Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

Imaging transcriptional dynamics

Bomyi Lim

Recent advances in imaging techniques have enabled visualizations of nascent transcripts or individual protein molecules at high spatiotemporal resolution, revealing the complex nature of transcriptional regulation. Here, we highlight recent studies that have provided comprehensive insights to transcriptional dynamics using such quantitative imaging techniques. Specifically, they demonstrated that transcriptional activity is stochastic, and such transcriptional bursting is modulated by multiple components like chromatin environments, concentration of transcription factors, and enhancer–promoter interactions. Moreover, recent studies suggested that regulation of transcriptional activity is more complex than previously thought, by showing that transcription factors and RNA polymerases also move within the cell with distinct kinetics and sometimes form dynamic clusters to mediate transcriptional initiation.

Address
Department of Chemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104, USA

Corresponding author: Lim, Bomyi (bomyilim@seas.upenn.edu)

Current Opinion in Biotechnology 2018, **52**:49–55

This review comes from a themed issue on **Tissue, cell and pathway engineering**

Edited by **David Schaffer** and **Stanislav Y Shvartsman**

https://doi.org/10.1016/j.copbio.2018.02.008

Introduction

Transcriptional regulation is a critical yet complex biological process that requires precise spatiotemporal regulation to ensure normal functioning of organisms. Despite its importance, transcriptional regulation is yet to be fully understood due to its complex nature. Also, transcription is not a static, but rather a dynamic process that involves multiple layers of DNA–DNA, DNA–protein, and protein–protein interactions (Figure 1a) [1]. Pre-initiation complexes are stably formed at the promoter on the order of seconds to minutes, and RNA polymerase II (Pol II) molecules elongate and produce mRNAs at a rate of a few kb per minute [2••,3]. The resulting gene expression is also dynamic in nature, where some genes exhibit oscillatory behaviors, while others show stable expressions [4]. In such dynamic environment, it is important to understand the kinetics of transcription machineries, such as transcription factors and RNA polymerases, and the effects of changes in transcription kinetics on cellular processes (Figure 1a,b).

Recently developed quantitative imaging methods, such as live-cell and single-molecule imaging techniques, have significantly transformed our view of transcriptional regulation [5,6]. Development of such techniques enabled visualizations of various components of transcription, which were not readily accessible previously due to technical challenges. Assays like the MS2-MCP detection system have allowed labeling of nascent transcripts in living cells, and advancement in microscopy techniques has allowed visualization of single molecules at high spatiotemporal resolution (Figure 2). This review highlights recent studies that take an advantage of such quantitative imaging to provide better insights on the dynamics of transcriptional regulation.

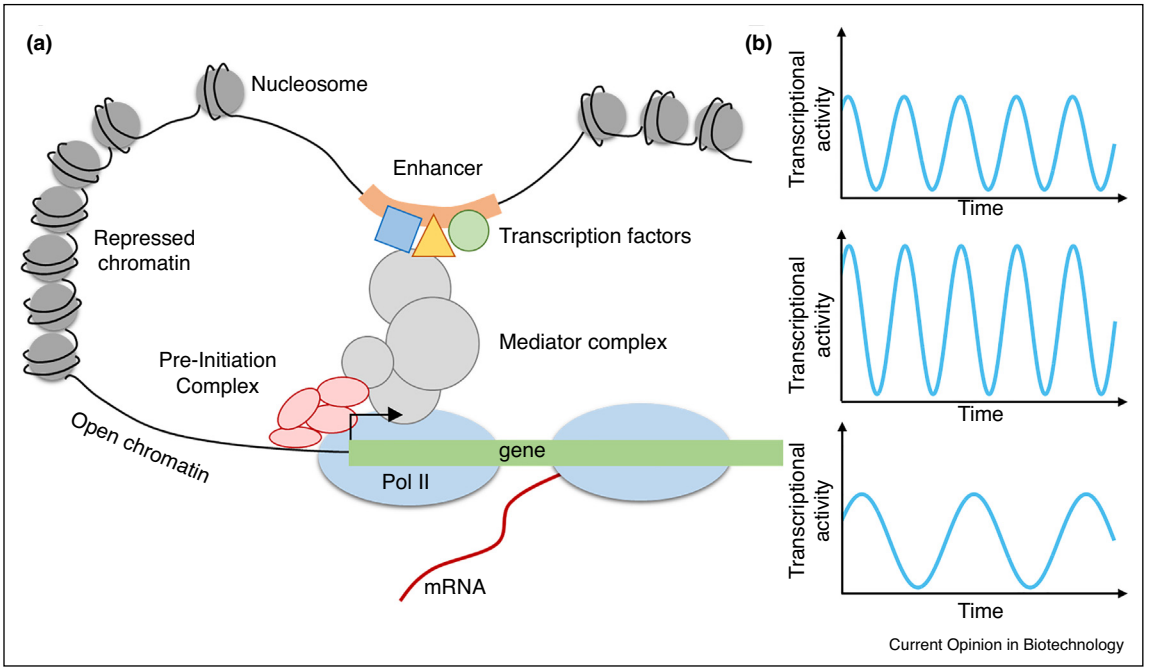
Visualization of transcriptional bursting in multicellular eukaryotes

