Microscopy TODAY

Coming Events

2013

Pittcon 2013
March 17–21, 2013
Philadelphia, PA
www.pittcon.org

Focus on Microscopy 2013
March 24–27, 2013
Maastricht, The Netherlands
www.focusonmicroscopy.org

2013 MRS Spring Meeting
April 1–5, 2013
San Francisco, CA
www.mrs.org/Spring2013

EMAS 2013
May 12–16, 2013
Porto, Portugal
www.emas-web.net

Scandem 2013
June 10–14, 2013
Copenhagen, Denmark
cfim.ku.dk/scandem2013

Microscopy & Microanalysis 2013
August 4–8, 2013
Indianapolis, IN
www.microscopy.org

Denver X-ray Conference
August 5–9, 2013
Westminster, CO
www.dxcicdd.com

EMAG 2013
September 3–6, 2013
University of York, UK
emag-iop.org

CIASEM 2013
September 24–28, 2013
Cartagena, Columbia
ciasem2013.com/index_ing.html

Neuroscience
November 9–13, 2013
San Diego, CA
www.sfn.org/am2013

2014

Microscopy & Microanalysis 2014
August 3–7, 2014
Hartford, CT
www.microscopy.org

2015

Microscopy & Microanalysis 2015
August 2–6, 2015
Portland, OR
www.microscopy.org

2016

Microscopy & Microanalysis 2016
July 24–28, 2016
Columbus, OH
www.microscopy.org

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

Actin Assembly Takes Off Like a Rocket!

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Actin filament assembly occurs in all eukaryotic cells and involves a delicate balance between factors that promote assembly and factors that inhibit assembly. Filament assembly begins with a process of nucleation and then proceeds via elongation. Filament assembly *in vivo* requires nucleation and elongation factors to overcome barriers that could either bind actin monomers to inhibit nucleation or "cap" the ends of elongating filaments. The formation of most cellular actin structures depends on two or more such factors, which may interact directly. The interaction between two factors that initiate nucleation and promote assembly has recently been demonstrated by Dennis Breitsprecher, Richa Jaiswal, Jeffrey Bombardier, Christopher Gould, Jeff Gelles, and Bruce Goode [1]. Interestingly, the model of these factors in action (Figure 1) resembles a rocket launcher!

In many cases actin assembly is thought to involve pairs of "assembly-promoting factors" (APFs), with one of the molecules being a "formin." The formin-homology 2 (FH2) domains of formins processively track the growing barbed end of the actin filament, protecting it from capping proteins. Actin monomers bind with profilin before assembly. The adjacent FH1 domains recruit profilin-actin monomer complexes and can increase the rate of elongation at barbed ends. Whereas profilin enhances formin-mediated filament elongation, it also strongly suppresses filament nucleation by formins. One APF is the tumor suppressor adenomatous polyposis coli (APC), which was known to directly interact with the forming mDial. Collaboration of formins with other APFs that bind actin monomers may be required to overcome the inhibitory effects of profilin and capping proteins. However, direct evidence for this mechanism has been lacking. To address this, Breitsprecher et al. reconstituted APC-formin-mediated actin

