



# The Ways of Actin: Why Tunneling Nanotubes Are Unique Cell Protrusions

Nina Ljubojevic, J. Michael Michael Henderson, Chiara Zurzolo

## ► To cite this version:

Nina Ljubojevic, J. Michael Michael Henderson, Chiara Zurzolo. The Ways of Actin: Why Tunneling Nanotubes Are Unique Cell Protrusions. Trends in Cell Biology, 2021, 31 (2), pp.130-142. 10.1016/j.tcb.2020.11.008 . pasteur-03167401

**HAL Id: pasteur-03167401**

**<https://pasteur.hal.science/pasteur-03167401>**

Submitted on 12 Mar 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

# **Title:** The ways of actin: why tunneling nanotubes are unique cell protrusions

Nina Ljubojevic<sup>1,2,#</sup>, J. Michael Henderson<sup>1,3,#</sup>, Chiara Zurzolo<sup>1\*</sup>

<sup>1</sup>Membrane Traffic and Pathogenesis, Institut Pasteur, UMR3691 CNRS, 75015 Paris, France

<sup>2</sup>Sorbonne Université, ED394 - Physiologie, physiopathologie et thérapeutique, 75005 Paris, France

<sup>3</sup>Laboratoire Physico-Chimie Curie, Institut Curie, PSL Research University, CNRS UMR168, 75005, Paris, France

<sup>#</sup>These authors contributed equally

<sup>\*</sup>Correspondence should be addressed to Chiara Zurzolo

(email: chiara.zurzolo@pasteur.fr)

**Keywords:** Cellular protrusions, tunneling nanotubes, filamentous actin, actin regulators, actin polymerization

## **Abstract**

Actin remodeling is at the **heart** of the cell's response to external or internal stimuli allowing a variety of membrane protrusions to form. Fifteen years ago, tunneling nanotubes (TNTs) were identified, bringing a novel addition to the family of actin-supported cellular protrusions. Their unique property as conduits for cargo transfer between distant cells, emphasizes the unique nature of TNTs among other protrusions. While TNTs in different pathological and physiological scenarios have been described, the molecular basis of how TNTs form is not well understood. This review addresses several actin regulators in the formation of TNTs and suggests potential players based on their comparison with other actin-based protrusions. New perspectives for discovering a distinct TNT formation pathway would enable us to target them in treating the increasing number of TNT-involved pathologies.

## Actin processes generate a diverse array of cell protrusions

Actin, one of the key cytoskeletal polymers of the cell, forms helix-shaped **polar filaments** (see Glossary) that are further assembled into highly-organized actin networks, such as branched and linear.[1] The spatial and temporal control of these actin networks is crucial in maintaining the integrity of the cell, contributing to its mechanical properties, and driving cell shape changes that enable a cell's response to various processes.[1] Cell protrusions **appear as** the most prominent **changes in cell shape**, whose growth and characteristics mainly rely on actin cytoskeleton structure.[1] **Filamentous actin (F-actin)** is formed through the polymerization of **globular actin (G-actin)** monomers, a process governed by a broad pool of actin regulators and/or actin-binding proteins. In order for a cell protrusion to form into a mature structure, actin remodeling processes—that comprise the steps of initiation, actin polymerization and stabilization of the actin filaments—need to take place in the cell in a **tightly** controlled manner. Among these protrusions, **filopodia**, **microvilli** and **dendritic filopodia/spines** (see Glossary) comprise a family of morphologically similar structures (see Figure 1).

An addition to the family of cell protrusions, tunneling nanotubes (TNTs), were first documented in 2004 by Rustom and colleagues.[2] They described TNTs as membranous **tubular extensions** connecting two remote cells, thus providing cytoplasmic continuity between them. Similar to canonical protrusions, they are thin (up to 700 nm)[3] and comprised of F-actin, but in contrast, they are non-surface adherent, and have an ability to reach extraordinarily long distances (up to 100  $\mu$ m).[4] First evidence of being actin-supported was shown by phalloidin staining in rat pheochromocytoma (PC-12) cells.[2]

More recently, an ultrastructural study employing correlative **cryogenic electron microscopy (cryoEM)**, shed light on the actin organization within TNTs **in two different neuronal cell lines**. [3] This work uniquely showed straight and continuous, hexagonally packed actin bundles that appear to run parallel along the entire length of the TNTs. **Apart from this actin-related feature, the morphological aspect of TNTs was further assessed; what was observed as a single TNT using low-resolution confocal microscopy, cryo-EM coupled with**

tomography showed that it was instead a bundle formed of individual TNTs (iTNTs). This implicates a more complex structural organization than initially anticipated [3], and necessitates further structure-function studies addressing whether the diverse array of TNT-like protrusions reported in different physio-pathological contexts have conserved or instead cell-type dependent structures (see [5,6] for discussion of specific morphological features of TNT-like protrusions).

Another unique property of TNTs is their ability to transfer cargo of various sizes, encompassing small ions and molecules, but also larger organelles such as lysosomes and mitochondria.[5] Our work supports the identifying property of TNTs as being open-ended conduits that directly connect the cytoplasm of two cells for organelle transport.[3] Thus, a key requirement to distinguish TNTs from other cellular protrusions is to confirm their functional ability to transfer cargo. In the field, a common way of this assessment is by performing a transfer experiment where donor cells, containing labeled cargo, are cocultured with differently labeled acceptor cells that are further analyzed (flow cytometry, confocal microscopy) for the presence of cargo.[7,8]. Our cryo-EM data support this TNT feature that vesicular cargo is transported on actin tracks, likely through **myosin motors** (see Glossary).[3] This is in agreement with previous studies looking for example at the presence of molecular motors inside TNTs.[8,9] However more effort is needed to identify the organelle-specific motors mediating transfer inside TNTs.

All these aforementioned differences have raised important questions about their disparate nature and role in the cell. Since their discovery, many studies followed that were eager to address their role in physiological and pathological conditions.[4,5,10] In contrast, what we still lack is a fundamental understanding about how these structures form. As many reviews have already highlighted the types of cargoes transferred by TNTs and the physiological and pathological implications that TNTs represent[5,10], the focus of this review is to discuss the less understood, but equally important mechanism of TNT formation. There are two proposed mechanisms of TNT formation: 1) a cell dislodgement one, where two cells, initially attached to each other, move apart leaving a membrane thread that eventually matures into an actin-supported TNT; and 2) an actin-driven one, where a cell

forms through actin assembly a precursor protrusion that subsequently fuses with a recipient cell, thus forming a TNT.[2,11,12]

As the scope of this review is to understand how TNTs differ from known cell protrusions formed through actin polymerization, we will focus here on discussing exclusively TNTs formed by the actin-driven mechanism. While the current body of evidence supports TNTs as unique protrusions, it is still not clear how the cell utilizes a common pool of actin regulators (see Box 1) to build such different but morphologically similar protrusions such as TNTs, filopodia, microvilli and/or dendritic filopodia/spines. Therefore, comparing TNTs with better investigated cellular projections will help clarify the still enigmatic actin-driven mechanism of TNT formation and allow us to precisely distinguish them from other communicating protrusions, such as cytonemes (see Glossary) and other filopodia-like structures.

Outstanding questions still need to be addressed to understand this process. What are the actin regulators orchestrating the formation of TNTs? Certain actin elongators (formins, etc.; see Box 1) and actin bundlers (fascin, fimbrin, epsin, etc.; see Box 1), and others might play a role in their biogenesis. Is the actin-driven mechanism of TNT formation initiated as a *de novo* process, or are TNTs matured from a filopodia-like precursor?

To better understand their distinct nature, in this review we will address and compare mechanisms of formation of several other better-understood cell protrusions such as filopodia, dendritic spines and microvilli. This will give the field insight into understanding how TNTs may form and consequently galvanize new experiments that are key and necessary in elucidating TNT assembly.

