Microscopy TODAY

Coming Events

2010

SPIE Scanning Microscopy 2010
May 17–19, 2010, Monterey, CA
www.spie.org

Electron Backscatter Diffraction Topical Conference
May 24–26, 2010, Madison, WI
www.microbeamanalysis.org/ebsd-2010

Lehigh Microscopy School
June 6–18, 2010, Bethlehem, PA
www.lehigh.edu/microscopy

E-MRS Spring Meeting
June 7–11, 2010, Strasbourg, France
www.emrs-strasbourg.com

3D Microscopy of Living Cells
June 12–24, 2010, Vancouver, Canada
www.3dcourse.ubc.ca/2010

Basic Confocal Microscopy
June 14–18, 2010, Columbia, SC
http://dba.med.sc.edu/price/irf/irf.htm

3-D Electron Microscopy 2010
June 20–25, 2010, Lucca, Italy
www.grc.org

Microscience 2010
June 29–July 1, 2010, London, UK
www.rms.org.uk

ACMM-21
July 11–15, 2010, Brisbane, Australia
www.microscopy.org.au

Inter/Micro 2010
June 12–16, 2010, Chicago, IL
www.mcri.org/home/section/101/inter-micro

Microscopy & Microanalysis 2010
August 1–5, 2010, Portland, OR
www.microscopy.org

International Microscopy Congress
September 19–24, 2010, Rio de Janeiro, Brazil
www.imc-17.com

2011

Microscopy & Microanalysis 2011
August 7–11, 2011, Nashville, TN

2012

Microscopy & Microanalysis 2012
July 29–August 2, Phoenix, AZ

2013

Microscopy & Microanalysis 2013
August 4–8, Indianapolis, IN

More Meetings and Courses
Check the complete calendar near the back of this magazine and in the MSA journal *Microscopy and Microanalysis*.

Carmichael's Concise Review

Formation of New Proteins Seen from the Beginning

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All cells have the ability to synthesize and secrete proteins. Although many details of this process are well-known, Martin Kampmann and Günter Blobel recently highlighted two "landmark papers" that used cryo-electron microscopy (cryoEM) to obtain information at subnanometer resolution, which provided direct visualization of nascent polypeptide chains in the tunnel with ribosomes [1]. It is known that the signal peptide (the first few amino acids on the amino terminal that do not become part of the final polypeptide) emerges from a ribosome and engages the signal recognition particle (SRP) in the cytoplasm, and this complex is directed to the SRP receptor on the endoplasmic reticulum (ER). The SRP is released, the signal peptide enters the protein-conducting channel (PCC), and the nascent polypeptide chain (that will become the protein) enters the lumen of the ER.

Thomas Becker, Shashi Bhushan, Alexander Jarasch, Jean-Paul Armache, Soledad Funes, Fabrice Jossinet, James Gumart, Thorsten Mielke, Otto Berninghausen, Klaus Schulten, Eric Westhof, Reid Gilmore, Elisbet Mandon, and Roland Beckmann visualized the interaction between a PCC called Sec61 and the eukaryotic ribosome [2]. Different Sec complexes are involved in different organisms (mammals, yeast, bacteria, etc.), but there has been controversy as to how many Sec molecules are needed for an active PCC and the actual path of the polypeptide chain. Becker et al. extracted a version of Sec from yeast (Ssh 1) with a detergent, and this complex is only active when it is bound to a ribosome. They reconstituted this complex with ribosomes that carried a nascent polypeptide chain and observed stable binding among these molecules. CryoEM analysis revealed a variety of configurations, but they sorted the sample to further analyze an active complex, an idle complex, and active ribosomes with only nascent polypeptides. The 3D reconstruction of these structures could be resolved at 6.1 Å.

As expected, the Ssh 1 complex was bound at the exit site of the ribosome. However, because of the apparent flexibility of the ribosome-PCC connection, the PCC could not be resolved as well as the ribosome. The idle complex revealed an empty ribosomal tunnel leading directly to the central pore of the PCC. In contrast, the pore in the active PCC was occupied, probably by the nascent polypeptide chain. Notably, the active complexes appeared to have a nascent polypeptide chain within the tunnel that in some cases could be traced from the transfer RNA to the tunnel exit. Additional experiments suggested that the Ssh 1 complex bound to the ribosome is likely to exist mainly as a single copy. This addresses the controversy as to how many Sec molecules are needed for an active PCC; the answer may be one. The pore of a single Sec complex may be used by the nascent polypeptide chain. Furthermore, experiments with a mammalian Sec complex yielded compatible results suggesting that the binding mode is well conserved and is basically the same in inactive and active complexes.

In a companion study involving many of the same scientists, Birgit Seidelt, Axel Innis, Daniel Wilson, Marco Gartmann, Jean-Paul Armache, Elizabeth Villa, Leonardo Trabuco, Thomas Becker, Thorsten Mielke, Klaus Schulten, Thomas Steitz, and Roland Beckmann used cryoEM to examine a different model at 5.8 Å resolution [3]. This system utilized the expression of a bacterial (*E. coli*) trytophanase operon that depends on ribosome stalling during translation of an upstream "leader" peptide, a process for which interaction between the nascent chain and the ribosomal exit tunnel are critical. In their experimental system, Seidelt et al. showed that the nascent

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chain was extended within the exit tunnel, making contacts with ribosomal components at distinct sites. The results of several experiments lead them to propose a model that involves interactions within the tunnel that could be relayed within the ribosome to inhibit translation. Moreover, they showed that nascent chains adopt distinct conformations within the ribosomal exit tunnel. Thus, it has been shown that the tunnel within the ribosome is not just a passive conduit; rather active interactions between the tunnel and the nascent chain are involved.

Taken together, these two "landmark papers" make important contributions to our understanding for the structural basis of the translation of proteins [4].

References

[1] M Kampmann and G Blobel, *Science* 326 (2009) 1352–53.
[2] T Becker, S Bhushan, A Jarasch, J-P Armache, S Funes, F Jossinet, J Gumbart, T Mielke, O Berninghausen, K Schulten, E Westhof, R Gilmore, EC Mandon, and R Beckmann, *Science* 326 (2009) 1369–73.
[3] B Seidelt, CA Innis, DN Wilson, M Gartmann, J-P Armache, E Villa, LG Trabuco, T Becker, T Mielke, K Schulten, TA Steitz, and R Beckmann, *Science* 326 (2009) 1412–15.
[4] The author gratefully acknowledges Drs. Elisabet Mandon and Thomas Steitz for reviewing this article.

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