# Imaging gene expression: tiny signals make a big noise

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**The hypothesis of ubiquitous fluctuations in gene expression has spurred the development of general methods for tracking temporal changes in protein concentrations in individual cells. The determination of protein levels with single-molecule sensitivity represents a significant advancement in the monitoring of cellular behavior that is driven by gene expression.**



Over the past decade, the notion of a 'noisy central dogma' has arisen from the convergence of live-cell measurement technologies and quantitative modeling aimed at describing gene expression. The underlying biochemical reactions involved in the dynamics of gene expression are inherently stochastic owing to the random nature of the binding and unbinding events in transcription and translation, along with the regulatory processes of activation and repression. These processes often involve a small number of reactant molecules, leading to relatively large fluctuations that can induce heterogeneous cellular responses during key biological processes[1]. Although several studies have demonstrated the existence and importance of expression variability arising from the small number of reactant molecules[2,3], detection limitations of cellular assays have precluded the quantification of gene expression noise in most contexts. Recently, two methods have been developed by Xie and colleagues for interrogating protein expression in individual living cells with single-molecule sensitivity[4,5]. Stochastic bursts of protein production in live cells were observed in both studies using ultrasensitive protein detection techniques.

Although previous single-cell experiments have used fluorescent reporters to track gene expression, high concentrations were a requirement for such studies because of the low sensitivity for detecting cytoplasmic fluorescent proteins with fast diffusion times. Yu *et al.* have circumvented this limitation by constructing a fusion protein consisting of a yellow fluorescent protein, Venus, and a membrane protein, Tsr, that can be detected with single-molecule sensitivity as a result of the slower diffusion times for membrane-bound protein molecules[4] (**Fig. 1a**). They expressed the membrane-bound fluorescent protein, Tsr-Venus, from a repressed *lac* promoter to allow for infrequent initiation of transcription such that mRNA transcripts were generated a single molecule at a time. Time-lapse fluorescence microscopy measurements from individual *Escherichia coli* cells revealed that a burst of Tsr-Venus fusion proteins were translated from each mRNA transcript. Further analysis of the protein expression bursts showed that the number of proteins produced from each mRNA transcript follows a geometric distribution which is consistent with a theoretical model for protein production.

Cai *et al.* have developed an alternative method based on the β-galactosidase (β-gal) reporter for monitoring protein production with single molecule sensitivity[5]. They modified the classic β-gal assay to achieve high sensitivity by using a microfluidic chamber to contain fluorescent product molecules that are actively pumped out of the cell (**Fig. 1b**). By measuring fluorescence signals from the microfluidic chambers over time, they were able to determine the integer number of β-gal molecules contained in live *E. coli* cells within the chamber. Using their gene expression assay, Cai *et al.* observed β-gal production from the *lac* promoter under highly repressed conditions and found that β-gal molecules are expressed in stochastic bursts, with the number of protein molecules per burst following an exponential distribution. Furthermore, their study demonstrated that the number of molecules per burst and the frequency of expression bursts can be found either using real-time protein production data or from an instantaneous measurement of steady-state protein copy number distributions.

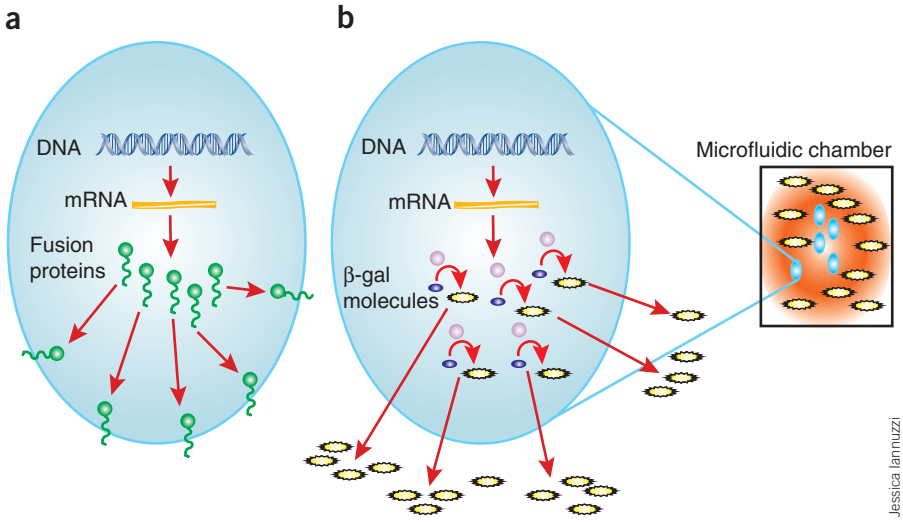


**Figure 1** Two novel techniques for monitoring gene expression with single-protein-molecule sensitivity. (**a**) Infrequent initiation of transcription results in the stochastic production of a single mRNA molecule at a time. Translation of the mRNA transcript generates a burst of multiple Tsr-Venus fluorescent membrane-binding proteins. Time-lapse fluorescence microscopy is used to detect the production of individual fluorescent fusion proteins. (**b**) mRNA molecules are transcribed from a highly repressed promoter. Translation of the mRNA produces a burst of β-galactosidase (β-gal) molecules. β-gal is an enzyme that hydrolyzes a synthetic substrate to produce a fluorescent product. The fluorescent molecules are expelled from the cell and accumulate in the surrounding microfluidic chamber. Time-lapse fluorescence microscopy is used to detect the fluorescence signal from the chamber, and the integer number of β-gal molecules is calculated from the fluorescence data.

