

Scientists first to see RNA network in live bacterial cells

October 22 2009

Scientists who study RNA have faced a formidable roadblock: trying to examine RNA's movements in a living cell when they can't see the RNA. Now, a new technology has given scientists the first look ever at RNA in a live bacteria cell—a sight that could offer new information about how the molecule moves and works.

Interest in RNA, which plays a key role in manufacturing proteins, has increased in recent years, due in large part to its potential in new drug therapies. RNA localization and movement in bacterial cell are poorly understood. The problem has been finding a way to mark RNA in a living cell so that scientists can track it, says Natasha Broude, a research associate professor at Boston University's Department of [Biomedical Engineering](#).

"You can label any [protein](#) within the cell and watch what it is doing," says Broude, a senior researcher on the new study, published in a recent issue of the [Proceedings of the National Academy of Sciences](#). "For RNA it was much more difficult because RNA is more mobile and less stable than both proteins and DNA."

Before now, scientists used [green fluorescent protein](#) (GFP) to label RNA in a cell. But proteins were also tagged with GFP and their fluorescence was so bright, it drowned out the glow from the RNA. "The initial idea was to do something to allow us to decrease background fluorescence," Broude says.

In 2007, Broude and her colleagues developed a system to persuade a cell to synthesize protein in two fragments rather than a whole, which made the protein inactive. They then modified an RNA molecule, adding a small tail of RNA sequence that works like a handle, grabbing the fragments and pulling them together, which makes the protein active—and glow bright green. The scientists can then follow the RNA as it moves through the cell.

"In our case, the protein becomes fluorescent because it binds to RNA," Broude says. "If there is no RNA, we don't see this protein."

In this new work, the team modified this system to allow for the controlled synthesis of RNA—allowing the researchers to track RNA as soon as it appears in the cell. For the study, they used live *Escherichia coli* cells, the simplest [bacteria](#) model, and a nonfunctional RNA. To monitor the RNA and capture images as it moved through the cell, the team used a sophisticated microscope and detection system developed by colleague Amit Meller, a co-author of the study and associate professor of biological engineering at Boston University. Meller's system made it possible to watch RNA in whole cells with high resolution. Their observations are not only the first of their kind, they also contradict previously held theories about RNA localization, which held that RNA was evenly distributed throughout the cell.

"The first thing we saw is that RNA is localized along mostly the periphery of the cell," Broude says. One possibility for this could be that the middle of the bacterial cell, which is occupied by DNA, is less accessible to the RNA.

The researchers also noted that the RNA appeared to form helical structures resembling those seen in proteins involved in producing the cell's cytoskeleton, which is involved in DNA replication, cell division and other important processes. "They are necessary structural elements

which rule all changes in bacterial life," Broude says. "But we need to learn more before we can say anything about the RNA helical structure's function."

With this new technology in place, Broude and her colleagues can learn more about the RNA network they've observed, examine the localization and movement of other types of RNA in live bacterial cells and, ultimately, mammalian cells.

Source: Boston University

Citation: Scientists first to see RNA network in live bacterial cells (2009, October 22) retrieved 23 April 2024 from <https://phys.org/news/2009-10-scientists-rna-network-bacterial-cells.html>

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