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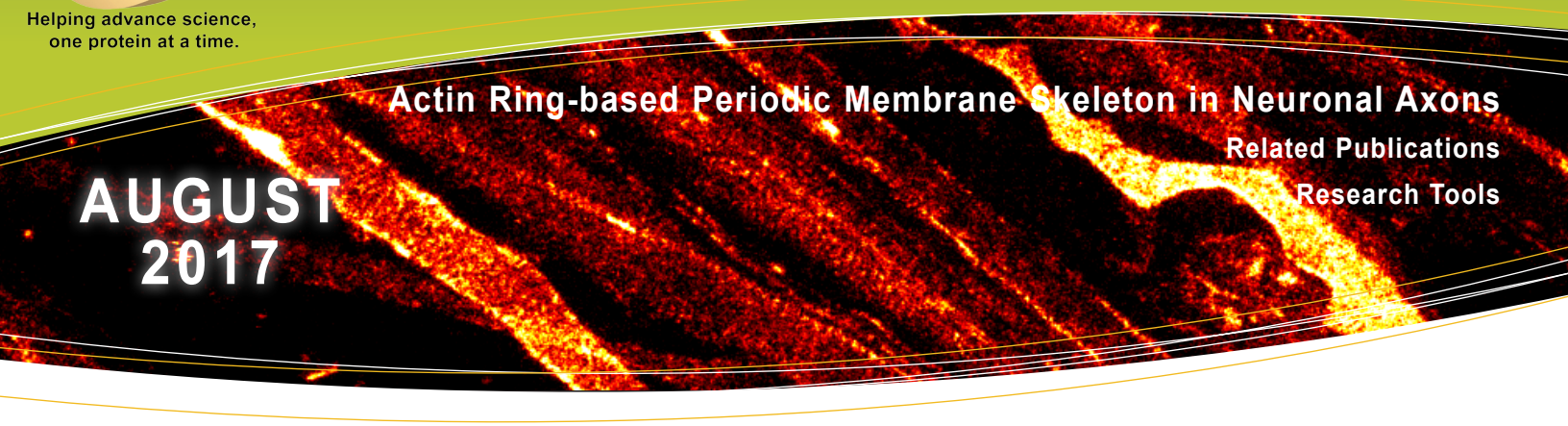

Actin Ring-based Periodic Membrane Skeleton in Neuronal Axons

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Actin Ring-based Periodic Membrane Skeleton in Neuronal Axons

Actin is an integral part of the neuronal cytoskeleton as it is involved in the regulation of neuronal polarization, cell morphology, the development of neuronal processes (i.e., growth cones with lamellipodial and filopodial extensions and dendritic spines), intracellular trafficking, and synaptic plasticity (dynamic changes in dendritic spine number and/or morphology)[1-3]. Actin's presence in growth cones and dendritic spines have garnered the attention of scientists for decades; however, actin is also found in neuronal axons, though its presence there has been described as the "black sheep of the neuronal actin family"[4]. This is because the exact details of actin's structure and role in the axon are unknown. Recently, significant advances have been made in unraveling the structure of axonal actin with the discovery of the periodic membrane skeleton (PMS) by nanoscopic microscopy[5] (Fig. 1). This newsletter discusses the discovery, structure, and possible functions of the PMS in axons.

PMS Discovery and Structure

Discovered in 2013, the PMS is a type of cortical actin and the primary component of the actin cortex, a mixture of F-actin and actin binding proteins which supports eukaryotic cells' plasma membrane and membrane-associated processes such as endo- and exocytosis and cell motility[4,5]. In neuronal axons, including the initial segment[6], the PMS consists of short actin filaments bundled into evenly spaced rings that wrap around the circumference of the axon with a periodicity of 180-190 nanometers[5-9] (Fig. 1). The short filaments are stabilized by an adducin cap which controls the diameter of actin rings and axons, as well as actin filament growth within the rings[6,10]. Adjacent actin rings are secured through cross-linkage by spectrin tetramers (βII in the axon proper and βIV in the axon initial segment)[6,8,11].

The PMS was described for the first time in fixed mammalian neurons and brain tissue slices using stochastic optical reconstruction microscopy (STORM)[5]. Shortly thereafter, these findings were confirmed by others using STORM, stimulated emission depletion [STED] nanoscopy, and structured illumination microscopy [SIM] in fixed mammalian and non-mammalian neurons[5,6,8,11,12], and most importantly, living mammalian neurons using either the F-actin live cell imaging probe SiR-actin[7,10,13] (silicon rhodamine actin; Fig. 1) or exogenous expression of fluorescently-labeled βII spectrin[8].

In cultured mammalian neurons, the PMS is established in the first few days of development in the axon proximal to the cell body before moving distally along the axon as the neuron develops[5,7,8,11]. In Drosophila primary neurons, development of the PMS begins within hours of plating[11]. Notably, the authors reported differences between the PMS of very young (hours to 2 days old) vs mature (≥3 days old) Drosophila neurons. For instance, the PMS in young, growing axons, but not older axons, depends upon actin polymerization (i.e., nucleation factors)[11].

The actin binding proteins involved in nucleation, assembly, and maintenance of the actin rings are relatively unknown. In Drosophila neurons, two nucleation factors, Arp2/3 and formin DAAM [disheveled-associated activator of morphogenesis], participate in PMS formation, likely nucleating the multiple short filaments that comprise the PMS[11].

