Trends in Genetics

What is a transcriptional burst?

--Manuscript Draft--

Manuscript Number:	TIGS-D-19-00210R1
Article Type:	Review
Keywords:	transcriptional bursting; single cell gene expression; single molecule imaging; stochastic gene expression; MS2; smFISH
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Abstract:	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.

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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

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2 **What is a transcriptional burst?**
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13 Keywords: transcriptional bursting, single cell gene expression, stochastic gene
14 expression, MS2, smFISH

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.

An Introduction

If we accept that genes can be 'on' or 'off' and that cells are able to change their gene expression, then it requires no major leap of faith to accept the possibility that transcription can be discontinuous over time. Indeed, direct visual evidence of discontinuous transcription emerged as early as the 1970s. 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) [1]. 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 regulatory components, by their very nature, detach gene regulation from normal cell physiology, making it difficult to arrive at meaningful models of the transcriptional process.

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 (Box 1). More specifically, the application of single molecule fluorescence *in situ* hybridization (smFISH) [2] on fixed cells and MS2 stem-loop-based detection (Figure 1B) [3] in living cells, corroborated the temporal transcriptional discontinuity inferred from Miller spreads [4-6]. 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 features such as developmental time and local environment. The study of this phenomenon has since expanded to many different organisms, both prokaryotic and

60 eukaryotic, with an increasing number of mechanistic models used to explain the
61 different dynamic behaviors observed across a variety of genes. Here we collate and
62 review these models, focusing on recent studies where features of transcriptional
63 mechanism have been explored through the study of bursts, and how these features
64 can be linked to specific molecular regulatory events.
65

66 **What is a Burst?**

67 The term 'transcriptional bursting' has been employed to explain a range of
68 potentially different phenomena. There is nothing implicit in the word 'burst' that
69 implies a specific model, mechanism or dynamic behavior, beyond being
70 discontinuous over time. Although the term is vague, the descriptions of bursting
71 have often been highly quantitative and integrated with simple models of gene
72 activity. Typically, a model applied to a bursting phenotype will focus on the number
73 of 'states' or levels of activity at which a gene can be transcribed (Figure 2, Key
74 Figure). Although a simple 'one-state' model based on a fixed initiation rate can give
75 rise to fluctuations in transcriptional activity [7, 8] and such a model can fit well to
76 distributions of smFISH RNA counts for a few genes [9], the complexity offered by
77 these models is not sufficient to explain the dynamic behavior of most genes that
78 have been studied. A two-state or random telegraph model [10] has been more
79 widely adopted. Here, a gene is only transcribed when in an 'active' configuration.
80 Fluctuations between this 'active' state and an 'inactive' state, result in short spurts
81 of mRNA production interspersed with periods of no activity [6]. This model is now
82 widely used to explain how pulsatile mRNA synthesis is controlled, particularly when
83 inferring dynamics from fixed-cell smFISH transcript distributions. It has also been
84 used in genome-wide studies to show how transcriptional dynamics can explain
85 developmental gene expression heterogeneity [11] and to understand broad
86 mechanisms of sequence-encoded regulation [12].

87 As a recent example, using a two-state model [13] uncovered a common
88 regulatory mechanism governing transcription of gap genes in *Drosophila*.
89 Comparisons between mRNA count distributions showed almost identical statistical
90 relationships for all four genes studied. Modulation of promoter occupancy alone was
91 found to be sufficient to explain the common regulation, with tight coupling of ON and
92 OFF switching rates resulting in the emergence of a unified pattern of transcriptional
93 control across the gene set [13]. A similar coupling of switching rates was found in

94 live imaging experiments using *even-skipped*, which is regulated by the gap gene
95 transcription factors (TFs) [14]. However, experiments on developmentally matched
96 gene sets in *Dictyostelium* do not exhibit such statistical similarities in bursting
97 activity [15] suggesting such unified control suits rigidly instructive forms of
98 development, such as in the *Drosophila* embryo, rather than more responsive
99 developmental systems.
100 Despite its widespread use, the assumptions of the standard two-state model
101 – constant rates for initiation, degradation, and switching between active and inactive
102 states – are unrealistic in many biological systems. Transcription changes in
103 response to a multitude of signals, yet the model does not easily account for this.
104 These assumptions rather marginalise bursting as a side issue of transcription,
105 failing to accommodate extrinsic sources of variation (such as signaling to
106 transcription), with bursting consigned to only those processes designated intrinsic
107 (molecular noise). Ideally a model should be an informed attempt to explain the
108 biology, rather than a device that inadvertently excludes much that is interesting.
109 This case of the model 'owning' the bursting phenomenon is widespread, but rather
110 unusual if one considers bursting as the dynamic manifestation of the complete
111 transcriptional process. Beyond these issues, it is now clear the two-state model
112 cannot accurately describe transcription kinetics for all genes, in all systems. The
113 use of fixed-cell approaches can be limiting when exploring alternative models of
114 regulation; theoretical work has shown how dynamic measurements, rather than
115 transcript counting by smFISH, must be made in order to distinguish between certain
116 promoter state conformations (such as two-state and some three-state models) [16,
117 17]. In keeping with this, a gene found by live imaging to show a spectrum of activity
118 states would be well-described by a two-state model if assayed by smFISH [7].
119
120 More Complexity, Less Consensus
121 If a two-state model is largely unsatisfactory as a description of transcription, what
122 alternatives are there? Models containing multiple promoter activity states have been
123 employed theoretically to account for experimental data in numerous cell types [18-
124 21], with imaging studies demonstrating more explicitly that an expansion of the two-
125 state model architecture could be appropriate [22, 23]. In yeast, a four-state model
126 with a single inactive state was identified as the best fit to smFISH data for a small
127 number of stress-response genes [24]. Multiple timescales of transcriptional bursting

