Rolling dice during development:
Timelapse microscopy of stochastic cell fate decisions in *C. elegans*

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***Short Abstract* — During development cell fate decisions are often stochastic, meaning that a cell randomly chooses one fate out of repertoire of different fates. It is hypothesized that such stochastic cell fate decisions are driven by molecular fluctuations that are amplified by feedback loops in the gene regulatory network in the cell. We are testing this hypothesis by studying a classic stochastic cell fate decision, the so-called AC/VU decision, in the nematode *C. elegans*. For this, we use a novel combination of microfrabrication and timelapse microscopy to image gene expression dynamics on the single-cell level in live *C. elegans* animals.**

***Keywords* — *C. elegans*, stochastic cell fate decision, feedback loop, gene regulatory network.**

I. Introduction

THE development of multicellular organisms is a highly reliable process. In order for this to happen properly, all the cells in the organism must assume the right fate at the right point in space and time. A fundamental open question is how such robust patterning is achieved even though many of the underlying processes on the molecular level are highly stochastic [1]. Surprisingly, developing organisms might actively use such random molecular fluctuations to drive cell fate decisions.

During development, patterning often occurs through the so-called stochastic cell fate decisions, in which a cell randomly chooses one cell fate out of a repertoire of different possible fates [2, 3]. It is hypothesized that all such stochastic cell fate decisions are driven by molecular fluctuations that are subsequently amplified by feedback loops in the underlying gene regulatory network. However, stochastic cell fate decisions have not been followed on molecular level and hence this hypothesis remains untested. As a consequence, fundamental questions about stochastic cell fate decisions are entirely unanswered:

What is the initial source of the molecular fluctuations? How are such random fluctuations reliably amplified into cell fate within the ~1-10hr time window provided by the ongoing development?

In order to address these questions, we study stochastic cell fate decisions in the small nematode *C. elegans*, focusing on the so-called AC/VU decision. During the development of the reproductive organ, two precursor cells randomly choose the anchor cell (AC) or the ventral uterine cell (VU) fate in a mutually exclusive way [4]. The gene regulatory network underlying the AC/VU decision is well-known but its stochastic dynamics has never been studied, making it an excellent system for the study of stochastic cell fate decisions.

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II. Results and Methods

Since the AC/VU decision is a stochastic process, it is strongly history dependent. For that reason, it is essential to study the gene expression dynamics over developmental timescales in live animals using timelapse microscopy. So far, the difficulty of constraining *C. elegans* under the microscope has severely limited timelapse microscopy in live animals and previous studies of AC/VU decision were limited to imaging a small number of fixed, i.e. dead, worms [5]. To address this, we developed microfabricated arrays of small, 200μm x 200μm, microchambers [6], in which we confine single animals during the ~40 hours of development. This allows us to study the developmental dynamics in many animals in a highly parallel fashion. Our results show that the development of animals in our microchambers is normal in terms of timing, behavior and growth. Moreover, combining confinement in microchambers with fluorescence timelapse microscopy, we have been able to quantify gene expression dynamics during the AC/VU decision on the single-cell level in live animals, allowing us a first look at the dynamics underlying a stochastic cell fate decision.

III. Conclusion and Outlook

Combining microfabrication and timelapse fluorescence microscopy is a powerful tool to study gene expression dynamics during development in live *C. elegans* animals. We expect our setup not only to be instrumental in understanding the stochastic gene expression dynamics during the AC/VU decision, but also in many other systems in *C. elegans* involving time-dependent processes, such as gene expression of core developmental regulators, cell cycle dynamics and cell migration.

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