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High-tailing it to the apical surface. Focus on "Apical targeting of the $P2Y_4$ receptor is directed by hydrophobic and basic residues in the cytoplasmic tail"

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EPITHELIAL CELL FUNCTION REQUIRES the generation and maintenance of polarized apical and basolateral membrane domains with distinct protein and lipid compositions. This asymmetry in membrane composition is essential for myriad cell functions that include substratum adhesion, cell-cell communication, nutrient uptake, ion transport, and signal transduction. Genetic mutations that interfere with the polarized targeting or routing of plasma membrane proteins can result in severe disease states (6). Identifying the signals and machinery that direct the initial and postendocytic delivery of proteins to either surface domain has been the subject of intense investigation over the past three decades. Nonetheless, many questions remain about how diverse classes of proteins with distinct membrane topologies are targeted, often with exquisite fidelity, to the apical or basolateral surface.

Apical sorting signals are diverse and can be found on the luminal, membrane-associated, or cytoplasmically disposed aspects of various proteins (10). For some proteins, the apical sorting determinants involve posttranslational modifications, including a glycosyl-phosphatidylinositol anchor or *N*- or *O*-linked glycans. In addition, there is a rapidly growing list of proteins sorted to the apical surface via peptide sequences within cytoplasmically oriented loops or tails of proteins. In most cases, these peptide-based motifs have been identified on polytopic proteins that span the membrane multiple times; however, apical sorting information on the single membrane-spanning receptor megalin has also been localized to its cytoplasmic tail (7, 8). To date, no similarity among these signals, either in length or in sequence, has been noted, and how these motifs are interpreted by cellular machinery to support precise cargo routing remains a mystery.

One important class of polytopic proteins that mediates cellular responses to external signals is the nucleotide P2Y receptors. These members of the G protein-coupled receptor (GPCR) family bind to extracellular ATP and nucleotides and regulate cellular processes via both paracrine and autocrine mechanisms (2). The P2Y receptor family includes eight non-sequentially named receptors that are variously coupled to heterotrimeric G proteins, such as to G_q or G_i that mobilize intracellular Ca^{2+} or inhibit adenylyl cyclase, respectively (9). The $P2Y_4$ receptor, which is the focus of the article by DuBose et al. (3) in this issue of *American Journal of Physiology-Cell Physiology*, is expressed in numerous tissues. In intestinal cells, activation of the receptor induces luminal Cl^- secretion, and the development of targeted $P2Y_4$ agonists and antagonists has been proposed as promising potential strategies for the treatment of diseases as diverse as cystic fibrosis and infectious diarrhea.

Individual P2Y receptors are typically distributed to either the apical or the basolateral surfaces of polarized lung, kidney, and intestinal epithelial cells (12). At steady state, $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors are expressed apically, whereas $P2Y_1$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{14}$ receptors are enriched at the basolateral membrane, and $P2Y_{13}$ is present at both domains. The differential distribution of these related family members thus provides a unique opportunity to dissect polarized sorting motifs on polytopic proteins using a chimera approach. Indeed, previous studies have identified the apical sorting determinant of the $P2Y_2$ receptor using this strategy (5). The apical sorting signal of $P2Y_2$ is striking in that it requires two stretches of amino acids localized within a luminally oriented loop of the protein, and may thus represent a new class of peptide-based apical targeting signal (5).

The article by DuBose et al. describes the systematic dissection of apical targeting information in the cytoplasmic tail of the $P2Y_4$ receptor (3). Previous studies by this group demonstrated that deletion of the 55 amino acid tail of $P2Y_4$