## **Initiation of TNT protrusions: from membrane deformation to actin growth cascades**

Globally, to form an F-actin based protrusion, two tightly regulated processes within the cell need to occur in concert—membrane deformation and growth of actin filaments at the membrane. What precedes the other is still not clearly defined; an outgrowth of a membrane protrusion can either be triggered by membrane deforming I-BAR proteins [13] (see Box 1) that would initiate and stabilize negative membrane curvature[14], or by

94 localized spots of newly formed actin filaments that can collectively generate enough force  
95 to overcome the membrane tension and initiate outward membrane extension.[15]

96  
97 Actin cascades necessary for protrusion formation are triggered by a set of proteins called  
98 Rho GTPases (see Box 1).[16] For example, CDC42 (see Box 1) promotes filopodia  
99 formation.[17] Downstream of these signaling molecules, protrusion initiation by I-BAR  
100 proteins, such as IRSp53 (see Box 1) seems to be a ubiquitous process in the formation  
101 mechanism of filopodia, microvilli and immature dendritic spines.[18–21] CDC42 can release  
102 the autoinhibition of IRSp53 by binding to its partial Cdc42- and Rac-interactive binding  
103 (CRIB) domain. [20] Upon activation, IRSp53's **barbed end** (see Glossary) capping behavior is  
104 inhibited and a direct interaction with VASP (see Box 1) is enabled.[22] IRSp53 then  
105 advantageously clusters VASP (see Box 1) molecules at the initiation site to promote actin  
106 filament elongation through VASP's anti-capping behavior necessary for filopodia  
107 outgrowth.[22]

108  
109 Importantly, acting within the same CDC42-dependent pathway, IRSp53 when in complex  
110 with Eps8 (see Box 1) localizes to the filopodia initiation site, where they synergize and  
111 enhance each other's likely role in the process of filopodia formation through Eps8's F-actin  
112 bundling activity[23–26] and IRSp53's ability to subsequently stabilize the topology of the  
113 initiated filopodia.[25–27] Recent super resolution microscopy work has revealed that I-BAR  
114 proteins, among them IRSp53, IRTKS and MIM (see Box1) were found to localize all along a  
115 filopodium's length.[27] This suggests that this family of proteins can assemble into higher  
116 order structures that can stabilize the formed protrusion. Indeed, recent in silico molecular  
117 dynamics simulations show such assembly of IRSp53 in tubular membranes.[28]

118  
119 Apart from IRSp53's well-documented role in filopodia, MIM and IRTKS have important roles  
120 in both, dendritic spine and microvilli formation. In dendritic spines, IRSp53 was important in  
121 the maintenance of their morphogenesis.[29] It was shown to maintain proper spine density,  
122 as well as their shape, width and length, all of which were negatively affected upon  
123 depletion of IRSp53. This might implicate the role of IRSp53 in the formation of curved  
124 initiation sites where dendritic spines will assemble, and/or several other possible roles in  
125 elongation/stabilization. Furthermore, MIM accumulated to membrane domains in order to

initiate the formation of a dendritic spine by promoting outward membrane deformation.[18] Upon actin polymerization blockage, this MIM-induced proto-protrusion devoid of actin was unable to grow further into a mature spine suggesting the role of MIM in the initiation step.

In contrast to filopodia, where IRTKS was observed throughout the protrusion[27], a recent study unraveled an important role of IRTKS in microvilli formation, demonstrating its localization exclusively to the tip of microvilli.[21] IRTKS was shown to positively regulate the density and length of microvilli, suggesting its role in initiation and elongation, possibly through the recruitment of its downstream partners. For example, Eps8 was targeted to the tip of microvilli by binding to the **SH3 domain** (see Glossary) of IRTKS, where it positively influenced the elongation of microvilli, likely through its actin bundling activity.[21]

Initiation of TNTs might depend on the cell type. In Box 2 we summarize specifically the actin-related proteins that have an opposite effect on TNT formation in differing cell types, e.g., neuronal vs. immune origin. A sole study using neuronal cells showed IRSp53 had a negative impact on the amount of TNT-connected cells and the transfer of vesicles in a CDC42-dependent manner, suggesting that activation of CDC42-dependent pathways would preferentially lead to the formation of canonical filopodia, rather than TNTs that are functional for cargo transport.[7] Expression of an IRSp53 mutant defective in its SH3 domain binding to its ligands, had no significant effect on the amount of TNT-connected cells and the functional transport of cargo, suggesting VASP (and perhaps other downstream partners) in the process of negatively regulating TNT formation.[7] In contrast, Eps8's bundling activity was identified as a positive regulator of TNT formation[7], suggesting its potential role in protrusion initiation when in complex with an I-BAR protein.[26] Therefore, this result does not completely negate the role of IRSp53 or other I-BAR proteins in TNT formation in neuronal or other cell types, because it is known from in vitro binding studies that IRSp53 has a high, nanomolar binding affinity for Eps8.[26] It does raise several questions, such as if IRSp53 is regulated in a different manner, redirecting it towards TNT formation, or if another I-BAR protein is responsible in TNT biogenesis. Recent studies show that regulation of IRSp53 in protrusions is indeed more complex than being solely activated by Rho GTPases. It was demonstrated that the regulatory molecule **14-3-3**[30] (see Glossary) coordinates the activity of IRSp53 and thus filopodia formation.[19] The work

showed that 14-3-3 binds to AMPK-phosphorylated IRSp53 and blocks its activation by CDC42 or by its several effectors such as VASP and Eps8.[19] While in the presence of 14-3-3, even though IRSp53 was expressed together with CDC42, VASP or Eps8, filopodia formation was largely inhibited, implicating that 14-3-3 has a substantial role in the fine tuning of filopodia formation through the inhibition of IRSp53 binding to the membrane.[19,31]

Apart from CDC42 and IRSp53 involvement in filopodia formation, the less investigated IRTKS induced the growth of attached filopodia.[21] Furthermore, an interaction between an atypical Rho GTPase Rif (see Box1) and IRTKS promoted dorsal filopodia assembly, which, similar to TNTs, are not surface adherent structures.[32] Moreover, in dendritic filopodia, when inactive Rif mutants were overexpressed, the length of these protrusions was significantly reduced, emphasizing the role of Rif in the formation of long non-adherent filopodia-like structures.[33] Another atypical Rho GTPase, RhoD (see Box1), was found to induce the formation of long filopodia-like protrusions.[34] These alternate mechanisms of protrusion initiation share striking similarities with TNTs and give us an insight into other potential avenues to explore in the field of TNTs.

While several recent reviews on TNTs have more thoroughly discussed the role of **M-Sec** (see Glossary), in this review we want to only highlight two possibilities through which this protein might function. Firstly, an interaction of M-Sec with **RalA** (see Glossary) was found to be necessary for the formation of TNTs.[35] Furthermore, RalA has been shown to interact with **filamin** (see Glossary) to promote the formation of filopodia[36], suggesting similar complexes that would influence actin crosslinking or other processes related to actin reorganization within TNTs. Secondly, given that the **exocyst complex** (see Glossary) is well known to be involved in vesicle trafficking[37], it is possible that the directed delivery of membrane to a growing TNT is needed, however, the exact mechanism of its involvement in TNT formation needs to be further characterized.

#### **How linear F-actin assembly leads to protrusion growth**

**TNTs as compared to other similar protrusions reach extraordinary long distances.**

Foundational theoretical work in the field of filopodia, for example, has shown that filopodia



growth is largely limited by the diffusion of G-actin to the polymerizing barbed end, which ultimately sets an upper threshold **on the order of 5  $\mu$ m** or less for the maximum length a filopodia can reach.[15] As previously described, TNTs likely contain uninterrupted, straight actin filaments organized in hexagonally packed bundles.[3] This actin organization suggests the involvement of actin nucleators, elongators and efficient delivery mechanisms that may supply required actin regulators, G-actin, etc. to the growing barbed end. Several actin nucleators and elongators have been assessed for their ability to extend actin filaments within different protrusions, but not much is known for TNTs. Given their length and actin architecture, only a potent elongator would be able to assemble such long actin filaments. In this section we will describe the involvement of different actin nucleators and elongators known to form filopodia, microvilli and dendritic filopodia, as they can be predictive for the types of actin regulators involved in the formation of TNTs.