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Both studies report stochastic bursts of protein expression from a repressed *lac* promoter, with the number of proteins per burst following an exponential distribution (or its discrete counterpart, the geometric distribution). Although such random bursts of gene expression have been previously observed, the protein detection techniques developed by Cai *et al.* and Yu *et al.* have provided a more detailed picture in the low-expression regime by detecting the integer number of proteins produced in each burst. In addition, these methods provide a general means of evaluating the significance of noise in important cellular processes where the number of molecules is difficult to detect using standard fluorescence microscopy techniques.

An important aspect of the Cai *et al.* study is the use of microfluidic devices to contain fluorescent product molecules in the extracellular environment. This approach sets the stage for coupling the single-molecule assay to other 'lab-on-a-chip' techniques. For example, microfluidic technology has recently demonstrated its potential in the context of cellular development within controlled environments. Gradients of diffusible chemicals are important in many biological processes, including biological pattern formation, cell chemotaxis and axon pathfinding. However, conventional assays for cell migration research have been traditionally limited in their ability to generate and maintain stable gradients. These assays have been replaced by microfluidic devices that can generate arbitrarily defined stable or dynamic concentration gradients[6]. Single-molecule resolution could be combined with these approaches to explore the effect of stochastic gene expression on cellular gradient sensors. Along the same lines, recent microfluidic chemostats have been developed for long-term cellular assays[7,8] and single-molecule resolution could lead to new insights into the consequences of gene expression variability over long periods of time.

The single-protein-molecule detection techniques presented by the Xie group have proved useful for examining the low-level expression of a single gene. Perhaps future studies will apply these approaches to more complicated systems involving a network of interacting genes. For example, a recent single-cell study has monitored the levels of multiple fluorescent reporter proteins to examine the dynamics of gene expression from a synthetic circuit containing alinear cascade of transcriptional repressors[2]. Performing similar experiments with single-protein-molecule sensitivity could provide information about noise propagation in a network in the low-expression-level regime. Such experiments would advance the development of quantitative modeling approaches that can be used to design synthetic gene regulatory networks[9] or to understand the role of fluctuations in native regulatory modules[10].

Single-cell gene expression experiments are necessary for observing cell-to-cell variations in protein production that are masked when monitoring population averages. The present studies represent a major step forward in such detection technology by demonstrating single-molecule resolution. This technology will likely lead to further insights into gene regulation as similar low-level gene expression assays are utilized in conjunction with breakthroughs in live-cell imaging and microfluidic technologies.


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# Orchestrating sulfur incorporation into RNA

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**The modified tRNA base 2-thiouridine is required for many steps in translation. Surprisingly, the biosynthesis of this simple derivative requires a minimum of four sulfur transfer reactions involving seven proteins.**

The biosynthesis of sulfur-containing metabolites was largely a mystery until the discovery of cysteine desulfurases by Dean and co-workers in 1993 (ref. 1). Since then, a rapid series of discoveries has implicated these enzymes in the biosynthesis of a wide range of sulfur-containing metabolites. These include enzyme cofactors such as thiamin, biotin and molybdopterin, thionucleosides in tRNA, and ubiquitous Fe-S clusters in proteins. Each of the biosynthetic pathways begins with the liberation of sulfur from cysteine by a cysteine desulfurase in the form of a cysteine persulfide, followed by its subsequent transfer to proteins that insert the sulfur atom into a specific substrate. In a recent issue of *Molecular Cell*, Ikeuchi *et al.*[2] have identified a surprisingly complicated series of sulfur transfers, involving multiple proteins, that is required for 2-thiouridine biosynthesis in bacterial tRNAs.

The 2-thiouridine modification is found at the wobble position of glutamyl, lysyl and glutaminyl tRNAs and is required for normal growth in many organisms, including eukaryotes. Studies on the related 4-thiouridine have shown that only the cysteine desulfurase IscS and a thiamin pathway protein, ThiI, are required *in vitro*[3]. Previous work had also shown that 2-thiouridine synthesis in *Escherichia coli* required IscS and MnmA, a homolog of ThiI (**Fig. 1**)[4]. Yet, in contrast to the 4-thiouridine system, 2-thiouridine activity *in vitro* was found to be very low, indicating a probable requirement for other factors. Because MnmA lacked a rhodanese sulfurtransferase domain found in some forms of ThiI[5], it seemed likely that one of the many known rhodanese-like proteins was involved. Suzuki and co-workers have now reported not only the missing sulfurtransferase, but an additional four proteins

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