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Origins and consequences of transcriptional discontinuity

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In both prokaryotes and eukaryotes, transcription has been described as being temporally discontinuous, most genes being active mainly during short activity windows interspersed by silent periods. In mammalian cells, recent studies performed at the single cell level have revealed that transcriptional kinetics are highly gene-specific and constrained by the presence of refractory periods of inactivity before a gene can be turned on again. While the underlying mechanisms generating gene-specific kinetic characteristics remain unclear, various biological consequences of transcriptional discontinuity have been unravelled during the past few years. Here we review recent advances on understanding transcriptional kinetics of individual genes at the single cell level and discuss its possible origins and consequences.

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Introduction

In a particular cell type protein-encoding genes can be expressed at widely different levels, producing from less than two mRNAs/cell to more than 10^5 mRNAs/cell [1]. Transcription rates are determined by sequence-specific and general transcription factors and in mammalian cells can vary over a very large range. The maximal possible transcription rate is reached when elongation becomes limiting for initiation; that is, when the polymerase initiated at the promoter sterically hinders the next polymerase to take its place. Since the elongation rate in mammalian cells is about 63 nucleotides per second [2•] and since an RNA polymerase II molecule may occupy a similar number of base pairs the maximal transcription rate is about one transcript/second. Some genes, for example immunoglobulin light and heavy chain mRNAs in plasmacytoma cells, may actually be transcribed at initiation frequencies approaching the maximal possible rate [3]. However, many if not most mRNAs accumulate to only a few copies per cell. For example, in liver the average number of mRNAs encoding the general transcription factor TATA-Binding Protein (TBP) has been estimated to be 4.6 per cell [4]. The half-life of *Tbp* mRNA in liver is not known, but even if one assumes a very short half-life of one hour, each of the two *Tbp* alleles would have to produce only one transcript every forty minutes to maintain the steady state concentration of *Tbp* mRNA. As the *Tbp* gene encompasses 18,528 base pairs, its transcription should be completed in five minutes at the elongation rate provided above. Therefore, although *Tbp* is an essential gene, it probably is in a silent state most of the time.

Although the mRNA output of a given gene is surprisingly precise and gene-specific if measured in a large population of cells, it is unlikely that the initiation frequencies of genes are controlled by precise metronomes that make the promoters fire at regular, gene-specific time intervals. Rather, transcription initiation is expected to be guided by stochastic processes at the single gene level. Over the recent years, studies in prokaryotic and eukaryotic systems have indeed described a wide range of transcriptional kinetic behaviours [5–8]. As speculated in the previous paragraph, transcription appears to occur during short time periods followed by silent intervals. These transcriptional bursts can result in discontinuous protein expression and thereby generate heterogeneity of protein accumulation in individual cells of an isogenic population [9–11]. In turn, such heterogeneity could generate phenotypic diversity [12], which in some cases may render a subpopulation of cells more adaptive to changing environmental conditions and in other cases may tune a cell's probability to enter a particular differentiation pathway [13,14]. Here we review recent advances in describing gene-specific transcriptional kinetics and its implication in a variety of biological processes.

