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Localization of SNAREs in the mouse urothelium

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Urothelium, an epithelium of the mammalian urinary bladder, is a perfect model for studying membrane traffic. In the process of differentiation, urothelial cells synthesize uroplakins, characteristic transmembrane proteins that are arranged into urothelial plaques in the post-Golgi compartments. Most prominent of these compartments are mature fusiform vesicles (FVs), which contain two plaques connected by thin hinge region. FVs store and transport urothelial plaques. During bladder filling FVs are fused with the apical plasma membrane of the superficial urothelial cells, where plaques contribute to the barrier function of the urinary bladder [1]. The mechanisms that regulate transport and fusions of the FVs are currently not completely understood, but might involve MAL proteins, Rab proteins (8a, 11a, 27b) and myosin 5b [2-4]. Data about SNARE proteins, which are major players in the final stage of the docking and the subsequent fusion of diverse vesicle-mediated transport events [4], are missing.

Our aim was to localizeze SNARE proteins syntaxin 6, 11, Vti1a and Vti1b in the mouse urothelial cells.

The experiments were performed on urinary bladders of 6-8 weeks old male C57B6 mice. Cryo- thin and ultrathin sections were prepared by modified Tokuyashu method and labelled with anti- syntaxin 6, 11, Vti1a, Vti1b mouse monoclonal antibodies (BD Transduction Lab.), and with anti-uroplakin rabbit polyclonal antibody, generated against mature bovine uroplakins (a kind gift from Prof. T.T. Sun, NYU, USA). Briefly, urothelium was fixed with 4% PA in 0.1M phosphate buffer for 48 hours, washed in PBS, embedded in 12% gelatine and cryoprotected by incubation in 2.3M saharose. Samples were frozen in LN_2 and cut with a Leica FCS cryo-ultramicrotom at -120°C. Semithin and ultrathin sections were cut for light and electron microscopy, respectively. Sections were incubated overnight with primary antibodies, washed in PBS, and incubated with secondary antibodies for 1.5 hours (AlexaFlour 555 goat anti-mouse and AlexaFlour 488 goat anti-rabbit for LM, goat anti-mouse conjugated with 16 nm colloidal gold for EM). Semithin sections were mounted with VectaShield (Vector laboratories) containing DAPI and visualized with a Nikon T300 light microscope. Ultrathin sections were counterstained with MC/UA and examined with a Philips CM100 transmission electron microscope.

Results showed that urothelium consist of basal, intermediate and superficial cells. Superficial cells were labelled with anti- uroplakin, syntaxin 6, 11, Vti1a and Vti1b antibodies (Figure 1). All these proteins were detected on some mature FVs. In the intermediate cell layer, cells were weakly labelled with anti-uroplakin antibody and contained more immature FVs (iFVs) which are characteristics of less differentiated urothelial cells. Nevertheless, more of studied SNARE proteins were found in the intermediate cells than in the superficial cells. SNAREs were associated with the hinge regions of the iFVs located beneath the plasma membrane (Figure 2). Basal cells were uroplakin-negative and contained no FVs.

The presence and location of SNARE proteins on hinge regions of FVs shows that hinge regions contain some key features enabling them to fuse with the apical plasma membrane. Observation that more SNAREs were found on iFVs in the intermediate cells than on terminally differentiated superficial cells, and since it is known that iFVs do not fuse with plasma membrane, it is possible that SNAREs in intermediate, less differentiated cells enable homo- and/or heterotypic fusions between iFVs during maturation of FVs.

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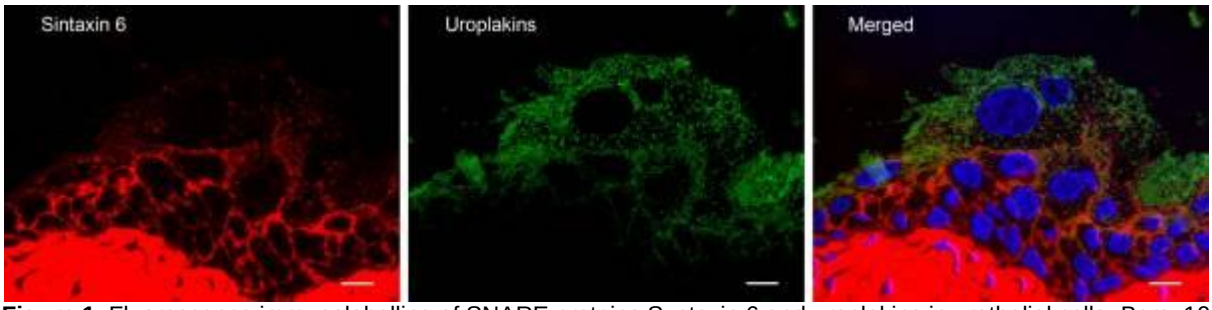

Figure 1. Fluorescence immunolabelling of SNARE proteins Syntaxin 6 and uroplakins in urothelial cells. Bars: 10 µm.

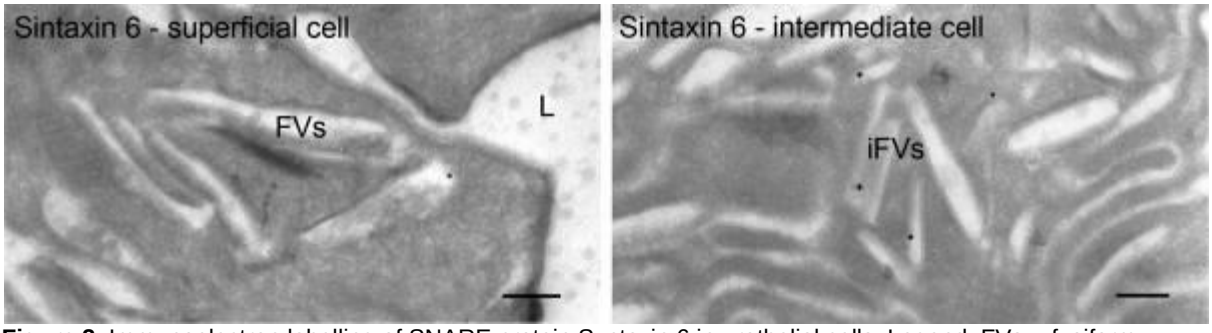

Figure 2. Immunoelectron labelling of SNARE protein Syntaxin 6 in urothelial cells. Legend: FVs – fusiform vesicles, L – lumen, iFVs – immature fusiform vesicles; bars: 200 nm.