There is emerging evidence that transcription is discontinuous, consisting of a series of stochastic bursts. McKnight and Miller showed the first evidence for transcriptional bursting by visualizing sequential clusters of irregularly spaced nascent RNAs, using transmission electron microscopy on chromatin spreads from *Drosophila* embryos [7]. Recent advances in imaging techniques have enabled further support for this concept of transcriptional bursting.

Studies with single molecule fluorescent *in situ* hybridization (smFISH) have revealed that only a fraction of cells in a population shows active transcription at any given time point (Figure 2a,b). Such heterogeneity in gene expression implies that transcription occurs in bursts rather than continuously. This has been inferred from the behavior of many genes across various cell types and organisms [6,8,9]. In the past three years, transcriptional bursting was exhibited by *Nanog* and *Oct4* in mouse embryonic stem cells [10–12], the *β-globin* in erythroid cells [13•], and the *Pck1* in intact mammalian livers [14].

Rather than inferring from a population of fixed tissues, nascent transcripts can now be directly monitored at single-cell resolution using live imaging techniques. Using the widely used MS2-MCP system as a detection method [5], transcriptional bursting was first observed in living bacteria in 2005 [15]. Since then, bursting has been visualized in living multicellular eukaryotes as well, both

Figure?1



Transcriptional regulation modules that affect transcriptional dynamics. **(a)** Schematic of regulatory modules that control transcription. **(b)** Example trajectories of transcriptional activities that can result from modulations on one of the transcriptional machineries.

in transgenic reporter genes and in endogenous genes (Figure 2c,d) [16–21,22••]. In all cases, bursting was visualized as fluctuating fluorescent signals, with the duration of bursts ranging from a few minutes (*Drosophila* embryos) up to an hour (human U2OS cells) (Figure 3a, b). Such observations support the view that transcriptional bursting is a general property of transcription.

Until recently, transcriptional bursting has been characterized in the context of a 'two-state' model, wherein a promoter stochastically switches between an *ON* state, where transcription occurs at a fixed rate, and an *OFF* state where no transcription happens (Figure 3c) [23,24]. This model serves as the simplest explanation for the observed heterogeneity of transcriptional activity. However, many live-imaging studies have recently suggested that the promoter at its *ON* state produces mRNA at changing rates rather than at a single, fixed rate. Using live imaging and modeling, Corrigan *et al.* showed that endogenous *actin5* in *Dictyostelium* exhibits continuous changes in the rates of active transcription before reaching an inactive state [22••]. Varying levels of transcription activation rates were shown in pituitary tissues as well as in early *Drosophila* embryos [25,26]. Featherstone *et al.* identified multiple levels of active transcription rates across individual cells in living pituitary tissues, where each cell showed distinct duration of *ON* states from each other [25]. Holloway *et al.* used live imaging data of *eve2*-mediated transcription by Bothma *et al.* and developed a stochastic model to demonstrate that there exist at least two distinct *ON* rates of transcription, in agreement with studies in *Dictyostelium* and in pituitary cells [26,27]. Such high-resolution imaging data combined with mathematical modeling have provided better understanding on the stochastic nature of transcriptional dynamics.

Modulation of transcriptional dynamics

With the availability of single-molecule sensitivity, extensive studies have been performed to understand how different transcription machineries can modulate the property of transcriptional bursting, such as bursting frequency, size, and durations (Figure 3d). Many studies have tried to elucidate the mechanism of the regulation of bursting kinetics by the level of transcription factors. For example, using live imaging, Larson *et al.* controlled the level of steroids through light activation, and showed that steroids mediate the level of steroid-responsive genes by modulating bursting frequency [20]. Similarly, Senecal *et al.* showed that concentration of ERK or p38 can be used to regulate bursting frequency of the target gene *c-Fos*, while not affecting other parameters such as burst size or duration [28]. Most recently, Kafri *et al.* showed that it is the rate of nuclear *β-catenin* accumulation that shapes the transcriptional output of downstream target gene *cyclinD1*, by modulating both bursting frequency and size [29•].