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Dynamics of transcription and mRNA export

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Abstract

Understanding the different molecular mechanisms responsible for gene expression has been a central interest of molecular biologists for several decades. Transcription, the initial step of gene expression, consists of converting the genetic code into a dynamic messenger RNA that will specify a required cellular function following translocation to the cytoplasm and translation. We now possess an in-depth understanding of the mechanism and regulations of transcription. By contrast, an understanding of the dynamics of an individual gene's expression in real time is just beginning to emerge following recent technological developments.

Introduction

Molecular events governing gene expression are among the best-studied and best-understood mechanisms in biology. In many cases, our understanding of these processes has reached the atomic level, especially with the recent crystallization of transcription complexes [1] and the unraveling of the atomic order within the nuclear pore complex [2]. This high-resolution picture of the transcription and export machineries contrasts with our lack of knowledge regarding the time scales of these reactions within live cells. While mechanistic approaches shed light on how these events are regulated, the consequences of this regulation for cell fate can only be understood by integrating these mechanisms into kinetic pathways, allowing an understanding of the inherent plasticity within living cells. In this review, we focus on the recent technological and conceptual advances that provide a better understanding of the kinetic rules that govern the molecular processes of mRNA transcription and export.

Dynamics of transcription initiation: few factors for many genes

Gene promoters can be viewed as static binding elements on which transcription factors assemble. It is the combinatorial variety of transcription factors in a cell that will presumably modulate the transcriptional activity of a specific gene. Recent approaches in which immunoprecipitation of chromatin using antibodies against transcription factors is followed by analysis of the bound sequences by DNA microarrays (‘ChIP-chip’ methods) have identified factor-specific yeast and mammalian promoter sequences [3, 4,5and 6••]. A complex network of interactions was revealed by a genome-wide ChIP-chip analysis of *S. cerevisiae* that identified 106 transcription factors bound to 2343 promoter sequences [6••]. In a recent study, 142 transcription factors interacting with 3420 target genes were linked to the expression of the genes they regulate, uncovering the underestimated dynamics of the

'transcription factor network' [7••]. One of the findings was that out of the 70 transcription factors that bind to transcription units during the cell cycle, 56 were also regulated by the cell cycle. Synthesis and binding of transcription factors could occur in the same phase of the cycle (as was the case for 13/56 genes) or also bind to genes expressed in subsequent phases (24/56 genes), providing a trigger to move to the next phase. The lag between transcription factor binding and gene expression, although highly reproducible, varied greatly from gene to gene and did not seem to depend on specific transcription factors but rather on the target gene [7••]. Although these findings correlating the binding of transcription factors to their expression can provide crucial information on the wiring of the transcriptional network, it is still unclear how the time lag separating transcription factor binding from gene expression is controlled at the molecular level. So far, the complexity of the mammalian genome has not allowed genome-wide ChIP-CHIP analysis [3 and 8]. However, new approaches where chromatin immunoprecipitation is analyzed by tandem cloning, known as serial analysis of chromosome occupancy (SACO), allow the genome-wide quantitative interrogation of the binding of specific transcription factors [9]. One way to extend this analysis would then be to investigate the correlation between transcription factor binding and polymerase loading of a gene. Technologies to address these questions now exist. For example, a time-course ChIP approach has shown that the binding of the estrogen receptor to its promoter is cyclical, defining permissive and non-permissive states for gene activation [10, 11 and 12]. These biochemical approaches can only address a population of cells at a fixed time determined by chemical fixation procedures, and often require cell synchronization [13]. Recent developments enable us to detect the transcriptional activity of individual genes in single cells by FISH (fluorescent in-situ hybridization) using color-coded probes for specific mRNAs at their transcription sites [14]. Ultimately, these experiments will have to be conducted in live cells and in real time, allowing the onset of gene activity to be correlated with upstream events of transcription factor binding and downstream (post-transcriptional) gene expression regulation events.

