In scientific first, researchers visualize proteins being born

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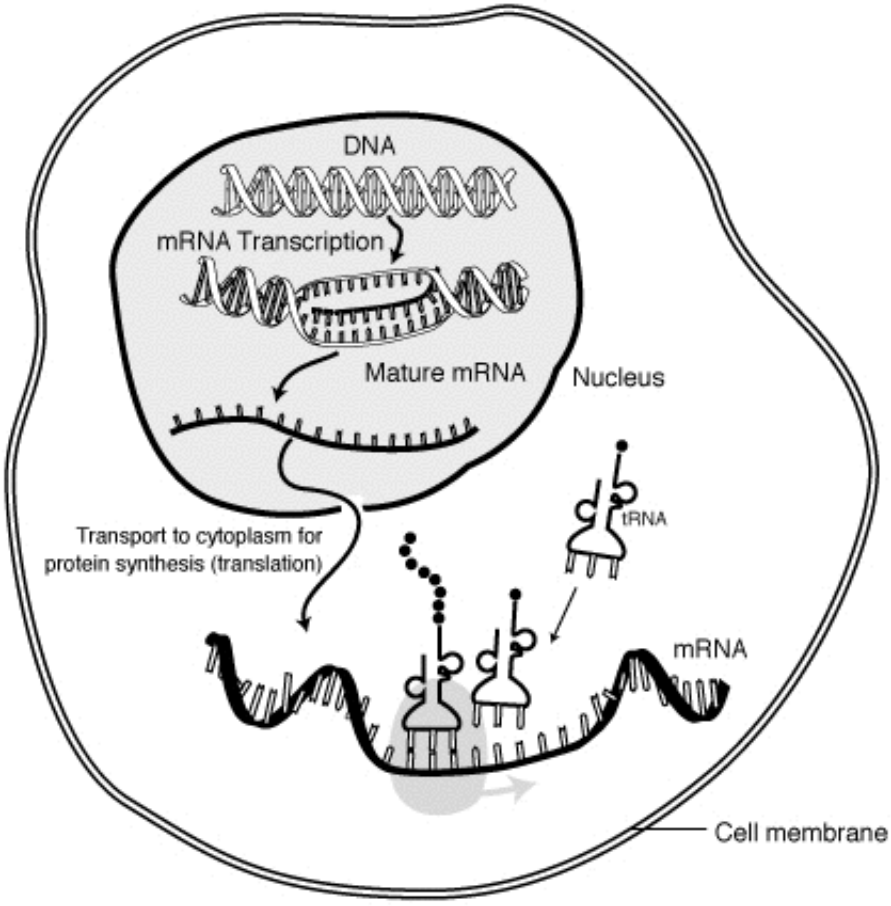

The "life cycle" of an mRNA in a eukaryotic cell. RNA is transcribed in the nucleus; processing, it is transported to the cytoplasm and translated by the ribosome. Finally, the mRNA is degraded. Credit: Public Domain

For the first time, scientists at Albert Einstein College of Medicine have developed a technology allowing them to "see" single molecules of messenger RNA as they are translated into proteins in living mammalian cells. Initial findings using this technology that may shed light on neurological diseases as well as cancer were published online today in *Science*.

"Translation is the fundamental biological process for converting mRNA's information into proteins," said Robert Singer, Ph.D., the paper's senior author and co-chair of anatomy & structural biology and co-director of the Gruss Lipper Biophotonics Center at Einstein. "We know from genome-wide studies that translation controls protein abundance in cells—crucially important to every single function that cells carry out. Using this technology, we can finally learn how translation is regulated and gain major insights into diseases that occur when translation is faulty." Dr. Singer also holds the Harold and Muriel Block Chair in Anatomy & Structural Biology at Einstein.

The production of the thousands of different proteins made by our cells starts in the nucleus, when protein-making information encoded in a gene's DNA is transcribed into molecules of messenger RNA (mRNA). These mRNA molecules exit the nucleus and migrate to specific regions of the cytoplasm. In the next step, called translation, the mRNA molecules hook up with molecular structures called ribosomes. Using mRNA as their blueprint, the ribosomes generate proteins by linking together amino acids one at a time. Researchers can use the Einstein technology to follow single mRNA molecules in real time as they arrive at their destination in the cytoplasm—and then to observe the proteins as they are being generated by the ribosomes.

The Einstein scientists observed the translation of single mRNA molecules in two types of cells: human cancer (osteosarcoma) cells and mouse neurons. The scientists made a surprising finding in neurons,

where mRNA translation into protein was found to occur in "bursts"—a phenomenon never before possible to observe.

"Neurons must control protein synthesis very closely, because nerve transmission depends on synthesizing the right amount of protein at precisely the right place: the synapses, where neurons form circuits," said Dr. Singer. "Bursts of translation activity may be the best way for neurons to control the amount and location of protein production—and neurological disease may result from neurons' inability to control that bursting. So our findings may have implications for intellectual disorders such as Fragile X Syndrome, which seem to involve too much protein production, and possibly for neurodegenerative disorders such as Alzheimer's in which clumps of beta-amyloid protein may block neuron-to-neuron signaling at synapses."

Another surprising observation occurred when the researchers looked at mRNA translation in cancer cells. In contrast to neurons, cancer cells displayed a striking inability to regulate the translation of mRNA. Instead, mRNA translation was a continuous process in these cells. Since proteins play crucial roles in controlling cell division, the uncontrolled translation of certain proteins may lead to certain types of cancer.

"With our technology, researchers can now study disease-causing protein aberrations at a very basic level that was never possible before," says Dr. Singer.

The Einstein technology for observing the translation of single molecules of mRNA was developed primarily by Bin Wu, Ph.D., the lead author of the study and research assistant professor of anatomy & structural biology. It involved two challenges: visualize single molecules of mRNA as well as single molecules of protein translated from the mRNA. In research published in *Molecular Cell* in 1998, Dr. Singer's lab had become the first to successfully visualize single molecules of mRNA in

living cells, so Dr. Wu adapted that technique here.

To the mRNA that codes for the protein actin, he added mRNA that codes for red fluorescent protein along with "membrane targeting sequence" mRNA that helps the mRNA molecule find its way to the endoplasmic reticulum (ER)—a membranous cellular structure that is a major focus of protein synthesis and that transports proteins and lipids throughout the cell. This package of mRNA was inserted into cells by attaching it to a retrovirus used to infect them. As the mRNA molecules diffused towards the ER, each synthesized a "nascent peptide" that tethered it to the ER—after which translation began in earnest.

Next he tackled the second challenge, which was finding a way to visualize the individual proteins being born as the mRNA was translated. Here Dr. Wu used a recently published technique in which genetically encoded single-chain antibodies fused to green fluorescent protein recognize the newly formed protein and bind to it, making the protein visible. While this first study looked at translation of single mRNA molecules in neurons and cancer cells, the technology can potentially be used to study translation in any type of cell.

More information: B. Wu et al. Translation dynamics of single mRNAs in live cells and neurons, *Science* (2016). DOI: 10.1126/science.aaf1084

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