

Trends in Genetics

What is a transcriptional burst?

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Corresponding Author:	Jonathan Chubb UNITED KINGDOM
First Author:	Ed Tunnacliffe
Order of Authors:	Ed Tunnacliffe Jonathan Chubb
Abstract:	<p>The idea that gene activity can be discontinuous will not surprise many biologists – many genes are restricted in when and where they can be expressed. Yet during the past 15 years, a collection of observations compiled under the umbrella term ‘transcriptional bursting’ has received considerable interest. Direct visualization of the dynamics of discontinuous transcription has expanded our understanding of basic transcriptional mechanisms and their regulation, and provides a real-time readout of gene activity during the life of a cell. In this review, we try to reconcile the different views of the transcriptional process emerging from studies of bursting, and how this work contextualizes the relative importance of different regulatory inputs to normal dynamic ranges of gene activity.</p>

What is a transcriptional burst?

Ed Tunnacliffe¹ and Jonathan R Chubb²

¹MRC Molecular Haematology Unit, MRC Weatherall Institute of Molecular Medicine,
University of Oxford, John Radcliffe Hospital, Headington, Oxford, OX3 9DS. ²MRC
Laboratory for Molecular Cell Biology, University College London, Gower Street,
London, WC1E 6BT.

Correspondence to edward.tunnacliffe@ndcls.ox.ac.uk

12 **Abstract**

13 The idea that gene activity can be discontinuous will not surprise many biologists –
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Introduction

Without even considering prominent themes such as the need for genes to be turned on and off at different events during development, the dynamic nature of cell signaling, and the metastability of many protein complexes, direct evidence of discontinuous transcription is not recent. When viewed under an electron microscope, Miller chromatin spreads from the fruit fly embryo showed unequal distribution of nascent transcripts along gene sequences (Figure 1A)(McKnight and Miller, 1979). The gaps between groups of multiple transcripts were interpreted as interruptions in transcription initiation events.

Attempts to directly visualize transcription were far from the mainstream for the next 25 years, with the emphasis instead on defining transcriptional regulatory components and their interactions. These reductionist strategies were essential for determining the molecular players involved, but lacked certain features necessary for building a more complete view of the transcriptional process. Firstly, the measurements were static, merging transcription and RNA degradation into a single RNA quantity. Secondly, samples were ensemble, usually the average of millions of cells, blurring the dynamics of the activity of individual genes. Finally, the biochemical and genetic strategies used to define transcriptional complexes take cells out of their normal dynamic range of signaling, making meaningful physiological models of the transcriptional process difficult.

Solving these issues of reductionism required the ability to see transcription of single genes, in single cells, in an appropriate physiological context. This needed improvements in fluorescence microscopy, to approach the speed and sensitivity required for single molecule imaging in living cells, combined with the development of appropriate RNA labeling strategies for transcript detection. More specifically, the application of single molecule fluorescence *in situ* hybridization (smFISH; Femino et al., 1998) and MS2 stem-loop-based detection (Figure 1B)(Bertrand et al., 1998) corroborated transcriptional discontinuity apparent in Miller spreads (Chubb et al., 2006; Golding et al., 2005; Raj et al., 2006). These approaches also highlighted the dynamics of transcriptional events. The ‘bursts’ or ‘pulses’ of transcriptional activity were found to operate over timescales of a few minutes (Figure 1C), and were measurably responsive to multiple features of cell context. This study of this phenomenon has since expanded to many different organisms, both prokaryotic and

eukaryotic, with an increasing number of mechanistic models used to explain the different dynamic behaviors observed across a variety of genes. Here we collate and review these models, focusing on recent studies where features of transcriptional mechanism have been explored through the study of bursts, and how these features can be linked to specific molecular regulatory events.

What is a burst?