A highly likely candidate in the formation of TNTs that fulfills the criteria of generating linear actin filaments is the formin protein family **(see Box1)**. [38] The ability of formins to processively incorporate G-actin to a growing filament is highly sensitive to both the applied forces on actin filaments where increasing loads can accelerate actin subunit incorporation[39], and geometrical constraints including filament bundling and formin attachment to membranes.[40] While no studies have explored the role of formins in microvilli, far more information has been obtained about their performance in the elongation of filopodia and dendritic filopodial precursors. Notably, mDia1-3 were all found to form filopodia, along with other formins such as formin-like protein 3 (FMNL3), and Disheveled-associated activator of morphogenesis 1 (DAAM1)[41–46]. Moreover, FMNL2 was also recently found to regulate dynamics of fascin in filopodia.[47] mDia3C was able to elongate extraordinarily long filopodia-like protrusions in a RhoD-dependent fashion where the protrusions reached lengths of 20-30  $\mu$ m that are on the same order found for TNTs.[34] Regarding dendritic filopodia, their elongation was driven by a Rif effector protein, mDia2[33], as well as by DAAM1.[48] Subsequently to the formation of the immature spine by mDia2, Arp2/3 complex **(see Box 1)** enabled the maturation of the spine through the formation of a branched actin array necessary for the spine head enlargement.[33]

Interactions of formins with I-BAR proteins and/or Rho GTPases are also important in filopodia biogenesis. In particular, IRSp53 associates with mDia1[41], while Rif activates mDia1 and mDia2.[42,44] Considering Rif also interacts with IRTKS[32], a possible association of IRTKS with some formins is highly likely to occur during protrusion formation, possibly through establishment of a protrusion tip complex.

As previously discussed, I-BARs interact with the Ena/VASP protein family to assemble protrusions such as filopodia.[22,26] VASP's highly organized tetrameric structure enhances actin polymerization during filopodia formation[49] and dendritic spine formation[50], while at the same time preventing the binding of actin-capping proteins to the barbed end.[49,51,52] In vitro experiments on fascin-induced bundling of actin filaments showed an increase of VASP's processivity. [49,52] Knowing that fascin, a well-established actin bundler in filopodia[53], has been shown to have an opposite, negative role in TNT formation[8], this negative impact of VASP on TNTs might be further explained through a synergistic activity with fascin. Fascin can increase the processivity of VASP and decrease the processivity of mDia1.[40,49] Considering that both VASP and fascin are negative regulators of TNTs and positive of filopodia, we might conclude that one of the formins might be indeed involved in the formation of TNTs and therefore represent an interesting path to explore.

Even though TNTs have been known since their discovery to be actin-based, why is there still no evidence for an actin nucleator/elongator involved in TNT formation? Many directions originating from what we know concerning filopodia, microvilli and dendritic spines might guide the scientific community to intensify its efforts in discovering the molecule(s) in charge of the assembly of actin filaments within TNTs.

#### **Stabilization of F-actin to give protrusions greater permanence**

Actin filament crosslinking represents a mechanism that cells employ in order to organize actin into different architectures and to stabilize the collection of actin filaments within a protrusion.[1] Primarily unlike single actin filaments, bundled filaments (on the order of 10 to 30 filaments)[54][55] have sufficient structural rigidity to overcome the resistance

imposed by the tension of the membrane, and also confer stability against buckling, necessary for outward growth and proper protrusion length.[15]

The canonical actin bundler fascin[53,56] was initially found to tightly bundle straight actin filaments in a filopodium.[53] It enables packing of actin filaments into hexagonal arrangements, with inter-filament distances on the order of 8-10 nm[57][58], thus providing necessary stability for the protrusion. Similar to fascin, fimbrin and espin bundle individual filaments in hexagonal structures, providing near identical distances between individual filaments.[58,59] Recent *in vitro* work suggests that these crosslinkers do not exclude each other and may mutually reside within the same actin network in the cell.[57] Indeed, the crosslinkers espin and fimbrin were both identified in microvilli.[60] Even after their depletion, the formation of microvilli was not completely abolished, implicating other crosslinkers such as Eps8 in the stabilization of microvilli.[61] In contrast, fascin acts as a negative regulator of functional TNT formation, further implicating the opposite regulation of filopodia and TNTs.[8]

Eps8 was able to promote filopodia formation in some cell models, highly likely through its interaction with an I-BAR protein that induces Eps8's crosslinking activity.[25,26] In contrast, in cells of neuronal origin, Eps8 had a negative impact on filopodia formation, as well on density of immature dendritic filopodia, possibly through its capping activity.[7,26,62] Similarly, in microvilli, overexpression of another Eps8 family member, Eps8L1a, controlled their overall length, preventing their excessive elongation, whereas bundling activity of Eps8 was crucial for maintaining microvillar shape.[63] However, in TNTs, Eps8's crosslinking property was found to be an important positive regulator of TNT formation, whereas its capping activity had no impact on the formation of TNTs.[7] We might hypothesize then that it is the bundling role that is enhancing the formation of TNTs, but this does not exclude other potential crosslinkers, other than fascin[8], creating hexagonally packed actin filament networks in TNTs.

Further evidence supports the important role of actin bundling and stabilization in TNT biogenesis. Dephosphorylated  $\beta$ CaMKII (see Glossary) was shown to prolong the half-life of TNTs observed in cell culture, implicating it has a positive effect on the lifetime of these

protrusions.[64] It has been shown that  $\beta$ CaMKII stabilizes F-actin, but also binds to G-actin preventing its nucleation.[65][66] Phosphorylating  $\beta$ CaMKII leads to its detachment from F- and G-actin, consequentially enabling actin polymerization from the free G-actin pool.[66]

Recently, it was demonstrated that  $\beta$ CaMKII can bind multiple actin filaments, aligning them in a mostly parallel manner.[67] In TNTs, when overexpressed,  $\beta$ CaMKII was localized at the base of the protrusion.[64] Considering  $\beta$ CaMKII assembles F-actin with an interfilament spacing of approximately 36 nm[67], and given that bundlers having different molecular sizes exclude one another within an actin network[57], we assume that  $\beta$ CaMKII will specifically segregate and will not co-localize with molecularly smaller actin bundlers that most likely give rise to the hexagonal arrangement of actin observed within the shaft of neuronal TNTs.[3] This segregation of  $\beta$ CaMKII is likely why it more resides at the base of TNTs where it might compete against **cofilin** (see Glossary) binding to the same hydrophobic pocket on F-actin[67], preventing **pointed end** (see Glossary) depolymerization and consequentially stabilizing and enhancing the permanence of linear F-actin within TNTs.[3,64]

### **Delivery of proteins necessary for the protrusion biogenesis and elongation**

How do cell protrusions, such as TNTs, reach long distances? How do they overcome the diffusion limit within the growing protrusion? One of the unconventional motors, Myosin-X (Myo10), which can link the plasma membrane—through phosphatidylinositol (3,4,5)-triphosphate lipid binding—with actin filaments[68], has been vastly investigated in the formation of protrusions, but not as much in the context of its contribution to direct the delivery of elongators, bundlers, etc. during protrusion growth. It was shown that Myo10 can recognize only straight actin filaments, known to be present within several protrusions, linking this property with cargo delivery for protrusion elongation.[69]

Notably, Myo10 was shown to induce dorsal and attached filopodia formation, and dendritic filopodia.[70–73] Myo10's head domain was sufficient to initiate short, but unstable filopodia[71,72], whereas the full-length Myo10 (FL-Myo10) was likely indispensable for the cargo-binding tail domain to exert its function in supplying the factors imperative for their

elongation.[72,74] It was observed that Myo10's **C-terminal tail-located FERM domain** (see **Glossary**) was responsible for  $\beta_1$ -integrin binding and FL-Myo10 relocation to the tip of a filopodium, implicating the importance of integrin activation in Myo10-induced filopodia.[74] Additionally, FL-Myo10 was able to form longer filopodia, a property F2- and/or F3-deleted Myo10 mutants did not have.[74] This suggested Myo10 could elongate and stabilize these protrusions, the latter explained through the ability of Myo10 to recruit  $\beta_1$ -integrin to form focal adhesions involved in the stabilization of filopodia, while the elongation was probably dependent on VASP[73,75] or other, still unknown binding partners of Myo10.