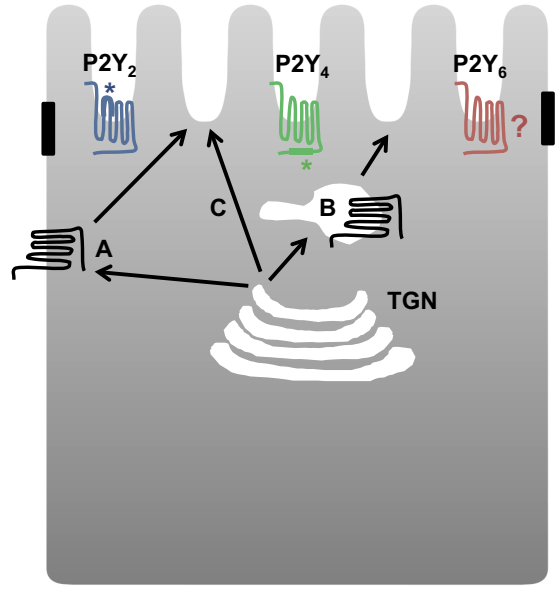

Fig. 1. Apical sorting and trafficking of P2Y receptors. Three of the eight P2Y receptors are localized apically in kidney, lung, and intestinal epithelial cells. The apical targeting signal (asterisk) of $P2Y_2$ (blue) is present within the first extracellular loop, whereas the closely related $P2Y_4$ (green) is targeted via a signal within the cytoplasmic tail of the receptor. Apical sorting information has not been identified in $P2Y_6$ (red). Several apical trafficking routes from the *trans*-Golgi network (TGN) have been identified for newly synthesized membrane proteins in polarized cells, including transcytosis from the basolateral surface (A), passage through endosomal compartments (B), and direct delivery (C). Whether all P2Y receptors follow the same itinerary or instead use alternative routes to the apical membrane is unknown.

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resulted in nonpolarized delivery of the receptor in polarized Madin-Darby canine kidney (MDCK) cells (5). In the current study, truncation analysis of the tail revealed that the terminal 22 amino acids were not required for apical localization of $P2Y_4$. Substitution of the remaining $P2Y_4$ tail sequence for the cytoplasmic tail of the structurally related BK2 channel confirmed that this region contains a transferable apical sorting signal, and subsequent deletion analysis pared down the critical $P2Y_4$ apical sorting signal to a 23 amino acid sequence (Cys321 to Asp343) roughly within the middle of the cytoplasmic tail. The authors then systematically tested the effect of mutating serines and threonine residues, a cysteine residue, basic residues, and hydrophobic residues within this sequence on the steady-state distribution of $P2Y_4$. Mutagenesis of two basic residues in the proximal half of the 23 amino acid sequence slightly disrupted apical polarity (to 61% apical compared with 85% for the wild-type protein). However, the most striking change was observed when a cluster of leucine and valine residues in the distal half of the targeting sequence were converted to alanine, resulting in redistribution of the receptor largely (64%) to the basolateral domain. These hydrophobic residues are highly conserved among mammalian orthologs. Interestingly, the targeting information within the $P2Y_4$ tail may be dominant over basolateral sorting signals in other receptors, as appendage of the 23 amino acid tract to a basolaterally targeted truncated version of the $P2Y_{12}$ receptor directed the chimera primarily to the apical domain.

Scientific advances always raise new questions, as is the case here. An obvious question is the mechanism of sorting. The authors found that inversion of the 23 amino acid sequence did not disrupt the apical targeting of $P2Y_4$ and suggest that a structural determinant rather than a defined sequence motif may be required for its apical sorting. Testing whether recognition of this determinant is saturable would provide additional clues as to the mechanism of $P2Y_4$ sorting. As an aside, the cytoplasmic targeting sequence of the basolaterally directed $P2Y_1$ receptor can also be inverted without disrupting polarized sorting (11). Additionally, while sorting was assessed using two independent approaches (indirect immunofluorescence and domain-selective surface biotinylation), both methods report only the steady-state distribution of $P2Y_4$. Thus, it is unclear whether the targeting signal represents a true biosynthetic targeting signal or is interpreted after initial membrane delivery. This is a relevant question because whereas newly synthesized membrane proteins in kidney cells are targeted apically primarily via direct pathways, many apical proteins in intestinal epithelial cells (a primary site of $P2Y_4$ expression) follow a transcytotic route to the apical domain. Indeed, there is evidence that some $P2Y_4$ exists at the basolateral surface of intestinal cells (4). Additionally, another GPCR (the M_2 muscarinic cholinergic receptor) has been shown to traffic to the apical domain of MDCK cells via the basolateral surface (1). Like $P2Y_4$, this receptor also contains cytoplasmically disposed apical targeting information, although in this case it is present within the third intracellular loop of the protein. Yet another possibility is that $P2Y_4$ could be delivered indiscriminately to both surface domains but selectively retained via this motif at the apical surface.

