

Sorbonne Université/China Scholarship Council program 2021

Thesis proposal

Title of the research project: **A structure-function analysis to decipher noise and robustness in the establishment of the Bicoid morphogen step-like response**

Keywords: morphogen gradient, transcription dynamics, live imaging

Joint supervision: no

Joint PhD (cotutelle): no

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Doctoral school (N°+name): ED 515 Complexité du Vivant

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1) Study context

This PhD project is multidisciplinary as it involves, in a highly complementary manner, molecular genetics and developmental biology, experimental biophysics (imaging) and advanced data analysis. It is integrated within the framework of the ANR Firefly consortium (2020-2024) coordinated by Nathalie Dostatni in partnership with Aleksandra Walczak (DR2, Physics Department at the ENS, UMR8023) and Cécile Fradin (Professor, Physics and Astronomy Department, McMaster University, Canada).

The PhD project will be conducted at the Institut Curie which hosts well-recognized scientific platforms, for animal maintenance and leading-edge live cell imaging. The biological material required for the project will be produced in the team “Epigenetic Plasticity and Polarity of the Embryo” (flyteam) of the UMR3664, which routinely performs molecular and genetic manipulations on fruit flies. Imaging of the MS2 signals will be performed on this biological material with the confocal LSM780 microscope at the PICT-IBISA Imaging facility (Pasteur Building, Institut Curie). Mathieu Coppey (DR2, Physics Department, UMR168, Institut Curie) will provide expertise in quantitative biophysics for advanced processing of the movies. The large amount of data collected will be saved on the Institut Curie server with support of the DEEP Labex. Data analyses will be performed on computers equipped with fast processors and high performance graphic cards in collaboration with theoreticians, Huy Tran (ANR post-doc, UMR3664 at Institut Curie) and Aleksandra Walczak. Running costs of the project (molecular genetics, animal maintenance and imaging) and training/mission of the PhD candidate will be supported by the ANR Firefly grant and an ARC Fondation grant attributed to N. Dostatni (2020-2021).

2) Details of the proposal

Morphogen gradients are used to establish polarity along embryonic axes or within organs. In these systems, positional information stems from the morphogen concentration detected by each cell in the target tissue and mediates the determination of cell identity through the expression of specific sets of target genes. Although the critical role of these gradients is now well described, how they can provide reproducible transcription patterns given the inherent stochastic nature of transcription remains unclear.

Recently, methods to observe the kinetics of the transcription process directly in living cells have been developed. These approaches combine fluorescent labeling of nascent mRNA with live-cell imaging at high spatial and temporal resolution. They provide an unprecedented access to the dynamics of the transcription process [1] and a powerful tool to understand the robustness of patterning in development [2]. The team has recently adapted these approaches to one of the best characterized model organism, the fruit fly embryo [3], aiming to better understand how cell identity is controlled by the Bicoid (Bcd) morphogen system along the antero-posterior (AP) axis [4]. Focusing on the expression of the main Bcd target, *hunchback*, at the onset of zygotic transcription, the team built an MS2 reporter (*hb-MS2*) reproducing endogenous expression of the gene (see: [movie](#)). Advanced data analysis of this *hb-MS2* reporter indicated that surprisingly, despite the absence of transcription during the frequent mitoses at this stage of development, it only takes 3 min at each interphase for the system to measure subtle differences in Bcd concentration and produce a very sharp boundary separating expressing from non-expressing nuclei (step-like response) [5]. Synthetic reporters carrying various number and combination of binding sites for Bcd and for its two partners Hunchback (Hb) or Zelda (Zld), indicated that *i*) Bcd alone is not able to fully endorse the *hb* expression pattern, *ii*) that at a given position along the AP axis, Hb increases the probability for a Bcd-responding locus to be ON and *iii*) that Zld promotes Bcd-dependent expression at Bcd concentration thresholds lower than the Bcd concentration threshold normally required for Bcd-dependent expression. These recent findings (in prep) reveal that the Bcd-dependent transcription process involves several mechanistic steps, which each can be optimized with the contribution of Hb or Zld for rapid and efficient expression.

The goal of this PhD project is to better understand how a step-like expression pattern is formed so rapidly under the control of Bcd and how threshold concentration limits of Bcd are setting the position of this boundary. Towards this goal, a structure-function analysis of the Bcd protein will be performed to determine, which of its domains are involved in the Bcd-dependent mechanistic steps uncovered using the MS2 approach. The Bcd protein has been shown to carry a number of partially redundant functional domains including 4 independent activation domains as well as 2 inhibition/repression domains [6]. However, how each of these domains contribute to the different mechanistic steps of the Bicoid response has not been determined with the precision that the MS2 approach permits. To answer these questions, genome editing will be used to engineer these deletion mutants directly at the *bcd* locus and thus control their transcription level. In order to identify, which mechanistic step of the transcription process involves the domains of Bcd that they are lacking, the ability of these deletion mutants to express the *hb-MS2* reporter and the synthetic reporters with various number and combination of binding sites for Bcd, Hb or Zld will be determined. In addition, the tagging of the mutant proteins with fluorescence will allow to control their expression, distribution (decay length of their gradient) and motility (in collaboration with C. Fradin). Finally, If the functional analysis of a Bcd deletion mutant points to an interesting property for one of the Bcd domain involved (i.e. at the level of DNA binding, cooperativity or interaction with a potential partner), this property will be further explored either in vitro (IP or gel-shift assays), in vivo (imaging) or at the genome wide level to determine for instance how the binding of Hb or Zld is altered in the Bcd deletion mutants (ChIP).

The work will be organized as follow:

- 1) Two deletion mutants (Bcd- Δ C and Bcd- Δ QC) which have been recently generated at the *bcd* locus in the team will be first analyzed. In these mutants of Bcd, the C activation domain (C) either alone or in combination with the Q-rich activation domain are deleted. When analyzed by in situ hybridization in these mutants, the boundary of the *hb* expression domain appears much less steep than in wild-type embryos [7]. The goal of this part of the project will be to

determine which of the mechanistic steps involved in the Bcd transcriptional response is altered in these mutants. The functional analysis of these two mutants using the MS2 system will help understand how the making of a steep step-like transcription response is achieved under the control of Bcd and to what extent it involves its partners Hb or Zld.

2) In parallel, new deletion mutants of Bcd will be engineered using genome editing at the *bcd* locus and analyzed. These mutants will be deleted from the other known activation domains of Bcd or from its repression domains. The transcriptional behavior of these new deletion mutants will be analyzed and compared to the behavior of the wild-type Bcd protein using the MS2 reporters described above.

This project is at the interface of molecular and developmental biology on the one hand, and biophysics and systems-biology on the other hand. The PhD candidate will be involved in the “wet” part of the project and also in the data extraction and analysis part. Interactions with the physicists and theoreticians will help us extract the quantitative data from our MS2 movies and use them for modeling.

3) References

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- [7] Schaeffer V, Janody F, Loss C, Desplan C, Wimmer EA. Bicoid functions without its TATA-binding protein-associated factor interaction domains. *PNAS.* 1999. 96:4461–6.

4) Profile of the Applicant (skills/diploma...)

The PhD candidate will have a Master degree in Biology with experimental skills in molecular biology, genetics, confocal imaging and image analysis. He/She will be open to interact with physicists involved in data analysis and modeling. The candidate should be fluent in English since scientific interactions within the international team are conducted in this language.

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