Gene-specificity of transcription kinetics

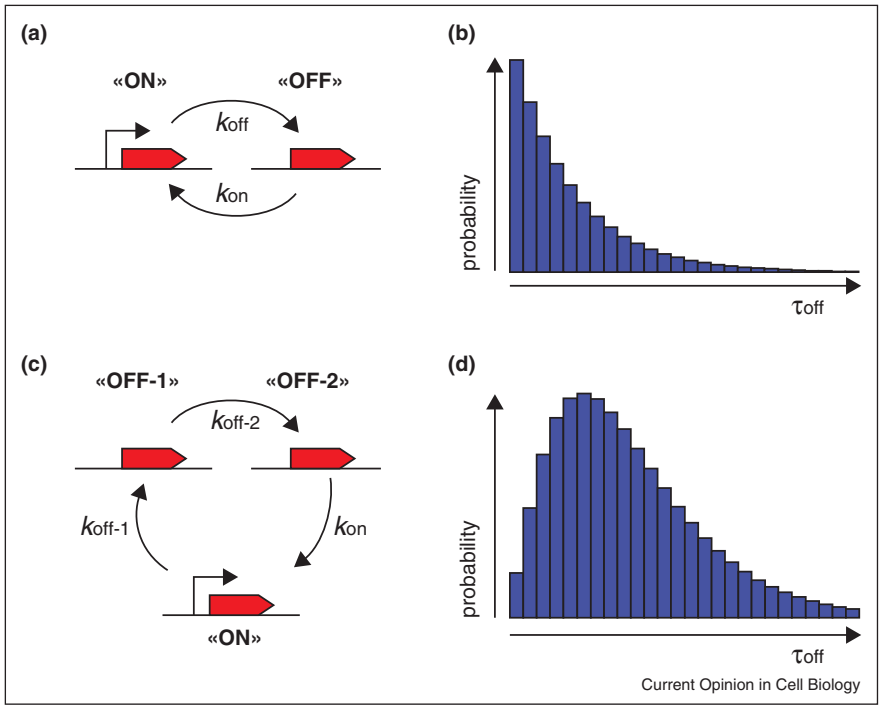
The first hint that transcriptional kinetics present characteristics which are intrinsic for a given gene came from an electron microscopy study on chromatin spreads by Miller and co-workers. Using this technique for the analysis of newly replicated transcription units during Drosophila development, McKnight and Miller observed that the

distances between elongating RNA polymerase molecules were irregular within a given gene [15]. Therefore the promoter of this gene must have fired at uneven time intervals. Interestingly, however, the spacing patterns of nascent transcripts were frequently similar on two homologous alleles that had just been replicated (i.e. found close to the replication fork). Perhaps, this similarity implies that owing to the close proximity of the replicated genes the transcription initiation machinery was acting simultaneously on both gene copies. More recently, Singer and colleagues showed that different genes in yeast have characteristic temporal patterns of mRNA accumulation [16]. Among four genes examined, three were transcribed in a relatively continuous manner and one was expressed in a burst-like fashion. This suggests the existence of gene-specific determinants of transcriptional kinetics. Shav-Tal and colleagues examined transcriptional kinetics of a transgene in which transcription of the cyclin D1 coding sequence is driven either by its own promoter or by the CMV promoter [7]. Both constructs contained tandem repeats of MS2-binding sites to follow transcription by live imaging and both were integrated in a determined genomic location, allowing to monitor transcriptional kinetics driven by different regulatory sequences independently of genomic context. Interestingly, the CMV promoter conferred continuous high-level transcription, whereas the endogenous promoter yielded discontinuous transcription. To better characterize transcriptional kinetics of endogenous mammalian genes, Suter *et al.* used a gene trap strategy to insert a short-lived luciferase under the control of endogenous regulatory sequences [17••]. Using a mathematical approach, they calculated kinetic parameters such as rates of on-switching and off-switching, allowing them to show that each gene can be defined by its own set of kinetic parameters.

A refractory period of gene inactivity

Many studies focusing on transcriptional kinetics use the Random Telegraph Model to describe transcriptional kinetics [18]. In this model, a gene can be in one of two different states; either it is transcriptionally inactive (off), or active (on). One important assumption is that the transitions between the on-states and off-states are stochastic and can thus be described by rate constants defining the probability of switching from one state to another. Such a simple model has been very useful in describing heterogeneity in mRNA numbers across isogenic cell populations at a given time point [19]. In particular, this model can account for higher variability in the accumulation levels of the gene products compared to what is expected from a model with constitutively active transcription, as suggested experimentally in [11]. However, fixed cell population analysis cannot catch dynamic information such as the regularity in bursting frequency or the distributions of 'on' and 'off'

