

JULY 20, 2000

Technique Shows Ratcheting Motion of Ribosomes

Using a technique called three-dimensional cryo-electron microscopy, researchers have detected a ratcheting rotation deep inside the cell's tiny protein-making "factory" at a key point in the protein construction process.

The ratcheting motion of the ribosome, a large protein-making organelle, consists of a rapid rotation of one of the ribosomal subunits relative to the other just as the messenger RNA (mRNA) and the attached molecules of transfer RNA (tRNA) are advanced by one codon. Howard Hughes Medical Institute investigator Joachim Frank and colleague Rajendra Kumar Agrawal at Health Research Inc., at the Wadsworth Center in Albany, New York, reported their discovery in the July 20, 2000, issue of the journal *Nature*.

The finding highlights the burgeoning ability of researchers to tease apart the details of protein synthesis a key biological process that occurs in every living cell. "This just gives us a flavor of what is yet to come," said Frank of his laboratory's efforts to understand the intricate contortions of the ribosome. "We have also seen other movements that play a key role in ribosomal function and we will continue to explore those."

The ribosome is a large molecular complex of RNA and protein. When ribosomes are isolated from cell extracts, two different fractions are obtained. One fraction consists of a smaller, 30S subunit, and the other, a larger 50S subunit. The 30S subunit binds the mRNA, as well as tRNA which carries each specific amino acid to be added to the growing chainlike protein molecule. As the 30S unit, thus, helps in "reading" the mRNA, the larger 50S subunit catalyzes the formation of the bond between each amino acid and the growing protein.

After each bond is made, a molecule called elongation factor G binds to the ribosome. This binding, along with the chemical reaction of the energy-containing molecule GTP, triggers the translocation, or movement, of the mRNA and the tRNAs attached to it by one unit, or codon. Once it is moved, the mRNA can be read to determine the next amino acid to be added.

A central question, said Frank, was whether the two ribosomal subunits underwent some sort of movement relative to one another to facilitate the translocation.

"There have been hypotheses about subunit movement for years," said Frank. "But there has never been a direct confirmation of this. The problem was that all the evidence was indirect. Scattering studies, for example, indicated changes in specific regions of subunits between one state and the other, but they were never directly observed. And it is only now, with cryo-electron microscopy, that we can visualize the ribosome with such clarity."

Three-dimensional cryo-electron microscopy (cryo-EM) is one of the few techniques capable of visualizing large, dynamic molecules. In preparing for cryo-EM, researchers first immerse the ribosomes in water solution and then abruptly freeze them in supercold liquid ethane. The rapid freezing imprisons the ribosomes in ice, thus preserving their native structure. Using an electron microscope with a low-intensity beam to avoid damaging the molecules, scientists obtained images of the thousands of captive ribosomes. The scientists employed sophisticated computerized image analysis to produce a detailed, three-dimensional map of ribosome motion from the otherwise low-contrast, noisy images produced by the electron microscope.

To capture the ribosome in the act of moving mRNA and tRNA, the scientists added a non-working analog of GTP along with elongation factor G to the ribosomes, effectively stopping protein synthesis dead in its tracks.

"Using this analog made it possible to capture a state in which the elongation factor is bound to the ribosome, but there is no further progress," said Frank. "So, the whole system is frozen by a chemical means."

Their analysis of the chemically frozen ribosomes revealed that when the elongation factor and GTP bind to the 30S subunit, it rotates about six degrees with respect to the 50S subunit. And after the GTP chemical reaction, the 30S subunit rotates back.

"This rotation goes along with other movements," said Frank. "If one looks at the entry channel for the mRNA into the ribosome, it normally appears to narrow and widen as the subunit moves back and forth. It is exactly the expected opening if one thinks that in one state the mRNA has to be free to move and in the other state it needs to be secured and prevented from moving."

According to Frank, further advances in cryo-EM together with detailed atomic-resolution analysis from x-ray crystallography studies should provide even greater insight into ribosomal movement during protein synthesis.

Frank and his colleagues are now developing a technique to synchronize ribosomal processes before freezing, so that the thousands of ribosomes that are frozen in a given preparation are halted at precisely the same point in protein synthesis. Such synchrony would enable the researchers to study the mass of ribosomes at any particular point, to further understand how ribosomes aid in the construction of proteins.