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SPOTLIGHT

Adding SNX to the mix: SNX9 drives filopodia biogenesis

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Filopodia are actin-rich protrusions important for sensing and responding to the extracellular environment, but the repertoire of factors required for filopodia formation is only partially understood. Jarsch et al. (2020. *J. Cell. Biol.* https://doi.org/10.1083/jcb.201909178) combine an in vitro system of filopodia biogenesis with a phage display screen to show that SNX9 drives filopodial assembly.

Filopodia are narrow membrane-encased protrusions that contain linear bundles of filamentous (F-) actin and are used in environmental sensing, morphogenesis, adhesion, and motility. Filopodia biogenesis is an intensely studied aspect of cytoskeletal biology, and several mechanisms have been proposed to drive filopodial assembly (1, 2). All models rely on the actions of the actin nucleation, elongation, and bundling machinery (Fig. 1). In many cases, small G-proteins from the Rho family (e.g., Cdc42, Rif, or Rac) are thought to activate members of the Wiskott-Aldrich Syndrome Protein (WASP) family (e.g., N-WASP or WAVE1/2/3), which promote actin nucleation via the Arp2/3 complex. A subset of Arp2/3-derived branched actin filaments that have their growing plus ends facing the plasma membrane are then reorganized by proteins that prevent capping and mediate filament elongation. Actin polymerases from the Ena/VASP family (e.g., VASP itself) and actin nucleators from the Formin family (e.g., mDia2, mDia1, FMNL2, DAAM1, or others) perform these functions. However, filopodial F-actin need not emerge from such convergent elongation or clustered outgrowth of Arp2/3-associated networks, as filopodia can also form de novo when Rho-family G-proteins activate Formins, which directly nucleate and elongate actin filaments to create filopodia. Irrespective of the origin of filopodial F-actin, cross-linking proteins including Fascin bundle the filaments within filopodia, while membrane-deforming proteins from the Bin-Amphiphysin-Rvs (BAR) family (e.g., IRSp53 or TOCA-1) link the filaments to the surrounding membrane. At steady-state, the actin within filopodia remains dynamic, as filaments undergo treadmilling, and filopodial lengths are modulated by the balance between actin assembly at the tip and disassembly at the base. Building upon this basic understanding of filopodial initiation, growth, and maintenance, Jarsch et l. (3) probed deeper into the mechanisms of filopodia biogenesis by integrating a powerful in vitro filopodial assembly platform with a phage display-based functional screening system.

Their cell-free assays for studying the formation of filopodia-like structures (FLSs) combine *Xenopus* egg extracts spiked with purified proteins, plus supported lipid bilayers supplemented with specific phosphoinositides (4). This biomimetic system allows for actin polymerization into structures that reflect the organization of filopodia and that contain the core molecular constituents responsible for their assembly in cells (5). In the current paper (3), Jarsch et al. sought to functionally dissect the requirements for FLS biogenesis through the addition of a library of phage displaying single-chain antibodies (6). After initially subtracting phage that recognized several known filopodial components, they selected for phage that bound to FLSs, and used a subset of the subsequently cloned/purified antibodies to screen for alterations in FLS formation. Remarkably, they observed both gain-of-function and loss-of-function phenotypes related to FLS abundance, length, thickness, and linearity.

To define a cause of FLS shortness, the authors then used proteomic and biochemical approaches to identify one of the bound antigens as SNX9, a member of the sorting nexin protein family of membrane trafficking coordinators. SNX9 was previously shown to participate in endocytosis (7), N-WASP-mediated actin assembly (8), G-protein-associated protrusion (9), and pathogen internalization (10). Using classical immunodepletion and add-back experiments, the authors verified a requirement for SNX9 in FLS assembly and observed its localization primarily at the filopodial tip. SNX9 has an SH3-PX-BAR domain organization, wherein the latter two portions of the protein interact with phospholipids, and the authors concluded that PX binding to PI(3)P may regulate SNX9 localization and actin assembly in FLSs. Finally, the authors finished flexing their experimental muscles by confirming that SNX9 is present in cellular filopodia and during the filopodial-based capture of the invasive bacterial pathogen *Chlamydia trachomatis*. Collectively, these

