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Myosin II Functions as a Direct Mechanosensor for Intercellular Invasion during Cell-Cell Fusion

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How cells sense and react to external mechanical stimuli is a fundamental question in cellular biophysics. Here, we describe a previously unrecognized mechanosensory response to a localized cellular protrusive force during cell-cell fusion. Our previous studies of myoblast fusion in Drosophila embryos and cell fusion in a reconstituted culture system revealed that cell-cell fusion is an asymmetric process, in which one fusion partner (the attacking cell) extends invasive finger-like protrusions into the other (the receiving cell) to promote plasma membrane juxtaposition and fusion. Here, we demonstrate that the Rho-Rok-Myosin II (MyoII) pathway is specifically activated in the receiving cell in response to the invasive force from the attacking cell. Disrupting the function of this pathway renders less cortical resistance in the receiving cell and defects in cell-cell fusion, despite deeper invasion of the attacking cell. Increasing the cortical tension in the receiving cell by overexpressing an actin crosslinker significantly rescued such fusion defect. We show that MyoII accumulates to the cell cortex earlier than its upstream biochemical regulators Rok and Rho in response to applied force, and that MyoII is required for the steady-state accumulation of Rok and Rho. Furthermore, the motor domain of MyoII is indispensible for its cortical accumulation triggered by intercellular invasion. These results strongly suggest that MyoII functions as a direct mechanosensor that feeds back to its upstream regulators, and that the mechanosensory function of MyoII is mediated by its binding to actin filaments under mechanical stress. This newly discovered mechanosensory system, consisting of a well-defined source of protrusive force and the corresponding activation of the Rho-Rok-MyoII pathway in the neighboring cell, highlights a central role of MyoII in the mechanosensory response that ultimately leads to cortical tension generation in animal cells.

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Electromechanical Model for Eukaryotic Cells

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Electromechanics is important in many cellular processes, including motility, cancer metastasis, wound healing, and embryogenesis. Experiments show that cells placed in direct current electric fields are able to sense the field and direct their motion. Other experiments show that cell volume changes are important in invading cancer cells. A computational model is necessary to understand how cells respond electro-mechanically to electro-mechanical changes in their environment. The model proposed in this study considers how ion flux and water flux across the cell membrane enable a cell to regulate its size, internal pressure, and membrane voltage. This model also studies how active ion pumps, voltage gated channels, mechanosensitive channels, and water transport allow a cell to change its size when its membrane voltage is fixed during a voltage clamp experiment. Specifically, the model predicts cell volume increases during hyperpolarization and decreases during depolarization. Preliminary voltage clamp experiments suggest that the predicted size changes are observed in eukaryotic cancer cells, which validates our model.

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Molecular Mechanisms Underlying the Inside-Out Signaling through Focal Adhesions

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Cell adhesion is key to many important processes such as cell differentiation and migration, which all involve bi-directional signaling across the plasma membrane. At sites of adhesion a large assembly of macromolecules, called focal adhesions, function together in order to orchestrate complex signaling events. It is not yet clear how molecules in focal adhesions communicate with one another causing the signals to be transmitted through their interaction. Cells sense and respond to both chemical and mechanical signals suggesting that individual focal adhesion molecules should act as mechanosensors. Mechanical forces from the extracellular matrix (ECM) are detected by integrin receptors on the plasma membrane and transmitted to actin cytoskeleton through focal adhesions, which is called out-side-in signaling. The reverse process is also possible in which acto-myosin forces are applied to the ECM. Some proteins play key roles in regulating focal adhesions and their function may shed light on the order of events in a certain signaling pathway. Talin, alpha-actinin and filamin are among a few molecules that directly bind to both actin and integrin, and thus focal adhesions are largely affected by their function. It has been shown that talin plays a significant role in integrin activation, which is essential for initiating focal adhesion formation, while filamin inhibits integrin activation. The role of alpha-actinin is somehow controversial, i.e. it is not yet clear whether it inhibits or promotes activation since both observations have been made. We use all-atom molecular dynamics simulations to investigate the role of each of these molecules both in isolation and in competition with one another. Our results reveal how the presence of one molecule would affect the interaction of others suggesting possible cooperative functions in some cases.

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Quantitative Determination of Cell Wall Mechanical Properties using Microfluidics

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Certain cell types have the intriguing ability to forcibly invade a neighboring tissue or a solid growth matrix. The purpose of this invasive growth activity depends on the cell type and ranges from establishing contact between remote locations in the organism (neurons), procuring nutrients and water (fungi, root hairs), to the delivery of gametes (pollen tubes). To invade a tissue or solid matrix, these cells exert significant penetrative forces generated either by the cytoskeleton or the hydrostatic turgor pressure. Using a microfluidic device we quantified the penetrative forces generated by pollen tubes, the fastest growing plant cells. The tubes were guided through microscopic gaps made of elastic polydimethylsiloxane (PDMS) material. Depending on the size ratio between tube and gap, the tubes either deformed the gap walls completely, became deformed themselves while passing, or stalled. Within a narrow range of size ratios, the tubes successfully passed the gap but subsequently burst raising the question whether sperm cell release in planta is triggered mechanically. Based on the deformation of the PDMS-gaps the extrusive force exerted by the elongating tubes was determined using reverse engineering and finite element modeling.

2902-Pos Board B594

Assessing the Influence of Electric Cues and Conductivity on Pollen Tube Growth via Lab-On-A-Chip Technology

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Pollen tubes are believed to react to a combination of chemical, mechanical, and electrical cues during its journey through the pistil in order to achieve fertilization. Despite extensive work dedicated to the subject it is still not clear how these exogenous guidance signals work or how they are processed internally. Using Lab-on-a-chip (LOC) technology, we assessed the influence of electric fields on pollen tube growth at the microscale. Microelectrodes were integrated into the LOC in order to enable the application of electric fields in a controlled manner. Due simulation of the LOC electrical configuration and characterization of the pollen growth medium conductivity were carried out. DC and AC electric fields were applied to batches of Camellia japonica pollen grains under various conditions. Results show that pollen tube growth is increasingly degraded as the applied DC electric field increases. Furthermore, germination is completely inhibited for sufficiently strong fields. AC electric fields, however, had a restoring effect as growth is promoted as frequency increases beyond 100 mHz, which suggests a significant role of the medium conductivity in enabling cell growth. Interestingly, no sign of pollen tube orientation was found under any tested condition, weakening the much debated argument for electrotropism in pollen tubes. When exposed to a highly localized field, pollen tubes did not deviate. This work suggests that both strength and frequency of an applied electric field influence pollen tubes, and most likely living cells in general, in a much more subtle way rather than being a macro scale exogenous guiding signal.

2903-Pos Board B595

Keratins Significantly Contribute to Cell Stiffness and Impact Invasive Behavior

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Cell motility and cell shape adaptations are crucial during wound healing, inflammation and malignant progression. These processes require the remodeling of the keratin cytoskeleton, to facilitate cell-cell and matrix adhesion.