128 were also inferred from measurements of HIV-1 promoter activity using the MS2
129 system in mammalian cells [25]. Here, TATA-binding protein (TBP) and mediator
130 were found to independently regulate gene activity on these alternate timescales,
131 and a three-state model of transcription was proposed, with inactive and active
132 states as well as an intermediate 'permissive' state.
133 Multi-state models containing a 'refractory' inactive state through which a
134 gene promoter must pass before reactivation can occur have also emerged, from
135 studies using destabilized reporter proteins (Box 1; Figure 2) [26-28]. Endogenous
136 promoters were typically found to pass through 5-7 sequential inactive steps before
137 reactivation, while synthetic or TATA-containing promoters had fewer inactive steps
138 which resulted in noisier gene expression [29]. Bartman *et al.* [30] combined PolII-
139 chromatin immunoprecipitation (ChIP) with smFISH and also identified refractory
140 period-based models as consistent with their data, although their preferred model
141 involved burst initiation and polymerase pause release as limiting steps in gene
142 activation. Refractoriness is often associated with a 'reset' of molecular components
143 in preparation for receiving a new stimulus. A less intuitive role for refractoriness in
144 transcription may be to enable rapid and sensitive responses to stimuli [31]. Models
145 of promoter progression, in which events at the promoter form an ordered sequence
146 of recruitment of different parts of the transcriptional machinery, may be consistent
147 with refractory behaviour [32]. An alternative view questions whether the refractory
148 period is a transcriptional phenomenon, or merely an adaptation response in the
149 upstream signaling, such as phosphatase activity or receptor down-regulation [33].
150 While refractoriness has now been described across several systems and genes, in
151 terms of information transmission, this type of system may be less favourable than a
152 simple two-state model of gene expression [16].
153 Rodriguez *et al.* [34] also found inefficient information transfer in multi-state
154 transcription while studying *TFF1* regulation in MCF7 cells. Here, a model containing
155 three 'gene states' (two inactive, one permissive) and two 'RNA steps' (activity levels
156 in the ON state) was the best fit to the data from an MS2 reporter cell line, with a
157 highly inactive state occupied for extremely long periods of time. By measuring
158 changes in chromatin contacts in response to an estradiol (E2) stimulus the authors
159 showed that while cells can effectively sense multiple levels of E2 dose, the
160 information transfer to transcriptional output is inefficient and slow. While it is unclear
161 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 [35] and core promoter sites [36]. 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 [37].

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) [7, 38]. 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, [39] 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 difficult to distinguish these states accurately. Along these lines, a fast switching model emerged as the most appropriate scenario to explain transcript output from the lysogeny maintenance promoter of lambda phage [40].

Finally, it is not the case that simply adding more activity states to a computational model provides a better fit to experimental data. Fritzsch *et al.* [41] 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

196 diversity in the genes and experimental systems, and the different methods that have
197 been employed? Diversity in bursting is clear even in more closely-related contexts.
198 Comparing separate detailed studies of bursting in oestrogen-inducible genes, where
199 similar regulation might be expected, highlights different regulatory regimes. As
200 previously mentioned, Rodriguez *et al.* [34] proposed a model containing five activity
201 levels, including a deep repressive state defined by long periods of inactivity for
202 *TFF1*, even at saturating E2 concentrations in MCF7 cells. On the other hand, in the
203 same cells with similar saturating induction conditions, *GREB1* showed near-
204 constant activity in most cells and a simple two-state model was preferable to those
205 with multiple states and circular architecture [41]. Despite their different cellular
206 functions, these genes previously showed similarly strong induction by E2
207 stimulation in multiple cell types [42, 43]. While this comparison is somewhat limited
208 in scope, it shows that even genes with superficially similar regulation can be subject
209 to very different dynamic control. Therefore, the regulation of bursting may well be
210 highly gene-specific and will depend, potentially to differing extents, on the multiple
211 different inputs to gene regulation. Despite the apparent convergence of regulatory
212 mechanisms in certain specific contexts [13], any substantial coherence between
213 models of transcriptional bursting will require a more detailed understanding of the
214 relative contributions of the processes affecting bursts.
215
216 **Making Bursts**
217 Cis-Regulation
218 As the scaffold for RNA polymerase loading onto a gene, the promoter represents an
219 important integration zone for transcriptional control [44]. Sequence diversity permits
220 enormous heterogeneity in transcriptional output [45] and individual promoter cis-
221 regulatory elements have been shown to influence transcriptional bursting at the
222 single-cell level [7, 46]. Even within a family of duplicated actin genes encoding
223 exactly the same protein, considerable diversity in bursting patterns was identified
224 [15]. The role of the upstream sequence was directly evaluated by a reciprocal
225 switching experiment exchanging around 500bp of the proximal 5' regulatory regions
226 of genes with different bursting patterns. This treatment revealed bursting dynamics
227 to be almost entirely instructed by the upstream regulatory sequence with only a
228 minor role for features specific to genomic context.

