and in vivo (6, 7, 13). Once internalized from the basolateral membrane, AQP2 is transcytosed in a microtubule-dependent fashion to the perinuclear recycling compartment (PNR) and the apical recycling endosomes (ARE). In response to VP, the exocytosis and recycling of AQP2 at the apical membrane are enhanced, resulting in a net increase of AQP2 at the apical membrane. Alternative pathways for AQP2 to the basolateral and apical membranes are indicated by thin orange arrows. AQP2 at the apical membrane regulates transepithelial water flux, while basolateral AQP2 may be involved in cell migration, epithelial tubulogenesis, and, possibly, transepithelial water flux. ald, aldosterone; BEE, basolateral early endosome; colch, colchicine; MT, microtubules.
a significant fraction of AQP2 is detectable at the basolateral membrane (which actually appears to be greater than 47% of the total surface-biotinylated AQP2), and this pool appears to be subsequently transcytosed to the apical membrane. Although both observations are totally unexpected, solid controls were in place to validate them. The condition that odd enough seems to “uncover” this basolateral pool of AQP2 is a technique that is commonly used ostensibly to halt cellular processes in a reversible fashion, the 4°C block. The rationale behind the 4°C block is that protein-catalyzed events, such as intracellular trafficking and membrane transport, are reversibly inhibited by cold-dependent inhibition of conformational changes of, and/or energy transduction by, proteins.

When localization experiments were performed without the 4°C block, this basolateral pool of AQP2 was not detectable. The detection of this basolateral pool does not appear to be an artifact of heterologous expression or overexpression of AQP2 or to be unique to MDCK cells, since in untransfected MDCK cells, which have a low level of endogenous AQP2 expression, as well as in AQP2-transfected rat inner medullary collecting duct cells, basolateral AQP2 is observed in both of these cases in response to the 4°C block. Thus, one possibility is that under the conditions of the 4°C block, a significant fraction of AQP2 relocates to the vicinity of the basolateral membrane and is inserted into the basolateral membrane; however, both steps would be contrary to established views of membrane trafficking and vesicle fusion. Alternatively, if the 4°C block does what it is supposed to do, which is to block endocytosis of AQP2 from the basolateral membrane, then under normal conditions, a significant fraction of AQP2 would have to be targeted to the basolateral membrane, rapidly endocytosed, and transcytosed, to establish the subapical and apical distribution of AQP2 observed at steady state and to render the basolateral pool of AQP2 effectively “invisible.” Unfortunately, attempts to elucidate the mechanism by which the 4°C block led to the uncovering of this basolateral pool of AQP2 were unsuccessful and must await further investigation.

Interestingly, with the 4°C block, clathrin also accumulates near the basolateral membrane, placing it in a position to mediate the rapid internalization of the basolateral pool of AQP2 once the cells are rewarmed, as a first step in the transcytosis of AQP2. Furthermore, the reestablishment of apical expression of AQP2 upon rewarining depends on intact microtubules, a well-known requirement for basal-to-apical transcytosis (1, 8). Thus, in MDCK cells, it appears that AQP2 is a bona fide transcytotic protein.

In MDCK cells, a number of other heterologously expressed proteins, such as the well-characterized polymeric immunoglobulin receptor (pIgR) (2, 5, 10, 11, 12), have been shown to undergo basal-to-apical transcytosis to varying extents in the establishment and maintenance of their predominantly apical membrane expression. However, AQP2 is the only one of these proteins that is also endogenously expressed in MDCK cells and localized predominantly apically in vivo. In addition, the basolateral targeting and transcytosis of AQP2 reported in this study, although quite unexpected, could provide a mechanism resulting in the dual apical and basolateral localization of AQP2 observed in vivo (6, 7). It is intriguing to speculate that the transcytotic pathway in MDCK cells extensively characterized by use of the pIgR as the model marker may have been already in place for endogenous AQP2. Collectively, these studies on transcytosis also suggest that basal-to-apical transcytosis may not be the sole domain of the hepatocyte, may be a more prevalent mechanism for polarized protein localization in epithelial cells than has been previously appreciated, and may provide for plasticity in polarized protein localization, when necessary (3).

As with any novel finding, more intriguing questions arise. Is nascent AQP2 sorted into direct apical and basolateral pathways, and, if so, what sorting motifs mediate its localization to these pools? Or, does all of the nascent AQP2 get sorted to the basolateral membrane, and its apical localization is generated entirely by transcytosis? Is the hypertonicity-dependent basolateral localization of AQP2 (13) related to the mechanism of cold-induced basolateral localization? Also, what triggers the transcytosis of AQP2, and how does the transcytosed AQP2 enter into the vasopressin-sensitive subapical storage compartment? Finally, and most importantly, to what extent are the mechanisms that regulate trafficking of AQP2 in MDCK cells similar to those in the distal part of the nephron in vivo? It is clearly worthwhile to care about the 100%.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author.

AUTHOR CONTRIBUTIONS

C.T.O. prepared the figure; drafted the manuscript; edited and revised the manuscript; approved the final version of the manuscript.

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