The term ‘transcriptional bursting’ has been employed to explain a range of potentially different phenomena. There is nothing implicit in the word ‘burst’ that implies a specific model, mechanism or dynamic behavior, beyond being discontinuous. Although the term is vague, the descriptions of bursting have often been highly quantitative and integrated with simple models of gene activity. Typically, a model applied to a bursting phenotype will focus on the number of ‘states’ or levels of activity at which a gene can be transcribed (Figure 2). Although a simple ‘one-state’ model based on a fixed initiation rate can give rise to fluctuations in transcriptional activity (Corrigan et al., 2016; Larson et al., 2011) and such a model can fit well to distributions of smFISH RNA counts for a few genes (Zenklusen et al., 2008), the complexity offered by these models is not sufficient to explain the dynamic behavior of most genes that have been studied. A two-state or random telegraph model (Peccoud and Ycart, 1995) has been more widely adopted. Here, a gene is only transcribed when in an ‘active’ configuration. Fluctuations between this ‘active’ state and an ‘inactive’ state, result in short spurts of mRNA production interspersed with periods of no activity (Raj et al., 2006). This model is now widely used to explain how pulsatile mRNA synthesis is controlled, particularly when inferring dynamics from fixed-cell smFISH transcript distributions. It has also been used in genome-wide studies to show how transcriptional dynamics can explain developmental gene expression heterogeneity (Antolović et al., 2017) and to understand broad mechanisms of sequence-encoded regulation (Larsson et al., 2019).

As a recent example, using a two-state model, Zoller et al. (2018) uncovered a common regulatory mechanism governing transcription of gap genes in *Drosophila*. Comparisons between mRNA count distributions showed almost identical statistical relationships for all four genes studied. Modulation of promoter occupancy alone was found to be sufficient to explain the common regulation, with

91 tight coupling of ON and OFF switching rates resulting in the emergence of a unified
92 pattern of transcriptional control for all genes (Zoller et al., 2018). A similar coupling
93 of switching rates was found in live imaging experiments using *even-skipped*, which
94 is regulated by the gap gene transcription factors (TFs) (Berrocal et al., 2018).

95 However, experiments on developmentally matched gene sets in *Dictyostelium* do
96 not exhibit such statistical similarities in bursting activity (Tunnacliffe et al., 2018)
97 suggesting such unified control suits rigidly instructive forms of development, such
98 as in the *Drosophila* embryo, rather than more responsive developmental systems.

99 Despite its widespread use, the assumptions of the standard two-state model
100 – constant rates for initiation, degradation, and switching between active and inactive
101 states – are unrealistic in many biological systems. Transcription changes in
102 response to a multitude of signals, yet the model does not easily account for this.
103 These assumptions rather marginalise bursting as a side issue of transcription,
104 failing to accommodate extrinsic sources of variation (such as signaling to
105 transcription), with bursting consigned to only those processes designated intrinsic
106 (molecular noise). This case of the model ‘owning’ the bursting phenomenon is
107 widespread, but rather unusual if one considers bursting as the dynamic
108 manifestation of the complete transcriptional process. Beyond these issues, it is now
109 clear the random telegraph model cannot accurately describe transcription kinetics
110 for all genes, in all systems. The use of fixed-cell approaches can be limiting when
111 exploring alternative models of regulation; theoretical work has shown how dynamic
112 measurements, rather than transcript counting by smFISH, must be made in order to
113 distinguish between certain promoter state conformations (such as two-state and
114 some three-state models) (Rieckh and Tkačik, 2014; Zhang and Zhou, 2014). In
115 keeping with this, a gene found by live imaging to show a spectrum of activity states
116 would be well-described by a two-state model if assayed by smFISH (Corrigan et al.,
117 2016).

