Phosphorylation of the carboxyl-terminal domain (CTD) of RNA polymerase II (RNAP2) occurs in a spatiotemporal organized manner at a single-copy gene in live-cells

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***Short Abstract* —We visualized, quantified, and modeled the spatiotemporal organization of RNAP2 phosphorylation at a single-copy gene. Using three-color fluorescence microscopy with antibody-based probes that specifically bind to different phosphorylated forms of endogenous RNAP2 in living cells at single-gene resolution. Applying this methodology in combination with computational models, we found live-cell evidence that genes contain a heterogenous distribution of RNAP2 along their length, with 5-40 copies per transcription site (predominantly phosphorylated in Serine 5 clusters near the promoter). RNAP2 fluctuations are spatially and temporally resolved from those of the nascent mRNA, with most RNAP2 being phosphorylated within 6 seconds, escaping the promoter within ~1.5 minutes, and completing transcription in 5 minutes [3].**

***Keywords* — Transcription dynamics, RNAP2 clusters, transcriptional bursting, single-copy gene, single-molecule microscopy, computational modeling.**

I. Background

Transcription in eukaryotic cells is regulated by dynamic phosphorylation of the CTD as RNAP2 progresses through the transcription cycle. In this cycle, RNAP2 is first recruited to the gene promoter in an unphosphorylated form (CTD-RNAP2), and it is then phosphorylated at the serine 5 (Ser5ph-RNAP2) upon initiation. RNAP2 clusters have been observed near to the promoter regions of genes [1], and these have been spatially correlated with CTD-RNAP2 and Ser5ph-RNAP2 forms [2]. Resolving the temporal dynamics of RNAP2 phosphorylation and clustering and subsequent transcription combined with multiple factors could shed new light on global mechanisms of transcription regulation.

Previous immunoprecipitation-based assays provide precise distribution of RNAP2 along the 1D genome but mask heterogeneity and limit temporal resolution. Here, we combine multicolor single-molecule fluorescent microscopy, complementary fluorescent antibody-based probes (Fabs), and rigorous stochastic model inference (Fig. 1) to determine endogenous RNAP2 phosphorylation dynamics during the transcription cycle at a single-copy gene in living cells [3]. Specifically, we employed an established cell line containing a reporter gene controlled by an HIV-1 promoter and tagged with an MS2 cassette that, when transcribed, synthesizes stem loops, which are bound by its coating protein MCP. mRNA is marked by MCP-GFP (mRNA, blue). The recruited and initiated RNAP2 are labeled by Fabs (conjugated with CF640 and Cy3) that bind unphosphorylated and phosphorylated CTD RNAP2 heptad repeats (CTD, red) and Serine 5 phosphorylated repeats (Ser5ph, green), respectively (Fig 1A-B).

II. Summary of the Results

We quantified the correlated temporal and spatial organization of RNAP and mRNA throughout the transcription cycle. We then fit our experimental data with a range of candidate computational models (e.g., Fig 1B) to find a minimal model that captures the CTD, Ser5ph, and mRNA signals' auto- and cross-correlation information (e.g., Figs 1C,D) and statistical distributions (e.g., Fig 1E). Model predictions for the concentration of RNAP2 along the reporter gene were validated against ChIP. By comparing model predictions assuming different perturbations to independent sets of live-cell experiments following application of transcription inhibitors, the models help to shed light on the mechanisms of transcription inhibition.

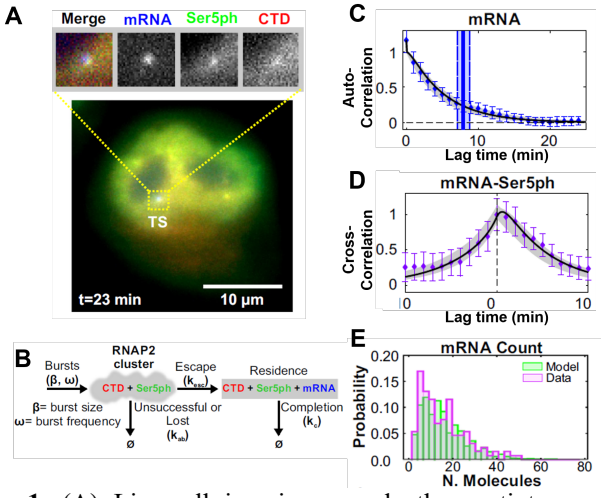

Fig. 1. (A) Live-cell imaging reveals the spatiotemporal organization of endogenous RNAP2 phosphorylation at a single-copy gene (Adapted from [3]). A simple discrete stochastic model (B) reproduces all signals' autocorrelations (C), cross-correlations (D) and intensity disctributions (E).

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