In TNTs, Myo10 was also found to be a positive regulator of their formation.[8,76] In contrast, neither of Myo10 head or tail mutants were able to induce the formation of functional TNTs.[8] Therefore, how FL-Myo10 induces TNT formation might be explained by Myo10's role as a cargo transporter, suggesting the delivery of necessary actin regulators to the growing tip of the TNT. Furthermore, it was demonstrated that the F2 lobe of the FERM domain was fundamental in the ability of Myo10 to induce TNTs.[8] In contrast, Myo10 completely devoid of the FERM domain was still able to form dorsal filopodia, while both F2 and F3 lobes were important in the formation of attached filopodia.[8,70,72,74] This highlights the subtle but important difference in the mechanism of formation and characteristics of dorsal filopodia and attached filopodia vs. TNTs. Furthermore, CDC42, a master regulator of filopodia[17], was found to act upstream of Myo10 to promote growth of dorsal filopodia.[70] While little is known about the F2 lobe and its cargo binding, one can still hypothesize that proteins specific for TNT formation might bind to the F2 lobe to be transported to the tip of a growing TNT. This avenue should be further explored, as it might lead to the identification of TNT-specific components.

### **Concluding remarks and future perspectives**

TNTs were described over 15 years ago, bringing a novel addition to the family of F-actin-composed cellular protrusions. Importantly, TNTs have been functionally characterized as conduits for the direct transfer of various cargoes and organelles between cells, emphasizing a key difference between TNTs and other actin-based cell protrusions. The ongoing identification of TNTs and TNT-like structures in a variety of cell types in culture and ex vivo

tissue slices have strengthened their involvement in normal physiological processes such as signal transduction, apoptosis, development and immune responses[5]. Furthermore, TNTs are also implicated in diseases, serving as a novel route for the propagation of infectious bacteria[77], viruses such as HIV-1[11], misfolded proteins involved in neurodegenerative diseases[78–80], and even in aggressive cancers[10]. Because of their pathological involvement, TNTs represent novel therapeutic targets that could offer a unique strategy to supplement the current therapeutics employed against pathogens and to fight presently incurable neurodegenerative diseases and therapy-resistant cancers[10,81].

As highlighted herein, formation of a TNT through actin-driven processes encompasses yet unknown signaling cascades that recruit membrane deforming and actin-regulating molecules that must work in concert to drive outward growth of a developing TNT towards a neighboring cell (Figure 2). Table 1 compares the few presently known actin-related molecules that promote (or inhibit) the formation of TNTs as compared to filopodia. However, still little to nothing is known about their underlying formation from the standpoint of which specific molecules are involved, leaving several aspects about their biogenesis, fusion with the recipient cell, and final maturation for cargo transport unknown (see Outstanding Questions).

For example, the identification of these TNT-specific molecules is necessary for directly targeting and impairing TNT formation in disease pathways. Future characterizations of TNTs in a robust manner must address their transfer ability in order to substantially distinguish them from other cell protrusions. This will supply the TNT community with indispensable information and insights on how to better understand and approach essential questions of tunneling nanotube identity, formation and structure-function relationships.

## Acknowledgments

N.L. is supported by Sorbonne Université (doctoral grant number 3210/2018), and J.M.H. is supported by a Pasteur Foundation Fellowship. This work was supported by grants to C.Z. from Equipe FRM (Fondation pour la Recherche Médicale) 2014 (DEQ 20140329557), Agence Nationale de la Recherche (ANR 16 CE160019-01 NEUROTUNN), Université Paris Sciences et Lettres-QLife Institute (ANR-17-CONV-0005 Q-LIFE), and the INCEPTION program-P2I (Investissement d'Avenir grant ANR-16).



## **BOX 1 – The cell’s molecular toolbox for generating actin-based protrusions**

Here we summarize important actin-regulating molecules that participate in the generation of cellular protrusions. **Figure I** below schematically depicts an interaction network between these molecules, identifying the kind(s) of protrusion(s) generated between pairs of molecules and providing the appropriate reference(s).

**I-BAR proteins:** A five-member family of inverted Bin/Amphiphysin/Rvs domain proteins that initiate and stabilize negative membrane curvature. Notable members include: IRSp53 (insulin receptor tyrosine kinase substrate of 53 kDa), IRTKS (insulin receptor tyrosine kinase substrate) and MIM (missing-in-metastasis).[13]

**Rho GTPases:** Signaling proteins that cycle between an ‘on’ and ‘off’ state depending on their GTP and GDP cycle, respectively, that trigger actin rearrangement. Notable members include CDC42 (cell division cycle 42), Rac1 (Ras-related C3 botulinum toxin substrate 1) involved in Arp2/3 activation, Rif (Rho in filopodia) and RhoD.[16]

**Arp2/3:** The actin-related protein 2/3 (Arp2/3) is a complex made of seven protein subunits that mediates the formation of dense, branched networks of filamentous actin. When properly activated by CDC42-stimulated **N-WASP** (Neural Wiskott-Aldrich syndrome protein), or by Rac-stimulated **WAVE** (WASP family verprolin-homologous protein), Arp2/3 binds on the side of a pre-existing actin filament and through its Arp2 and Arp3 subunits, which closely resemble the structure of monomeric actin, nucleates the formation of a new, ‘daughter’ filament oriented 70° relative to the pre-existing ‘mother’ filament.[82][83]

**VASP:** Vasodilator-stimulated phosphoprotein – member of the Enabled/vasodilator-stimulated phosphoprotein (Ena/VASP) family of actin nucleators that elongates straight actin filaments.[51]

**Eps8:** Epidermal growth receptor substrate 8 – actin-binding protein with a dual function – when it interacts with an adaptor protein Abl Interactor 1 (Abi1) it acts as a cap to limit



protrusion extension; when it interacts with an I-BAR protein, e.g., IRSp53, it promotes bundling of actin filaments and thus the stabilization of the formed protrusion.[23–26]

**Formins:** A family of fifteen actin nucleators that elongate straight actin filaments through the processive addition of G-actin to the growing barbed end. Formins are autoinhibited, a state reverted by their interaction with Rho GTPases. Most notable members include mDia1-3 also known as Diaphanous-related formins (Drfs) formins, FMNL2 and FMNL3 (formin-like protein 2 and 3) and DAAM1 (Disheveled-associated activator of morphogenesis 1).[38][84]

**Fascin, Fimbrin, Espin:** Common actin crosslinkers that bundle straight actin filaments hexagonally into parallel arrangements.[53,56,58,59]

**Figure I.** Direct interactions of actin-related proteins found to be indispensable in protrusion formation. These interactions were characterized by immunoprecipitation and/or fluorescence resonance energy transfer (FRET) assays. Note: This schematic does not show interactions of actin-related proteins in other cell processes (e.g., secretory pathways).

