PhD Student (m/f/d) in

"Understanding the Influence of eRNA Association Modes on Transcriptional Condensates"

Scientific Background

Multicellular organisms rely on differential gene expression to create cellular diversity. Precise gene regulation is crucial, as failures can lead to reduced fitness, developmental defects, and disease. Enhancers, DNA elements with binding sites for transcription factors, play a key role in this process. They regulate gene activation by coming into physically proximity of promoters and transferring some of the bound components. Recently, it has become clear that enhancer activity requires the formation of sub-micrometer-sized transcriptional condensates.
Enhancers also produce enhancer RNAs (eRNAs), and their transcriptional activity correlates with their ability to activate nearby genes. While proteins have been extensively studied in transcriptional condensate formation, the role of RNAs has received less attention. However, previous studies suggest that short RNAs like eRNAs can trigger phase separation of proteins in transcriptional condensates, and transcription factors can interact directly with RNAs. Given that most eRNAs only act locally (in *cis*), the presence of eRNAs at enhancer sites, often forming **R-loop** structures, may be more important than the specific eRNA molecule itself. This leads to the hypothesis that **eRNAs play a crucial role in the formation and function of transcriptional condensates**, with their impact on transcriptional condensate formation remaining largely unexplored.

PhD Project: "Understanding the Influence of eRNA Association Modes on Transcriptional Condensates"

Join our dynamic research group at IMB, established in September 2023, and contribute to the exciting field of gene regulation. We are seeking a motivated PhD student to contribute to our investigations of how transcriptional condensates form and regulate gene expression. Using cutting-edge single-molecule assays coupled to fluorescence microscopy (read more in Golfier et al. and Morin et al.), we reconstite different stages of gene activation on single DNA molecules. In this project, you will focus on the role of eRNAs in condensate formation by exploring different RNA association modes (Fig. 1). You will be integrated into the 4R-RTG (more information here) and benefit from a like-minded community of PhD students. By combining

classic phase separation assays and single molecule reconstitution, you will address the following questions:

- How does the eRNA association mode impact transcription factor condensation in solution?
- How does the eRNA association mode affect the formation of condensates on single molecules of DNA?
- How does the eRNA association mode influence DNA:DNA interaction?

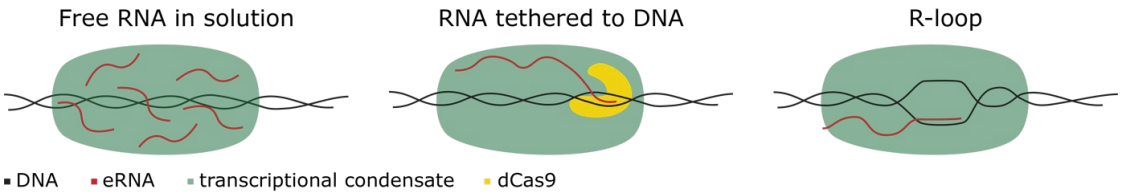

Fig. 1: The following eRNA association modes will be tested in the proposed PhD project. *(Left) Freely diffusing RNA, emulating eRNA released after transcription has terminated. (Middle) RNA tethered to DNA via enzymatically dead Cas9 (dCas) emulating nascent eRNA during transcription. (Right) RNA tethered to DNA via a DNA:RNA hybrid structure called R-loop, as commonly found at enhancers.*

Publications relevant to this project

Morin JA, Wittmann S, Choubey S, Klosin A, Golfier S, Hyman AA, Jülicher F, Grill SW. (2022) **Surface condensation of a pioneer transcription factor on DNA.** Nat Physics, 18: 271–276 Link

Golfier, S., Quail, T. & Brugués, J. (2024) **Single-Molecule Approaches to Study DNA Condensation.** Methods Mol Biol 2740, 1–19 Link

Chen, Q. et al. (2023) **Enhancer RNAs in transcriptional regulation: recent insights**. Front Cell Dev Biol 11, 1205540 Link

Wittmann S, Alberti S. (2019) **ERα condensates: chronic stimulation is hard to ignore**. Nat Struct Mol Biol 26(3): 153-154 Link

Ryu, K., Park, G. & Cho, W. K. (2024) **Emerging insights into transcriptional condensates**. Experimental & Molecular Medicine 2024 56:4 56, 820–826 Link

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