229 At least superficially, this result goes against the grain of some earlier ideas
230 on the origins of bursts, which suggested switching between ON and OFF states
231 reflects chromatin remodeling [47]. Clearly, chromatin regulation is an important part
232 of transcriptional control, and several studies have shown that disruption of the
233 normal chromatin landscape can affect bursting [29, 48-51]. Recent live imaging
234 studies directly showed an increase in H3K27ac levels immediately prior to the
235 appearance of active forms of RNA pol II at transcriptionally active nuclear
236 compartments in early zebrafish development [52, 53]. This is consistent with a
237 prominent role for the chromatin environment in influencing transcriptional decisions,
238 although it is not clear if the sensitivity of detecting the different chromatin and
239 polymerase modifications is equivalent. Similarly, histone acetylation was also found
240 to regulate burst frequency-mediated changes in circadian clock gene expression
241 [54]. A role for chromatin modification and remodeling is evident – chromatin is the
242 substrate, it is close to the action- it is almost expected that experimentally
243 perturbing chromatin will affect transcription. But to what extent do chromatin
244 changes drive bursting dynamics? Given the direct demonstration that actin gene
245 bursts can be dominated by the promoter region [15] as well as other data showing
246 similar bursting patterns at multiple genomic loci [29, 55], our current view is that
247 although chromatin is crucial for the functional integrity of the bursting process, it
248 does not instruct the dynamic behaviour.
249 Bursting is influenced by distal enhancers as well as proximal promoters, with
250 these elements directly involved in regulating transcriptional bursts, predominantly by
251 modulating the frequency of these events [31, 34, 41, 56-58]. Genome wide
252 inferences from single cell RNAseq data suggest regulation of burst frequency is the
253 most widespread method of modulating transcription during developmental
254 progression [11], with enhancers likely to be a major control point for this regulation
255 [12]. However, enhancer regulation by modulating burst frequency is not universal,
256 with burst size regulation predominating in response to Notch signalling [59, 60].
257 Further complexity arises when considering the combined effects of multiple
258 enhancers at different times and places during embryogenesis [61, 62].
259 The importance of enhancer-promoter proximity for bursts has recently been
260 evaluated using dual labelling of both DNA and nascent RNA in live cells. Dynamic
261 transcription was found to be both correlated [63] and uncorrelated [64] with
262 enhancer-promoter proximity, suggesting a number of models are required to explain

263 enhancer activity. Indeed, such a dichotomy exists even at a single locus, as
264 different tissue-specific enhancers of the *Shh* gene showed both increased [65] and
265 decreased [66] enhancer-promoter proximity concomitant with gene activation. The
266 rules governing enhancer-mediation of dynamic transcription in tissues are
267 seemingly complex, and will likely depend on the specific transcription and structural
268 factors bound there at any particular time, in addition to higher order features of the
269 nuclear microenvironment [67, 68]. Current excitement for potential roles of liquid-
270 liquid phase separation (LLPS) in forming compartments that enhance the efficiency
271 of transcriptional reactions has been discussed elsewhere [69], although at the time
272 of writing, there is a lack of convincing experimental evidence that compartments
273 formed by LLPS bring any functional benefits [70].
274
275 Transcription Factors
276 The binding of transcription factors (TFs) to target motifs at both promoter and
277 enhancer elements is key to activation of a gene yet, until recently, it has not been
278 clear how TF binding events are dynamically related to transcriptional activity.
279 Residence times of TF binding at target sites are typically on the order of seconds
280 [71, 72], which contrasts the timescales of minutes usually associated with bursts.
281 While single-molecule tracking (SMT) methods have enabled the study of
282 individual TF molecule binding dynamics, it has remained challenging to assess the
283 importance of these events to transcription of a specific gene of interest, given the
284 many other potential binding sites for the TF within the genome. New imaging
285 methods have made headway in solving this issue. One approach uses 3D orbital
286 tracking (3DOT) to simultaneously monitor transcriptional dynamics from a PP7
287 reporter together with binding of individual Halo-tagged TF molecules. Unlike
288 conventional confocal microscopy, 3DOT only collects intensity information from the
289 site of transcription via orbital scanning of the sample, limiting the amount of
290 photobleaching [73-75]. This method explicitly revealed the temporal coupling
291 between TF binding and initiation of transcriptional bursts [76, 77]. In yeast, for
292 example, an average TF (GAL4) binding time of 34 seconds initiates a mean burst
293 duration of around 2.5 minutes. An analogous approach to computationally 'fix' the
294 transcription site during imaging is target-locking 3D STED [78]. This live cell super-
295 resolution technique was used for simultaneous molecular quantitation and spatial
296 mapping of protein factors at the transcription site. A number of surprising features of

297 gene regulation were revealed for pluripotency markers. In particular, the gene
298 encoding Oct4 (*Pou5f1*) appears to have around 20 molecules of the TF Sox2
299 clustered nearby when active, contrasting the textbook view of a single or dimeric TF
300 binding and triggering a cascade of events. In addition, echoing the potential for
301 transcription in the absence of enhancer-promoter communication [64], Sox2 TFs
302 were spatially distinct from the active transcription site (Figure 1D). These
303 approaches are a significant advance for the field, and will allow a more detailed
304 understanding of the molecular interplay driving a transcriptional burst. In particular,
305 a detailed dissection of the relative contributions of different proteins and complexes
306 to multi-state models of dynamic transcription is now seemingly within reach.
307
308 **Concluding Remarks**
309 As bursting has finally entered mainstream thought, the challenge for the future is
310 the same challenge faced by the entire study of transcription (see 'Outstanding
311 Questions'). How can we possibly formulate realistic models of dynamic
312 transcriptional activity given the sheer number of factors influencing the process?
313 The ability to directly visualize the interaction of different regulatory factors with
314 transcriptional activity at loci of interest is a big step towards building such models.
315 Limitations that need to be overcome include the restrictions on the number of
316 different components that can be imaged in healthy living cells. Transcriptional
317 regulation is often discussed in terms of complexes, but if one can only see a single
318 component of a complex, then detailed mechanistic insight will remain elusive. Our
319 impression is that the brute force approaches of drug treatment and genetics need to
320 be superseded if we are to make more effective models. Optogenetics potentially
321 provides a more subtle way of perturbing a system [79-81], although again, this
322 takes the system outside its normal dynamic range, albeit in a potentially more
323 sensitive manner. For all the reservations expressed here about the applicability of
324 two state models, applying the new tools described above to genes which fit more
325 simple regulatory regimes may yet provide the most straightforward route to a more
326 complete understanding of bursting.
327