Figure 1



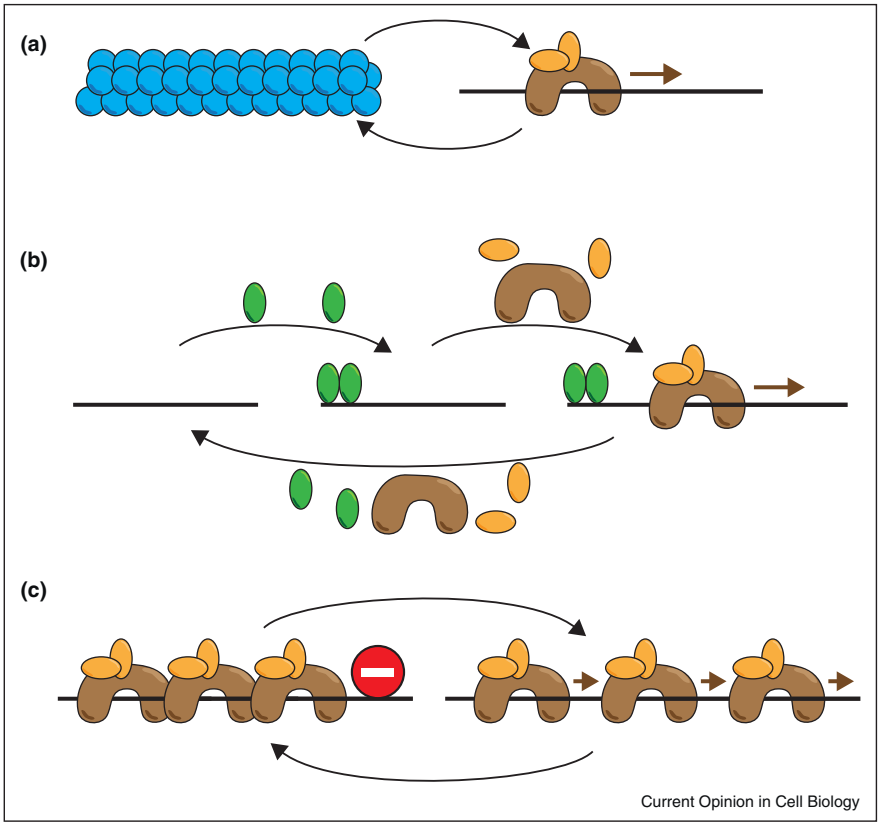
(a) Random Telegraph Model, in which a gene can be either in the 'on' or 'off' state. Switching between the two states is stochastic, but occurs with defined rates. **(b)** Waiting time (τ_{off}) distributions derived from the Random Telegraph Model. **(c)** Three-state model, in which a gene has to go through at least two different 'off'-states before it can be switched on again. **(d)** Waiting time (total τ_{off}) distributions derived from the three-state model.

time periods. Importantly, the Random Telegraph Model predicts that the time intervals during which a gene will stay in one state follows an exponential distribution, that is, that the most frequent situation will be the shortest measurable time interval before switching to the other state (Figure 1). Naef and colleagues [17••] and another study by White and colleagues [20] found that switching from the 'on' to the 'off' state could be well-described by a single first order process. However, the time spent in the off state was constrained by refractory periods of gene inactivity, that is, genes need a certain amount of time in the 'off' state before they can be switched on again. Thus, these analyses suggested that a more general and accurate model should include at least one additional off state (or refractory state). So why is there a refractory period in the 'off' state? A close look at the process of Pol-II mediated transcription could provide an explanation. When the transcription machinery is in the elongation phase, one can hypothesize that a single and rapid biochemical event can perturb the transcriptional process and thereby stop mRNA production. However, turning on transcription of a gene requires several consecutive steps that must be tightly coordinated in order to prepare the transcription machinery to be active (Figure 2) [21]. Consecutive sequences of first order reaction lead to a total off time that follows peaked distributions (hypoexponential distribution). Recently, Singer and colleagues reported on an elegant fluorescence fluctuation approach to record the history of single pre-mRNAs initiated from the yeast *POL1* and *MDN1* promoters in real time [22••]. In parallel, these authors measured the dynamics of mobility of an Mbp1p-GFP fusion protein by two-photon fluorescence correlation spectroscopy – Mbp1p being an essential transcription factor of cell cycle-specific *POL1* transcription. Based on these results, they propose that the search time of Mbp1p is determining the kinetics of initiation at the *POL1* promoter.

