HHS Public Access

Author manuscript

Curr Opin Biotechnol. Author manuscript; available in PMC 2019 August 01.

Published in final edited form as:

Curr Opin Biotechnol. 2018 August ; 52: 49–55. doi:10.1016/j.copbio.2018.02.008.

Imaging transcriptional dynamics

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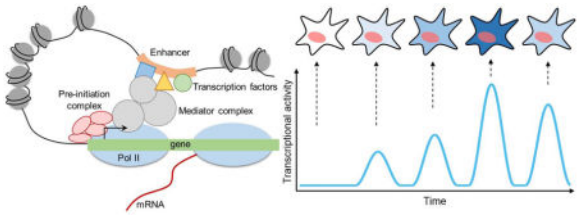
Abstract

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.

Graphical Abstract



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

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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 in transgenic reporter genes and in endogenous genes (Figure 2C–D) [16–22]. 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 *cyclin D1*, by modulating both bursting frequency and size [29].

Other studies have examined the role of nucleosome occupancy, chromatin states, or mediators on transcriptional regulation [30–33]. Dey et al. used the HIV-1 promoter to show that promoters with more repressed chromatin produce fewer bursts and exhibit more noise, suggesting the effects of nucleosome occupancy on gene expression heterogeneity [30]. It has been shown that the local chromatin environment affect transcriptional dynamics as well, by changing bursting frequency or size. When reporter genes were integrated into random locations in the genome, distinct stochastic gene expressions were observed in different genetic loci [30–32]. In addition, a study with the HIV-1 promoter showed that the time scale of bursting is mediated independently by Mediator and by TBP-TATA box interaction, showing modulation of transcriptional dynamics at the level of the pre-initiation complex [33].

Lastly, two recent studies examined how enhancers control transcriptional bursting [13,34]. By forcing looping between the *β-globin* enhancer and the *β-globin* promoter in murine and human erythroid cells, Bartman et al. showed that forced tethering between the enhancer and the promoter increased burst frequency, but not burst size [13]. Using MS2-MCP-based live imaging in early *Drosophila* embryos, Fukaya et al. showed that different enhancers produce transcriptional bursts with similar size, but with different frequencies, such that strong

enhancers produce more bursts than weak enhancers [34]. Their results also support the idea that bursting frequency is a key parameter of gene control in development.

Given all these different modes of modulating transcriptional dynamics, one would wonder why cells would exhibit such stochastic behaviors. A previous study demonstrated that heterogeneity in single-cell organisms could help overall fitness of the population [35]. Recently, it was suggested that transcriptional bursts might sensitize cells for repression. Esposito et al. showed that Snail repressor silences the target gene expression during the refractory period between bursts, implying that the repressor can act more effectively during the inactive state [36]. Similarly, Antolovic et al. showed that genes that will eventually be downregulated show more stochasticity, supporting the idea that discontinuous transcription might be beneficial for repression [37]. This indicates that transcriptional bursting can be used as a mechanism to facilitate dynamic repression of gene expression.

Kinetics of transcription factors and RNA polymerases

In addition to extensive imaging-based studies on transcription dynamics, many researchers have tried to understand the kinetics of proteins that comprise transcriptional machineries, such as transcription factors and RNA polymerases (Figure 1A). Recent advances in microscopy and fluorophores made single-molecule imaging readily available in living tissues. The Betzig lab recently developed the lattice light-sheet microscope, which has allowed quantitative imaging of single molecules with high spatiotemporal resolution [38]. Moreover, traditionally used fluorophores like rhodamine dyes were improved to obtain a few-fold increase in brightness and sensitivity [39,40].

Through these improved imaging techniques, the dynamic nature of protein-DNA interactions has been visualized. Many studies showed how different transcription factors move with different kinetics to find their target DNA. Izeddin et al. demonstrated that the transcription factor c-Myc sweeps an entire nucleus in a diffusive manner, while the elongation factor P-TEFb explores only a compact domain within a nucleus sub-diffusively [41]. Chen et al. and Xie et al. also showed that the transcription factors Sox2, Oct4, STAT3, and ESRRB, all of which bind to the same enhancer, actively search for their target DNA with distinct kinetics [42,43]. Moreover, general transcription factors bind to the promoters in a very dynamic fashion. Zhang et al. showed that general transcription factors TFIIA and TFIID bind to the promoter stably, while TFIIB-promoter interaction is transient [2]. These studies have provided valuable insights into the dynamics of the search process between transcription factors and target DNAs.

