PhD PROJECT PROPOSAL

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EndoEmbryo: Hybrid AI to understand how intracellular trafficking shapes embryo morphogenesis.

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Abstract

The ability of cells and tissues to change shape during development relies on patterns of force-transmitting molecules at the cell surface, such as adhesion molecules (e.g. E-Cadherin), that anchor and regulate cell contractions. Those patterns emerge from the dynamics of hundreds of intracellular organelles that transport adhesion molecules within the cell. Organelle dynamics can be observed in vivo through time-lapse fluorescence microscopy (Fig. 1A,B), however, the understanding of how their collective dynamics give rise to the observed patterns of cell adhesion has been limited by difficulties in measuring the complex motions of organelles in the noisy images typical of developing 3D tissues. In this project, we will build upon our recent breakthroughs in multi-particle tracking (MPT) to study how study of intracellular trafficking regulates patterns of adhesion during tissue morphogenesis. Specifically, we will combine the robustness of neural networks used in large language model (ChatGPT, Gemini etc …) and the interpretability of conventional Markov modeling to study the intracellular trafficking of E-cadherin during the epithelial morphogenesis in Drosophila embryos. The project will test the hypothesis that specific trafficking fluxes of E-cadherin sustain different cell shape changes and emerge from specific organization and dynamics of the endocytic system in the cell (Fig.1C,D).

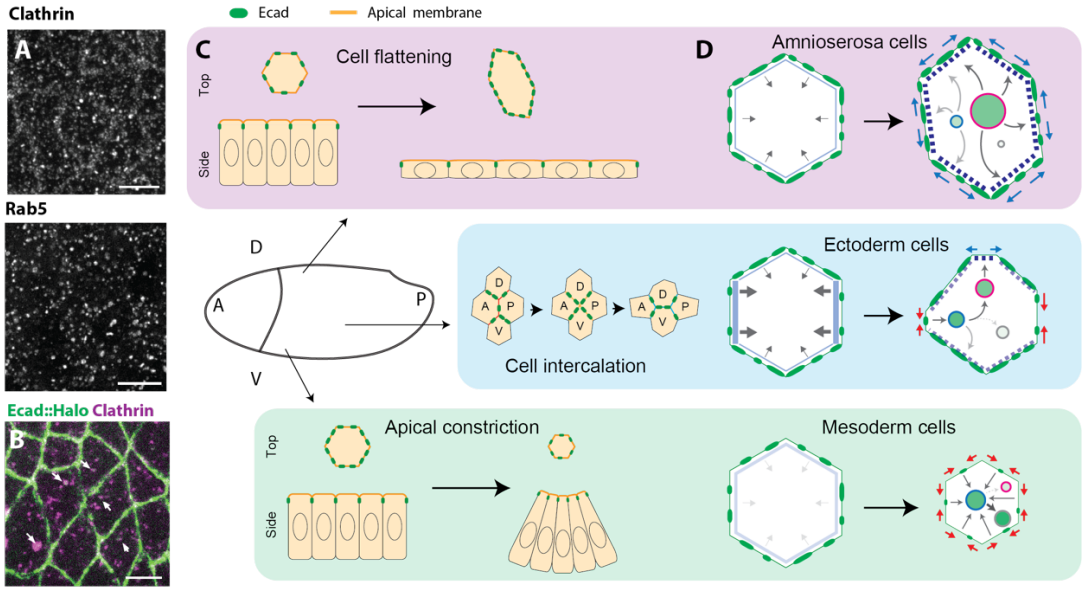

Fig. 1: (**A**) Confocal images of the indicated endocytic makers labelled with NeonGreen in cells of *Drosophila* embryos. **(B)** Ecad tagged with the far-red dye JF646 has been combined with clathrin marker tagged with NeonGreen. (**C**) Different types of cell and tissue shape changes occrring in *Drosophila* embryos during gastrulation. (**D**) Observed and predicted patterns of endocytosis in the indicated cells: Right: observed patterns of Ecad, endocytic machinery components and of the endocytic influx rate of dextran. The ligher shade in the mesoderm indicates lack of observations. Left: hypothetical traffking fluxes of Ecad across the endocytic pathway accompanying the observed cell chape changes.

