PhD PROJECT PROPOSAL

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Change in transcription factor dynamics during the suspension of development induced by a lack of oxygen

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Abstract (10 lines)*

Due to environment changes or pathologies (such as ischemia or tumors), cells of aerobic animals can experience stressful hypoxia conditions (low oxygen levels) or even transient anoxia (no oxygen). While the response of cells to hypoxia is well characterized, how cells adapt to anoxia is less understood. Interestingly, it has been observed that embryos of several animal species adapt to anoxia by entering a state of reversible suspended animation, during which divisions and differentiation stop. Once oxygen levels return to normal, development resumes. The molecular mechanisms regulating this intriguing phenomenon remain poorly characterized. Here we will analyze how cells rapidly suspend their differentiation program in anoxia, using the *C. elegans* embryo. We have observed that following entry into anoxia, a key fate-determining transcription factor is trapped in a few nuclear condensates. We will characterize the biophysical nature of these condensates and their functional consequences using a combination of genetics, quantitative live imaging and modeling. Our project will reveal how anoxia regulates the activity of key developmental transcription factors via segregation in molecular condensates, and therefore suspends cell differentiation programs.

Keywords*
Development, anoxia, cell fate specification, transcription factors, condensates, live quantitative imaging

Scientific question and Objectives (10 lines)*

Cells of aerobic animals can experience stressful fluctuations in oxygen levels due to changes in their environment. For example, during ischemia or inside solid tumors, cells can be subjected to low oxygen levels (hypoxia) or even no oxygen transiently (anoxia). While the response of cells to hypoxia has been well studied, how cells adapt to anoxia is less characterized. Interestingly, it has been observed that, when subjected to transient anoxia, the embryos of several species, such as Zebrafish or *C. elegans*, adapt by entering a reversible state of suspended animation. Cells quickly stop dividing, moving and differentiating. When oxygen is back to normal levels (normoxia), development rapidly resumes. However, the molecular mechanisms regulating the entry and exit from suspended animation remain poorly characterized. In particular, it is unclear how cells halt their differentiation process in anoxia and subsequently resume their developmental program at the right point when back to normoxia. In this project, we will analyze the effect of anoxia on the partitioning of a key fate-determining transcription factor into condensates and its functional consequences on the differentiation program.

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Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

To address this question, we will use *C. elegans* as a model organism. Interestingly, we have observed that entry into suspended animation in anoxia is accompanied by a rapid change in the distribution of a critical fate-specifying transcription factor CEH-10 (Figure). Under normal oxygen levels, CEH-10 is uniformly located in the nucleus. When subjected to anoxia, CEH-10 proteins rapidly relocate into a few bright dots (termed here condensates) within a few minutes. When embryos are put back to normal oxygen levels, these condensates quickly disappear. One attractive hypothesis is that, following entry in anoxia, the recruitment of CEH-10 proteins into condensates sequesters CEH-10 proteins away from their normal target genes, stopping the specification and differentiation program. To test this hypothesis we will:

1) Determine the biophysical nature of CEH-10 condensates. We will characterize the biophysical nature of CEH-10 condensates (liquid, solid …) as this can impact CEH-10 protein function. We will first analyse the dynamics of CEH-10 condensates formation following oxygen deprivation and disappearance upon re-exposure to normal oxygen levels. The dynamics of CEH-10 condensates will be followed by time-lapse imaging of endogenous fluorescently-tagged versions of CEH-10. It will be, for example, interesting to see whether CEH-10 condensates can undergo fusions or fissions, which would suggest formation by a liquid-liquid phase separation process. We will also analyse whether CEH-10 proteins can dynamically exchange between CEH-10 condensates and the rest of the nucleoplasm using FRAP (Fluorescence Recovery After Photobleaching) as well as photoconversion experiments. The experimental results obtained will be compared to a mathematical model in order to inform us on the liquid or solid nature of CEH-10 condensates. A solid nature would suggest a form of storage of biologically non-active CEH-10, while in a liquid condensate CEH-10 could keep some biological activity.