118 *More complexity, less consensus*

119 If a two-state model is largely unsatisfactory as a description of transcription, what
120 alternatives are there? Models containing multiple promoter activity states have been
121 employed theoretically to account for experimental data in numerous cell types
122 (Blake et al., 2003; Coulon et al., 2010; Sanchez et al., 2011; Sanchez and Kondev,
123 2008), with imaging studies demonstrating more explicitly that an expansion of the
124

two-state model architecture could be appropriate (Bothma et al., 2014; Senecal et al., 2014). A four-state model with a single inactive state was identified as the best fit to smFISH data for a small number of stress-response genes in yeast (Neuert et al., 2013). Multiple timescales of transcriptional bursting were also inferred from measurements of HIV-1 promoter activity using the MS2 system in mammalian cells (Tantale et al., 2016). Here, TATA-binding protein (TBP) and mediator were found to independently regulate gene activity on these alternate timescales, and a three-state model of transcription was proposed, with inactive and active states as well as an intermediate 'permissive' state.

Multi-state models containing a 'refractory' inactive state through which a gene promoter must pass before reactivation can occur have also emerged (Figure 2) (Cesbron et al., 2015; Harper et al., 2011; Snyder et al., 2011; Suter et al., 2011). Endogenous promoters were typically found to pass through 5-7 sequential inactive steps before reactivation, while synthetic or TATA-containing promoters had fewer inactive steps which resulted in noisier gene expression (Zoller et al., 2015). Bartman et al. (2019) combined PolII-chromatin immunoprecipitation (ChIP) with smFISH, and also identified refractory period-based models as consistent with their data, although their preferred model involved burst initiation and polymerase pause release as limiting steps in gene activation. Refractoriness has also been proposed to enable rapid, sensitive responses to stimuli, where the concentration of a particular TF regulates frequency of transcriptional bursting (Li et al., 2018). While such a refractory period has now been described across several systems and genes, in terms of information transmission, which is presumably vital for fast responses, this type of system may be less favourable than a simple two-state model of gene expression (Rieckh and Tkačik, 2014). Another consideration is whether the refractory period is a transcriptional phenomenon, or merely an adaptation response in the upstream signaling, such as phosphatase activity or receptor down-regulation (Sorkin and von Zastrow, 2009).

Rodriguez et al. (2019) also found inefficient information transfer in multi-state transcription while studying *TFF1* regulation in MCF7 cells. Here, a model containing three 'gene states' (two inactive, one permissive) and two 'RNA steps' (activity levels in the ON state) was the best fit to the data from an MS2 reporter cell line, with a highly inactive state occupied for extremely long periods of time. By measuring changes in chromatin contacts in response to an estradiol (E2) stimulus the authors

showed that while cells can effectively sense multiple levels of E2 dose, the information transfer to transcriptional output is inefficient and slow. While it is unclear why such regulatory schemes have evolved in this way, it could represent a similar process of robustness through sub-optimisation of the network, a concept also applied to sub-optimal binding of TFs to developmental enhancers (Farley et al., 2015) and core promoter sites (Ravarani et al., 2016). Alternatively, these observations may imply that a coherent transcriptional response is only likely in the presence of the full complement of signals available in a normal tissue niche, with measurement of these additional signals likely to provide more explanatory power (Corrigan and Chubb, 2014).

Although the inclusion of additional activity states can improve the fit between a model and experimental data, how far should one go with this? Is there an upper limit to the descriptive benefits of increased model complexity? A continuum or spectrum of activity states, rather than a discrete number, can provide the best fit to dynamic expression data from genes in diverse systems (Figure 2)(Corrigan et al., 2016; Featherstone et al., 2016). Intuitively, this makes sense given the myriad of molecular inputs influencing gene transcription. Whether a continuum actually represents many discrete activity states which simply cannot be resolved is unclear and such distinctions may remain elusive. In their paper describing a general multi-state mathematical framework for transcriptional bursting, Klindziuk and Kolomeisky (2018) show that it may be difficult to determine the precise number of activity states, particularly if the time spent in each is very short. If the number of regulatory inputs (and therefore perhaps the number of activity states) of transcription is high, and the relative time spent in individual regulatory conformations is low, it will be very difficult to distinguish these states accurately. Along these lines, a fast switching model emerged as the most appropriate scenario to explain transcript counts from the lysogeny maintenance promoter of lambda phage (Sepulveda et al., 2016).

