Exploring *Drosophila* Myosin-ID's Possible Interaction with DE-Cadherin

Authors: Jakub A. Kochanowski*, Faviolla A. Baez-Cruz, E. Michael Ostap

Introduction: Chirality is the property of structures to hold shapes in which a rotation cannot produce its mirror image, seen in human hands or positioning of the heart. Chirality exists on all scales, from molecules all the way to the structure of full organisms. During development, chirality is essential for the proper positioning and twisting of organs, enabling their function. The understanding of its emergence and establishment is crucial to understand cellular mechanics, a field which has recently shown much growth in interest and discoveries. However, the underlying mechanisms that achieve chirality are still widely unknown.

Previous studies have shown that *Drosophila* myosin-I isoforms myosin-1C (myo1C) and myosin-1D (myo1D) are involved in establishing chirality of certain organs (Petzoldt). In addition, the overexpression of either isoform into a non-chiral tissue induces chirality, with the expression two myosin-I isoforms resulting in organs with opposite handedness (Lebreton). To add on, a possible interaction between myo1D and cell-cell adhesion proteins such as DE-cadherin and/or beta-catenin have been suggested, where myo1C is thought to inhibit this interaction. In this project, we study the possible role of *Drosophila* myo1D at the molecular level that could translate to organ chirality.

The potential of myo1D binding to DE-cadherin could not only imply that the motor directly applies forces between cells, but also induces directional cues that induce chirality. Ongoing biochemical studies of these myosin-Is suggest that myo1D is a high-duty ratio motor, meaning that it dwells in strong-binding states for most of its ATPase cycle. This kinetic feature points to the possibility that myo1D binds to cell-cell adhesion sites and creates a twisting torque that could translate to neighboring cells to promote tissue/organ chirality. Our goal is to directly test the possibility that myo1D can bind DE-cadherin and power the motility of actin filaments.

Materials and Methods: Using an *in vitro* motility technique we call actin gliding assays, we reconstituted actin movement on supported lipid bilayers. Experiments were performed with bilayers containing PtdIns(4,4)P_2 (PIP2) and/or the cytoplasmic domain of hist-tagged DE-cadherins bound DGS-NTA (Ni) lipids which bind to our His-tagged cytoplasmic DE-cadherin construct. We added myo1D and a final solution containing fluorescently labeled actin filaments to detect and control for myosin-cadherin attachment by observing the attachment and movement of the actin filaments.

Results and Discussion: To build this intricate *in vitro* system, a control condition previously done in the lab was performed, composed of a lipid bilayer of PIP2 which binds to the tail domain of the myosin-Is. We reproduced binding and motility of actin filaments in these conditions. Preliminary experiments with DGS-NTA (Ni) with and without PIP2 lipids show limited actin filament binding, but further experimentation is needed to confirm this finding as mixed results were obtained. Though several attempts at introducing out Hist-tagged DE-cadherin into the DGS-NTA (Ni) system have been made, no reliable results have yet been established.

Conclusions: More troubleshooting and experimentation is needed to make accurate conclusions about our experiments.

References

Petzoldt, A. G., Coutelis, J.-B., Géminard, C., Spéder, P., Suzanne, M., Cerezo, D., & Noselli, S. (2012). DE-Cadherin regulates unconventional Myosin ID and Myosin IC in Drosophila left-right asymmetry establishment. In Development (Vol. 139, Issue 10, pp. 1874–1884). The Company of Biologists. https://doi.org/10.1242/dev.047589

Lebreton, G., Géminard, C., Lapraz, F., Pyrpassopoulos, S., Cerezo, D., Spéder, P., Ostap, E. M., & Noselli, S. (2018). Molecular to organismal chirality is induced by the conserved myosin 1D. In Science (Vol. 362, Issue 6417, pp. 949–952). American Association for the Advancement of Science (AAAS). https://doi.org/10.1126/science.aat8642