Keywords

Tissue morphogenesis, intracellular trafficking, cell-cell adhesion
Multiple Object Tracking, Large Language Modeling, Markov Modeling.

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Scientific question and Objectives

Computational perspective: The key challenge will be the robust measurement of unknown collective dynamics (endocytic organelle movements, fusion/fission events etc.) through photon-limited signals. To tackle this problem, we will shift the *a priori* knowledge from the biophysics to the information we have on the image formation process, the limitations of sensors and detectors, and the optical properties of the tissue. Based on our hybrid AI methods for MPT[1], the main goal will be to generalize the training of a tracking transformer to endocytic events combined with the use of conventional Markov modeling for the validation and physical interpretation of results. One of the key challenges will be to ensure training convergence and scalability with an increasing number of objects in the field of view.

Biological perspective: The global trafficking kinetics of adhesion molecules depend on the local densities and dynamics of hundreds of individual organelles constituting the endocytic pathway. The key hypothesis addressed in this project is that different organization and collective dynamics of endocytic organelles underlie different types of cell and tissue morphogenesis (Fig. 1C-D). We first will infer the global E-cadherin trafficking rates (e.g. internalization, recycling, degradation) from endocytic organelles trajectories and then measure key differences in cells undergoing different types of morphogenetic events in the fly embryo during gastrulation.

Proposed approach (experimental / theoretical / computational) and research plan

Computational framework: The quantification of endocytic turnover is based on stochastic modeling of both the biophysics of endocytic vesicle formation and the noisy nature of fluorescence imaging. We propose a new approach that enables the decrease of apparent motion magnitude by increasing the framerate/SNR ratio. This regime of acquisition requires the registration of many false targets in combination with the targets of interest (the endocytic vesicles). Such cluttered scenarios were previously out of reach of conventional approaches due to the very high number of possible trajectories that must be evaluated ($\boldsymbol{N}!^{\boldsymbol{T}}$ trajectories, with N number of particles). We showed that attention-based large language model significantly pushed the boundaries of existing methods when a large number of hypothesis has to be considered. However, this was demonstrated only in a 2-particle scenario where the challenge comes from high particle diffusion and low inter-particle distance. Our key strategy will first focus on scaling this method to hundreds of molecules and the simulation of realistic endocytic events including the simulation of heterogeneous clutter and contractile events. In a second step, will focus on the generalization of those techniques, notably to the unpredictable number of molecules present in a movie.

Biological Framework: We will study epithelial morphogenesis and E-cadherin trafficking in the fly embryo where different processes of cell and tissue shape changes occur simultaneously (Fig. 1C). Specifically, we will study: 1) the ventrolateral ectoderm where cells undergo polarized cell intercalation and drive tissue extension, 2) the ventral mesoderm where cells undergo apical constriction driving tissue invagination and 3) the dorsal amniosierosa where cells undergo apico-basal flattening driving tissue expansion. Confocal imaging data of endocytic cargo (E-cadherin) together with markers of endocytosis (Clathrin, AP-2), exocytosis (Sec5, Sec15) and intracellular endocytic organelles (Rab5, Rab11, Rab7) will be analyzed to estimate the rates of transport in and out of specific endocytic compartments. Furthermore, MPT will be performed to estimate global trafficking rates from measurements of particle intensity and movements at the entire organelle population scale. We will first analyze intercalating cells where initial measurements of dextran endocytosis and steady state distribution of endocytic markers [2,3] suggest specific patterns to be observed. The analysis will then be extended to the mesoderm and the amnioserosa to measure E-cadherin endocytosis in cells undergoing apical constriction and flattening.