## **BOX 2 – TNT formation mechanism differs depending on the cell type**

The same Rho GTPase pathways activated in neuronal cells might have a different role in TNTs depending on the cell type (e.g., cells of immune origin). For example, in macrophages, two pathways converging on Arp2/3, one that is dependent on CDC42-mediated activation of N-WASP (see Box 1), and the other dependent on Rac1 activation of WAVE2 (see Box 1), were found to participate in the formation of TNTs.[85] While the inhibition of Arp2/3 in macrophages led to a decrease in the number of TNTs[85], suggesting the importance of branched actin network formation during TNT biogenesis, in neuronal cells Arp2/3 blockage had an opposite effect—it increased the percent of TNT-connected cells and the vesicle transfer they conduct.[3] Consistent with these observation, in neuronal cells TNTs were found to be composed of exclusively straight actin filaments[3], suggesting that the inhibition of Arp2/3 probably led to the reorganization of actin cytoskeleton through redirection of available G-actin towards straight F-actin formation, subsequently inducing TNT biogenesis in these cells. Considering cells of immune and neuronal origin are different,

the conclusion for this discrepancy probably lies in cell-specific mechanisms utilized to form TNTs. In order to fully assess the role of these proteins in macrophages, it is crucial to elucidate the actin architecture within macrophage TNTs and to confirm their functionality by employing transfer-based experiments, similar to what was shown in neuronal cells[7]. A similar observation regarding Arp2/3 inhibition was observed in microvilli.[86] Blocking Arp2/3-dependent branched network assembly stimulated the growth of longer microvilli, highly likely through the reorganization the F-actin assembly from the cortical towards straight actin network in microvilli.[86] In contrast, inhibition of Arp2/3 led to a decrease in the formation of **tumor microtubes (TMs)** (see Glossary) between pancreatic cancer cells, implicating a similar regulation of TM biogenesis as compared to macrophage TNTs, but an opposite one as compared to neuronal TNTs.[87]

## **GLOSSARY**

**14-3-3:** A family of regulatory molecules that bind phosphorylated serine/threonine motifs to protect phosphorylated residues from phosphatases, block downstream protein binding, and provide a scaffold for promoting direct protein-protein interactions, for example.

**βCaMKII:** A serine/threonine-specific  $\text{Ca}^{2+}$ -Calmodulin-dependent protein kinase II (CaMKII) enzyme highly expressed in the brain that mediates synaptic structures through binding and bundling of F-actin, and by sequestering G-actin.

**Cofilin:** A member of the actin depolymerizing factor (ADF) family that disassembles actin filaments at their pointed end through severing.

**Cytonemes:** Long (up to 700  $\mu\text{m}$ ) actin-based extensions that specifically allow for direct protein-protein interactions involved in growth factor and morphogen signaling over long distances.

**Dendritic spines:** Neuronal protrusions emerging from dendrites that receive excitatory inputs from axons. Immature 'dendritic filopodia' adopt the characteristic mushroom shape of the mature spine that is supported by branched actin.

**Exocyst complex:** An eight-subunit complex involved in vesicle trafficking where it facilitates the tethering of vesicles to the plasma membrane for exocytosis prior to membrane fusion.

**FERM domain:** Is a module originally identified in the four-point one/ezrin/radixin/moesin protein family that mediates plasma membrane binding by interacting with phosphatidylinositol (4,5) biphosphate lipids.

**Filamins:** A family of actin-binding proteins that crosslink actin filaments into orthogonal networks.

**Filopodia:** Dynamic, closed-ended finger-like protrusions containing parallel bundles of F-actin reaching typical lengths on the order of 1–5  $\mu\text{m}$ . They can be found on the dorsal side of cultured cells, but more commonly they are observed attached to the substrate.

**Microvilli:** Epithelial protrusions on the order of 1–2  $\mu\text{m}$  in length that form a dense array known as the 'brush border.' Similar to filopodia they contain a core of bundled actin filaments.

**M-Sec:** Also known as Tumor necrosis factor alpha-induced protein 2 (TNFAIP2) acts as a platform that connects RalA (a Ral GTPase subfamily member) and the exocyst complex.

**Myosins:** Motor protein family that bind actin and move along actin filaments. Conventional class II myosins form microfilaments and produce contractile forces, while non-class II myosins comprise notable motors for organelle transport (e.g., Myosin-V, Myosin-X).

**Polar filaments:** Actin filaments are polarized, i.e. having different ends, referred to as the **barbed** (i.e., plus) and **pointed** (i.e., minus) end. Actin monomers preferentially incorporate at the barbed end, while filament disassembly occurs preferentially at the pointed end. In protrusions, the barbed end is oriented towards the plasma membrane such that polymerization can help outward growth of the protrusion.

**SH3 domain:** Src homology 3 domain is an adapter module that mediates the assembly of multi-protein complexes by recognizing short PXXP peptide motifs (P, proline; X, any amino acid) that adopt a polyproline type II helix.

**Tumor microtubes (TMs):** Membrane protrusions forming networks between cancer cells. TMs are thicker than TNTs and apart from actin they contain microtubules. They are close-ended protrusions with GAP junction channels at their ends that permit intercellular transfer of electrical signals and small molecules.

**Table 1. Summary of actin-related proteins and their effect in the formation of TNTs vs. filopodia.**

Actin and membrane-related proteins	TNT formation	Filopodia formation
IRSp53	Inhibits[7]	Promotes[19,20]
IRTKS	n.d.	Promotes[21,32]
CDC42	Inhibits/Promotes[7,85]	Promotes[17,19,20]
Rac1	Promotes[85]	n.d.
Rif	n.d.	Promotes[32]
RhoD	n.d.	Promotes[34]
VASP	Inhibits[7]	Promotes[7,22]
mDia1-3	n.d.	Promotes[41-44]
DAAM1	n.d.	Promotes[45]
FMNL3	n.d.	Promotes[46]
Fascin	Inhibits[8]	Promotes[53]
Eps8	Promotes[7]	Inhibits/Promotes[7,25,26]
Filamin	n.d.	Promotes[36]
Myosin-X	Promotes[8,76]	Promotes[70-72]

\* n.d. – not defined

## Figure legends

**Figure 1.** Schematics of canonical **actin-based** protrusions. **A.** Microvilli (1-2  $\mu\text{m}$  in length) form a brush border on the apical surface of epithelial cells and contain straight actin filaments bundled by several actin crosslinkers. **B.** Various cells form dorsal and attached filopodia ( $< 10 \mu\text{m}$  in length) that are comprised of straight actin bundles crosslinked by fascin. **C.** Immature dendritic spines (i.e., dendritic filopodia) found in neurons share similarities with microvilli and filopodia; in contrast, apart from straight actin they are supported by Arp2/3-formed branched actin filaments. A common feature amongst these protrusions, apart from their morphological resemblance, is the presence of I-BAR proteins necessary for initial protrusion formation through membrane curvature sensing and induction.

**Figure 2.** Proposed model of TNT formation **between two cells.** A cell in its inactive state (1) is stimulated by various signals (2) that activate a Rho GTPase which subsequently releases I-BAR inhibition. Activated I-BAR proteins sense and induce negative membrane curvature necessary for TNT formation (2); actin polymerization is triggered to form an initial actin bundle that can overcome membrane resilience further enabling TNT extension for example by formins **or another actin elongator** (3). The growing TNT from cell 1 reaches the recipient cell 2 and fuses with its membrane through an unknown fusion mechanism **(not depicted here)** (4). A functional TNT, containing straight actin filaments bundled, for example by Eps8, is formed between cell 1 and cell 2 which can now exchange large cargo such as vesicles **(by not yet known motors, not depicted here)** (5).

