Proposition de stage de M2 et de thèse
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Mechanotransduction at the Golgi apparatus

Cells can sense and respond to external forces and mechanotransduction events appear to be critical for most cellular functions. While mechanotransduction has been extensively studied at the plasma membrane and at the nucleus, the impact of forces on other organelles is still not clear. Our M2/PhD project will study mechanotransduction at the **Golgi apparatus** (GA), a central organelle for intracellular transport pathways. We have previously shown that a force directly applied on the GA using internalized beads manipulated by optical tweezers induce a **delay in membrane fission** resulting in **tubulation** of Golgi membranes. More recently, we have shown that a hypoosmotic shock has the opposite effect and **accelerates post-Golgi trafficking** by increasing the size of transport vesicles and facilitating membrane fission (Figure). These results suggest that the GA can respond to both internal and external forces and thus displays mechanosensitive properties.

Until now our studies were limited to cells plated on rigid (glass) substrates in 2D. In this project we want to study the role of the **rigidity (soft vs. stiff) and dimensionality (2D vs. 3D)** of the extracellular matrix in mechanotransduction to the GA. We will ask whether external forces induced by the matrix can propagate to the GA and impact on Golgi mechanical properties and tension and on post-Golgi trafficking. At the molecular level, we will focus on the role of the **small G protein Cdc42** in regulating actin dynamics and, possibly, Golgi tension at Golgi membranes. To achieve these goals, the candidate will use mammalian RPE-1 cells expressing the fluorescent Golgi marker GFP-Rab6 and plated on soft or stiff substrates in 2D or 3D. He/she will use or develop fluorescent tools to visualize Golgi tension and Cdc42 activity in living cells. Golgi mechanics will be probed using intracellular optical tweezers. Our project combines micromanipulation and live cell imaging to address fundamental questions on mechanotransduction.

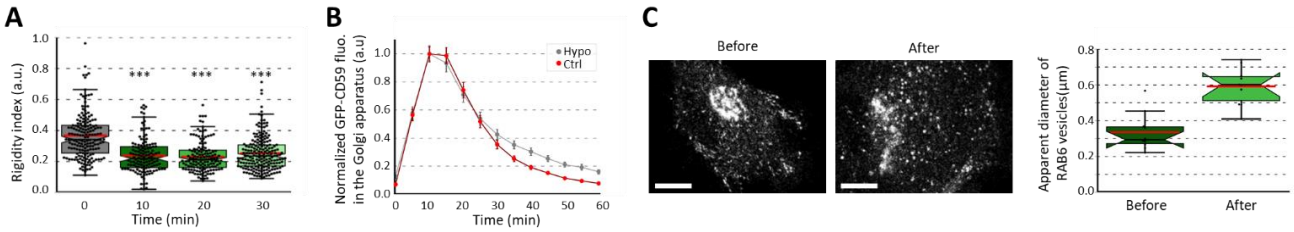

Figure: Mechanosensitivity of the Golgi apparatus (GA) and post-Golgi trafficking upon application of hypoosmotic shocks. A hypoosmotic shock was applied to RPE-1 cells plated on glass by adding 50% water in the external culture medium. A. The GA softens upon application of a hypoosmotic shock. The rigidity was measured by optical-tweezers based viscoelastic relaxation experiments. B. Cargo exit from the Golgi apparatus is accelerated upon application of a hypoosmotic shock (grey points) compared to control (red points). C. The size of RAB6-positive transport intermediates increases upon application of a hypoosmotic shock. Scale bars, 10 µm.

Key words: optical tweezers; soft and stiff matrices; 3D substrates; FRET; membrane tension, cytoskeleton; actin; RhoGTPases; Golgi matrix; microrheology; intracellular trafficking; extracellular matrix; cancer.

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