Application note

Studying the architecture of actin waves with cryo-ET

Revealing the underlying wave propagation

Actin filaments are a fundamental component of the cytoskeleton in eukaryotic cells[1]. They play a role in regulating cell shape and in enabling cells to protrude, polarize, and move. In the late 1990s, evidence emerged that actin possesses wave-like dynamics through which filaments propagate within cells[2]. Since then, studies have observed these "actin waves" in a variety of cells, including Dictyostelium cells, neurons, leukocytes, melanomas, osteosarcoma cells, oocytes, and embryos. The role of these waves has been demonstrated in a number of processes like cell migration, cytokinesis, adhesion, and neurogenesis.[2,3] However, until recently, the actin architecture underlying wave propagation was unknown.

An incomplete picture

Previous research has illustrated that actin waves propagate by actin polymerization on the substrate-attached cell membrane at the front of the wave, combined with disassembly at the back.4 Proteins in the Arp2/3 complex initiate this polymerization, branching off new filaments from the sides of existing ones. There are two possibilities for how this could take place:

1) by elongating filaments that point in the direction of wave propagation, and
2) by the nucleation of new filaments in front of the wave[3].

Deconvoluting the wave architecture

Researchers led by Marion Jasnin and Günther Gerisch at the Max Planck Institute of Biochemistry in Martinsried, Germany harnessed cryo-focused ion beam (cryo-FIB) sample preparation and in-situ cryo-electron tomography (cryo-ET) to study the actin wave architecture of Dictyostelium cells[3].

Cryo-FIB sample preparation provides access to the cell interior with minimal artifacts; in-situ cryo-ET allows for the high-resolution 3D visualization of proteins in their undisturbed cellular environment. These technologies, together with cryo-fluorescence microscopy, were used to explore the role of the Arp2/3 complex in the branch nucleation of actin filaments. The direction of the branches potentially relates to the direction of wave propagation and how filaments are oriented in relation to the substrate-attached cell membrane on which the waves propagate.

Results

The direction of branch junctions could not be related to a favored alignment of mother/daughter filaments relative to wave propagation. This indicates that the mechanism is unlikely to be based on the elongation of filaments in the direction of wave propagation and that filament nucleation is the more likely mechanism.

The angle between the direction of the mother/daughter filaments and the substrate-attached cell membrane was explored in order to relate branch-nucleation geometry to the membrane on which the waves propagate. 64-74% of daughter filaments were found facing the membrane, indicating that the Arp2/3 complex clearly favors nucleation towards the membrane. Meanwhile, the majority of mother filaments ran parallel to the membrane. However, there was also a small fraction of mother filaments that did not grow parallel to the membrane, but rather build-up, along with their daughter filaments, into tent-like arrays.

Additionally, live-imaging data was able to show that VASP, a nucleation and elongation factor, appears to cooperate with the Arp2/3 complex, generating the part of the filaments from which the Arp2/3 complex nucleates branches.

A new mechanism

A mechanism for wave progression was proposed in which actin polymerization is initiated at the membrane by VASP along with other nucleation and elongation factors[3]. At these sites of dense actin assembly, the Arp2/3 complex then accumulates and nucleates branches. Filaments grow from these sites towards the membrane and assemble into tent-like arrays. This process then repeats, creating new generations of these arrays as the wave propagates, lifting up the previous generations together with the mother filaments.

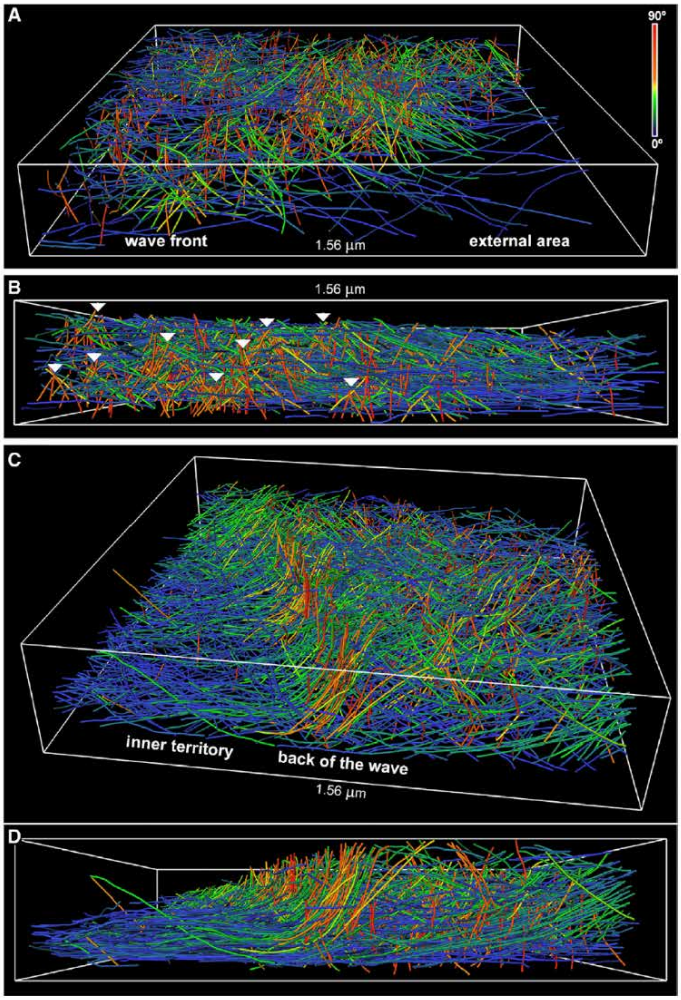

Figure 1. Tomography 3D reconstructions, showing actin filaments organized in actin waves with tent-like arrays. A) View from the front of the wave. B) Side view of the wave; white arrows indicate the tent-like arrays at different distances from the membrane. C) Slanted and D) side views of the back of a wave; the tent-like arrays disappear when the wave has passed[3].

"Altogether, the quantitative analysis of branch organization in traveling actin waves reflects the ordered progression in space and time of an actin network from nucleation to depolymerization," the authors conclude in ***Structure***.[3]

Thermo Scientific technology

In this study, a Quanta 3D DualBeam, formerly produced by FEI, was used in combination with Thermo Scientific™ Maps Software. Focused ion beam scanning electron microscopy (FIB-SEM) allows you to uncover subsurface structural detail by combining precise FIB cuts (i.e., ion milling) with high-resolution SEM imaging of the exposed sample surface. Maps Software is a complementary correlative imaging software suite that is compatible with both Thermo Scientific SEM and FIB-SEM platforms. Maps Software offers an integrated approach to data acquisition, annotation, and storage, combining multi-scale imaging automation, correlative microscopy, and integrated analytics. Thermo Scientific™ Titan™ Krios™ or Tecnai™ G2 Polara™ Transmission Electron Microscopes (TEMs) were used to acquire the tomographic tilt series. Thermo Scientific™ Amira™ Software was used to perform automated filament segmentation. This involved non-local means filtering of the tomograms followed by tracing of the actin filaments using an automated segmentation algorithm.

References

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