586

587 **References**

- 588 1 Svitkina, T. (2018) The Actin Cytoskeleton and Actin-Based Motility. *Cold Spring Harb.*  
589 *Perspect. Biol.* 10, a018267
- 590 2 Rustom, A. *et al.* (2004) Nanotubular highways for intercellular organelle transport.  
591 *Science* 303, 1007–1010
- 592 3 Sartori-Rupp, A. *et al.* (2019) Correlative cryo-electron microscopy reveals the structure of  
593 TNTs in neuronal cells. *Nat. Commun.* 10, 342
- 594 4 Korenkova, O. *et al.* (2020) Fine intercellular connections in development: TNTs,  
595 cytonemes, or intercellular bridges? *Cell Stress* 4, 30–43
- 596 5 Yamashita, Y.M. *et al.* (2018) Specialized Intercellular Communications via Cytonemes and  
597 Nanotubes. *Annu. Rev. Cell Dev. Biol.* 34, 59–84
- 598 6 Cordero Cervantes, D. and Zurzolo, C. (2020) Peering into Tunneling Nanotubes — The  
599 Path Forward. *EMBO J.* (in press)
- 600 7 Delage, E. *et al.* (2016) Differential identity of Filopodia and Tunneling Nanotubes  
601 revealed by the opposite functions of actin regulatory complexes. *Sci. Rep.* 6, 39632
- 602 8 Gousset, K. *et al.* (2013) Myo10 is a key regulator of TNT formation in neuronal cells. *J.*  
603 *Cell. Sci.* 126, 4424–4435
- 604 9 Kolba, M.D. *et al.* (2019) Tunneling nanotube-mediated intercellular vesicle and protein  
605 transfer in the stroma-provided imatinib resistance in chronic myeloid leukemia cells. *Cell*  
606 *Death Dis.* 10, 1–16
- 607 10 Pinto, G. *et al.* (2020) Tunneling Nanotubes: The Fuel of Tumor Progression? *Trends*  
608 *Cancer* 6, 874-888
- 609 11 Sowinski, S. *et al.* (2008) Membrane nanotubes physically connect T cells over long  
610 distances presenting a novel route for HIV-1 transmission. *Nat. Cell Biol.* 10, 211–219
- 611 12 Dupont, M. *et al.* (2018) Tunneling Nanotubes: Intimate Communication between  
612 Myeloid Cells. *Front. Immunol.* 9, 43
- 613 13 Zhao, H. *et al.* (2011) I-BAR domain proteins: linking actin and plasma membrane  
614 dynamics. *Curr. Opin. Cell Biol.* 23, 14–21
- 615 14 Prévost, C. *et al.* (2015) IRSp53 senses negative membrane curvature and phase  
616 separates along membrane tubules. *Nat. Commun.* 6, 8529

617 15 Mogilner, A. and Rubinstein, B. (2005) The Physics of Filopodial Protrusion. *Biophys. J.* 89,  
618 782–795

619 16 Sadok, A. and Marshall, C.J. (2014) Rho GTPases. *Small GTPases* 5, e983878

620 17 Nobes, C.D. and Hall, A. (1995) Rho, Rac, and Cdc42 GTPases regulate the assembly of  
621 multimolecular focal complexes associated with actin stress fibers, lamellipodia, and  
622 filopodia. *Cell* 81, 53–62

623 18 Saarikangas, J. *et al.* (2015) MIM-Induced Membrane Bending Promotes Dendritic Spine  
624 Initiation. *Dev. Cell* 33, 644–659

625 19 Kast, D.J. and Dominguez, R. (2019) IRSp53 coordinates AMPK and 14-3-3 signaling to  
626 regulate filopodia dynamics and directed cell migration. *Mol. Biol. Cell* 30, 1285–1297

627 20 Krugmann, S. *et al.* (2001) Cdc42 induces filopodia by promoting the formation of an  
628 IRSp53:Mena complex. *Curr. Biol.* 11, 1645–1655

629 21 Postema, M.M. *et al.* (2018) IRTKS (BAIAP2L1) Elongates Epithelial Microvilli Using EPS8-  
630 Dependent and Independent Mechanisms. *Curr. Biol.* 28, 2876–2888

631 22 Disanza, A. *et al.* (2013) CDC42 switches IRSp53 from inhibition of actin growth to  
632 elongation by clustering of VASP. *EMBO J.* 32, 2735–2750

633 23 Disanza, A. *et al.* (2004) Eps8 controls actin-based motility by capping the barbed ends of  
634 actin filaments. *Nat. Cell Biol.* 6, 1180–1188

635 24 Hertzog, M. *et al.* (2010) Molecular Basis for the Dual Function of Eps8 on Actin  
636 Dynamics: Bundling and Capping. *PLOS Biol.* 8, e1000387

637 25 Disanza, A. *et al.* (2006) Regulation of cell shape by Cdc42 is mediated by the synergic  
638 actin-bundling activity of the Eps8–IRSp53 complex. *Nat. Cell Biol.* 8, 1337–1347

639 26 Vaggi, F. *et al.* (2011) The Eps8/IRSp53/VASP Network Differentially Controls Actin  
640 Capping and Bundling in Filopodia Formation. *PLoS Comput. Biol.* 7, e1002088

641 27 Sudhakaran, T. *et al.* (2019) Superresolution microscopy reveals distinct localisation of  
642 full length IRSp53 and its I-BAR domain protein within filopodia. *Sci. Rep.* 9, 2524

643 28 Jarin, Z. *et al.* (2019) Unusual Organization of I-BAR Proteins on Tubular and Vesicular  
644 Membranes. *Biophys. J.* 117, 553–562

645 29 Choi, J. (2005) Regulation of Dendritic Spine Morphogenesis by Insulin Receptor  
646 Substrate 53, a Downstream Effector of Rac1 and Cdc42 Small GTPases. *J. Neurosci.* 25,  
647 869–879



648 30 Cornell, B. and Toyooka, K. (2017) 14-3-3 Proteins in Brain Development: Neurogenesis,  
649 Neuronal Migration and Neuromorphogenesis. *Front. Mol. Neurosci.* 10, 318

650 31 Kast, D.J. and Dominguez, R. (2019) Mechanism of IRSp53 inhibition by 14-3-3. *Nat.*  
651 *Commun.* 10, 483

652 32 Sudhaharan, T. *et al.* (2016) The Rho GTPase Rif signals through IRTKS, Eps8 and WAVE2  
653 to generate dorsal membrane ruffles and filopodia. *J. Cell Sci.* 129, 2829–2840

654 33 Hotulainen, P. *et al.* (2009) Defining mechanisms of actin polymerization and  
655 depolymerization during dendritic spine morphogenesis. *J. Cell Biol.* 185, 323–339

656 34 Koizumi, K. *et al.* (2012) RhoD activated by fibroblast growth factor induces cytoneme-  
657 like cellular protrusions through mDia3C. *Mol. Biol. Cell* 23, 4647–4661

658 35 Hase, K. *et al.* (2009) M-Sec promotes membrane nanotube formation by interacting  
659 with Ral and the exocyst complex. *Nat. Cell Biol.* 11, 1427–1432

660 36 Ohta, Y. *et al.* (1999) The small GTPase RalA targets filamin to induce filopodia. *Proc.*  
661 *Natl. Acad. Sci. U.S.A.* 96, 2122–2128

662 37 Mei, K. and Guo, W. (2018) The exocyst complex. *Curr. Biol.* 28, 922–925

663 38 Courtemanche, N. (2018) Mechanisms of formin-mediated actin assembly and dynamics.  
664 *Biophys. Rev.* 10, 1553–1569

665 39 Yu, M. *et al.* (2017) mDia1 senses both force and torque during F-actin filament  
666 polymerization. *Nat. Commun.* 8, 1650

667 40 Suzuki, E.L. *et al.* (2020) Geometrical Constraints Greatly Hinder Formin mDia1 Activity.  
668 *Nano Lett.* 20, 22–32

669 41 Goh, W.I. *et al.* (2012) mDia1 and WAVE2 Proteins Interact Directly with IRSp53 in  
670 Filopodia and Are Involved in Filopodium Formation. *J. Biol. Chem.* 287, 4702–4714

