Timing is everything: First step in protein building revealed

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Timing is everything, it seems, even in science. A team led by Johns Hopkins scientists has unraveled the first step in translating genetic information in order to build a protein, only to find that it's not one step but two.
In a series of experiments, the scientists found that when yeast's protein-building machinery recognizes the starting line for a gene's instructions, it first alters its structure and then releases a factor known as eIF1, a step necessary to let it continue reading the assembly instructions. Even though yeast are the most primitive relatives of humans, the protein-building machinery, or ribosomes, of each are quite similar.

"The idea is to really know at the molecular level how life is put together," says Jon Lorsch, Ph.D., professor of biophysics and biophysical chemistry, one of the departments in Johns Hopkins' Institute for Basic Biomedical Sciences. "We see disease largely as an incorrect timing event -- the wrong thing happening at the wrong time, or the lack of the right thing."

As a result, Lorsch studies the timing of how the ribosome complex itself assembles and how other factors come and go as it translates genetic information to build proteins, the workhorses of cells. If the ribosome doesn't start in the right place along a gene's instructions, it will make the wrong protein, which can kill the cell or lead to disease.

"The ribosome is the end stage of gene expression, and gene expression keeps us alive and causes disease," says Lorsch. "If we can better

understand how the ribosome works, perhaps we can harness it to help us fix disease."

Already, scientists knew that without eIF1, the ribosome can start reading the gene's RNA instructions at places other than a particular three-block piece of RNA known as the "start codon." And excessive amounts of eIF1 are associated with cardiac hypertrophy, or an enlarged heart.

While eIF1's role in cardiac hypertrophy remains a mystery, the new discovery reveals exactly how eIF1 regulates the ribosome's activity. The research team has demonstrated that eIF1's mere presence on the yeast ribosome prevents the machinery from getting started. Only after its release from the complex can the ribosome start making proteins.

"No one had any idea when eIF1 was released from the ribosome, or that its release might serve an important purpose, so this was a completely unexpected result," says graduate student David Maag, first author of the paper.

"It's impossible to know for sure whether eIF1 is released completely in living creatures, but in our laboratory experiments that is clearly the case," adds Lorsch. "Even if it isn't released completely in intact cells, our results would indicate that it must be very loosely associated for translation [protein building] to begin."

To monitor what was happening to eIF1, the researchers tagged it and a related part of the ribosome with different fluorescent chemicals. When two fluorescently labeled molecules are near one another, the fluorescent chemicals subtly interact, which changes the color or wavelength of light that is given off. If the distance between the fluorescent molecules changes, the color of the emitted light changes as well.

The researchers successfully used this phenomenon, known as fluorescence resonance energy transfer or FRET, to monitor the relationship between eIF1 and its relative as the ribosome complex assembled and after RNA was added to the mix.

"We weren't even sure the two fluorescent molecules would be close enough together to create a FRET signal at all," says Maag. "We were very pleased just to be able to monitor it, and then we were surprised and pleased by what we saw next."

They had expected -- or at least hoped -- to see a shift in the color of light once the RNA was mixed in. Instead, they saw two shifts in the color given off. First, there was a slight shift, indicating a small change in the distance between eIF1 and its relative, and then a much larger shift, indicating a much bigger separation.

To prove eIF1 was being released from the ribosome complex, the researchers examined how fast the pieces of the ribosome come together, and how long it takes them to fall apart under various circumstances. Their results support the idea that two separate steps take place once the instruction's starting point is found: first a structural change in the ribosome complex, and then release of eIF1.

Lorsch's goal is know the five "Ws" and one "H" that affect timing of all of the ribosome's pieces and activities. But unraveling every what, when, where, why, who and how is no small task -- roughly 27 bits like eIF1 play a role at one point or another. To tackle the problem, Lorsch and his colleagues move between "timing" studies of the ribosome's molecular comings and goings, and genetic studies that create mutant ribosome parts, which likely affect ribosome function -- and change its timing.

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Development; and Zygmunt Gryczynski of the University of Maryland School of Medicine.

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