2) Analyze the effect of condensation on CEH-10 activity. To analyze the consequences of condensation on CEH-10 activity, we will induce CEH-10 condensate formation under normal oxygen level using the light-activatable Cry2 system (Shin et al. 2017) and analyze the effect on cell differentiation and CEH-10 target gene expression. The strategy of artificial condensation of CEH-10 (light intensity, pulses, …) will be chosen to mimic the dynamics observed during anoxia.

Interdisciplinarity and Implication of the two labs (15 lines)*

This project is based on a collaboration between a biologist (Vincent Bertrand) and a physicist (Pierre Recouvreux). The two partners have complementary expertise: 1) experimental biology of *C. elegans* (Vincent Bertrand); 2) quantitative cell imaging, biophysics and modeling (Pierre Recouvreux). Merging these skills will be crucial to analyze the nature and function of the transcription factor condensates that form during anoxia. The generation and maintenance of new *C. elegans* lines required for the project will be done in the lab of Vincent Bertrand. The quantitative imaging analysis (FRAP, photoconversion, Cry2 light activation …) and modeling will be done under the supervision of Pierre Recouvreux. The analysis on the effect on cell differentiation and CEH-10 target gene expression will be done using single-molecule fluorescence in situ hybridization and fluorescent reporters under the supervision of Vincent Bertrand. As the project requires a tight interconnection of the expertise of the two labs, weekly meetings will be organized between the recruited PhD student, Vincent Bertrand and Pierre Recouvreux.

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

The recruited PhD student will collaborate with Vincent Bertrand and members of his lab on the generation of *C. elegans* lines and analysis of cell differentiation programs and gene expression. He or she will interact with Pierre Recouvreux and members of his lab on quantitative imaging analysis and modeling.

PhD student's expected profile*

The student recruited on this project should have a background in experimental biology with a strong interest in quantitative biology and biophysics.

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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 (5 lines)*

This is a new project.

Two to five references related to the project*

- Bertrand and Hobert (2009). Linking asymmetric cell division to the terminal differentiation program of postmitotic neurons in *C. elegans*. Dev Cell 16, 563-75.
- Bhat et al. (2021). Nuclear compartmentalization as a mechanism of quantitative control of gene expression. Nat Rev Mol Cell Biol 22, 653-670.
- Padilla et al. (2002). Dephosphorylation of cell cycle-regulated proteins correlates with anoxia-induced suspended animation in *Caenorhabditis elegans*. Mol Biol Cell 13, 1473-1483.
- Shin and Brangwynne (2017). Liquid phase condensation in cell physiology and disease. Science 357. 10.1126/science.aaf4382.
- Shin et al. (2017). Spatiotemporal control of intracellular phase transitions using light-activated optoDroplets. Cell 168, 159-171.

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

Vincent Bertrand

- Bordet G., Couillault C., Soulavie F., Filippopoulou K. and Bertrand V. (2022). PRC1 chromatin factors strengthen the consistency of neuronal cell fate specification and maintenance in *C. elegans*. PLoS Genetics 18, e1010209.
- Kaur S., Mélénec P., Murgan S., Bordet G., Recouvreux P., Lenne P.F. and Bertrand V. (2020). Wnt ligands regulate the asymmetric divisions of neuronal progenitors in *C. elegans* embryos. Development 147, dev183186.

Pierre Recouvreux

- Recouvreux P., Pai P., Dunsing V., Torro R., Ludanyi M., Mélénec P., Boughzala M., Bertrand V. and Lenne P.F. (2024). Transfer of polarity information via diffusion of Wnt ligands in *C. elegans* embryos. Current Biology 34, 1853-65.
- Recouvreux P. (2024). Locally fast, globally slow. Biophys J., S0006-3495(24)00481-8.

Project's illustrating image

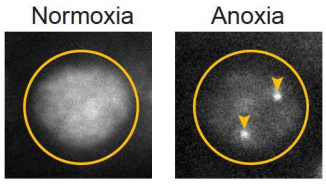

Figure: Under normoxia the CEH-10 protein is homogenous in the nucleus (circled), while under anoxia it condenses into a few dots (arrowheads).

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