328 ## References

329 1. McKnight, S.L., and Miller, O.L. (1979). Post-replicative nonribosomal transcription
330 units in D. melanogaster embryos. Cell *17*, 551-563.
331 2. Femino, A.M., *et al.* (1998). Visualization of single RNA transcripts in situ. Science
332 *280*, 585-590.
333 3. Bertrand, E., *et al.* (1998). Localization of ASH1 mRNA particles in living yeast. Mol.
334 Cell *2*, 437-445.
335 4. Golding, I., *et al.* (2005). Real-time kinetics of gene activity in individual bacteria. Cell
336 *123*, 1025-1036.
337 5. Chubb, J.R., *et al.* (2006). Transcriptional pulsing of a developmental gene. Curr. Biol.
338 *16*, 1018-1025.
339 6. Raj, A., *et al.* (2006). Stochastic mRNA synthesis in mammalian cells. PLoS Biol. *4*,
340 e309.
341 7. Corrigan, A.M., *et al.* (2016). A continuum model of transcriptional bursting. Elife *5*,
342 631.
343 8. Larson, D.R., *et al.* (2011). Real-time observation of transcription initiation and
344 elongation on an endogenous yeast gene. Science *332*, 475-478.
345 9. Zenklusen, D., *et al.* (2008). Single-RNA counting reveals alternative modes of gene
346 expression in yeast. Nat. Struct. Mol. Biol. *15*, 1263-1271.
347 10. Peccoud, J., and Ycart, B. (1995). MARKOVIAN MODELING OF GENE-PRODUCT
348 SYNTHESIS. Theor. Popul. Biol. *48*, 222-234.
349 11. Antolović, V., *et al.* (2017). Generation of Single-Cell Transcript Variability by
350 Repression. Curr. Biol. *27*, 1811-1817.e1813.
351 12. Larsson, A.J.M., *et al.* (2019). Genomic encoding of transcriptional burst kinetics.
352 Nature *424*, 147.
353 13. Zoller, B., *et al.* (2018). Diverse Spatial Expression Patterns Emerge from Unified
354 Kinetics of Transcriptional Bursting. Cell *175*, 835-847.e825.
355 14. Berrocal, A., *et al.* (2018). Kinetic sculpting of the seven stripes of the Drosophila
356 even-skipped gene. bioRxiv, 335901.
357 15. Tunnacliffe, E., *et al.* (2018). Promoter-mediated diversification of transcriptional
358 bursting dynamics following gene duplication. Proc. Natl. Acad. Sci. U.S.A. *115*, 8364-
359 8369.
360 16. Rieckh, G., and Tkačik, G. (2014). Noise and Information Transmission in Promoters
361 with Multiple Internal States. Biophys. J. *106*, 1194-1204.
362 17. Zhang, J., and Zhou, T. (2014). Promoter-mediated transcriptional dynamics. Biophys.
363 J. *106*, 479-488.
364 18. Sanchez, A., and Kondev, J. (2008). Transcriptional control of noise in gene
365 expression. Proceedings of the National Academy of Sciences *105*, 5081-5086.
366 19. Blake, W.J., *et al.* (2003). Noise in eukaryotic gene expression. Nature *422*, 633-637.
367 20. Coulon, A., *et al.* (2010). On the spontaneous stochastic dynamics of a single gene:
368 complexity of the molecular interplay at the promoter. BMC Syst Biol *4*, 1-18.
369 21. Sanchez, A., *et al.* (2011). Effect of promoter architecture on the cell-to-cell
370 variability in gene expression. PLoS Comput. Biol. *7*, e1001100.
371 22. Bothma, J.P., *et al.*. (2014). Dynamic regulation of eve stripe 2 expression reveals
372 transcriptional bursts in living Drosophila embryos. Proc. Natl. Acad. Sci. U.S.A. *111*,
373 10598-10603.

