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## Monitoring Cell-Wide Protein Production with Ribosome Profiling

Scientists have developed a powerful tool that can quickly tell them the identity and quantity of every protein produced by a living cell at any given time.

With the new high-throughput technique, researchers can capture and quantify protein synthesis--effectively providing a new way to take a "protein census" of living cells. This advance may help scientists pinpoint which proteins drive specific diseases, and which of those proteins might be the best target for new drugs.

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The research, which was led by Howard Hughes Medical Institute investigator Jonathan S. Weissman and his colleagues at the University of California, San Francisco (UCSF), is reported in the February 12, 2009, *Science Express*.

Microarray technology--one of the best known and most heavily used techniques to quantify protein production--is quick, thorough and has revolutionized biomedical research. But it focuses on molecular evidence that is one step shy of actual protein production. As a result, while microarrays are used to identify messenger RNA, the key molecule that is ready to participate in new protein production, they do not measure the payoff – the actual assembly of proteins.

In contrast, Weissman's new technology, called ribosome profiling, is more precise and reliable in identifying which proteins a cell produces in response to changing conditions. It also provides a way to measure how much of any given protein is produced.

"We recognized that ribosome profiling could provide a powerful complement to microarray technology, taking the next step beyond

identifying messenger RNA to measure actual protein synthesis – the ultimate payoff of gene expression,” said Weissman, who is a Howard Hughes Medical investigator at UCSF.

It’s as if the microarray gives a snapshot of all the cars on the road, Weissman explains, but ribosome profiling can identify which cars are actually moving and even how fast they’re going. Ultimately, the “moving” proteins are the ones that matter, and they are the targets of drugs.

“The ability to monitor precisely which proteins a cell makes should broadly inform many aspects of biology including developmental biology, learning and even aging. Ribosome profiling could ultimately also have important clinical applications. For example, knowing the spectrum of proteins a cancer cell is producing could give a detailed blueprint of its pathology, which in turn will inform the prognosis and treatment of the disease,” Weissman said.

Ribosomes are molecular machines that bind to messenger RNA and translate the RNA message into protein. Ribosome profiling takes advantage of a recent revolution in gene sequencing technology, called deep sequencing. Weissman and his team decided to adapt this technology for their use because it is astoundingly fast and precise. In a single experiment, deep sequencing can identify 50-100 million RNA sequences--a 100-fold increase over the tools used widely in labs today. This power allowed the scientists to measure the assembly of each of the 4,500 different proteins produced in yeast cells.

"Ribosome profiling is really a marriage of a classic observation about ribosomes with the rapid advances in sequencing," said Nicholas Ingolia, lead author of the article and a postdoctoral fellow in Weissman’s lab.

“The observation is that the ‘footprint’ of the ribosome on messenger RNA tells you exactly which part of which protein it’s currently producing. The deep sequencing technology let us count tens of millions of ribosome footprints, and let us see every protein being made by the cell," Ingolia said.

The researchers analyzed protein production in yeast cells because yeast’s simpler genome and well-characterized complement of proteins made it an excellent test case for ribosome profiling. The scientists don’t expect to run into any significant hurdles when they begin to use ribosome profiling on human cells.

In the experiments reported in *Science Express*, the HHMI scientists compared protein synthesis in normal yeast and those that have been starved of amino acids, a form of stress. They showed that nearly one-third of all protein production is regulated at the level of translation--regulation that is invisible to microarray measurements.

The key to ribosome profiling lies in capturing each messenger RNA molecule in its union with ribosomes. The RNA-ribosome unit is the site of protein synthesis. Ribosomes normally start near one end of the RNA sequence and move down the line, assembling amino acid chains according to instructions encoded in the sequence. Weissman likens the ribosome to

beads on the RNA chain.

Depending on the amount of protein to be made, more than 20 ribosomes may be bound to any RNA molecule, or just one. Ribosome profiling identifies the protein being synthesized by determining exactly which part of the mRNA is being read by the ribosome, and it identifies the abundance by the number of ribosomes bound to the RNA.

The ribosome profiling technique involved lysing yeast cells to collect all the messenger RNA molecules with ribosomes bound to them. Using standard techniques, the scientists then cut away all of the RNA not directly bound to the ribosome; isolated each of the RNA-ribosome units by centrifugation; and then recovered the messenger RNA fragments. The RNA was then copied back into DNA and analyzed by deep sequencing.

Weissman and Ingolia think that high-throughput sequencing for measuring protein synthesis may gain widespread use in research and pharmaceutical labs, much as the powerful microarray technology has. Cells in the body tightly control protein translation to prevent conditions such as diabetes. The new tool would be vital to aid researchers trying to understand how cells control or fail to control protein translation when under disease-induced stress. The understanding should aid diagnosis, treatment and development of new drugs, Ingolia said.