To determine cell fate, transcription factors must be present at limiting concentrations so that changes in their levels can lead to differential gene expression. Using cell lines with tandem repeated genes has allowed observations of transcription in live cells. The recruitment of the glucocorticoid receptor to such gene arrays revealed that binding was transient, exhibiting half-residence of a few seconds [15, 16 and 17]. These findings were extended to other components of the transcription recruitment machinery [17 and 18] and suggest that a single transcription factor molecule can affect the transcription of several genes through successive interactions. Findings that gene expression is stochastic in single cells [19 and 20] would be explained by a model whereby transcription activation can be achieved by a small number of molecules. The probability of interaction defines the 'transcriptional noise' within the system. Differences in noise levels can arise from different transcription initiation activities [19 and 20] and it has been proposed that transcription processivity [21] or translation efficiency [22] could significantly influence the stochasticity of the system. Genes that are essential for cellular viability or proteins involved in macromolecular complexes are biased toward noise reduction mechanisms [22]. While transcriptional activation ('promoter firing') represents the input for gene expression, the many control points downstream in the transcriptional process will modulate the output.

Dynamics of post-initiation polymerases

Following assembly of factors on the promoter, the polymerase initiates transcription and then elongates the nascent pre-mRNA. The switch from the post-initiation stage to elongation (termed promoter clearance and promoter escape, respectively) is believed to be a limiting step leading to high levels of aborted short RNA molecules [23 and 24]. The instability of the polymerase on its DNA substrate results in aborted promoter clearance and it is unclear how different transcription factors can influence its processivity. Promoter usage could provide a positive regulation loop selecting for a conformation more favorable to polymerase progression. A more active control operates at the level of promoter escape, where the polymerase enters a paused state under the control of factors that inhibit elongation. Release from pausing is, at least partly, triggered by a combination of maturation events including capping of the nascent pre-mRNA and the action of transcription elongation factors. Genes controlled at the promoter escape stage, for example heat shock genes in *Drosophila*, can react rapidly to a stimulus since processive elongation-competent polymerases are available [25 and 26]. Paused polymerases have been detected in the proximal regions of genes including c-Myc [27]. Recently, the activity of TFIIH on c-Myc transcription was investigated using cell lines deficient in one of TFIIH's components, XPB. While c-Myc protein levels were only moderately affected in the population, cell-to-cell variance in protein level increased by a factor of three. Although the function of TFIIH is not completely understood, it plays an important role in the initial stages of transcription that occur before promoter clearance. Mathematical modeling showed that TFIIH might function as an integrator of promoter firing events, therefore establishing stable expression levels of c-Myc from a stochastic input [28•].

Dynamics of elongation

Single bacterial polymerases tethered to a fixed substrate and used to drag a DNA-conjugated bead enabled the determination of elongation speeds for single polymerases [29 and 30], and demonstrated that RNA polymerases have an intrinsic propensity to pause. The conservation between bacterial core polymerases and mammalian RNA polymerase II makes it likely that pausing is a property of the mammalian enzyme as well. It is difficult to address the synthesis speed of an mRNA as the kinetics will be determined by the limiting step in the transcriptional process (initiation, promoter escape or elongation). The human dystrophin gene (2.3 Mb) provides a model where initiation and promoter escape rates are minimal relative to elongation time. The measured transcription rates for this gene were 1.7–2.5 kb/min [31]. Other approaches using FISH to detect nascent pre-mRNAs at their locus of transcription following activation have found elongation speeds of 1.1–1.4 kb/min for the *Drosophila* UBX mRNA [32] and mammalian β-actin mRNA [33]. Similar rates were observed using nuclear run-on assays on the HSP70 gene following heat shock in insect cells [25].