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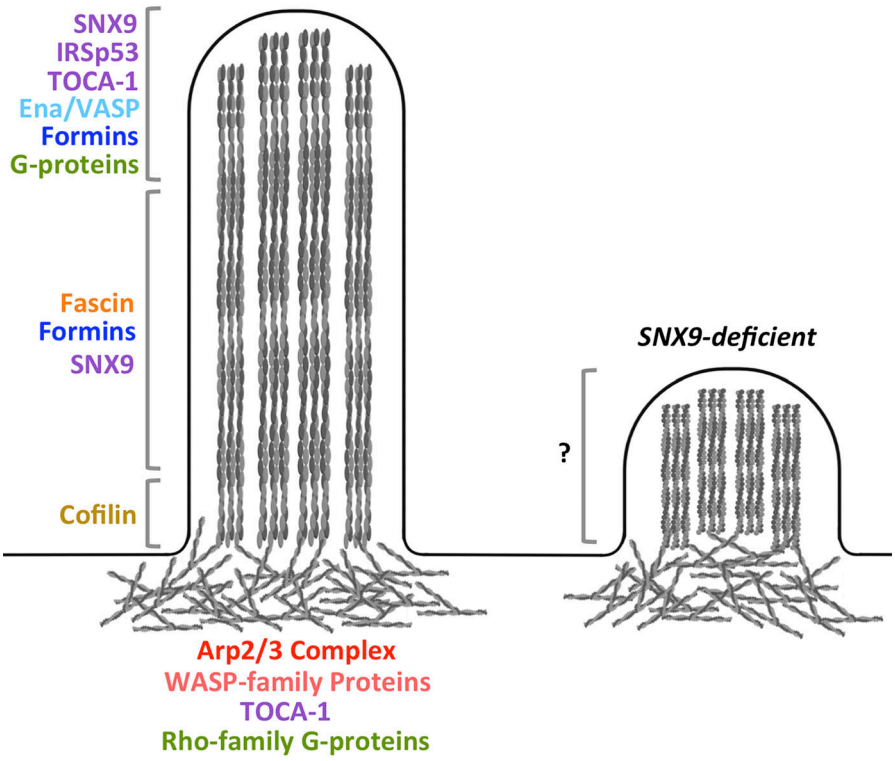

Figure 1. **SNX9 joins the ranks of filopodia formation factors.** On the left, normal filopodia biogenesis in cells employs Rho-family G proteins (green), which activate WASP-family proteins (salmon) to promote actin nucleation and branching by the Arp2/3 complex (red) at the filopodial base. Formins (blue) and Ena/VASP proteins (teal) elongate linear filaments (gray) that are bundled by Fascin (orange) within the protrusion. Filopodial dynamics are balanced through sustained actin assembly via Formins and VASP at the tip coupled with actin severing and disassembly by Cofilin (gold) at the base. BAR-domain proteins (purple) deform the plasma membrane for elongation, but can also interact with nucleation factors at the filopodial base. Jarsch et al. showed that SNX9 (purple) localizes to the tip and shaft of filopodia and regulates their assembly. Based on their biomimetic system, depletion of SNX9 results in shorter filopodia, as shown on the right.

findings suggest that SNX9 is a core component of the filopodial biogenesis apparatus (Fig. 1).

But what is SNX9's actual function within filopodia? The ability of SNX9 to interact with the plasma membrane through its PX and BAR domains, coupled with its localization at the filopodial tip, imply a role in membrane deformation that allows for filopodial elongation. Perhaps SNX9 coordinates cytoskeletal dynamics with membrane remodeling in a manner similar to that of IRSp53, which can enable VASP-associated actin elongation near the tip while using its BAR domain to deform the membrane (1, 2). The SH3 domain of SNX9 may also have an important activity, as this part of the protein binds N-WASP to promote actin assembly via the Arp2/3 complex (8). This is reminiscent of how IRSp53 and TOCA-1 both respond to G-protein signaling and use their SH3 domains to stimulate N-WASP, although it remains unclear if such events take place specifically at the branched base of the FLS. It would be interesting to tease out which domains and inter- (or perhaps intra-) molecular interfaces are perturbed by the single chain SNX9 antibodies discovered in the authors' initial screen. In addition, a clearer understanding of the filopodial components which are, and are not, recruited to the short SNX9-deficient FLSs (Fig. 1) might shed some light on the filopodial elongation process. A better characterization of SNX9 binding partners, both proteins and phospholipids, will help answer the question of SNX9's functions.

The identification of SNX9 as an important filopodial biogenesis factor is consistent with recent observations (4, 9, 10), but the technical achievements of the current work are particularly impressive. They reflect a powerful experimental pipeline for future molecular dissections of FLS assembly in vitro, which can then be translated to the study of filopodial dynamics in more complex cellular environments. However, it is just as important to note that the converse also holds true; working backward from studies of filopodia in broad cellular contexts, this in vitro system can be tailored to decipher the precise functions of various filopodial components with superb spatiotemporal resolution. While a core filopodial biogenesis machinery may exist, increasing evidence suggests that filopodia formed in diverse in vivo settings likely employ distinct molecular constituents. Different cell types use filopodia for specialized purposes, including migratory pathfinding, neuronal branching morphogenesis, and phagocytosis. Beyond the common players in filopodial assembly, how do factors from the more specialized structures impact FLS assembly in vitro? What are the contributions of specific Formin proteins in the cellular and in vitro systems? In addition to positive filopodial biogenesis factors, what molecules negatively regulate this process? Though much work is still needed to define all the factors present in filopodia and their mechanisms of action, the technical prowess enabled by the in vitro system ensures that these discoveries are likely to be achieved in the not-so-distant future.

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