Finally, it is not the case that simply adding more activity states to a computational model provides a better fit to experimental data. Fritzsche et al. (2018) explored the E2-regulated *GREB1* gene in MCF7 cells and found that despite sampling several multi-state models (with up to 10 discrete levels), a two-state model gave the best fit to their data. Therefore, while use of a two-state model to describe transcriptional bursting of a gene should not be the default position, equally, a multi-state architecture of some form is not guaranteed to be more descriptive.

With so many different models describing gene regulation, is it possible to derive general principles of transcriptional bursting? Which, if any, of the conformations described above could be relevant more generally to describe transcription? Should we even expect consensus, especially considering the diversity in the genes and experimental systems, and the different methods that have been employed? Diversity in bursting is clear even in more closely-related contexts. Comparing separate detailed studies of bursting in oestrogen-inducible genes, where similar regulation might be expected, highlights different regulatory regimes. As previously mentioned, Rodriguez et al. (2019) proposed a model containing five activity levels, including a deep repressive state defined by long periods of inactivity for *TFF1*, even at saturating E2 concentrations in MCF7 cells. On the other hand, in the same cells with similar saturating induction conditions, *GREB1* showed near-constant activity in most cells and a simple two-state model was preferable to those with multiple states and circular architecture (Fritzsche et al., 2018). Despite their different cellular functions, these genes previously showed similarly strong induction by E2 stimulation in multiple cell types (Lefebvre et al., 1996; Rae et al., 2005). While this comparison is somewhat limited in scope, it shows that even genes with superficially similar regulation can be subject to very different dynamic control. Therefore, the regulation of bursting may well be highly gene-specific and will depend, potentially to differing extents, on the multiple different inputs to gene regulation. Despite the apparent convergence of regulatory mechanisms in certain specific contexts (Zoller et al., 2018), any substantial coherence between models of transcriptional bursting will require a more detailed understanding of the relative contributions of the processes affecting bursts.

Making bursts

Local DNA sequences

As the scaffold for RNA polymerase loading onto a gene, the promoter represents an important integration zone for transcriptional control (Haberle and Stark, 2018). Sequence diversity permits enormous heterogeneity in transcriptional output (Weingarten-Gabbay et al., 2019) and individual promoter cis-regulatory elements have been shown to influence transcriptional bursting at the single-cell level (Corrigan et al., 2016; Hendy et al., 2017). Even within a family of duplicated actin genes encoding exactly the same protein, considerable diversity in bursting patterns

was identified (Tunnacliffe et al., 2018). The role of the upstream sequence was directly evaluated by a reciprocal switching experiment exchanging around 500bp of the proximal 5' regulatory regions of genes with different bursting patterns. This treatment revealed bursting dynamics to be almost entirely instructed by the upstream regulatory sequence with only a minor role for features specific to genomic context.

At least superficially, this result goes against the grain of some earlier ideas on the origins of bursts, which suggested switching between ON and OFF states reflects chromatin remodeling (Golding and Cox, 2006). Clearly, chromatin regulation is an important part of transcriptional control, and several studies have shown that disruption of the normal chromatin landscape can affect bursting (Chen et al., 2019a; Dunham et al., 2017; Mehta et al., 2018; Muramoto et al., 2010; Zoller et al., 2015). Recent live imaging studies directly showed an increase in H3K27ac levels immediately prior to the appearance of active forms of RNA pol II at transcriptionally active nuclear compartments in early zebrafish development (Hadzhiev et al., 2019; Sato et al., 2019). This is consistent with a prominent role for the chromatin environment in influencing transcriptional decisions, although it is not clear if the sensitivity of detecting the different chromatin and polymerase modifications is equivalent. Similarly, histone acetylation was also found to regulate burst frequency-mediated changes in circadian clock gene expression (Nicolas et al., 2018). A role for chromatin modification and remodeling is evident – chromatin is the substrate, it is close to the action, it is to be expected that damaging it will affect transcription. But to what extent does it drive the dynamics? Given the direct demonstration that actin gene bursts can be dominated by the promoter region (Tunnacliffe et al., 2018) as well as other data showing similar bursting patterns at multiple genomic loci (Hocine et al., 2015; Zoller et al., 2015), our current view is that although chromatin is crucial for the functional integrity of the bursting process, it does not instruct the dynamic behaviour.