The development of genetically encoded fluorescent markers such as GFP (green fluorescent protein), together with sophisticated imaging, have allowed kinetic measurements of many biochemical processes in live cells, including gene expression [34]. rRNA transcription is confined to the nucleolus, providing a system for studying a homogenous population of co-

regulated genes. The kinetics of pol I transcription in live mammalian cells were calculated using FRAP (fluorescence recovery after photobleaching) and iFRAP (inverse FRAP) experiments. The kinetics of recruitment of this enzyme to the rDNA genes were correlated with the time it takes to transcribe one gene. This approach allowed the dissociation of the different steps of transcription (polymerase association with the promoter and elongation), and the experimental measurements were compared to simulations based on mathematical kinetic models and provided an *in vivo* kinetic model for Pol I transcription [35[and] 36••].

Pol II transcription was addressed in live cells by complementing a non-functional endogenous copy of the large subunit with a GFP-tagged functional version [37]. Pol II recruitment to nuclear transcription units comprised two detectable components, a fast component presenting a half life in the order of several seconds that was attributed to Pol II diffusion and binding to the promoter, and a slow component showing a half life of 14 to 20 min, resulting from engaged polymerases. The average transcription unit length in mammalian cells (~14 kb) and the previously reported polymerase speeds (1.1–2.5 kb/min) would predict an average elongation time in the order of 6–13 min, significantly shorter than that observed [38••]. This discrepancy could be explained if the polymerases were engaged on the DNA longer than the elongation process, raising the possibility that promoter escape, termination or pausing could add a rate-limiting step within the transcription unit. Ultimately, kinetic analyses at the single gene level will resolve these issues.

To follow the activity of specific genes in single living cells, cell lines have been generated where a particular gene is integrated as multiple tandem repeats in the genome (Figure 1) and can be visualized either using the lac operator repeat system (lacO/LacI) [39,40 and 41] or by the recruitment of fluorescent chimeric transcription factors to higher levels than the nucleoplasmic background [15, 16, 17 and 18]. Using this technology, a GFP–RNA-pol-II subunit was used to monitor the transcriptional activity of an MMTV tandem gene array: it was found that its activation was transient even in the constant presence of dexamethasone, showing a maximal activity 30 min post-activation [17]. Observing the loading of the RNA polymerases on the tandem gene array over time revealed variability among individual cells. The lacO/LacI system combined with a live-cell approach to RNA visualization [42 and 43] has provided information on the dynamics of chromatin at the locus of transcriptional activity and on the temporal resolution of gene activation (Figure 2) [44••]. Applying such techniques to the study of transcription kinetics within a gene will undoubtedly lead to a more precise understanding of this process.

Dynamics of mRNA within the nuclear environment

The mechanisms that regulate RNA flow from the transcription site are not well understood. It is now clear that mRNAs traverse the nucleoplasm by a diffusion-based mechanism. Measurements of nuclear RNAs labeled either with fluorescent probes [45,46 and 47] or with specific GFP-fused protein tags [48••[and] 49] have yielded a range of diffusion coefficients. Single-particle tracking of messenger ribonucleoprotein particles (mRNPs) in the nuclei of living cells allowed the detection of random movements [48••]. This motion was energy-independent and was not directed, although corralled motion implied the presence of intra-nuclear structures hindering the movements; mRNPs could be seen

'bouncing off' nucleoli, suggesting the presence of chromatin domains inaccessible to mRNPs [50 and 51]. Saturation of the export machinery by RNA overexpression revealed a reticular network throughout the nucleus space [52], corroborating previous studies showing mRNA movement within inter-chromatin channels [46]. A simplistic view of chromatin domains separated from inter-chromatin channels has been complicated by the fact that large macromolecule complexes can move through condensed chromatin regions [53•]. Chromatin regions were completely accessible to fluorescent dextrans with sizes of 3–10 kDa, but 70-kDa dextrans could not penetrate highly condensed areas. The radius of gyration of these inert molecules was calculated and found to be 4, 6 or 10 nm, values which correspond to either spherical proteins with molecular weights of approximately 400, 1400 and 6600 kDa or to ellipsoid proteins of 55, 180 and 850 kDa, respectively. Therefore, large macromolecule complexes such as RNA polymerases could gain access to most chromatin areas, whereas larger complexes in the megadalton range, such as RNP complexes (~50 nm diameter [54]), would appear to be excluded.

