Learning the rules of gene regulation: from reporters to endogenous expression

Gonçalo Fernandes[1], Huy Tran[1,2], Maxime Andrieu[1], Julie Takissian[1], Mathieu Coppey[3], Aleksandra M. Walczak[2], Nathalie Dostatni[1]
[1]Institut Curie, Université PSL, Sorbonne Université, CNRS, Nuclear Dynamics, France;
[2]Laboratoire de Physique de l'École Normale Supérieure, CNRS, Université PSL, Sorbonne Université and Université de Paris, France;
[3]Institut Curie, PSL Research University, CNRS, Sorbonne Université, Physico Chimie, Paris, France

In many developmental systems, cell identity is determined by morphogen gradients providing concentration-dependent positional information along polarity axes. Although the critical role of these gradients is well recognized, it is unclear how they can provide reproducible expression patterns despite the stochastic nature of transcription. To address this question, we studied the response downstream of the Bicoid (Bcd) morphogen gradient in fruit fly embryos, focusing on its main and earliest target gene, *hunchback* (*hb)*.
Using the MS2-MCP system to fluorescently tag nascent mRNA, transcription dynamics were analysed at high spatiotemporal resolution in living embryos. Adapting this approach to synthetic MS2 reporters with various combinations of DNA binding sites, we highlighted the roles of Bcd and its partners, Hb and Zelda (Zld), in the transcription mechanism. In addition, a biophysical model of Bcd-dependent expression was developed providing a theoretical framework for the experimental data. Finally, reducing the dose of Bcd by half and quantifying the corresponding shifts of Bcd-dependent reporter boundaries confirmed that Bcd is the main source of positional information for *hb* expression.
We are now further exploring this system to ask new questions about the organization of DNA binding sites in enhancers and bridging the gap between transgenic reporters and endogenous expression.