671 42 Goh, W.I. *et al.* (2011) Rif-mDia1 Interaction Is Involved in Filopodium Formation  
672 Independent of Cdc42 and Rac Effectors. *J. Biol. Chem.* 286, 13681–13694

673 43 Block, J. *et al.* (2008) Filopodia formation induced by active mDia2/Drf3. *J. Microsc.* 231,  
674 506–517

675 44 Pellegrin, S. and Mellor, H. (2005) The Rho Family GTPase Rif Induces Filopodia through  
676 mDia2. *Curr. Biol.* 15, 129–133

677 45 Jaiswal, R. *et al.* (2013) The Formin Daam1 and Fascin Directly Collaborate to Promote  
678 Filopodia Formation. *Curr. Biol.* 23, 1373–1379

679 46 Young, L.E. *et al.* (2018) Roles for Ena/VASP proteins in FMNL3-mediated filopodial  
680 assembly. *J. Cell Sci.* 131, jcs220814

681 47 Pfisterer, K. *et al.* (2020) FMNL2 regulates dynamics of fascin in filopodia. *J. Cell Biol.* 219,  
682 e201906111

683 48 Kawabata Galbraith, K. *et al.* (2018) MTSS1 Regulation of Actin-Nucleating Formin  
684 DAAM1 in Dendritic Filopodia Determines Final Dendritic Configuration of Purkinje Cells.  
685 *Cell Rep.* 24, 95-106

686 49 Harker, A.J. *et al.* (2019) Ena/VASP processive elongation is modulated by avidity on actin  
687 filaments bundled by the filopodia cross-linker fascin. *Mol. Biol. Cell* 30, 851–862

688 50 Lin, W.-H. *et al.* (2010) Vasodilator-stimulated Phosphoprotein (VASP) Induces Actin  
689 Assembly in Dendritic Spines to Promote Their Development and Potentiate Synaptic  
690 Strength. *J. Biol. Chem.* 285, 36010–36020

691 51 Brühmann, S. *et al.* (2017) Distinct VASP tetramers synergize in the processive elongation  
692 of individual actin filaments from clustered arrays. *Proc. Natl. Acad. Sci. U.S.A.* 114, 5815–  
693 5824

694 52 Winkelman, J.D. *et al.* (2014) Ena/VASP Enabled is a highly processive actin polymerase  
695 tailored to self-assemble parallel-bundled F-actin networks with Fascin. *Proc. Natl. Acad.*  
696 *Sci. U.S.A.* 111, 4121–4126

697 53 Vignjevic, D. *et al.* (2006) Role of fascin in filopodial protrusion. *J. Cell Biol.* 174, 863–875

698 54 Jasnin, M. *et al.* (2013) Three-dimensional architecture of actin filaments in *Listeria*  
699 *monocytogenes* comet tails. *Proc. Natl. Acad. Sci. U.S.A.* 110, 20521–20526

700 55 Ohta, K. *et al.* (2012) Helical arrangement of filaments in microvillar actin bundles. *J.*  
701 *Struct. Biol.* 177, 513–519

702 56 Jansen, S. *et al.* (2011) Mechanism of actin filament bundling by fascin. *J. Biol. Chem.* 286,  
703 30087–30096

704 57 Winkelman, J.D. *et al.* (2016) Fascin- and  $\alpha$ -Actinin-Bundled Networks Contain Intrinsic  
705 Structural Features that Drive Protein Sorting. *Curr. Biol.* 26, 2697–2706

706 58 Volkmann, N. *et al.* (2001) An Atomic Model of Actin Filaments Cross-Linked by Fimbrin  
707 and Its Implications for Bundle Assembly and Function. *J. Cell Biol.* 153, 947–956

708 59 Purdy, K.R. *et al.* (2007) Structural Polymorphism of the Actin-Espin System: A  
709 Prototypical System of Filaments and Linkers in Stereocilia. *Phys. Rev. Lett.* 98, 058105

710 60 Crawley, S.W. *et al.* (2014) Shaping the intestinal brush border. *J. Cell Biol.* 207, 441–451

711 61 Revenu, C. *et al.* (2011) A new role for the architecture of microvillar actin bundles in  
712 apical retention of membrane proteins. *Mol. Biol. Cell* 23, 324–336

713 62 Menna, E. *et al.* (2013) Eps8 controls dendritic spine density and synaptic plasticity  
714 through its actin-capping activity. *EMBO J.* 32, 1730–1744

715 63 Zwaenepoel, I. *et al.* (2012) Ezrin regulates microvillus morphogenesis by promoting  
716 distinct activities of Eps8 proteins. *Mol. Biol. Cell* 23, 1080–1095

717 64 Vargas, J.Y. *et al.* (2019) The Wnt/Ca<sup>2+</sup> pathway is involved in interneuronal  
718 communication mediated by tunneling nanotubes. *EMBO J.* 38, e101230

719 65 Lin, Y.-C. and Redmond, L. (2008) CaMKII $\beta$  binding to stable F-actin in vivo regulates F-  
720 actin filament stability. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15791–15796

721 66 Sanabria, H. *et al.* (2009)  $\beta$ CaMKII regulates actin assembly and structure. *J. Biol. Chem.*  
722 284, 9770–9780

723 67 Wang, Q. *et al.* (2019) Assemblies of calcium/calmodulin-dependent kinase II with actin  
724 and their dynamic regulation by calmodulin in dendritic spines. *Proc. Natl. Acad. Sci.*  
725 *U.S.A.* 116, 18937–18942

726 68 Plantard, L. *et al.* (2010) PtdIns(3,4,5)P<sub>3</sub> is a regulator of myosin-X localization and  
727 filopodia formation. *J. Cell Sci.* 123, 3525–3534

728 69 Ricca, B.L. and Rock, R.S. (2010) The Stepping Pattern of Myosin X Is Adapted for  
729 Processive Motility on Bundled Actin. *Biophys. J.* 99, 1818–1826

730 70 Bohil, A.B. *et al.* (2006) Myosin-X is a molecular motor that functions in filopodia  
731 formation. *Proc. Natl. Acad. Sci. U.S.A.* 103, 12411–12416

732 71 Tokuo, H. *et al.* (2007) The motor activity of myosin-X promotes actin fiber convergence  
733 at the cell periphery to initiate filopodia formation. *J. Cell Biol.* 179, 229–238

734 72 Watanabe, T.M. *et al.* (2010) Myosin-X Induces Filopodia by Multiple Elongation  
735 Mechanism. *J. Biol. Chem.* 285, 19605–19614

736 73 Lin, W.-H. *et al.* (2013) Myosin X and its motorless isoform differentially modulate  
737 dendritic spine development by regulating trafficking and retention of vasodilator-  
738 stimulated phosphoprotein. *J. Cell Sci.* 126, 4756–4768

739 74 Zhang, H. *et al.* (2004) Myosin-X provides a motor-based link between integrins and the  
740 cytoskeleton. *Nat. Cell Biol.* 6, 523–531

741 75 Tokuo, H. and Ikebe, M. (2004) Myosin X transports Mena/VASP to the tip of filopodia.  
742 *Biochem. Biophys. Res. Commun.* 319, 214–220

743 76 Tasca, A. *et al.* (2017) Regulation of Osteoclast Differentiation by Myosin X. *Sci. Rep.* 7, 1–  
744 10

745 77 Onfelt, B. *et al.* (2006) Structurally distinct membrane nanotubes between human  
746 macrophages support long-distance vesicular traffic or surfing of bacteria. *J. Immunol.*  
747 177, 8476–8483

748 78 Abounit, S. *et al.* (2016) Tunneling nanotubes spread fibrillar  $\alpha$ -synuclein by intercellular  
749 trafficking of lysosomes. *EMBO J.* 35, 2120–2138

