Researchers 'watch' formation of cells' protein factories for first time

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A team from The Scripps Research Institute has revealed the first-ever pictures of the formation of cells' "protein factories." In addition to being a major technical feat on its own, the work could open new pathways for development of antibiotics and treatments for diseases tied to errors in ribosome formation. In addition, the techniques developed in the study can now be applied to other complex challenges in the understanding of cellular processes.

Identifying and observing the molecules that form ribosomes—the cellular factories that build the proteins essential for life—has for decades been a key goal for biologists but one that had seemed nearly unattainable. But the new Scripps Research study, which appears in the October 29, 2010 issue of the journal *Science*, yielded pictures of the chemical intermediate steps in ribosome creation.

"For me it was a dream experiment," said project leader James Williamson, Ph.D., professor, member of the Skaggs Institute for Chemical Biology, and dean of graduate and postgraduate studies at Scripps Research, who credits collaborators at the Scripps Research National Resource for Automated Molecular Microscopy (NRAMM) facility for making it possible. "We have great colleagues at Scripps to collaborate with who are willing to try some crazy experiments, and when they work it's just beautiful."

Past studies of the intermediate molecules that combine to form ribosomes and other cellular components have been severely limited by

imaging technologies. Electron microscopy has for many years made it possible to create pictures of such tiny molecules, but this typically requires purification of the molecules. To purify, you must first identify, meaning researchers had to infer what the intermediates were ahead of time rather than being able to watch the real process.

"My lab has been working on ribosome assembly intensively for about 15 years," said Williamson. "The basic steps were mapped out 30 years ago. What nobody really understood was how it happens inside cells."

Creating a New View

The NRAMM group, led by Scripps Research Associate Professors Clinton Potter and Bridget Carragher and working with Scripps Research Kellogg School of Science and Technology graduate students Anke Mulder and Craig Yoshioka, developed a new technique, described in the Science paper and dubbed discovery single-particle profiling, which dodges the purification problem by allowing successful imaging of unpurified samples. An automated data capture and processing system of the team's design enables them to decipher an otherwise impossibly complex hodgepodge of data that results.

For this project, second author Andrea Beck, a research assistant in the Williamson laboratory, purified ribosome components from cells of the common research bacterium Escherichia Coli. She then chemically broke these apart to create a solution of the components that form ribosomes. The components were mixed together and then were rapidly stained and imaged using electron microscopy. "We went in with 'dirty' samples that contained horribly complex mixtures of all different particles," said Williamson.

Mulder, who is first author on the paper, collected and analyzed the particles that were formed during the ribosome assembly reaction. Using

the team's advanced algorithms, they were able to process more than a million data points from the electron microscope to ultimately produce molecular pictures.

The Pieces Fit

The team produced images that the scientists were able to match like puzzle pieces to parts of ribosomes, offering strong confirmation that they had indeed imaged and identified actual chemical intermediates in the path to ribosome production. "We always saw the same thing no matter how we processed the data, and this led us to believe this was real," said Williamson.

Further confirmation came as the researchers imaged components from different timeframes. After breaking down ribosome components, the scientists prepared samples at various stages allowing enough time for the molecular mix to begin combining as they do during ribosome creation in cells.

Imaging this time series, the team was able to show higher concentrations of larger, more complex molecules and fewer smaller molecules as time elapsed. These results fit with the limited information that was already available about the timing of formation steps, providing further confirmation of the team's success.

Interestingly, this work also confirmed that there are more than one possible paths in ribosome formation, a phenomenon known as parallel assembly that been suggested by prior research but never definitively confirmed.

Long-Term Potential

Williamson says that with the information now at hand, they will be able to move forward with studies of which additional molecules might be present in cells and essential for ribosome formation. Such data could offer exciting medical potential.

All bacteria contain and are dependent on ribosomes. Identification of molecules required for ribosome assembly could offer new targets for antibiotic drugs aimed at killing bacteria. "If we can figure out how to inhibit assembly, that would be a very important therapeutic avenue," said Williamson.

There are also indications that some diseases such as Diamond Blackfan Anemia might be caused, at least in some cases, by errors in ribosome production. Better understanding of that production could also reveal ways such errors might be repaired to cure or prevent disease.

At the more basic level, this successful project has also proven techniques that Scripps Research scientists and other researchers can apply to allow similar imaging and understanding of other complex but critical cellular processes.

More information: "Visualizing Ribosome Biogenesis: Parallel Assembly Pathways for the 30S Subunit," *Science*, October 29, 2010.

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