374 23. Senecal, A., *et al.* (2014). Transcription factors modulate c-Fos transcriptional bursts.
375 Cell Rep. *8*, 75-83.
376 24. Neuert, G., *et al.* (2013). Systematic identification of signal-activated stochastic gene
377 regulation. Science *339*, 584-587.
378 25. Tantale, K., *et al.* (2016). A single-molecule view of transcription reveals convoys of
379 RNA polymerases and multi-scale bursting. Nat Commun *7*, 12248.
380 26. Harper, C.V., *et al.* (2011). Dynamic analysis of stochastic transcription cycles. PLoS
381 Biol. *9*, e1000607.
382 27. Cesbron, F., *et al.* (2015). Transcriptional refractoriness is dependent on core
383 promoter architecture. Nat Commun *6*, 6753.
384 28. Suter, D.M., *et al.* (2011). Mammalian genes are transcribed with widely different
385 bursting kinetics. Science *332*, 472-474.
386 29. Zoller, B., *et al.* (2015). Structure of silent transcription intervals and noise
387 characteristics of mammalian genes. Mol. Syst. Biol. *11*, 823-823.
388 30. Bartman, C.R., *et al.* (2019). Transcriptional Burst Initiation and Polymerase Pause
389 Release Are Key Control Points of Transcriptional Regulation. Mol. Cell *73*, 519-
390 532.e514.
391 31. Li, C., *et al.* (2018). Frequency Modulation of Transcriptional Bursting Enables
392 Sensitive and Rapid Gene Regulation. Cell Syst *6*, 409-423.e411.
393 32. Coulon, A., *et al.* (2013). Eukaryotic transcriptional dynamics: from single molecules
394 to cell populations. Nat. Rev. Genet. *14*, 572-584.
395 33. Sorkin, A., and von Zastrow, M. (2009). Endocytosis and signalling: intertwining
396 molecular networks. Nat. Rev. Mol. Cell Biol. *10*, 609-622.
397 34. Rodriguez, J., *et al.* (2019). Intrinsic Dynamics of a Human Gene Reveal the Basis of
398 Expression Heterogeneity. Cell *176*, 213-226.e218.
399 35. Farley, E.K., *et al.* (2015). Suboptimization of developmental enhancers. Science *350*,
400 325-328.
401 36. Ravarani, C.N.J., *et al.* (2016). Affinity and competition for TBP are molecular
402 determinants of gene expression noise. Nat Commun *7*, 10417.
403 37. Corrigan, A.M., and Chubb, J.R. (2014). Regulation of transcriptional bursting by a
404 naturally oscillating signal. Curr. Biol. *24*, 205-211.
405 38. Featherstone, K., *et al.* (2016). Spatially coordinated dynamic gene transcription in
406 living pituitary tissue. Elife *5*, e08494.
407 39. Klindziuk, A., and Kolomeisky, A.B. (2018). Theoretical Investigation of
408 Transcriptional Bursting: A Multi-State Approach. J. Phys. Chem. B *122*,
409 acs.jpcb.8b09676-11977.
410 40. Sepulveda, L.A., *et al.* (2016). Measurement of gene regulation in individual cells
411 reveals rapid switching between promoter states. Science *351*, 1218-1222.
412 41. Fritzsch, C., *et al.* (2018). Estrogen-dependent control and cell-to-cell variability of
413 transcriptional bursting. Mol. Syst. Biol. *14*, 2025.
414 42. Lefebvre, O., *et al.* (1996). Gastric Mucosa Abnormalities and Tumorigenesis in Mice
415 Lacking the pS2 Trefoil Protein. Science *274*, 259-262.
416 43. Rae, J.M., *et al.* (2005). GREB 1 is a critical regulator of hormone dependent breast
417 cancer growth. Breast Cancer Res. Treat. *92*, 141-149.
418 44. Haberle, V., and Stark, A. (2018). Eukaryotic core promoters and the functional basis
419 of transcription initiation. Nat. Rev. Mol. Cell Biol. *19*, 621-637.

420 45. Weingarten-Gabbay, S., *et al.* (2019). Systematic interrogation of human promoters.
421 Genome Res. *29*, 171-183.
422 46. Hendy, O., *et al.* (2017). Differential context-specific impact of individual core
423 promoter elements on transcriptional dynamics. Mol. Biol. Cell *28*, 3360-3370.
424 47. Golding, I., and Cox, E.C. (2006). Eukaryotic transcription: what does it mean for a
425 gene to be 'on'? Current biology : CB *16*, R371-373.
426 48. Dunham, L.S.S., *et al.* (2017). Asymmetry between Activation and Deactivation
427 during a Transcriptional Pulse. Cell Syst *5*, 646-653.e645.
428 49. Mehta, G.D., *et al.* (2018). Single-Molecule Analysis Reveals Linked Cycles of RSC
429 Chromatin Remodeling and Ace1p Transcription Factor Binding in Yeast. Mol. Cell *0*,
430 875-887.e879.
431 50. Chen, L.-F., L *et al.* (2019). Enhancer Histone Acetylation Modulates Transcriptional
432 Bursting Dynamics of Neuronal Activity-Inducible Genes. Cell Rep. *26*, 1174-
433 1188.e1175.
434 51. Muramoto, T., *et al.* (2010). Methylation of H3K4 Is required for inheritance of active
435 transcriptional states. Current biology : CB *20*, 397-406.
436 52. Hadzhiev, Y., *et al.* (2019). A cell cycle-coordinated Polymerase II transcription
437 compartment encompasses gene expression before global genome activation. Nat
438 Commun *10*, 67.
439 53. Sato, Y., *et al.* (2019). Histone H3K27 acetylation precedes active transcription during
440 zebrafish zygotic genome activation as revealed by live-cell analysis. Development
441 *146*, dev179127.
442 54. Nicolas, D., *et al.* (2018). Modulation of transcriptional burst frequency by histone
443 acetylation. Proceedings of the National Academy of Sciences *115*, 7153-7158.
444 55. Hocine, S., *et al.* (2015). Promoter-Autonomous Functioning in a Controlled
445 Environment using Single Molecule FISH. Sci Rep *5*, 9934.
446 56. Fukaya, T., *et al.* (2016). Enhancer Control of Transcriptional Bursting. Cell *166*, 358-
447 368.
448 57. Bartman, *et al.* (2016). Enhancer Regulation of Transcriptional Bursting Parameters
449 Revealed by Forced Chromatin Looping. Mol. Cell *62*, 237-247.
450 58. Larson, D.R., *et al.* (2013). Direct observation of frequency modulated transcription
451 in single cells using light activation. Elife *2*, e00750.
452 59. Falo-Sanjuan, J., *et al.* (2019). Enhancer Priming Enables Fast and Sustained
453 Transcriptional Responses to Notch Signaling. Dev. Cell *50*, 411-425.e418.
454 60. Lee, C., *et al.* (2019). Dynamics of Notch-Dependent Transcriptional Bursting in Its
455 Native Context. Dev. Cell *50*, 426-435.e424.
456 61. Scholes, C., *et al.* (2019). Signal Integration by Shadow Enhancers and Enhancer
457 Duplications Varies across the Drosophila Embryo. Cell Rep. *26*, 2407-2418.e2405.
458 62. Lim, B., *et al.* (2018). Temporal dynamics of pair-rule stripes in living Drosophila
459 embryos. Proceedings of the National Academy of Sciences *115*, 8376-8381.
460 63. Chen, H., *et al.* (2018). Dynamic interplay between enhancer-promoter topology and
461 gene activity. Nat. Genet. *50*, 1296-1303.
462 64. Alexander, J.M., *et al.* (2019). Live-cell imaging reveals enhancer-dependent Sox2
463 transcription in the absence of enhancer proximity. Elife *8*, e41769.
464 65. Williamson, I., L *et al.* (2016). Shh and ZRS enhancer colocalisation is specific to the
465 zone of polarising activity. Development *143*, 2994-3001.