750 79 Sharma, M. and Subramaniam, S. (2019) Rhes travels from cell to cell and transports  
751 Huntington disease protein via TNT-like protrusion. *J. Cell Biol.* 218, 1972–1993

752 80 Gousset, K. *et al.* (2009) Prions hijack tunnelling nanotubes for intercellular spread. *Nat.*  
753 *Cell Biol.* 11, 328–336

754 81 Victoria, G.S. and Zurzolo, C. (2017) The spread of prion-like proteins by lysosomes and  
755 tunneling nanotubes: Implications for neurodegenerative diseases. *J. Cell Biol.* 216, 2633–  
756 2644

757 82 Stradal, T.E.B. *et al.* (2004) Regulation of actin dynamics by WASP and WAVE family  
758 proteins. *Trends Cell Biol.* 14, 303–311

759 83 Svitkina, T.M. (2020) Actin Cell Cortex: Structure and Molecular Organization. *Trends Cell*  
760 *Biol.* 30, 556–565

761 84 Kühn, S. and Geyer, M. (2014) Formins as effector proteins of Rho GTPases. *Small*  
762 *GTPases* 5, e983876

763 85 Hanna, S.J. *et al.* (2017) The Role of Rho-GTPases and actin polymerization during  
764 Macrophage Tunneling Nanotube Biogenesis. *Sci. Rep.* 7, 8547

765 86 Faust, J.J. *et al.* (2019) Profilin-Mediated Actin Allocation Regulates the Growth of  
766 Epithelial Microvilli. *Curr. Biol.* 29, 3457–3465

767 87 Latario, C.J. *et al.* (2020) Tumor microtubes connect pancreatic cancer cells in an Arp2/3  
768 complex-dependent manner. *Mol. Biol. Cell* 31, 1259–1272

769

770

Figure 1

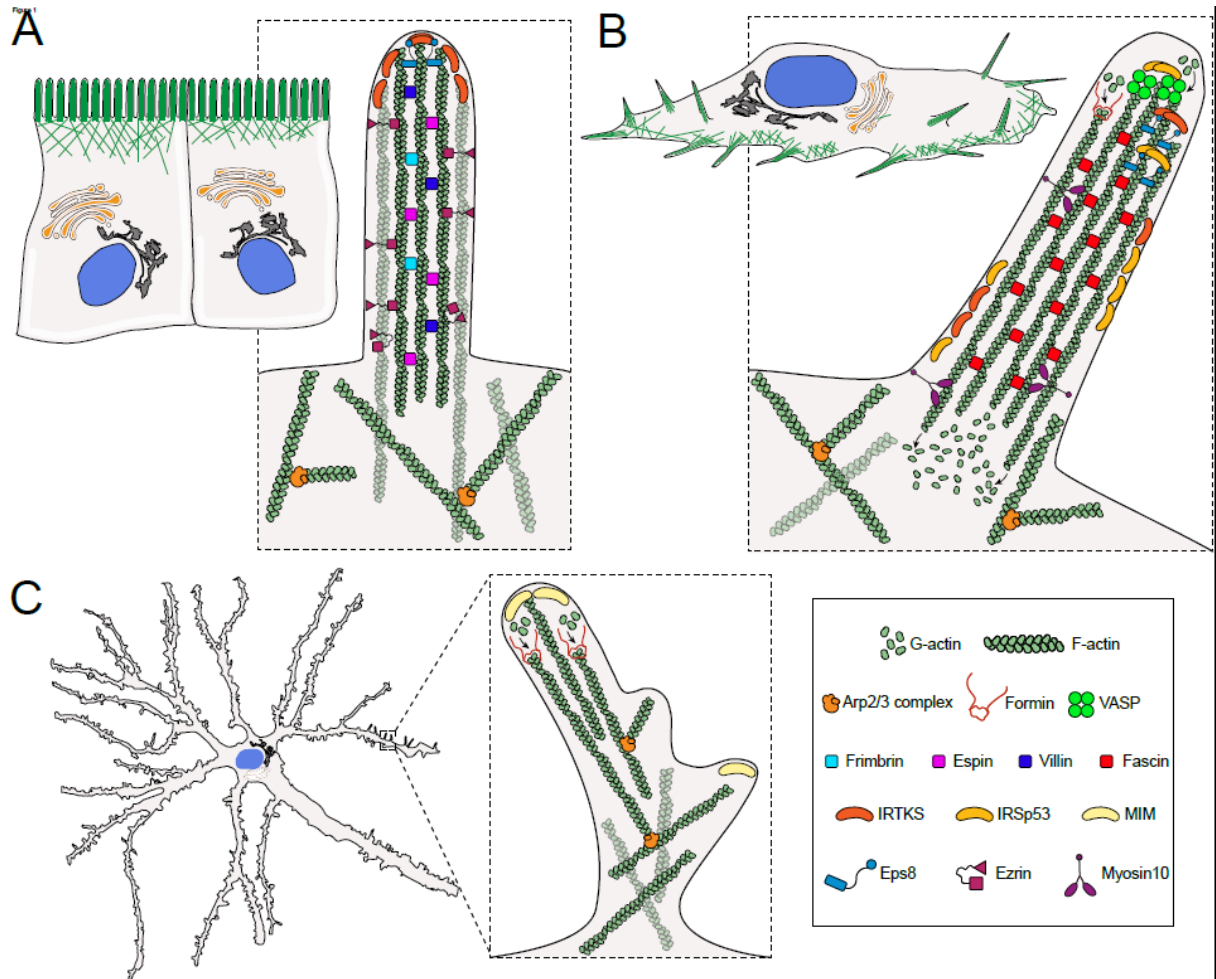


Figure 2

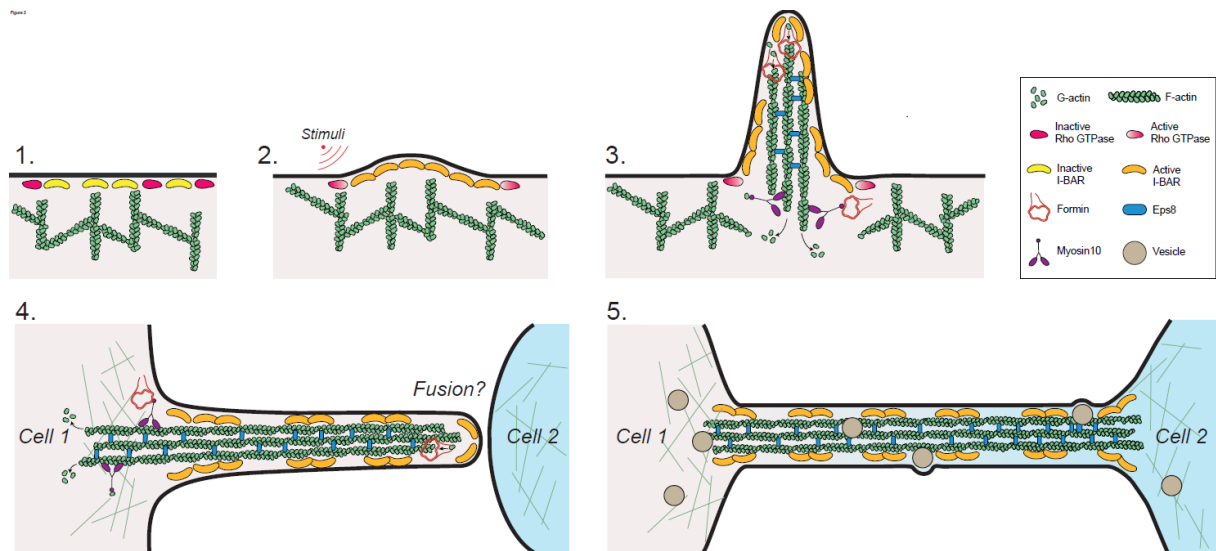


Figure i - Box 1