Elements shaping transcriptional kinetics

Different steps of Pol II-mediated transcription could potentially contribute to generate a characteristic kinetic profile, starting from chromatin opening into a permissive state for transcription, binding of transcription factors to the promoter and enhancer regions, assembly of the transcription machinery, isomerisation, and escape from promoter proximal pausing. Here we examine the possible contributions of genomic context, promoter/enhancer sequences

Figure 2



Possible steps shaping transcriptional kinetics in eukaryotes. **(a)** Chromatin transitions between transcription-permissive and non-permissive states. **(b)** Assembly and disassembly of the transcription machinery. **(c)** Promoter-proximal pausing and release of a cluster of RNA polymerase II (Pol-II) complexes. Blue: nucleosomes. Brown: Pol-II. Light brown: general transcription factors. Green: gene-specific transcription factors.

and promoter proximal pausing in shaping transcriptional kinetics (Figure 2).

Genomic context: Several studies showed that reporters inserted into the same locus tend to burst simultaneously, whereas insertion in different genomic locations yields asynchronous transcription [19,23]. One explanation of these findings is that the genomic environment itself is determining the timing of transcriptional bursts. Alternative explanations include the existence of some form of cooperativity between spatially close transcription units, resulting in increasing the probability of simultaneous bursting, or the synchronous recruitment to specialized Pol II-rich compartments called transcription factories [24]. This scenario may also apply for genes that have just been replicated, as outlined above [15]. More recently, the work of Arkin and colleagues [25•] suggested that the temporal accumulation of mRNA is relatively independent of chromosomal location; however they found that burst size (i.e. the number of mRNA produced during a transcriptional burst) is location-dependent. These results were corroborated by Naef and colleagues [17••] who demonstrated that histone hyperacetylation induced by trichostatin A treatment does not dramatically affect transcriptional kinetics, but clearly had a significant effect on the mean burst sizes. Taken together, these studies suggest that genomic context is not the dominant player in shaping transcriptional kinetics, even though neighbouring transcription units could cooperate to synchronize gene expression.

Promoter sequence: Work from different laboratories has shown that the presence and nature of the TATA box is also an important determinant of transcriptional kinetics [26,27], suggesting a role for transcription machinery assembly in shaping temporal profiles of mRNA production. To examine the possible role of promoter sequences, Naef and colleagues generated different cell lines carrying a reporter inserted in a defined locus and differing only by the number and/or affinity of CCAAT boxes binding the transcriptional activator NF-Y [17••]. Very different transcriptional kinetics patterns were observed, depending both on the number and affinity of CCAAT boxes. This indicated that promoter architecture is a strong determinant of transcriptional kinetics and that the assembly of factors necessary for transcription initiation or to enter active elongation play an important role in shaping transcriptional kinetics. It is tempting to speculate on a possible link between the huge varieties in transcription factor combinations involved in transcription initiation and the broad range of transcriptional kinetics observed for mammalian genes.

Promoter proximal pausing: It has been argued that many active mammalian genes are subject to transcriptional pausing, as demonstrated by their high density Pol II coverage just after the transcription start site [28]. If one assumes a synchronous release of several Pol II complexes into active elongation phase, this could in principle generate transcriptional bursting. General factors regulating pausing are relatively well known [28], but to date the only transcription factor having shown to play a direct role in pause release is c-Myc [29•]. Further work is required to identify other regulators of transcriptional pausing possibly involved in this process. Moreover, a single-allele analysis of pausing dynamics will be needed to consider its possible role in shaping transcriptional kinetics.