466 66. Benabdallah, N.S., *et al.* (2019). Decreased Enhancer-Promoter Proximity
467 Accompanying Enhancer Activation. Mol. Cell *76*, 1-12.
468 67. Mir, M., *et al.* (2018). Dynamic multifactor hubs interact transiently with sites of
469 active transcription in Drosophila embryos. Elife *7*, 8088.
470 68. Dufourt, J., *et al.* (2018). Temporal control of gene expression by the pioneer factor
471 Zelda through transient interactions in hubs. Nat Commun *9*, 400.
472 69. Hnisz, D., *et al.* (2017). A Phase Separation Model for Transcriptional Control. Cell
473 *169*, 13-23.
474 70. McSwiggen, D.T., *et al.* (2019). Evaluating phase separation in live cells: diagnosis,
475 caveats, and functional consequences. Genes & development *33*, 1619-1634.
476 71. Mazza, D., *et al.* (2012). A benchmark for chromatin binding measurements in live
477 cells. Nucleic Acids Res. *40*, e119-e119.
478 72. Chen, J., *et al.* (2014). Single-Molecule Dynamics of Enhanceosome Assembly in
479 Embryonic Stem Cells. Cell *156*, 1274-1285.
480 73. Annibale, P., and Gratton, E. (2015). Single cell visualization of transcription kinetics
481 variance of highly mobile identical genes using 3D nanoimaging. Sci Rep *5*, 9258.
482 74. Levi, V., *et al.* (2003). Scanning FCS, a novel method for three-dimensional particle
483 tracking. Biochem. Soc. Trans. *31*, 997-1000.
484 75. Kis-Petikova, K., and Gratton, E. (2004). Distance measurement by circular scanning
485 of the excitation beam in the two-photon microscope. Microscopy Research and
486 Technique *63*, 34-49.
487 76. Donovan, B.T., *et al.* (2019). Live-cell imaging reveals the interplay between
488 transcription factors, nucleosomes, and bursting. EMBO J., e100809.
489 77. Stavreva, D.A., *et al.* (2019). Transcriptional Bursting and Co-bursting Regulation by
490 Steroid Hormone Release Pattern and Transcription Factor Mobility. Mol. Cell *75*,
491 1161-1177.e1111.
492 78. Li, J., *et al.* (2019). Single-Molecule Nanoscopy Elucidates RNA Polymerase II
493 Transcription at Single Genes in Live Cells. Cell *178*, 491-506.e428.
494 79. Rullan, M., *et al.* (2018). An Optogenetic Platform for Real-Time, Single-Cell
495 Interrogation of Stochastic Transcriptional Regulation. Mol. Cell *70*, 745-756.e746.
496 80. Wilson, M.Z., *et al.* (2017). Tracing Information Flow from Erk to Target Gene
497 Induction Reveals Mechanisms of Dynamic and Combinatorial Control. Mol. Cell *67*,
498 757-769.e755.
499 81. Chen, S.Y., *et al.* (2019). Optogenetic control reveals differential promoter
500 interpretation of transcription factor nuclear translocation dynamics. bioRxiv,
501 548255.
502 82. Chao, J.A., *et al.* (2008). Structural basis for the coevolution of a viral RNA-protein
503 complex. Nat Struct Mol Biol *15*, 103-105.
504 83. La Manno, G., *et al.* (2018). RNA velocity of single cells. Nature *560*, 494-498.
505 84. Erhard, F., *et al.* (2019). scSLAM-seq reveals core features of transcription dynamics
506 in single cells. Nature *571*, 419-423.
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511 **Figure 1. Approaches to Visualize Transcription.**

512 A) Chromatin spreads from *Drosophila* embryos (image reproduced from [1]). The
513 image shows a pair of sister chromatids aligned in parallel, with inferred initiation
514 sites marked by α and β. Note the increasing size of the fibres (transcripts)
515 extending from the central axis of each chromatid with increasing distance from the
516 initiation sites (scale bar 1μm). Also note the fibre-free gaps (marked by arrows). B)
517 Schematic of the MS2/MCP system for visualizing nascent transcripts. MS2 arrays
518 are targeted into the gene of interest. The MS2 RNA forms stem loops, and can be
519 detected at the site of transcription, as a fluorescent spot, by the MCP-GFP fusion
520 protein. C) Transcription visualized using the MS2/MCP system, with stills from a
521 movie sequence showing nascent RNA detected in bursts from the *act5* gene of
522 *Dictyostelium* (scale bar 5μm). Normalised spot intensity values are shown in the
523 plot below the film strip, with yellow dots corresponding to the images. D) Combining
524 imaging of transcription, using MS2/MCP, with imaging of transcription regulators
525 (image taken from Li et al. 2019). Images show nascent transcript foci from the
526 mouse *Pou5f1* gene detected alongside different SNAP-tagged transcription factors
527 (scale bars 300nm).