Interdisciplinarity and Implication of the two labs

The PhD student will be co-hosted by the group of Philippe Roudot (Inst. Fresnel, an engineering laboratory) and the group of Thomas Lecuit (IBDM, a developmental biology institute), where Claudio Collinet is integrated. This will ensure interdisciplinary training to the candidate thanks to the different expertise (computer vision in the Roudot group and cellular biology, genetics and live-microscopy in the Lecuit group). The project is deeply interdisciplinary and will require close interactions between the two groups. First, the optimal alignment of techniques must be explored to optimize the measure of molecular events at scale: setting the blueprint for optimal fluorescent labelling, illumination level and sampling adjustment as well as algorithm selection and parameterization. Second, the design

of the motion model must be chosen as a trade-off between physical accuracy and computational feasibility, requiring strong mentoring in complex systems physics and computer vision. Similarly, the interpretation of biological measurements across scales to understand the organization of intercellular trafficking and setting the next experiment also requires expertise in cellular biology and live microscopy. Finally, the development of perennial tools to study those complex 3D systems involves significant training in numerical methods for stochastic inferences.

Specify with whom the person recruited will collaborate and on what aspects

- Philippe Roudot (CR CNRS at Fresnel): mentorship on quantitative approaches for bioimaging
- Piyush Mishra (2nd year PhD Student at Fresnel): collaborating on large language modeling and their application to dynamic inference
- Claudio Collinet (CR CNRS at IBDM): mentorship on imaging data collection, quantification and biological interpretation.

PhD student's expected profile

The PhD student will have a formal training in applied mathematics, computer science or biophysics with a keen interest in cell biology and the study of complex systems.

Is this project the continuation of an existing project or an entirely new one? In the case of an existing project, please explain the links between the two projects

It is a follow up study on a preliminary benchmark on tracking approach for the developing embryo.

Two to five references related to the project*

R. Levayer, A. Pelissier-Monier, and T. Lecuit, "Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis," Nat Cell Biol, vol. 13, no. 5, Art. no. 5, May 2011, doi: 10.1038/ncb2224.

R. Levayer and T. Lecuit, "Oscillation and Polarity of E-Cadherin Asymmetries Control Actomyosin Flow Patterns during Morphogenesis," Developmental Cell, vol. 26, no. 2, pp. 162–175, Jul. 2013, doi: 10.1016/j.devcel.2013.06.020.

Sigismund, S., Lanzetti, L., Scita, G. & Di Fiore, P. P. Endocytosis in the context-dependent regulation of individual and collective cell properties. Nat. Rev. Mol. Cell Biol. 22, 625–643 (2021).

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Y. Xie , H. Miao , J.T. Blankenship, "Membrane trafficking in morphogenesis and planar polarity", Traffic 2018 May 14:10.1111/tra.12580. doi: 10.1111/tra.12580

Two main publications from each PI over the last 5 years*

P. Roudot et al., (2023) u-track3D: Measuring, navigating, and validating dense particle

trajectories in three dimensions. Cell Reports Methods. Doi: 10.1016/j.crmeth.2023.100655.

Mishra, Piyush, and Philippe Roudot. "Comparative Study of Transformer Robustness for Multiple Particle Tracking without Clutter." In *EUSIPCO*. Lyon, France, 2024. https://hal.science/hal-04619330.

Collinet C.*, Bailles A., Dehapiot B. and Lecuit T. "Mechanical regulation of substrate adhesion and de-adhesion drives a cell-contractile wave during Drosophila tissue morphogenesis", Dev Cell 2024 Jan 8;59(1):156-172.e7. doi: 10.1016/j.devcel.2023.11.022. Epub 2023 Dec 15.

Bailles A., Collinet C., Philippe J-M., Lenne P-F., Munro E., Lecuit T. "Genetic induction and mechanochemical propagation of a morphogenetic wave" Nature 2019 Aug;572(7770):467-473. doi: 10.1038/s41586-019-1492-9.

Project's illustrating image

See first page.

Reference

1. Mishra, P. & Roudot, P. Comparative study of transformer robustness for multiple particle tracking without clutter. in *EUSIPCO* (Lyon, France, 2024).
2. Levayer, R., Pelissier-Monier, A. & Lecuit, T. Spatial regulation of Dia and Myosin-II by RhoGEF2 controls initiation of E-cadherin endocytosis during epithelial morphogenesis. *Nat. Cell Biol.* **13**, 529–540 (2011).
3. Jewett, C. E. *et al.* Planar polarized Rab35 functions as an oscillatory ratchet during cell intercalation in the Drosophila epithelium. *Nat. Commun.* **8**, 476 (2017).