Paradoxically, the common feature among apical sorting determinants identified to date for polytopic proteins is their utter uniqueness. The studies described here add yet another twist to the mystery of how these and other multi-pass receptors find their way to the apical surface. The $P2Y_2$ and $P2Y_4$ receptors are very similar (52% identical) yet they use entirely different sorting motifs that are oriented on different sides of the membrane. This raises the strong possibility that these receptors use different mechanisms and possibly even distinct trafficking routes to reach the same membrane target (Fig. 1). Perhaps the presence of divergent apical sorting signals reflects functional constraints of each receptor in the same or different epithelial cells: another mystery to be solved.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

O.A.W. prepared the figure, drafted the manuscript, edited and revised the manuscript, and approved the final version of the manuscript.

REFERENCES

1. **Chmelar RS, Nathanson NM.** Identification of a novel apical sorting motif and mechanism of targeting of the M_2 muscarinic acetylcholine receptor. *J Biol Chem* 281: 35381–35396, 2006.
2. **Corriden R, Insel PA.** Basal release of ATP: an autocrine-paracrine mechanism for cell regulation. *Sci Signal* 3: re1, 2010.
3. **DuBose DR, Wolff SC, Qi AD, Naruszewicz I, Nicholas RA.** Apical targeting of the $P2Y_4$ receptor is directed by hydrophobic and basic residues in the cytoplasmic tail. *Am J Physiol Cell Physiol* (October 10, 2012). doi:10.1152/ajpcell.00251.2012.
4. **Ghanem E, Robaye B, Leal T, Leipziger J, Van Driessche W, Beauwens R, Boeynaems JM.** The role of epithelial $P2Y_2$ and $P2Y_4$ receptors in the regulation of intestinal chloride secretion. *Br J Pharmacol* 146: 364–369, 2005.
5. **Qi AD, Wolff SC, Nicholas RA.** The apical targeting signal of the $P2Y_2$ receptor is located in its first extracellular loop. *J Biol Chem* 280: 29169–29175, 2005.
6. **Stein M, Wandinger-Ness A, Roitbak T.** Altered trafficking and epithelial cell polarity in disease. *Trends Cell Biol* 12: 374–381, 2002.
7. **Takeda T, Yamazaki H, Farquhar MG.** Identification of an apical sorting determinant in the cytoplasmic tail of megalin. *Am J Physiol Cell Physiol* 284: C1105–C1113, 2003.
8. **Tan CM, Brady AE, Nickols HH, Wang Q, Limbird LE.** Membrane trafficking of G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* 44: 559–609, 2004.
9. **Vallon V.** P2 receptors in the regulation of renal transport mechanisms. *Am J Physiol Renal Physiol* 294: F10–F27, 2008.
10. **Weisz OA, Rodriguez-Boulan E.** Apical trafficking in epithelial cells: signals, clusters and motors. *J Cell Sci* 122: 4253–4266, 2009.
11. **Wolff SC, Qi AD, Harden TK, Nicholas RA.** Charged residues in the C-terminus of the $P2Y_1$ receptor constitute a basolateral-sorting signal. *J Cell Sci* 123: 2512–2520, 2010.
12. **Wolff SC, Qi AD, Harden TK, Nicholas RA.** Polarized expression of human P2Y receptors in epithelial cells from kidney, lung, and colon. *Am J Physiol Cell Physiol* 288: C624–C632, 2005.