528

529 **Figure 2. Key Figure. Models of Transcriptional Dynamics.**

530 A selection of different model architectures used to describe transcriptional bursting
531 dynamics.

532

533 **Box 1. Popular Approaches to Measure Single Cell Transcription Dynamics**
534
535 1. Live cell imaging of nascent RNA
536 These approaches use live cell RNA detection systems based upon stem-loop motifs
537 from the genomes of RNA bacteriophages MS2 or PP7 [3, 82]. The distinct stem-
538 loops structures have a high affinity interaction with the cognate coat proteins of the
539 phages (MCP or PCP, respectively). By fusing fluorescent proteins to MCP or PCP,
540 the stem loops recruit the fluorescent reporter, allowing live cell detection of the
541 RNA. For imaging dynamic nascent transcript production, a sequence encoding an
542 array of the stem loops (up to 128 repeats have been used) is targeted into the gene
543 of interest. Upon transcription, the loops are incorporated into the nascent RNA and
544 rapidly bind the fluorescent coat protein, allowing the nascent RNA to be visualized
545 at the site of transcription as a fluorescent spot (see Figure 1B). The high specificity
546 of these systems means MS2 and PP7 can be used together in the same cell to
547 monitor activity of different genes, or to determine kinetic parameters of the
548 transcriptional process, such as elongation rate, at a single gene.
549
550 2. Destabilised protein reporters
551 An alternative technique is to use protein reporters such as GFP or luciferase to
552 observe activity of a particular gene. While these methods enable measurement of
553 the output of a gene over time, and therefore provide dynamic information, using a
554 protein rather than RNA reporter to model transcription requires the addition of
555 several assumptions about intervening processes such as mRNA export and
556 translation. Recent studies have tended to corroborate findings using other
557 techniques, such as smFISH.
558
559 3. Single molecule RNA FISH (smFISH) and single-cell RNA sequencing
560 (scRNAseq)
561 Fixed-cell measurements from methods such as smFISH produce distributions of
562 both nascent and mature mRNA counts in single cells. From these data, parameters
563 such as the frequency of burst initiation as well as the number of transcripts initiated
564 (burst size) can be inferred. The approach of extracting dynamic behaviours from
565 static measurement distributions has recently been extended to genome-wide
566 approaches such as scRNAseq [11, 12]. In both cases, certain assumptions about

567 the regulation of the gene (i.e., whether it can be modelled as one-state, two-state or
568 multi-state, see main text for further details) must be made which can limit the
569 accuracy of such methods. More recent scRNAseq methods and analysis tools can
570 give a coarse view of the changing gene expression of a cell [83, 84], adding an
571 element of temporal detail onto otherwise static measurements.
572

Figure 1

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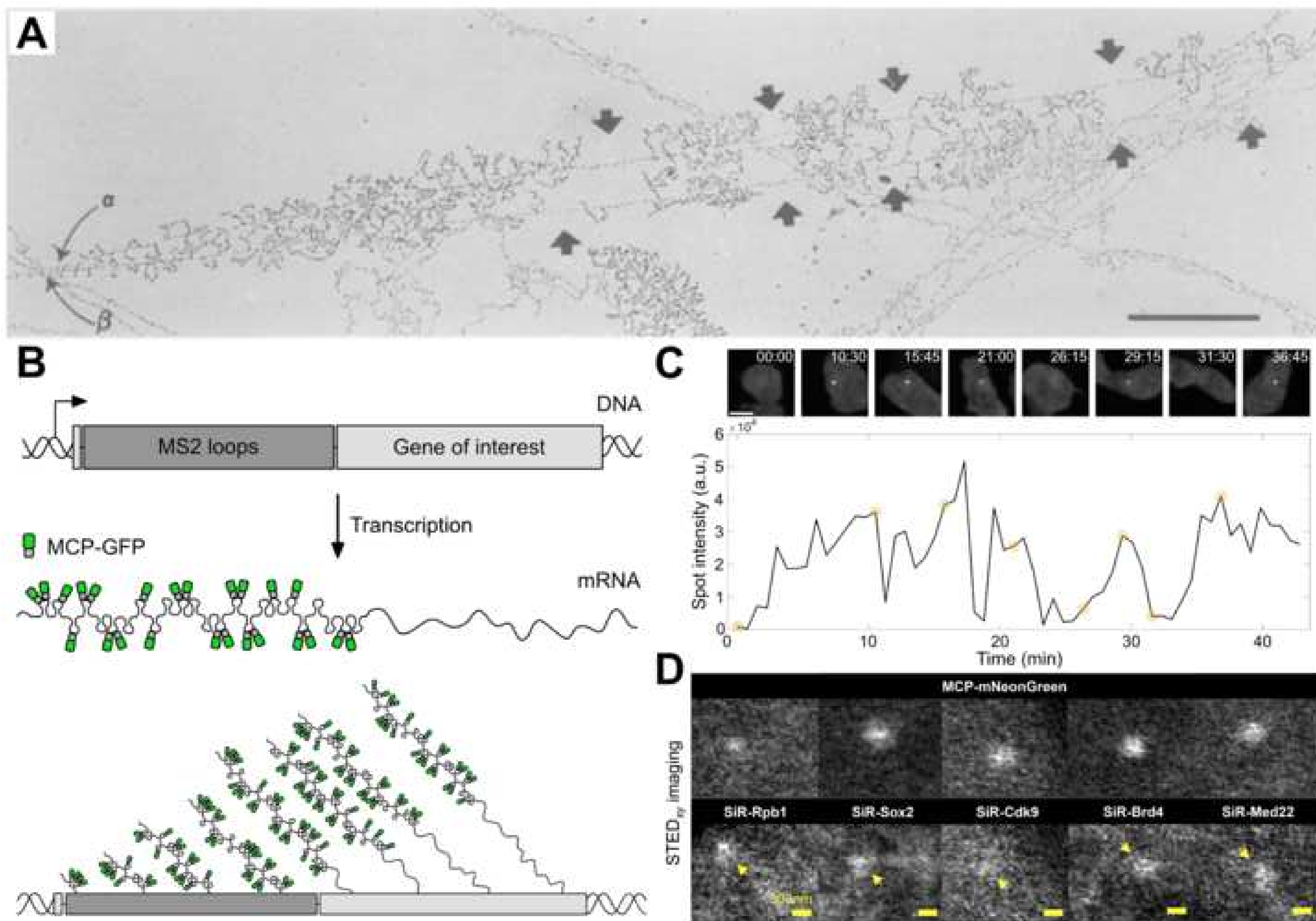

