MEETING NEWS

Rajendrani Mukhopadhyay reports from the **50th Annual Meeting of the Biophysical Society**—*Salt Lake City, Utah.*

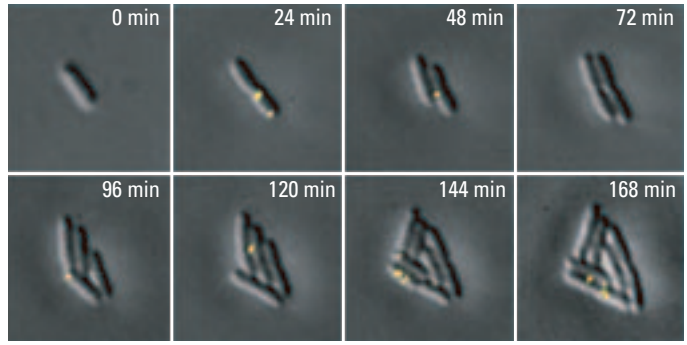
Going solo: Observing single-protein expression

It's like the ideal reality TV show for biologists: For the first time, X. Sunney Xie and colleagues at Harvard University have watched the birth of individual protein molecules in real time inside single, living cells. According to Xie, the ability to monitor the generation of lone protein molecules in live cells has "opened up ways of probing gene expression, the fundamental processes in biology, in a quantitative way at the single-molecule level."

Transcription and translation make up the two steps of gene expression. Transcription passes genetic blueprints from DNA to mRNA; translation uses the mRNA to build protein molecules. Gene expression is a stochastic event that is difficult to observe at the single-molecule level. The haphazard production of proteins makes observations a challenge, and many proteins aren't churned out in large, easily detectable quantities.

To overcome the challenges, Xie and colleagues used molecular biology tools to monitor the expression of proteins one at a time. As a first example, Xie, along with his graduate student Long Cai and postdoctoral fellow Nir Friedman, built a PDMS microfluidic device that trapped single *E. coli* cells in 100-pL volumes with the substrate fluorescein-di-β-D-galactopyranoside (FDG) (*Nature* **2006**, *440*, 358–362). The *E. coli* cells contained the gene for the enzyme β-galactosidase, which normally converts lactose to glucose. For their experiments, Xie and colleagues monitored the activity of the enzyme by watching it convert FDG to fluorescein.

However, the expression of the β-galactosidase gene is controlled by lactose. The absence of lactose causes a protein called the Lac repressor to bind to the DNA and shut off the transfer of information from the β-galactosidase gene to mRNA. But, even when lactose was absent, Xie and colleagues still saw abrupt bursts of fluorescence in the 100-pL volumes. These bursts occurred because the Lac repressor occasionally released its grip on the DNA, allowing some mRNA molecules to be created from the β-galactosidase gene. The mRNA molecules then survived long enough for a few β-galactosidase protein molecules to be made.



Images of individual YFP–Tsr proteins as they are expressed in *E. coli* cells over time. (Adapted with permission. Copyright 2006 American Association for the Advancement of Science.)

By measuring the amount of fluorescence generated by β-galactosidase, the investigators characterized the stochastic bursts of protein production by two parameters—the average frequency of bursts per cell cycle and the average number of protein molecules produced per burst. They determined that the frequency of bursts for the β-galactosidase gene was 0.11 ± 0.03 per cell cycle and that 5 ± 2 β-galactosidase molecules were produced per burst. The microfluidic-based assay wasn't limited to the examination of protein production in *E. coli* cells—Xie and colleagues also used it to monitor the low expression levels of proteins in yeast and mouse embryonic stem cells.

As a second example of single-protein production in individual, live *E. coli* cells, Xie, along with postdoctoral fellows Ji Yu and Jie Xiao, created a fusion protein with a yellow fluorescent protein (YFP) tag (*Science* **2006**, *311*, 1600–1603). YFP, a tag that rapidly folds into its mature form, was attached to a membrane protein called Tsr. The expression of the YFP–Tsr fusion protein was once again controlled by the absence of lactose and the Lac repressor.

By immobilizing the YFP–Tsr fusion protein at the cell membrane, the investigators could avoid the difficulties of rapid diffusion and cellular autofluorescence, which thwart fluorescence measurements in the cytoplasm. The proteins could be imaged on an individual basis because the tag's fluorescence was localized to a few pixels. To continuously monitor the proteins as they were generated, Xie and colleagues photobleached the YFP tag with a laser pulse once a protein was detected.

The investigators found that fusion-protein molecules were also generated in bursts. They observed ~1.2 bursts per cell cycle with an average of 46 min between 2 consecutive bursts. Xie and colleagues determined that, in a given burst, the Lac repressor released the DNA long enough to allow one mRNA molecule to be created from the gene for the YFP–Tsr protein. And from the single mRNA molecule, the investigators calculated that 4.2 ± 0.5 protein molecules were produced.

The stochastic behavior of gene expression for both β-galactosidase and the YFP–Tsr fusion protein demonstrates that each event is seemingly random. "You can never record an identical time trace even though the statistical properties of the time traces are reproducible," says Xie. "It is this intrinsic randomness at the single-molecule level that is partially responsible for different phenotypes in genetically identical cells or organisms."