Other studies have provided explanations for the mechanism of how DNA-bound transcription factors and RNA polymerases initiate transcription. One possible mechanism is that transcription factors and Pol II form a cluster at active transcription loci. Cisse et al. first reported the formation of Pol II clusters in live human cells by using exogenously labeled Pol II, and it was later found that endogenous Pol II also forms clusters [44,45]. Following up on these findings, Cho et al. showed that the lifetime of Pol II clusters are correlated with nascent mRNA output, suggesting a function of the clustering [46]. Moreover, protein clustering has also been observed for transcription factors. By tracking dynamics of the

transcriptional activator Msn2 and the repressor Mig1 in yeast, Wollman et al. suggested that clustering of proteins facilitates the search process for target DNAs [47]. In the *Drosophila* embryo, the transcription factor Bicoid is reported to form a cluster, which helps increase its local density and facilitates binding to target DNAs [48]. Altogether, single-molecule live imaging has enabled the exploration of the kinetics of transcriptional machineries with a high spatiotemporal resolution, which helped reveal dynamic nature of complex transcription processes.

Future perspectives

One of the biggest advantages of live imaging techniques is that transcriptional activity of a single cell can be directly monitored over time. Utilizing these techniques, it was shown that early developmental enhancers in early *Drosophila* embryos drive dynamic gene expression patterns, such that transcripts were expressed very broadly across the embryo and refined gradually over time to form a final pattern [27,49]. Other examples of gene expression dynamics include anterior shift of *Krüppel* gene expression patterns and a gradual activation of *T48* along the dorsoventral axis [50,51]. These interesting findings from *in vivo* studies of multicellular eukaryotes, however, were mostly limited to those using *Drosophila*. It will be interesting to see how dynamics of transcriptional activity can affect patterning or other key developmental processes in various other multicellular organisms.

Live imaging assays can also be used to understand long-range chromatin dynamics. Through chromosome conformation capture assays, it has been suggested that non-neighboring parts of a chromosome interact to form chromosomal loop domains [52]. However, kinetic information is missing from these models. For example, is loop domain formation static or dynamic? Is gene expression significantly affected if the loop formation is delayed or expedited? Recent studies have already shown that loop domain formation is indeed dynamic [53,54]. Moreover, Hansen et al. characterized the kinetics of CTCF and Cohesin, the two key molecules for the loop formation, providing better understanding on the chromatin loop dynamics [55]. More of such imaging studies *in vivo* will provide helpful insights into chromatin dynamics and its effects on transcriptional regulation.

Advancement in imaging technique has brought unforeseeable changes to the field of transcriptional regulation, especially through visualization of transcriptional dynamics at a remarkable spatiotemporal resolution that was unfeasible before. This enabled many interdisciplinary works, including molecular biology, imaging, and modeling, which highlighted an important aspect of gene control: the critical role of kinetics in transcriptional regulation. Further advances in imaging techniques and interdisciplinary works will provide more sophisticated and comprehensive insights to the complex world of transcriptional regulation.

Acknowledgments

BL would like to thank Sang Beom Kim, Tyler Heist, Yuji Yamazaki, Takashi Fukaya, Kai Chen, and other Levine Lab members for helpful discussions. This work was supported by the National Institutes of Health [F32GM122186].

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Highlights

- Imaging-based studies demonstrate that transcription is a dynamic process
- Quantitative imaging methods have revealed transcriptional bursting
- Changes in transcription machineries can modulate transcriptional dynamics
- Transcription factors search through target DNA with distinct kinetics

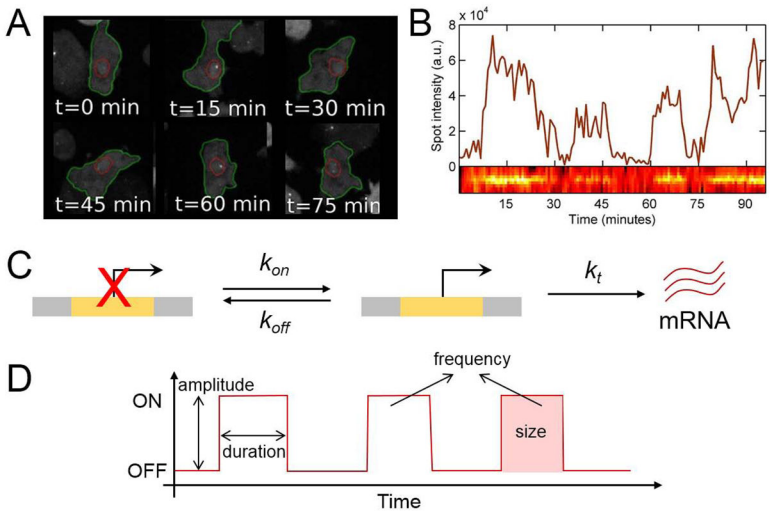

Figure 1. Transcriptional regulation modules that affect transcriptional dynamics

(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.