Bursting is influenced by distal enhancers as well as proximal promoters, with these elements directly involved in regulating transcriptional bursts, predominantly by modulating the frequency of these events (Bartman et al., 2016; Fritzsche et al., 2018; Fukaya et al., 2016; Larson et al., 2013; Li et al., 2018; Rodriguez et al., 2019). Genome wide inferences from single cell RNAseq data suggest regulation of burst frequency is the most widespread method of modulating gene regulation during

developmental progression (Antolović et al., 2017), with enhancers likely to be a major control point for this regulation (Larsson et al., 2019). However, enhancer regulation by modulating burst frequency is not universal, with burst size regulation predominating in response to Notch signalling (Falo-Sanjuan et al., 2019; Lee et al., 2019). Further complexity arises when considering the combined effects of multiple enhancers at different times and places in the embryo (Lim et al., 2018; Scholes et al., 2019).

The importance of enhancer-promoter proximity for bursts has recently been evaluated using dual labelling of both DNA and nascent RNA in live cells. Dynamic transcription was found to be both correlated (Chen et al., 2018) and uncorrelated (Alexander et al., 2019) with enhancer-promoter proximity, suggesting a number of models are required to explain enhancer activity. Indeed, such a dichotomy exists even at a single locus, as different tissue-specific enhancers of the *Shh* gene showed both increased (Williamson et al., 2016) and decreased (Benabdallah et al., 2019) enhancer-promoter proximity concomitant with gene activation. The rules governing enhancer-mediation of dynamic transcription in tissues are seemingly complex, and will likely depend on the specific transcription and structural factors bound there at any particular time, in addition to higher order features of the nuclear microenvironment (Dufourt et al., 2018; Mir et al., 2018).

Transcription factors

The binding of transcription factors (TFs) to target motifs at both promoter and enhancer elements is key to activation of a gene yet, until recently, it has not been clear how TF binding events are dynamically related to transcriptional activity. Residence times of TF binding at target sites are typically on the order of seconds (Chen et al., 2014; Mazza et al., 2012), which contrasts the timescales of minutes usually associated with bursts.

While single-molecule tracking (SMT) methods have enabled the study of individual TF molecule binding dynamics, it has remained challenging to assess the importance of these events to transcription of a specific gene of interest, given the many other potential binding sites for the TF within the genome. New imaging methods have made headway in solving this issue. One approach uses 3D orbital tracking (3DOT) to simultaneously monitor transcriptional dynamics from a PP7 reporter together with binding of individual Halo-tagged TF molecules. Unlike

conventional confocal microscopy, 3DOT only collects intensity information from the site of transcription via orbital scanning of the sample, limiting the amount of photobleaching (Annibale and Gratton, 2015; Kis-Petikova and Gratton, 2004; Levi et al., 2003). This method explicitly revealed the temporal coupling between TF binding and initiation of transcriptional bursts (Donovan et al., 2019; Stavreva et al., 2019). In yeast, for example, an average TF (GAL4) binding time of 34 seconds initiates a mean burst duration of around 2.5 minutes. An analogous approach to computationally ‘fix’ the transcription site during imaging is target-locking 3D STED (Li et al., 2019). This live cell super-resolution technique was used for simultaneous molecular quantitation and spatial mapping of protein factors at the transcription site. A number of surprising features of gene regulation were revealed for pluripotency markers. In particular, the Oct4 gene appears to have around 20 molecules of the TF Sox2 bound when active, contrasting the textbook view of a single TF binding and triggering a cascade of events. In addition, echoing the potential for transcription in the absence of enhancer-promoter communication (Alexander et al., 2019), Sox2 TFs were spatially distinct from the active transcription site (Figure 1D). These approaches are a significant advance for the field, and will allow a more detailed understanding of the molecular interplay driving a transcriptional burst. In particular, a detailed dissection of the relative contributions of different proteins and complexes to multi-state models of dynamic transcription is now seemingly within reach.

