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PhD Project Title

The role of protein aggregation in mammalian transcription

Project Description

Transcription is of central importance in biology, but many unresolved questions remain, in particular for mammalian systems. Contentious topics include action-at-a-distance type of regulation of gene expression by enhancers, the observation of clusters of transcribing polymerases in the nucleus, or the apparent lack of structures that spatially arrange these things. Apart from these topological issues, transcription itself is a highly fluctuating, stochastic process that is little-understood and includes many puzzling phenomena, such as the frequent 'pausing' of polymerase at certain intragenic positions.

Recent discoveries suggest that the aggregation of proteins with certain structural features has an important role in linking many of these phenomena. The aggregates produce liquid-liquid phase transitions (LLPT) that result in 'droplets' with unique biophysical properties. Notably, this applies to RNA polymerase II (PolII) itself (Nat Struct Mol Biol. 2018;25(9):833-840) and pioneering studies indeed suggest connections between transcription and such phase transitions (Science. 2018 Jul 27;361(6400):329-330).

The aim of the project will be to investigate how genome-wide dynamics of transcription are affected upon perturbing the aggregation properties of PolII. To this end, we will replace endogenous PolII with mutant versions that display reduced and increased LLPT induction compared to wildtype PolII, respectively, followed by single-cell RNA-sequencing. Analysis of the resulting data in conjunction with additional existing genomics data will produce mechanistic insights. Additional experiments based on imaging will characterize LLPT formation, PolII clustering, and nuclear topology in parallel, along with standard molecular biology assays such as western blotting to verify findings.

Key experimental skills involved

Next generation sequencing, imaging, mammalian cell culture, transfection, standard molecular biology (DNA cloning, qPCR, western blotting etc).

Subject Areas

Biochemistry

Bioinformatics

Genetics

Molecular Biology

Data Analysis

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