Application Note

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# Depletion of apical transport proteins perturbs epithelial cyst formation and ciliogenesis

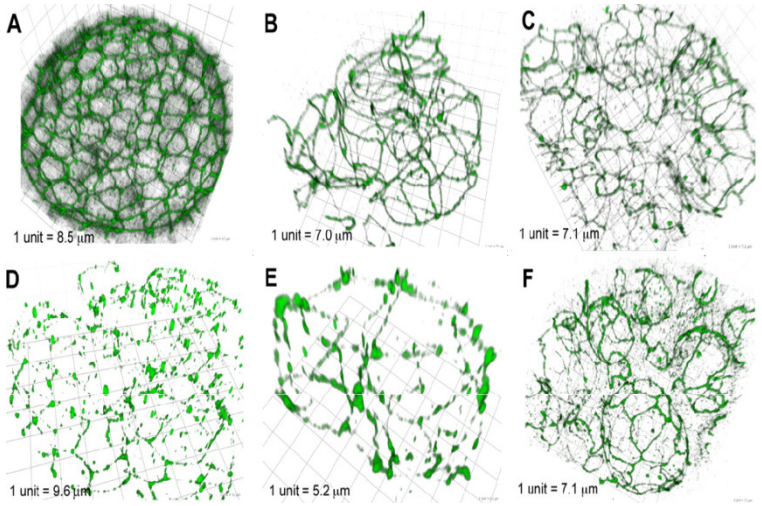
Dr Torkko and colleagues at the Max Planck Institute of Molecular Cell Biology and Genetics are focused on researching the fundamental process's involved in cyst formation and ciliogenesis. A cyst is a closed sac having a distinct membrane and division on the nearby tissue, and may contain air, fluids, or semi-solid material.

Epithelial cells form layers that constitute boundaries between the interior of vertebrates and invertebrates and the outside environment. In humans these epithelial layers can form cysts such as acini in the pancreas or follicles in the thyroid or, by branching, build tubes similar to in the intestine or in nephrons. The apical surface is the unique membrane domain characteristic of epithelia that defines the lumen in cysts and tubes. How apical membrane domains are generated remains a key issue in cyst development.

Dr Torkko and colleagues used a retrovirus-mediated RNA interference (RNAi) to generate a series of knockdowns (KDs) for proteins implicated in apical transport: annexin-13, caveolin-1, galectin-3, syntaxin-3, syntaxin-2 and VIP17 and/or MAL. Cyst cultures were then employed to study the effects of these KDs on epithelial morphogenesis.

To analyze the various KDs, the team used the following set of characteristic features to decide whether or not cyst formation was disturbed: (1) the formation of a single spherical lumen, (2) the polarized distribution of apical and basolateral markers, (3) the polarized distribution of the Tight Junctions (TJs) and, (4) the concentration of actin beneath the apical surface.

ZO-1 labeled with AlexaFluor 488 marked the TJs which appeared in a chicken-wire-like pattern and also defined the borderline between the apical and the basolateral membrane. 0.2 µm *z*-stack sections were acquired (0.4 µm or 0.6 µm sections were also acquired for some samples) using laser scanning confocal microscopy. The confocal stacks were 3D rendered using **Volocity® Visualization** to understand the 3D organization of the cysts by showing the distribution of the TJs (ZO-1 staining) in the different KDs.




**Volocity** 3D imaging of control and different KD cysts stained for ZO-1. AlexaFluor 488 staining reveals the overall structure of the cysts, showing the variable organization of the TJs related to the positioning of individual cells in the cysts. (A) Control, (B) Anx-13 KD, (C) Gal-3 KD, (D) Cav-1 KD, (E) Stx-3 KD, (F) VIP17-KD. Scaling of the different cysts is shown as volumetric units (in µm) and is indicated separately for each of the image panels.

**Volocity Visualization** allows the user to change the background color of the images, to better display the details of interest. In this case a white background provided a better view of the cyst structure than a more commonly used black background.

Depletion of the proteins by RNAi stalled the development of the apical lumen in cysts and resulted in impaired ciliogenesis. The most severe ciliary defects were observed in annexin-13 and syntaxin-3 KD cysts. Although the phenotypes demonstrate the robustness of the formation of the polarized membrane domains, they indicate the important role of apical membrane biogenesis in epithelial organization.

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