Gene positioning and mRNA export

An efficient and rapid path of mRNA export could result if active genes were associated with nuclear pores. Although studies have indicated that the nuclear periphery is actually involved in gene silencing, a screen operated on yeast has shown that classes of highly active genes, for instance genes involved in glycolysis or protein biosynthesis, are preferentially associated with nucleoporins, karyopherins and nuclear-pore-associated proteins [55••]. Moreover, transcriptional induction of the *GAL* genes caused their relocation towards the nuclear envelope, suggesting a mechanism for 'express shipping' of highly required transcripts. In another study, an active gene region was tethered to the nuclear pore by export proteins, including the transportin Cse1 [56]. Live imaging of GFP-tagged lac repressor protein (LacI) fused to the C-terminus of Cse1 showed not only the preferential relocation of a lacO locus to the nuclear periphery, but the occasional penetration of these chromosomal domains into the cytoplasm. The proximity of genes to the pore might be different in mammalian cells where the nucleus is much larger and chromosome organization more complex. Inactive genes in mammalian cells have been found to be associated with heterochromatin and the nuclear periphery, whereas active genes were sometimes interiorly positioned [57]. Yet the nuclear distribution of a specific mRNP was not influenced by the relative position of the transcription site in comparison to the nuclear envelope, implying that transcriptional proximity to nuclear pores might have more subtle or indirect effects [48••]. Genes distally separated by large DNA regions on the same chromosome had a high probability of sharing the same nuclear transcription space, demonstrating a high degree of spatial linkage within chromosome territories [58•], and other studies have shown that genes adopt preferential locations during differentiation [59, 60 and 61]. An analysis of the travels of endogenous mRNAs with respect to their distance from the nuclear envelope may reveal whether gene positioning can influence mRNA export in mammalian cells.

Nucleo-cytoplasmic transit of mRNA

Distinct RNA transport mechanisms are responsible for the export of different species of nuclear RNAs (mRNAs, spliceosomal U snRNAs and tRNAs). This implies the existence of

mechanisms that are able to distinguish between RNA species while in transit. For example, mRNAs and spliceosomal U snRNAs are both produced by RNA polymerase II transcription, yet are preferentially exported by different pathways. Interestingly, RNA length was found to be a parameter measured by the export machinery [62 and 63]. If shortened to <120 nt, mRNAs were exported by the U snRNA pathway, whereas U1 snRNA with a 300 nt insertion behaved like an mRNA. It remains to be defined whether the commitment to a certain pathway occurs during the formation of an mRNP at the transcription site or during its travels to the nuclear pore [64]. If the mRNP is indeed remodeled during transit, it would be of interest to see whether this occurs in a specific nuclear compartment.

The time necessary for transport of an mRNA molecule from its transcription site to the cytoplasm is still an open question due to the lack of suitable living cell systems for measuring the rates of transport of endogenous RNAs. The study of mRNA export in purified nuclei from *Xenopus* oocytes has yielded export times ranging from 10 min to 1 h. These biochemical studies depend on viral RNA systems or on microinjection of mRNAs that have been transcribed *in vitro*. The rabbit β-globin gene expressed under the control of a doxycycline-controlled inducible promoter allowed the kinetic analysis of mRNP assembly and transport following induction [65]. This approach yielded nuclear residency half-lives of 2.5–4.4 min, which obeyed first-order kinetics. Kinetic modeling of these data showed that the nuclear pool of mRNA built up slowly, whereas cytoplasmic accumulation was exponential. Differences in the nuclear dwell-times of the mRNAs were not observed if actin polymerization was inhibited or if cells were plated at low or high densities. The availability of new methods to label endogenous mRNAs [66] and the development of live cell systems for the study of mRNA transport, combined with kinetic modeling approaches, have been successfully applied to nuclear protein transport [67,68 and 69], and will provide new insights into the process of mRNA export.

