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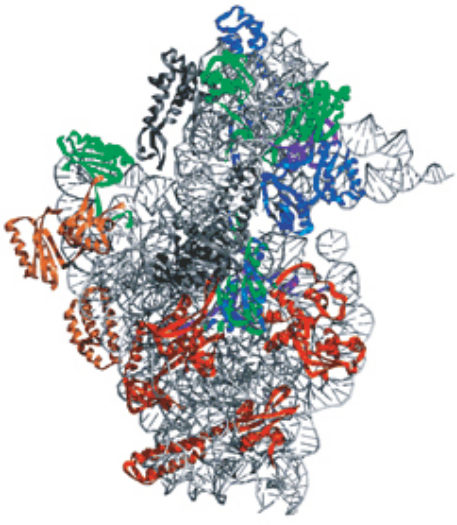
Tracking Cellular Machine Assembly

Technique observes how parts of a macromolecular complex bind in real time

Amanda Yarnell

By combining isotopic labeling and mass spectrometry, researchers have devised a way to study how huge cellular macromolecular complexes assemble in real time (*Nature* **2005,** *438*, 628).



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ASSEMBLY LINE Twenty different proteins must bind to ribosomal RNA (gray) to assemble the 30S ribosome. Here, these proteins are colored according to their binding rates: red (fastest), orange, green, blue, and purple (slowest).

James R. Williamson, Megan W. T. Talkington, and Gary Siuzdak of Scripps Research Institute demonstrate the power of their technique on the bacterial 30S ribosome. The 30S ribosome is part of the bacterial protein-making machinery and contains a large RNA molecule and 20 different proteins. Using their technique, the team measured the rates at which 17 of the 20 proteins bind to the RNA during 30S ribosome assembly.

"The elegance of their experimental design should allow it to be adapted to a wide range of such complexes," comments Sarah A. Woodson of Johns Hopkins University in an accompanying *Nature* commentary. A clearer picture of how large cellular complexes assemble should improve our understanding of how such complexes evolved and may guide the development of materials that mimic their properties, she adds.

To track assembly, the Scripps team introduced isotopically labeled components during a certain time window during complex assembly. They then measured the isotopic ratios of the resulting complexes and their individual protein components by matrix-assisted laser desorption ionization mass spectrometry. By varying the length of the isotopic "pulse," the researchers were able to calculate the rates at which each protein binds to the complex.

By repeating the experiment at different temperatures, Williamson and coworkers obtained results allowing them to conclude that, contrary to previous observations, assembly of the 30S ribosome doesn't irreversibly stall under less-than-perfect conditions. "This suggests that the assembly of key macromolecular complexes such as the ribosome might proceed via an energetic landscape of multiple pathways," a situation that might have evolutionary advantages, Talkington says.

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