PMS Functions

Neuronal axons are the means by which neurons communicate and transport cargo anterogradely and retrogradely between the cell body and axon terminal. Maintaining healthy axons is necessary for normal brain function as axonal loss is permanent

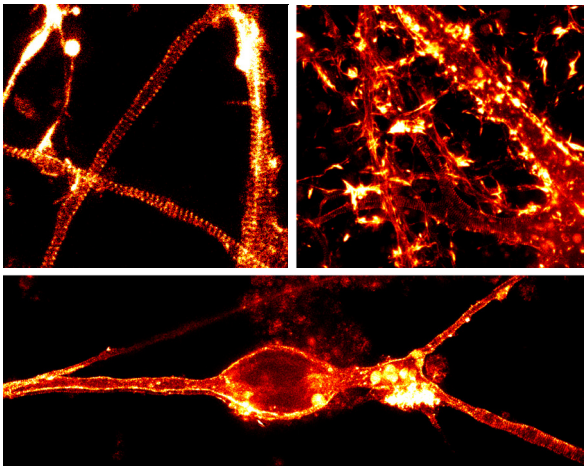

Figure 1. Live cell imaging in neurons with STED microscopy and 100 nM SiR-actin. Upper left: Hippocampal rat neuron, 8 DIV. Upper right: Hippocampal rat neuron, 17 DIV. Bottom image: Rat cerebellar granule neuron, 21 DIV. All images courtesy of Elisa D'Este, MPI Biophysical Chemistry, Göttingen.

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and underlies both normal aging deficits and various neurodegenerative disorders and diseases[14]. Functionally, the PMS has been hypothesized to serve as a scaffold for the axonal plasma membrane; spatially organize molecules important for axonal structure and action potential generation, such as ankyrin and sodium channels, respectively, into a periodic distribution in axons[5,8]; assist in the docking of motor-associated cargo vesicles, especially along the distal part of the axon[4,9,15]; and organize transmembrane proteins along the axon[11,15-18].

Actual functional data are sparse; however, a recent report using Drosophila primary neurons fixed and stained with SiR-actin concluded that the PMS is important in maintaining axon integrity by stimulating the polymerization of axonal microtubules (MTs), another primary cytoskeletal component of axons[11]. Cytochalasin D-induced PMS disassembly resulted in breaks in MT bundles, reduced MT polymerization, and reduced axon numbers[11]. Other MT-associated functional roles for PMS might involve assembling MTs into bundles[19-21]; serving as anchors for the minus end of MTs[22,23]; and supporting dynein-mediated sliding of MTs and transport[24].

Summary

The recent discovery of actin rings that comprise a sub-membranous lattice in neuronal axons offers an exciting opportunity to better understand the role of axonal actin. Moreover, actin rings have also been described in the nodes of Ranvier in the peripheral nervous system and in at least some dendrites and dendritic spines[7,25]. Similar to actin rings, actin waves and trails are other actin structures that have received little attention[4]. Without super-resolution microscopy (e.g., STORM, STED, SIM) and the development of live cell imaging probes such as SiR-actin, the existence and composition of the PMS would remain undiscovered. To help scientists further discover and define the composition, assembly, maintenance, and function of actin and other cytoskeletal structures, Cytoskeleton, Inc., provides SiR live cell imaging probes for F-actin, microtubules, DNA, and lysosomes, along with Acti-stain phalloidins for use in fixed cells.

Spirochrome™ Live Cell Imaging Probes

Description	Ex / Em	Cat. #	Amount
SiR700-Actin Kit Includes Verapamil	690 / 720 nm	CY-SC013	35 nmol
SiR-Actin Kit Includes Verapamil	630 / 680 nm	CY-SC001	50 nmol
SiR700-Tubulin Kit Includes Verapamil	690 / 720 nm	CY-SC014	35 nmol
SiR-Tubulin Kit Includes Verapamil	630 / 680 nm	CY-SC002	50 nmol
SiR-Lysosome Kit Includes Verapamil	630 / 680 nm	CY-SC012	50 nmol
SiR700-Lysosome Kit Includes Verapamil	690 / 720 nm	CY-SC016	35 nmol
SiR-DNA Kit Includes Verapamil	630 / 680 nm	CY-SC007	50 nmol
SiR700-DNA Kit Includes Verapamil	690 / 720 nm	CY-SC015	35 nmol
Cytoskeleton Kit Includes SiR-Actin, SiR-Tubulin, and Verapamil	630 / 680 nm	CY-SC006	50 nmol each

Actin Proteins

Actin Protein	Cat. #	Amount
Rhodamine Actin Protein Human Platelet, Non-Muscle, >99% Pure	APHR-A APHR-C	4 x 10 µg 20 x 10 µg
Rhodamine Actin Protein Rabbit Skeletal Muscle, >99% Pure	AR05-B AR05-C	10 x 20 µg 20 x 20 µg

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Acti-stain Phalloidins

Acti-stain Phalloidin	Cat. #	Amount
Acti-stain™ 488 Phalloidin	PHDG1	500 µl
Acti-stain™ 555 Phalloidin	PHDH1	500 µl
Acti-stain™ 670 Phalloidin	PHDN1	500 µl
Phalloidin (rhodamine)	PHDR1	500 µl

Actin Activation Assay Biochem Kit

Actin Activation Assay	Cat. #	Amount
G-Actin/F-Actin In Vivo Assay Biochem Kit	BK037	30-100 Assays