Conclusions

mRNA transcription and export constitute a chain of molecular events offering many points of control. Although there are many combinations of promoter–transcription-factor associations, the downstream cellular responses cannot be explained by promoter firing alone. To understand the subtleties of these transcriptional pathways, single cell approaches are necessary. Recent developments in our ability to probe single cells in real time have yielded new information on the dynamics of gene expression. These studies will ultimately take us to the complex task of unraveling the dynamics of transcription within the live organism.

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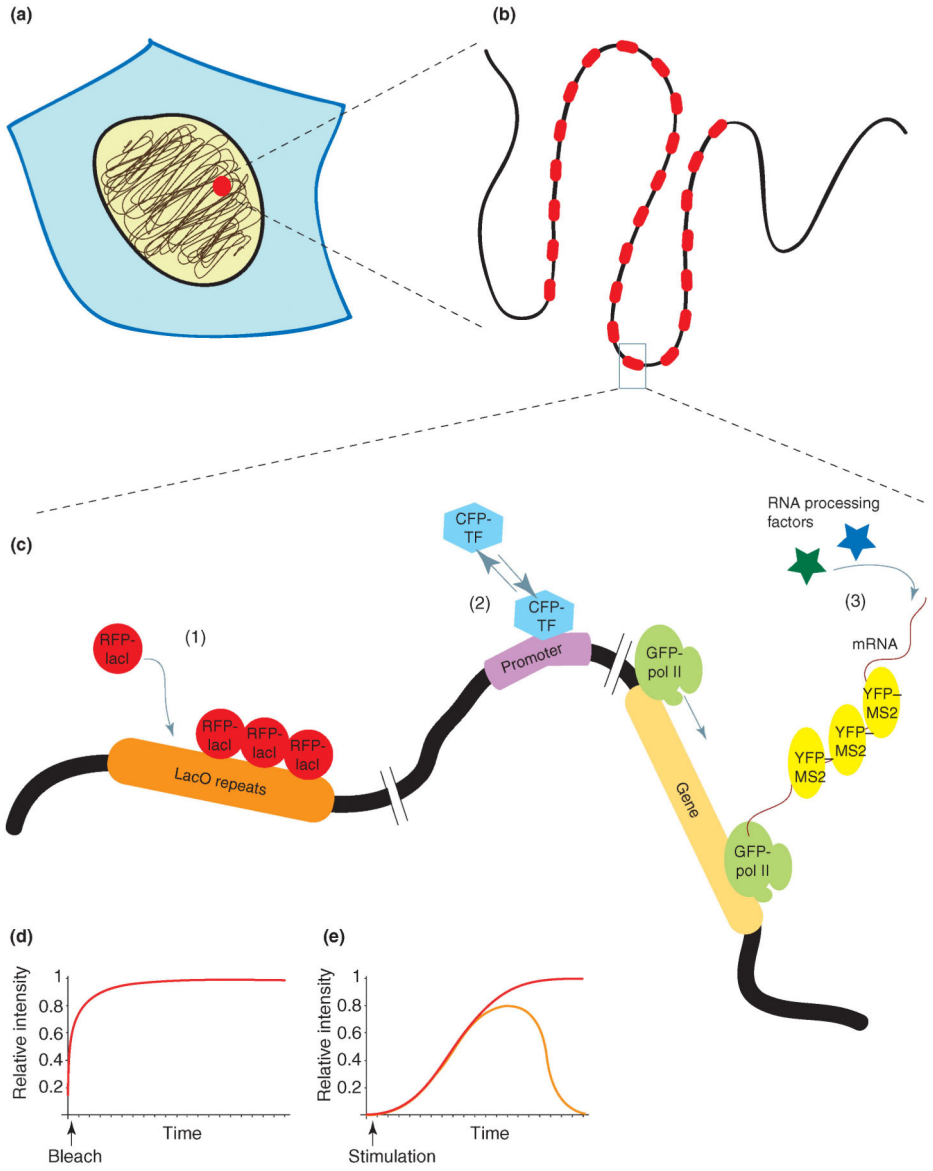

Figure 1.