Phenotypic consequences of transcriptional discontinuity

As transcriptional discontinuity generates fluctuations in the amount of transcripts present in a cell over time, it also causes heterogeneity of mRNA numbers between individual cells in an isogenic population. This is of obvious interest in order to generate phenotypic diversity in unicellular organisms, provided that heterogeneity of mRNA numbers is propagated to the proteins they encode. This is the case in bacteria and yeast, where the half-lives of both mRNAs and proteins are very short [30–32]. It is therefore not surprising that the distribution of many proteins in *E. coli* is relatively heterogeneous across an isogenic cell population [11], and in both bacteria and yeast, a significant fraction of heterogeneity at the protein level has been linked to variance in the number of mRNA molecules [33]. In unicellular organisms, a number of stochastic processes regulate the switching between different cell phenotypes [14,16,34–38], and in some cases this has been directly related to discontinuous transcription. In *E. coli*, it has been shown that stochastic gene transcription is responsible for the occasional entry of bacteria into a competent state [14,38], thereby providing the opportunity of a small subpopulation to gain new potentially useful adaptive states without compromising whole population survival. The regulation of lysogen stability of phage lambda in *E. coli* is another example of phenotypic consequences of the transcriptional kinetic pattern. An elegant study by Golding and colleagues has shown that the frequency of transcriptional bursts of the cI gene regulates the transition between lysogenic and lytic phases of the host bacteria [39]. In yeast, Singer and colleagues have shown that several housekeeping genes are transcribed in a continuous manner [16], in contrast to the inducible TATA box-containing *PDR5* gene. As yeast cells have very short generation times as well as short-lived mRNAs and proteins, it is plausible that continuous transcription has to be maintained in order to keep the products of housekeeping genes at constant levels. It is also possible that the selective pressure on transcriptional kinetics of other genes like *PDR5* is lower, allowing them to be expressed in a more burst-like manner.

In higher eukaryotes, mRNAs and proteins are more stable and the fluctuations of proteins amounts are

relatively low [40]. Also, the advantages of generating phenotypic diversity in multicellular organisms are less obvious. However, developmental processes such as retina morphogenesis [13], differentiation programs of many cell types including neurons [41], myoblasts [42], hematopoietic cells [43], or intestinal cells [44•] are subject to stochastic cell fate choices. In the intestine of the nematode C. elegans, Van Oudenaarden and colleagues established a direct link between discontinuous transcription of the gene encoding the ELT-2 transcription factor and cell fate choice [44•]. Transcription factors are key regulators of cell differentiation, and interestingly, many of them are short-lived [45]. Therefore, they have the potential to be expressed at different levels across cells and hence to lead to different cell fates within an isogenic cell population. However, in the case of most genes it is likely that transcriptional bursts are buffered at the protein level, and this raises questions about the necessity to keep such a mode of gene expression. A potential advantage of producing a high number of mRNAs within a short time period is to provide both a fast response to a stimulus and a large dynamic range. Other potential benefits of the limited time during which mammalian genes seem to be transcribed [17••] are the maintenance of a big pool of transcription factors and Pol II molecules available for new rounds of initiation, and, perhaps, limiting the time during which DNA is accessible and therefore more susceptible to genotoxic stresses. Finally, it might be advantageous to transcribe multiple mRNAs to maximize the yield of the potentially energy-consuming assembly of the transcriptional machinery.

Conclusion

The detailed study of transcriptional kinetics has been possible over the past years thanks to the emergence of new single-cell and single gene techniques to monitor gene expression. It is becoming clear that different genes carry their own kinetic signatures, which are determined to a great extent by their regulatory sequences. However, the mechanisms by which regulatory elements determine transcriptional kinetics remain unclear, and future studies are needed to decipher which steps set the pace of transcriptional activity. Finally, the development of novel strategies allowing to modulate transcriptional kinetics without affecting global transcriptional outputs is required in order to scrutinize the phenotypic consequences of kinetic changes in transcription.

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