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TRANSCRIPTION

Getting close to the action

Technological advances are increasing the temporal and spatial resolution with which RNA can be studied — down to the single-cell and even single-molecule level — and are thereby allowing researchers to unpick the dynamic details of gene expression. Four recent papers illustrate how high-resolution methods can provide insights into the rate and regulation of distinct stages of transcription.

Levels of mRNA are determined by a complex combination of transcription initiation, elongation, RNA processing and degradation, but the relative contribution of each stage has been difficult to assess. Rabani and colleagues explored the contributions of transcription versus degradation in stimulated mouse dendritic cells by using metabolic labelling of RNA coupled to high-throughput RNA sequencing to provide high temporal resolution of RNA regulation. Through computational modelling based on their data, they found that most changes in mRNA levels come from altering the transcription rate, but a regulated change in degradation has a role when a sharp 'peak' of expression is needed. Also, although generally constant across time, different genes have distinct rates of RNA degradation and processing, which could influence gene-specific regulation.

Larson *et al*. looked at the contributions of initiation and elongation to rates of RNA production. Their strategy involved inserting cassettes that encode multiple binding sites for the bacteriophage protein PP7 into target genes. As the cassettes are transcribed, the RNA forms stem–loops that are bound by a PP7–GFP fusion protein; this means that transcription of single molecules can be monitored over time in individual, living yeast cells. Interestingly, these authors found that elongation rates can vary during the cell cycle and their data are consistent with a model in which genes do not retain a 'memory' of transcription initiation and in which recruitment of a transcription factor is rate-limiting.

Transcription in eukaryotes and bacteria can occur in intermittent 'bursts' with stochastic switching of genes between 'off' and 'on' states. Two studies recently analysed this aspect of transcriptional kinetics using single-cell approaches. Harper *et al*. explored real-time dynamics in rat cells by using fluorescence microscopy to monitor expression of reporter genes driven by the prolactin promoter. By following two identical reporters in the same cell, they showed that the transcription cycles of the reporters were independent and that there was a period of ~3 hours between cycles that was refractory to initiation. The authors suggest that their data are consistent with independent chromatin cycles occurring at each gene. So *et al*. quantified the number of copies of various mRNAs by single-molecule fluorescence *in situ* hybridization in *Escherichia coli*. They show that the degree of 'burstiness' increases with gene expression level and that expression level seems to be varied by modulation of the gene off rate (with a constant gene on rate and transcription rate). It will be interesting to explore the extent to which mammals and bacteria share both transcription kinetics and molecular mechanisms.

In addition to their insights into transcription dynamics, all of these studies demonstrate the value of integrating experimental and mathematical approaches to address biological problems.

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ORIGINAL RESEARCH PAPERS Rabani, M. *et al.* Metabolic labeling of RNA uncovers principles of RNA production and degradation dynamics in mammalian cells. *Nature Biotech.* **29**, 436–442 (2011) | Larson, D. R. *et al.* Real-time observation of transcription initiation and elongation on an endogenous yeast gene. *Science* **332**, 475–478 (2011) | Harper, C. V. *et al.* Dynamic analysis of stochastic transcription cycles. *PLoS Biol.* **9**, e1000607 (2011) | So, L. *et al.* General properties of transcriptional time series in *Escherichia coli*. *Nature Genet.* 1 May 2011 (doi:10.1038/ng.821)