Technology Review
AN MIT ENTERPRISE

Tuesday, March 28, 2006

Proteins' Baby Pictures

A technique for imaging freshly made proteins is giving researchers a unique window on the working of genes and cells.

By Katherine Bourzac

For the first time, scientists have made movies of the birth of individual proteins in single cells. The ability to track protein production with such precision in vivo may make it easier to study the expression of important genes with low activity levels such as those that turn other genes on and off.

[Click **here (http://bernstein.harvard.edu/images/1119623clip.mov)** for video of proteins (yellow flashes) being made in dividing *E. coli* cells.]

Led by Sunney Xie, professor of chemistry and chemical biology at Harvard University, the scientists studied real-time protein production in two ways. In one set of experiments, they genetically engineered a gene for a fluorescent protein that moves to cells' membranes and becomes immobile; since the protein is still, the researchers could take its picture. In a second set of experiments, they created a microfluidic device with a series of holding chambers that they used to capture fluorescent molecules expelled from cells.

[**Click here (#)** for images from the experiments.]

Imaging individual proteins inside cells has traditionally been difficult because all cells have a small amount of background fluorescence, and cameras cannot track a single moving fluorescent protein over this faint glow.

"The cool thing about these experiments is that the tools they developed allow the measurement of transcription levels [gene expression] with single molecule accuracy," says **Alexander van Oudenaarden (http://openwetware.org/wiki/Van_Oudenaarden_Lab)**, systems biologist and associate professor of physics at MIT. "You can count single protein molecules."

Counting single proteins is powerful because many genes are not very active, and their products are effectively invisible using conventional techniques for studying gene expression. The gold standard in the field, the DNA microarray, is very good for studying genes with high activity levels but is less useful in looking at low-activity genes. Much of the genome, however, is not highly active. In his experiments, Xie could see very low levels of genetic activity across populations of living cells and in individuals.

"This research is really addressing a problem," says **Jeremy Berg (http://www.nigms.nih.gov/About/Director/BioSketch.htm)**, director of the National Institute of General Medical Sciences, who studies protein chemistry. "It's more the rule than the exception that proteins are made in relatively low copy numbers."

Xie says the microfluidic chambers could be used by other scientists to study genes of interest because they rely on a very commonly used enzyme called β-galactosidase. The Harvard researchers knew when cells were expressing β-galactosidase genes because the enzyme activates a fluorescent molecule. Levels of

fluorescence can be analyzed to determine how many molecules of the enzyme a cell made, and when. But the fluorescent molecule cleaved by β-galactosidase is quickly pumped out of cells. Xie addressed this problem with his microfluidic chambers, collecting the expelled molecule.

The technique could be applicable for studying a wide range of genes. Libraries of β-galactosidase genes attached to other genes are widely available; interested biologists could make bacteria or yeast cells with the enzyme's gene attached to their gene and study its expression using Xie's system.

But Xie's interest in getting a closer look at real-time gene expression goes beyond looking at genes with low activity. He studies the small but significant differences in the genetic activities of identical cells. Using conventional techniques, Xie says, "You just get average behavior." This wouldn't be a problem if genetically identical cells in identical environments behaved identically -- but, strangely, they don't. At the single-cell, single-molecule level, genetic activity is governed by randomness.

"Normally you think about gene expression or chemical reactions in general as very smooth phenomena, but it turns out they're very noisy, almost unpredictable," says van Oudenaarden, who in 2002 was one of the first scientists to provide quantitative evidence for the randomness of gene expression. "It's very apparent that there is a lot of fluctuation and variability in gene expression."

With Xie pushing the limits of resolution to single protein molecules, scientists can now do more quantitative studies of how individual cells can behave differently.