

## **RAB11A DEPENDENT STRETCH-INDUCED EXOCYTOSIS IN BLADDER UMBRELLA CELL**

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The proper function of the epithelial cells that lines the kidney depends on highly polarized membrane domains at the apical and basolateral poles of the cell. Intracellular trafficking maintains distinct protein and lipid content of each domain, by regulated vectorial transport of ions, solutes and proteins. Any disturbance in the polarized membrane traffic of proteins like the epithelial sodium channels, aquaporin-2, occludin and E-cadherin can lead to various diseases such as Liddle's syndrome, diabetes insipidus, hyper- or hypotension, and renal failure. Thus, it is important to understand how exocytic and endocytic pathways are distributed in the kidney-associated epithelial cells and how they are regulated in normal and disease state. Umbrella cells, the outermost cell layer of the uroepithelium, line the renal pelvis, ureter, and mucosal surface of bladder provides a useful model system to understand apical membrane traffic in the kidney-associated epithelial cells. Umbrella cells forms a selective barrier and accommodate large variations in mechanical stress by modulating their apical surface area by exocytosis and fusion of subapical discoidal/fusiform-shaped vesicles (DFV) and internalization of membrane by endocytosis. The molecular basis of DFV exocytosis is poorly understood and little is known about their biogenesis. We observed that Rab11a was expressed in umbrella cells (but not Rab11b or Rab25) and was associated with DFV. Using adenovirus-mediated delivery we transduced umbrella cells in situ with either dominant active (DA) or dominant negative (DN) mutants forms of Rab11a. The over expression of DA-Rab11a stimulated an increase in apical surface area in the absence of stretch, while DN-Rab11a inhibited stretch-induced changes. Endocytosed fluid and membrane markers had little access to Rab11a-positive DFV, but virally expressed human growth hormone (hGH), a secretory protein, was packaged into DFV. While expression of DA-Rab11a stimulated release of hGH into the bladder lumen, expression of DN-Rab11a had the opposite effect. Our results indicate that DFV may be biosynthetic in nature and that their exocytosis depends on the activity of the Rab11a GTPase.