Institute of General Physiology

Cellular and Molecular Lung Physiology

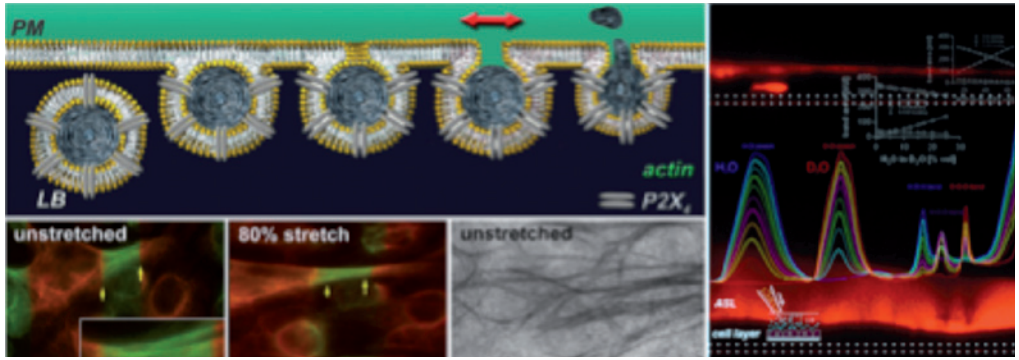
Head: Paul Dietl

Understanding the molecular physiology of the lung is essential for understanding disease and developing new treatment strategies. Within the institute, we focus on fundamental cellular mechanisms that are crucial for lung function. Employing a range of high-resolution imaging techniques compared with molecular biology and biochemistry, we study surfactant secretion, epithelial fluid transport and mechanical forces affecting cellular function.

The Team:
Head of Institute/Professor: P. Dietl
Group Leaders/Postdocs: M. Frick, E. Felder, O. Wittekindt, P. Miklavc
PhD Students: K. Thompson, K. Ehinger, K. Neuland
Additional Members of Thesis Advisory Committees: R. Tarran (Chapel Hill), P. Gierschik (Ulm)

a) Exocytosis and secretion (Dr. Frick/Dr. Miklavc)
Surfactant, a lipid-rich and lipoprotein-like substance, is the secretory product of type II pneumocytes stored in vesicles called lamellar bodies (LBs). Surfactant secretion is essential for life and occurs through regulated exocytosis of LBs. In addition, LB exocytosis is a good model for studying the exocytotic process using life cell imaging techniques.

We have recently developed several new microscopy techniques to study essential steps during this process. Combining live cell imaging techniques (darkfield microscopy, fluorescence microscopy, LSM, FRET, FRAP, TIRF etc.) with molecular and biochemical tools, we aim to elucidate cellular and molecular



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mechanisms of hemifusion, fusion pore formation, fusion pore expansion and content release. These experiments aim to improve mechanistic insights into membrane merger, lipid and content mixing, signaling and trafficking, and to understand basic pathogenic mechanisms of pulmonary disease.

b) Transepithelial transport (Dr. Wittekindt)
Transepithelial transport along the conducting and respiratory epithelium is essential for lung function. Its deregulation is a major pathomechanism in many inflammatory lung diseases like bronchitis, asthma and chronic obstructive pulmonary disease (COPD). We recently developed a new technique to study water transport and apical volume homeostasis in respiratory epithelia. This technique, in combination with electrophysiological measurements (impedance, Ussing-Chamber), enables us to investigate the effect of noxae on epithelial transport function in order to understand basic pathomechanisms in lung diseases.

c) Intermediate filaments in stretched cells (Dr. Felder)
Mechanical forces can modify cellular functions in various ways. Obviously, only limited levels of mechanical stress can be tolerated by a cell and intermediate filaments (IF) play a crucial role in protecting the cell from tensile strain. However, surprisingly little is known about the behavior of IF in stretched living cells. The lack of information about mechanical effects of IF crosslinks with other cytoskeletal components further complicates our understanding.

We are addressing these questions by stretching cells on elastic silicone membranes with different stretch devices. This allows life cell imaging experiments, preparation of stretched cells for electron microscopy as well as harvesting the cells for biochemistry or molecular biology. A particular focus of our work is the role of IF phosphorylation, the best studied modification of IF. Despite the drastic effects of phosphorylation on the IF in static cell cultures, our studies are among the very few that also demonstrate an effect on their mechanical properties.

Selected Publications:

- *Thompson K, Korbmacher J, Hecht E, Hobi N, Wittekindt O.H, Dietl P, Kranz C, Frick M (2013): FACE (fusion-activated Ca2+-entry) in alveolar type II epithelial cells couples surfactant secretion and lung fluid homeostasis. FASEB J.; 27(4):1772-83.*
- *Fois G, Weimer M, Busch T, Felder ET, Oswald F, von Wichert G, Seufferlein T, Dietl P, Felder E (2013): Effects of keratin phosphorylation on the mechanical properties of keratin filaments in living cells. FASEB J.; 27(4):1322-9.*
- *Neubauer D, Korbmacher J, Frick M, Kiss J, Timmler M, Dietl P, Wittekindt OH and Mizaikoff B (2013): Deuterium Oxide Dilution: A Novel Method to Study Apical Water Layers and Transepithelial Water Transport. Anal. Chem.; April 5.*
- *Miklavc P, Hecht E, Hobi N, Wittekindt OH, Dietl P, Kranz C and Frick M (2012): Actin coating and compression of fused secretory vesicles are essential for surfactant secretion: a role for Rho, formins and myosin II. J Cell Sci.; 125(11):2765-74.*
- *Miklavc P, Mair N, Wittekindt OH, Haller T, Dietl P, Felder E, Timmler M. and Frick M (2011): Fusion-activated Ca2+entry via vesicular P2X4 receptors promotes fusion pore opening and exocytotic content release in pneumocytes. Proc. Natl. Acad. Sci.; 108(35):14503-8.*
- *Miklavc P, Frick M, Wittekindt OH, Haller T, Dietl P (2010): Fusion-activated Ca2+ entry: an "active zone" of elevated Ca2+ during the postfusion stage of lamellar body exocytosis in rat type II pneumocytes. PLoSOne. 5:e10982.*