Figure 2- Key Figure

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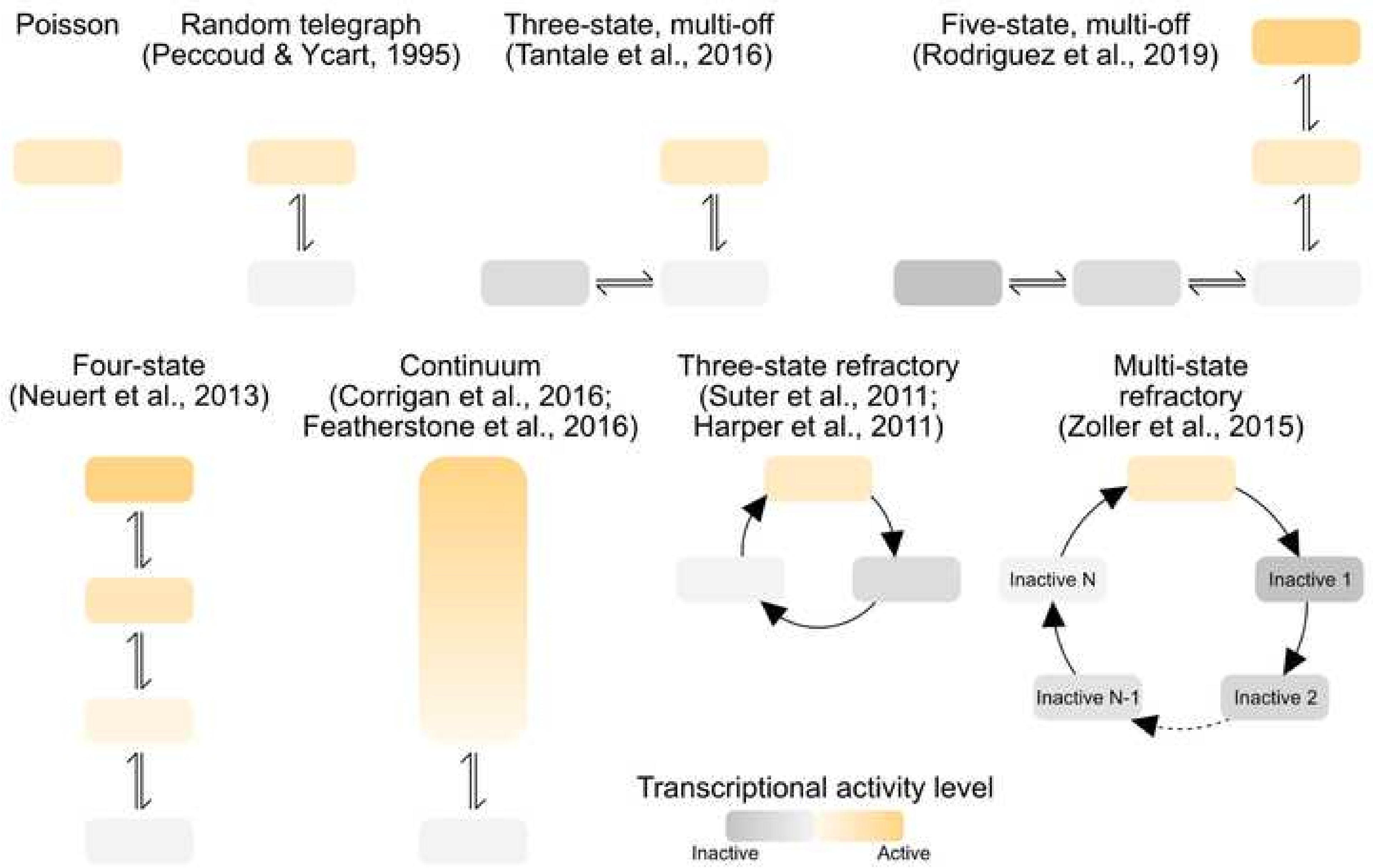

Outstanding questions:

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?