Tandem gene arrays for the analysis of nuclear factor dynamics in living cells. The establishment of integrated tandem gene arrays in the genome provides a platform on which a signal from a fluorescently labeled DNA- or RNA-binding protein can be amplified, detected and analyzed kinetically. **(a)** The locus of integration occurs randomly in the genome and **(b)** many copies of the gene are inserted in tandem at this locus. **(c)** Different types of gene arrays have been used. (1) lac operator repeats (lacO) are bound by the lac repressor protein (LacI), which can either be tagged with a fluorescent protein to mark the locus of integration or can be fused to a protein of interest, thus tethering it to the chromatin. (2) Tandem arrays of promoters can serve for the analysis of transcription factor (TF) binding dynamics. (3) The production of mRNA can be followed using gene arrays that transcribe mRNAs containing MS2 repeats, which are bound by the YFP–MS2 protein. The kinetics of RNA pol II or RNA processing factors can also be analyzed on such arrays. **(d)**

Photobleaching methods such as FRAP are used to measure transcription-factor binding to gene arrays. **(e)** The onset of transcription can be measured upon cellular stimulation (red line shows a classical activation with saturation; orange line shows activation with a negative feedback).

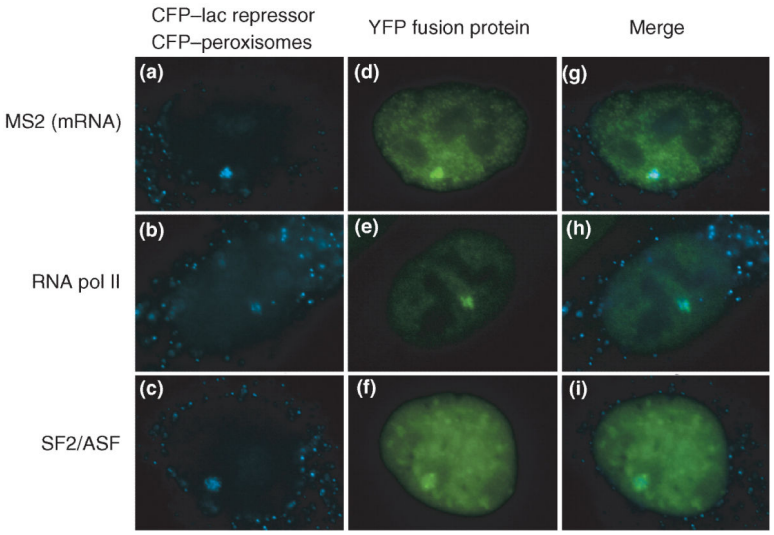

Figure 2.

Visualizing the binding of nuclear factors to tandem gene arrays. Human cells containing a tandem gene array (200 copies) were used for real-time detection of the recruitment of different factors to the site of active transcription. The tetracycline-inducible gene module gives rise to an intron-containing mRNA coding for a peroxisome-targeted CFP (cyan fluorescent protein) and containing 24 MS2 repeats in the 3′UTR. Each gene module is flanked by 256 repeats of the lacO binding site. **(a,b,c)** After induction of transcription, the gene locus is detected by the binding of the CFP–LacI protein to the lacO repeats and the peroxisome-targeted CFP protein product is observed in the cytoplasm. **(d)** The production of the mRNA at the gene locus is visualized by the binding of the yellow fluorescent protein (YFP)–MS2 protein to the MS2 stem-loops in the mRNA. **(e)** The recruitment of the transcription machinery to this active site of transcription is seen by the enrichment of YFP–RNA pol II at the locus. **(f)** The pre-mRNA splicing factor YFP–SF2/ASF is also enriched at the locus. **(g**,**h**,**i)** Merge. (Adapted from Janicki *et al.* [44••]).