Molecule by molecule, new assay shows real-time gene activity

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Chemists at Harvard University have developed the first technique providing a real-time, molecule-by-molecule "movie" of protein production in live cells. Their direct observation of fluorescently tagged molecules in single cells -- providing striking real-time footage of the birth of individual new protein molecules inside -- greatly increases scientists' precision in probing genetic activity.

Using the new assay, described this week in the journal *Science*, researchers led by Harvard's X. Sunney Xie counted, one by one, protein molecules generated in small bursts within cells as multiple ribosomes bound to single copies of mRNA complete the process by which DNA, an organism's long-term genetic repository, yields its crop of proteins. These random, or stochastic, bursts of protein expression are described in detail in a separate paper Xie and colleagues present this week in *Nature*.

"Although central to life processes, the expression of many important genes takes place at very low levels, making it difficult or impossible to observe using current technologies," says Xie, professor of chemistry and chemical biology in Harvard's Faculty of Arts and Sciences. "Our experiments provide the most sensitive means to date of observing real-time activity of single molecules inside cells. This new technique could provide us with unprecedented insights into gene expression and many other fundamental biological processes in living cells." The central dogma of molecular biology holds that DNA is transcribed into mRNA, which is then translated into proteins. But several technical hurdles have

hampered study of these key processes. Researchers' current understanding of this two-step pathway is built upon their averaging of genetic and biochemical activity across large populations of cells and molecules, masking the essential randomness of the process at the cellular level. Furthermore, much of our knowledge on the workings of the molecular machinery involved in gene expression comes from experiments done in vitro, rather than in living cells. Finally, the low sensitivity of current techniques for detecting gene expression has restricted analysis to highly expressed genes.

Xie's new assay addresses all three limitations. He and his colleagues melded a yellow fluorescent protein called Venus with Tsr, a hydrophobic membrane protein. The inclusion of the Tsr domain serves to anchor the fused protein to a cell's membrane, sidestepping the longstanding difficulty of imaging single proteins zipping about in cell cytoplasm, where diffuse fluorescent signals tend to be overwhelmed by background noise.

The gene coding for this combined protein was substituted for the well-studied lacZ gene in the Escherichia coli chromosome. When lacZ's regulatory machinery allows the modified gene to be converted into a handful of protein molecules, these Tsr-Venus hybrids migrate to the cell membrane, where each attaches firmly. The clearly visible flash from each Tsr-Venus molecule -- which when viewed across a population of cells looks somewhat akin to a sea of cellular paparazzi -- serves as an indication of that single protein molecule's production.

"Dr. Xie's experiments are the first to obtain quantitative, real-time information on protein expression in living cells at the single-molecule level," says Jeremy M. Berg, director of the National Institute of General Medical Sciences, which funded the work in part. "His imaging methods open up new possibilities for addressing fundamental questions about the precise events and factors involved in regulating these essential

processes. This is exactly the sort of highly innovative research with broad applicability that the National Institutes of Health Director's Pioneer Award was created to support."

Source: Harvard University