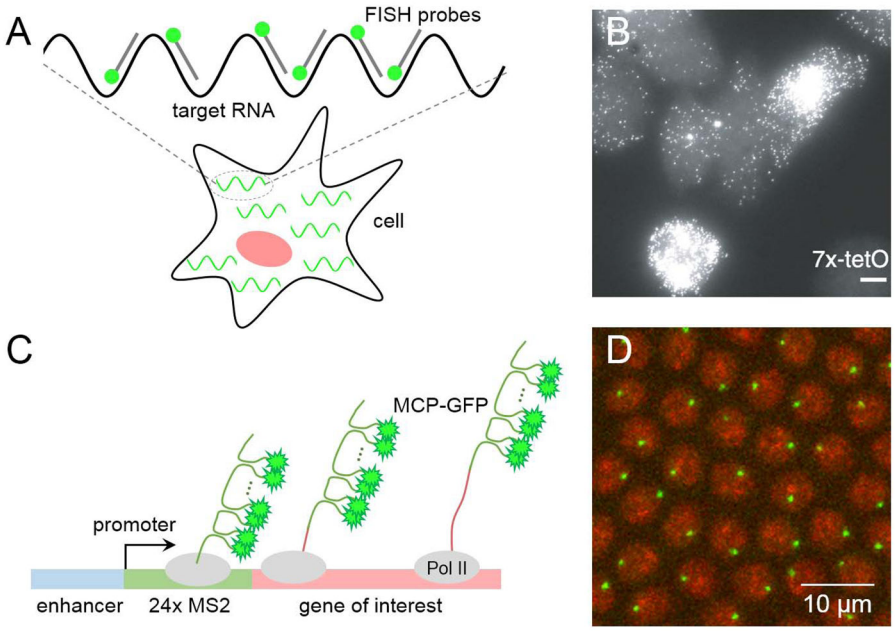

Figure 2. Imaging techniques for visualizing nascent transcription activity

(A) Schematic of single molecule fluorescent *in situ* hybridization (smFISH). Several short oligo probes that are complementary to the target RNA are designed to detect individual RNA molecules. Binding of multiple fluorescently labeled probes to the target RNA provides enough sensitivity and allows detection of single nascent RNA transcripts within cells.

(B) A representative image of cells from clonal cell lines where each mRNA is hybridized to smFISH probes (image from [6]).

(C) Schematic of MS2-MCP imaging method. The system uses bacteriophage MS2 and MS2 coat proteins (MCP) that is labeled with fluorescent proteins such as GFP. Typically, 6–24 tandem repeats of MS2 sequences are being inserted to the 5′ or 3′ untranslated region (UTR) of the gene of interest. Upon transcription, MS2 forms a stem loop and dimeric MCP-GFP binds to each stem loop, allowing the detection of *de novo* transcripts through fluorescent imaging.

(D) A representative snapshot of an early *Drosophila* embryo, where nascent transcripts are visualized with the MS2-MCP system. Nuclei are shown in red and nascent transcripts are shown in green.

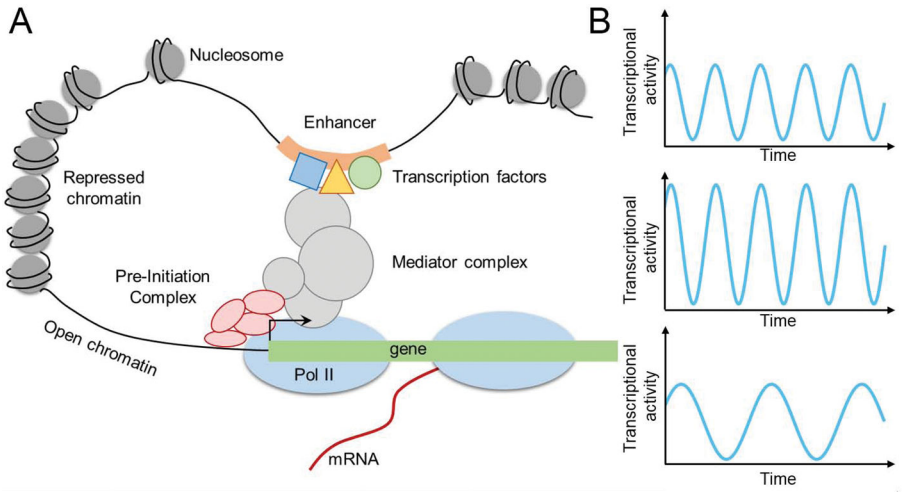

Figure 3. Visualization of transcriptional bursting and characterization of bursting parameters

(A) Snapshots of living *Dictyostelium* cells where transcription activity of *actin5* is detected as a fluorescent spot. (adapted from [22])

(B) Fluorescent intensity for the cells shown in (A), which reveals transcriptional bursting of *actin5*. (Image from [22]).

(C) Schematic of the "two-state" model of transcription. A promoter is either in an *OFF* state or in an *ON* state with the probability k_{on} and k_{off}, respectively. Only when the promoter is in an *ON* state, it can produce *mRNA* at a rate of k_t.

(D) Description of bursting parameters such as bursting amplitude, duration, frequency, and size.