

Progress Report

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1 Introduction

Eukaryotic transcription is episodic, consisting of a series of transcriptional bursts. Bursty transcriptional dynamics are well-exemplified by the transient expression of pro-inflammatory guanylate binding proteins (GBPs) - a group of interferon-inducible GTPases that restrict the replication of intracellular pathogens [XXX]. Classical models of gene regulation explain transcriptional bursts by invoking stochastic binding and unbinding of transcription factors, RNA polymerase and mediator proteins at enhancer or promoter sequences. However, more recent studies have pointed towards a more cooperative picture of transcriptional control where phase-separated aggregates of DNA, RNA, and proteins form higher-order structures to control gene expression. For example, both chromatin immunoprecipitation and super-resolution imaging have captured the phase separation of super-enhancer-binding proteins MED1 and BRD4 in transcriptional condensates at the *Essrb* genomic locus [XXX]. Fluorescence microscopy techniques have also colocalized MED1 and BRD4 with the GBP gene cluster alongside a reduction in the degree of disorder of 3D chromatin structure in murine macrophages after infection with *Mycobacterium tuberculosis*. Taken together, these results suggest that phase separation plays a role in the reorganization of chromatin structure during transcriptional control of innate immune response genes [XXX]. Here, we hypothesize that phase separation reduces the entropy of chromatin structure in order to induce bursty gene expression. Using single molecule localization microscopy (SMLM) to obtain super-resolution images of the H2B protein, we demonstrate simultaneous (i) loss of disorder in chromatin structure (ii) formation of transcriptional condensates containing MED1 and BRD4 and (iii) non-Poissonian gene expression. The following sections discuss recent biological evidence in more detail and summarize the single molecule microscopy techniques and biophysical models we employ to study the interactions between transcriptional condensates with the chromatin scaffold.

2 Interferon- γ reorganizes 3D chromatin architecture

3 Single molecule localization microscopy

Most detectors used for imaging have many elements (pixels) so that we can record an image projected onto the detector by a system of lenses. In fluorescence imaging, this is usually a relay consisting of an objective lens and a tube lens to focus the image onto the camera. Due to diffraction, any point emitter, such as a single fluorescent molecule, will be registered as a diffraction limited spot. The profile of that spot is often described as a Gaussian point spread function (Richardson and Wolf)

4 Langevin dynamics of a Rouse-like polymer embedded in a transcriptional condensate

Rouse-like chromatin scaffold with Monte Carlo simulations and analytical treatment

The formation of these transcriptional condensates driven by MED1 and BRD4 alongside decreased entropy of the chromatin scaffold have been previously demonstrated with super-resolution microscopy. Yet,

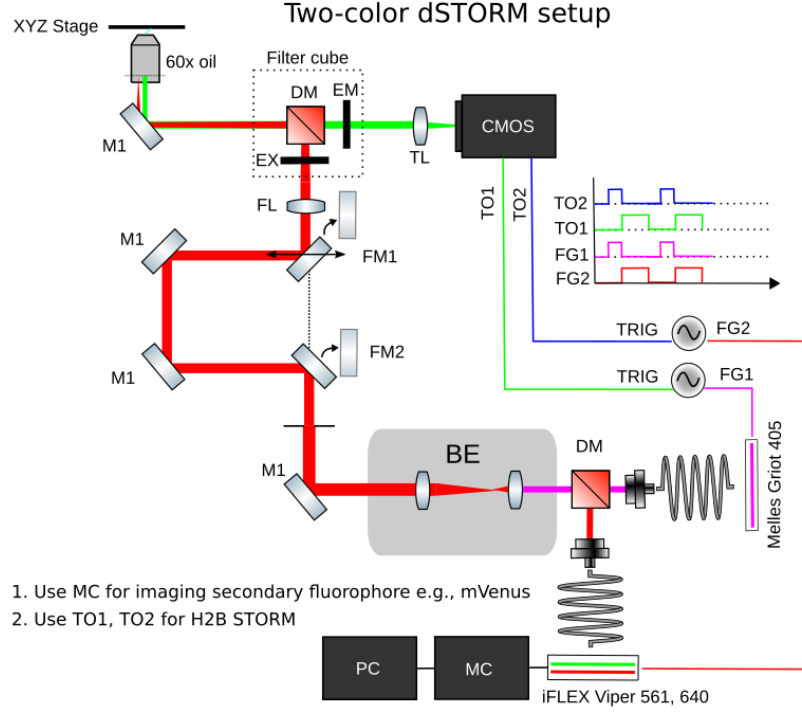


Figure 1: Direct stochastic optical reconstruction microscope

the biophysical descriptions of the interaction between transcriptional condensates with the chromatin fiber and the mechanisms by which they evoke bursty gene expression remain mere speculation.

Here, we intend to address these outstanding questions by modeling transcriptional condensate formation within a Rouse-like polymer model parameterized by constraints measured in single particle tracking experiments. These models serve to address the stability of transcriptional condensates as a function of their stoichiometry and the relationship between condensate formation and transcriptional bursting by integrating their associated Langevin dynamics with Monte Carlo simulations.

The general properties of transcriptional condensates can be investigated using the stochastic simulation algorithm [Gillespie].

References

- [1] Muir Morrison. Statistical Mechanics of Problems in Transcription Regulation. Technical report, 2021.