Concluding remarks

As bursting has finally entered mainstream thought, the challenge for the future is the same challenge faced by the entire study of transcription. How can we possibly formulate realistic models of dynamic transcriptional activity given the sheer number of factors influencing the process? The ability to directly visualize the interaction of different regulatory factors with transcriptional activity at loci of interest is a big step towards building such models. Limitations that need to be overcome include the restrictions on the number of different components that can be imaged in healthy living cells. Transcriptional regulation is often discussed in terms of complexes, but if one can only see a single component of a complex, then detailed mechanistic insight will remain elusive. Our impression is that the brute force approaches of drug treatment and genetics need to be superseded if we are to make more effective

models. Optogenetics potentially provides a more subtle way of perturbing a system (Chen et al., 2019b; Rullan et al., 2018; Wilson et al., 2017), although again, this takes the system outside its normal dynamic range, albeit in a potentially more specific manner. For all the reservations expressed here about the applicability of two state models, applying the new tools to genes which fit more simple regulatory regimes may yet provide the most straightforward route to a more complete understanding of bursting.

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Figure Legends

Figure 1: Approaches to visualize discontinuous transcription

A) Chromatin spreads from *Drosophila* embryos (image taken from McKnight and Miller, 1979). The image shows a pair of sister chromatids aligned in parallel, with inferred initiation sites marked by α and β . Note the increasing size of the fibres (transcripts) extending from the central axis of each chromatid with increasing distance from the initiation sites (scale bar $1\mu\text{m}$). Also note the fibre-free gaps (marked by arrows). B) Schematic of the MS2/MCP system for visualizing nascent transcripts. MS2 arrays are targeted into the gene of interest. The MS2 RNA forms stem loops, and can be detected at the site of transcription, as a fluorescent spot, by the MCP-GFP fusion protein. C) Transcription visualized using the MS2/MCP system, with stills from a movie sequence showing nascent RNA detected in bursts from the *act5* gene of *Dictyostelium* (scale bar $5\mu\text{m}$). Normalised spot intensity values are shown in the plot below the film strip, with yellow dots corresponding to the images. D) Combining imaging of transcription, using MS2/MCP, with imaging of transcription regulators (image taken from Li et al. 2019). Images show nascent transcript foci from the mouse Oct4 gene detected alongside different SNAP-tagged transcription factors (scale bars 300nm).

Figure 2: Models of transcriptional dynamics.

A non-exhaustive guide to different modeling frameworks used to describe transcriptional bursting dynamics. See text for details.

