IMAGING AND VISUALIZATION

Snapshots of gene expression

There is increasing evidence that the dynamics of gene expression are more complicated than previously thought. Sensitive snapshots of mRNA expression in mammalian cells highlight dramatic random temporal variations in gene expression in the absence of external stimuli.

Live measurements offer many advantages, particularly when you want to examine dynamic changes over time. Sometimes, however, it is easier to obtain detailed temporal data by taking very precise snapshots rather than by making less precise live recordings.

Arjun Raj, Sanjay Tyagi and their colleagues at New York University and the Public Health Research Institute have taken this approach to show that mammalian cells transcribe genes in large, intrinsically random bursts of transcriptional activity. Previous studies examined the temporal kinetics of gene expression in live bacteria or yeast using GFP and fluorescence microscopy. Such studies indirectly indicated that transcription occurs in bursts. Alternatively, a series of binding sites for the MS2 RNA-binding protein can be added to a target gene. A GFP-MS2 fusion protein is expressed constitutively in the cells and changes in the level of target mRNA can be observed by looking at changes in GFP localization. This technology has provided evidence of mild burst-like transcription in bacteria.

There are drawbacks to these approaches, however. "The problem with fluorescent proteins is that they don't give you a very good indication of the actual transcription," remarks Raj. Because GFP is very stable, it actually represents a moving average of the level of transcription. Although the GFP-MS2 binding assay can provide real-time information about mRNA, it is plagued by high background, which limits the sensitivity.

Raj and his colleagues thought they could overcome these drawbacks by forgoing live-cell imaging and instead making very precise and sensitive measurements of mRNA molecules in fixed whole cells. They placed 32 sequence repeats at 3′ ends of reporter genes and integrated them in the genome of mammalian cells. They then performed RNA fluorescence *in situ* hybridization (FISH) using probes containing up to five fluorophores. They found they could detect individual mRNAs as single spots in fluorescent microscopy snapshots (**Fig. 1**). Raj wrote software to automatically recognize these FISH spots and to analyze the data.

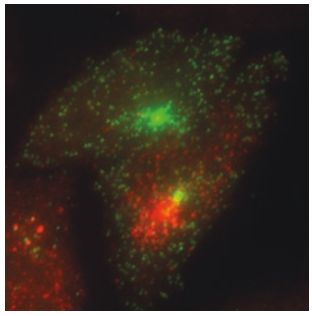

Figure 1 | A snapshot of single molecules of two different mRNAs in individual cells detected by FISH. Image courtesy of A. Raj.

One might think that this would not be very useful for determining temporal changes in expression, but it turns out to be quite powerful. Raj says, "You can infer individual cell behavior from a whole population if you assume that all the single-cell snapshots in the population are representative of what happens in one cell over time."

They observed very large variations in the FISH spots from cell to cell, indicative of pronounced uncoordinated bursts of gene activation. "Live imaging is sort of less useful for these kinds of studies," remarks Raj. "The main advantage is that we get very robust signals in comparison to other methods." Using statistical analysis, the authors determined both the frequency of the transcriptional bursts and their average size.

They exploited this high sensitivity to examine the expression of an endogenous gene that contained natural sequence repeats. Because it is easy to add differently colored fluorophores to the FISH probes, they were even able to examine mRNA from multiple genes at once.

Although there is ample room for methodological development in this area before the 'holy grail' of reliable live-cell single mRNA detection is achieved, this work shows that tweaks of older techniques combined with careful analysis can be very powerful.

Daniel Evanko

RESEARCH PAPERS
Raj, A. *et al.* Stochastic mRNA synthesis in mammalian cells. *PLoS Biology* **4**, e309 (2006).