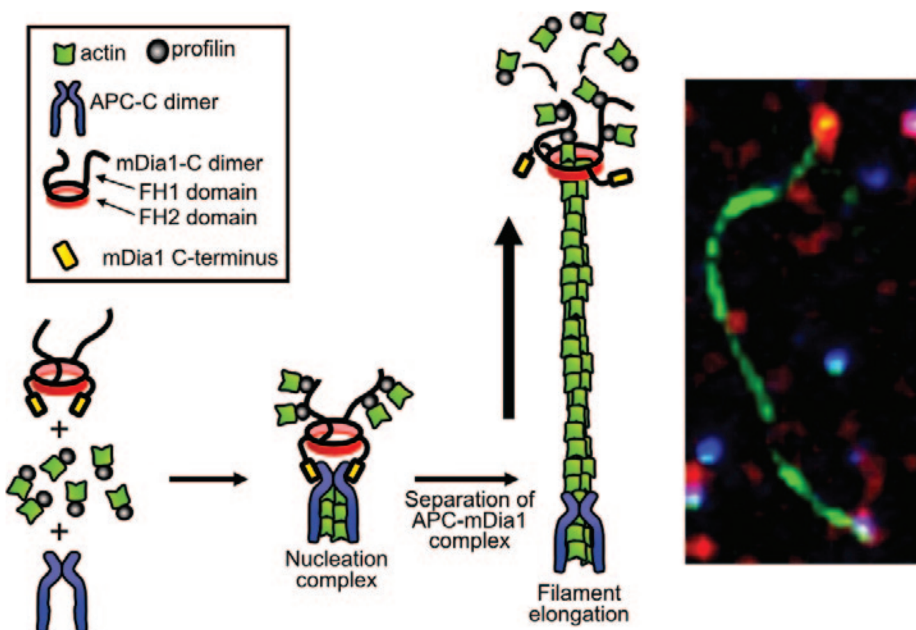

Figure 1: Factors involved in the assembly of actin filaments. Schematic of the factors and their assembly process (left). Fluorescently tagged proteins revealed by total internal refection fluorescence microscopy (right).

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assembly with purified, fluorescently tagged proteins and used multi-wavelength single-molecule total internal refection fluorescence microscopy (TIRFM) to directly visualize and define the mechanisms promoting collaborative filament assembly.

Dual-color TIRFM was used to directly visualize the process of actin filament formation by a SNAP-tagged dye-labeled C-terminal fragment of APC (APC-C) that catalyzes actin nucleation. The dimeric APC-C molecules appeared as discrete spots during assembly of filaments with tagged actin molecules. From some of the spots, Breitsprecher et al. observed actin filaments emerge and grow primarily from their barbed ends, and APC-C did not alter the filament elongation rate in the presence or absence of profilin. Furthermore, APC-C remained at the site of filament nucleation but did not cap the pointed ends, which suggests that it stays bound to the filament side at the site of nucleation. In their model, this forms the base of the rocket launcher. Other experiments with a dye-labeled SNAP-tagged formin (with FH1 and FH2 domains) showed that it moved processively with the growing barbed ends of filaments, both in the presence and absence of profilin. Their observations provide the first direct visualization of single formin molecules processively tracking the growing ends of filaments and confirm that processive movement occurs independent of profilin, whereas accelerated filament elongation requires profilin.

Breitsprecher et al. performed many other experiments using differentially labeled formin and APC molecules in the same reaction, which revealed a mechanism in which APC-C dimers recruit actin monomers and bind the formin to form a tripartite nucleation complex. At the onset of actin polymerization, the complex separates, leaving the APC-C at the base of the rocket (the pointed end of the filament) while the formin ring is propelled away on the rapidly growing barbed end as profilin-bound actin subunits are added. Profilin is thought to dissociate from actin upon subunit addition to the filament. This collaborative mechanism involving two different APFs may result in site-specific actin assembly in cells. This may in turn serve to promote directed cell migration or other functions that require a coordinated reorganization of the actin cytoskeleton.

References

[1] D Breitsprecher, R Jaiswal, JP Bombardier, CJ Gould, J Gelles, and BL Goode, *Science* 336 (2012) 1164–68.

[2] The authors gratefully acknowledge Drs. Bruce Goode, Dennis Breitsprecher, and Jeff Gelles for reviewing this article.

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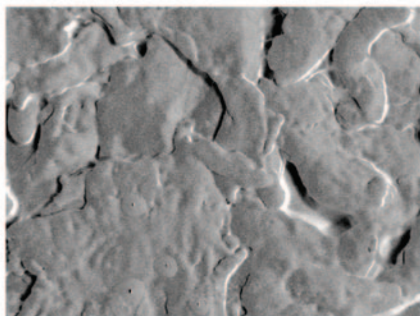
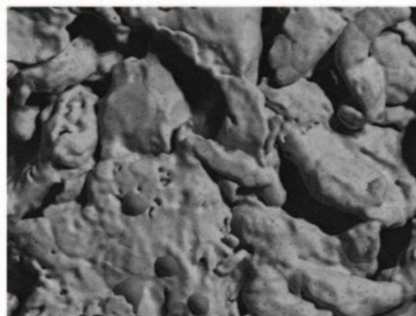
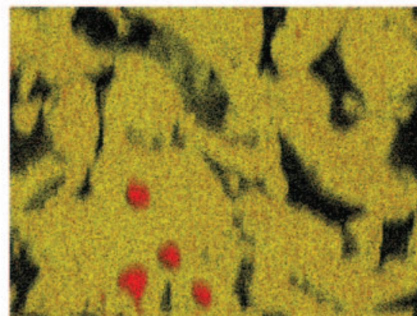


Typical Topographic Mode from Annular BSED



ON-X Take-Off Angle Image Sintered Stainless Steel



ON-X X-ray map showing Fe and Si (red)

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