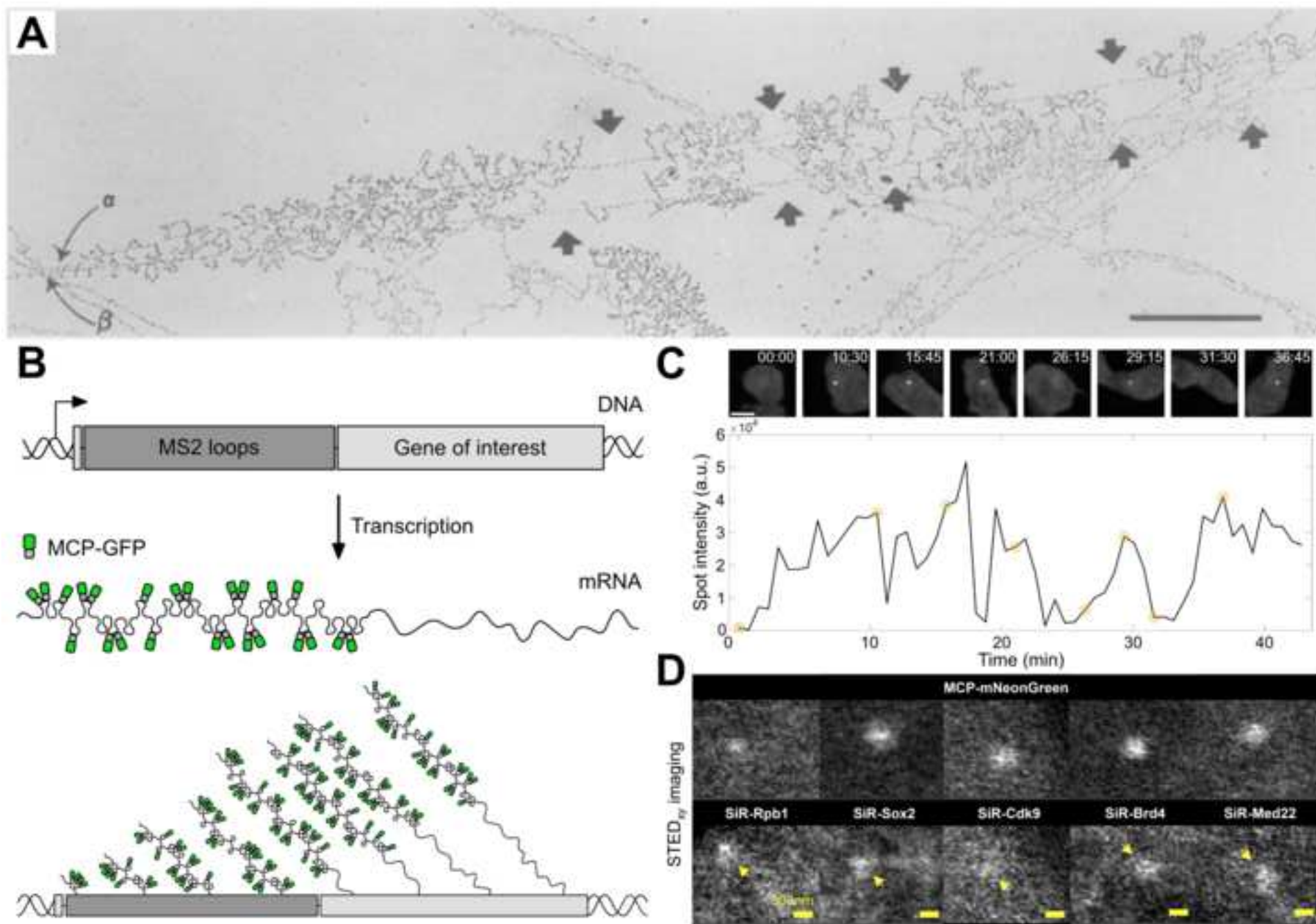
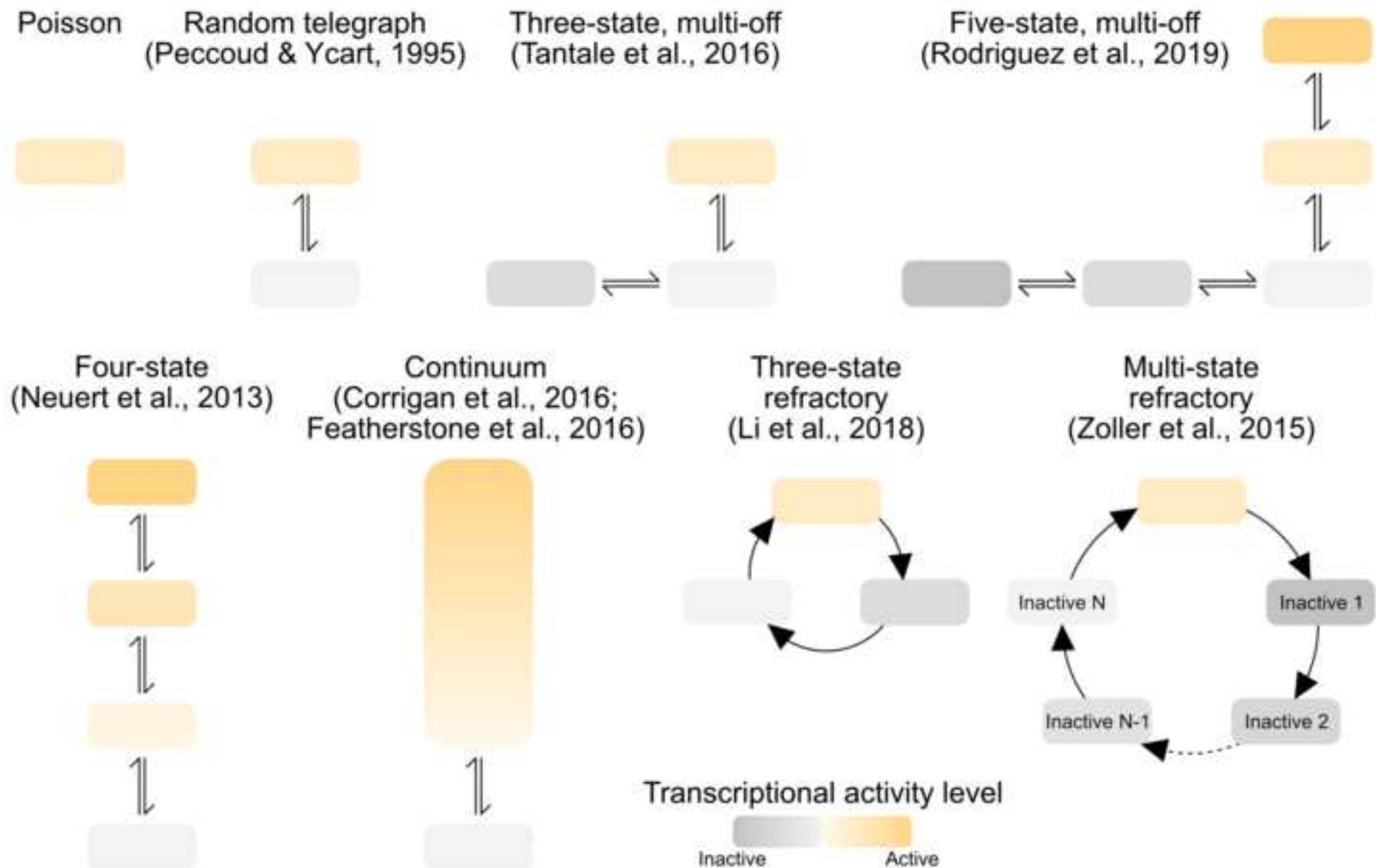


Figure 2



Highlights

- Demystification of the term “transcriptional bursting”
- Models with one or two gene states are unable to accurately describe dynamic transcription for many genes
- Many alternative multi-state models have been proposed but these are likely to be highly context-specific
- Understanding the contributions of numerous different cellular features and processes to bursting is required to build more accurate and general models of transcription dynamics
- Emerging imaging technologies are beginning to facilitate the monitoring of these diverse sources of regulation

Outstanding questions:

1. What are the relative contributions of the numerous regulatory inputs – involving tens, if not hundreds of cellular components – to transcriptional bursting?
2. How are the effects of these inputs integrated to generate the bursting patterns we observe?
3. What are the barriers to information transfer from cellular signalling to transcriptional apparatus? Is it really chromatin, or is the barrier function distributed through the regulatory network of the cell?
4. Does the apparently haphazard nature of transcription have any benefit for the organism, or is it simply a tolerable level of disorder?