

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.gtc.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.mcrl.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

Stephen W. Carmichael

Mayo Clinic, Rochester, MN 55905

carmichael.stephen@mayo.edu

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*) tryptophanase 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

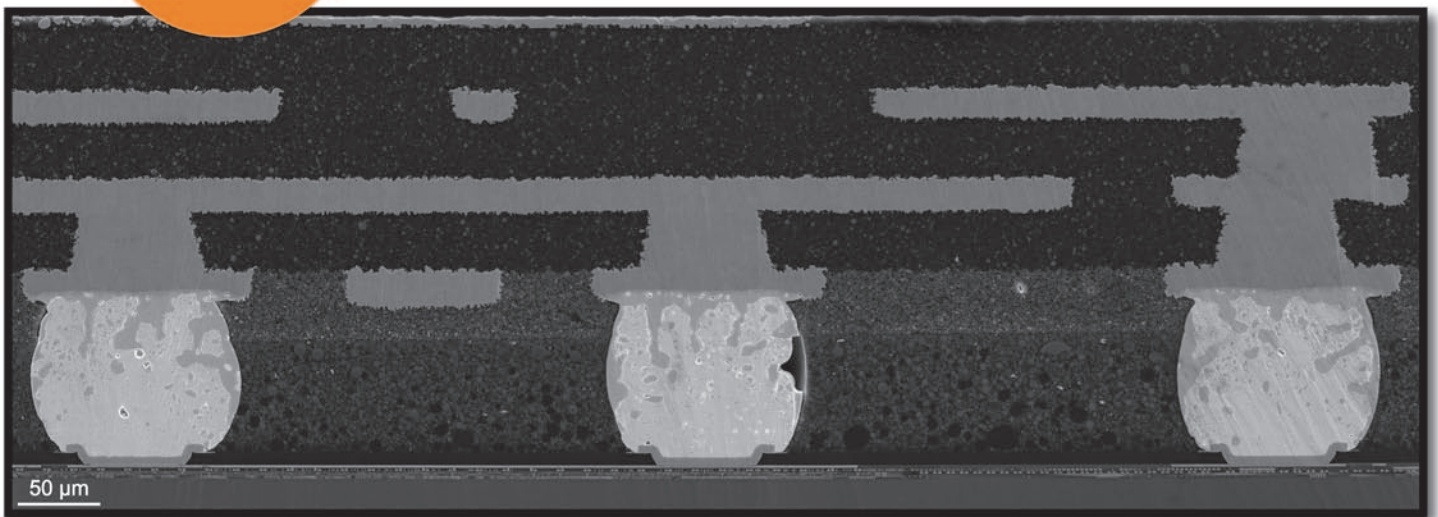
gatan gets it

Can you
prepare large area
SEM cross sections
quickly from your
samples?

To learn how the
NEW Ilion⁺
accomplishes this,
visit us at
www.gatan.com/answers

The Ilion⁺
masked ion beam
milling technique is
a powerful cross
section sample
preparation method
for even the
most demanding
materials.

Ilion⁺



SEM cross-sectional image of C4 bump prepared at 6 keV, in 3 hours using Gatan Ilion⁺.



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.

MT

INTER/MICRO 2010

◆ International Microscopy ◆
Symposium & Workshop

Conducted at
MCCRONE RESEARCH INSTITUTE
A Not-for-Profit Corporation
2820 S. Michigan Ave, Chicago, IL
www.mcri.org

July 12–16, 2010

◆ Techniques and Instrumentation
◆ Environmental and Industrial Microscopy
◆ Forensic and Chemical Microscopy

5



1960—2010

Workshop
July 15-16, 2010
**Identification of
Animal Hair**

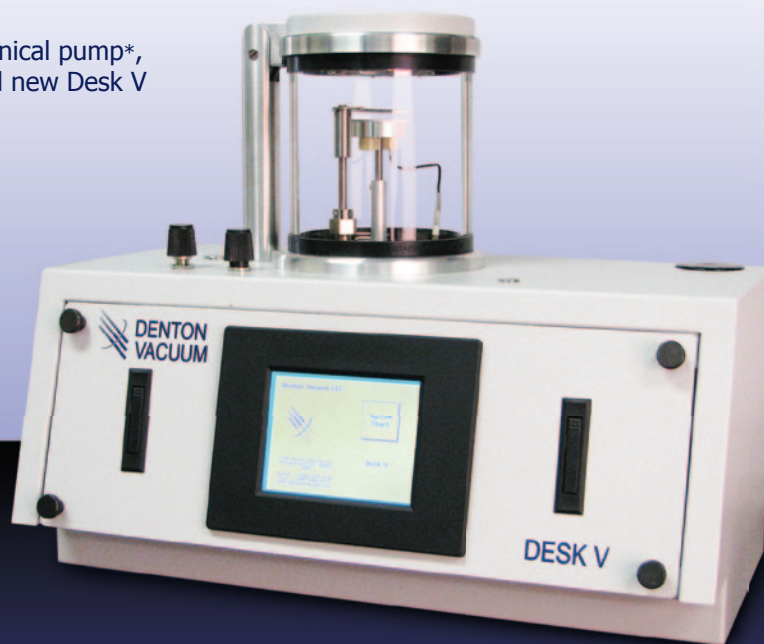
FIVE GENERATIONS OF PERFORMANCE...REFINED

The all new Desk V sputtering tool.

With an enhanced sputter head, larger mechanical pump*, a more powerful PLC and a lower price, the all new Desk V is generations ahead of the competition.

Features:

- Short deposition times
- Consistent deposition parameters
- Enhanced touchscreen controls
- Film thickness control
- Etch mode for sample cleaning
- Wide variety of coating materials
- Compact benchtop design



DENTON VACUUM
Quality • Support • Value

Call us today at 800-666-6004
or visit us online at www.dentonvacuum.com

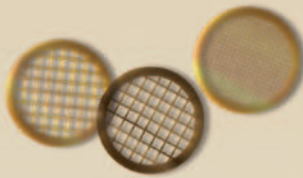
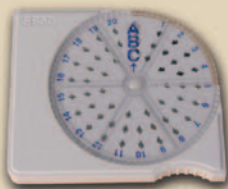
*mechanical pump is optional to allow a third party pump to be used at customer's discretion

SPI Supplies.

The complete source for all your microscopy needs...

just a click away.

2spi.com



Visit SPI Supplies to view the complete on-line catalog with up-to-the-minute product and pricing information.



SPI Supplies Division of **STRUCTURE PROBE, Inc.**

P.O. Box 656 • West Chester, PA 19381-0656 USA

Phone: 1-610-436-5400 • 1-800-2424-SPI (USA and Canada) • Fax: 1-610-436-5755 • E-mail: sales@2spi.com

