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GMF as an actin network remodeling factor

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Abstract

Glia Maturation Factor (GMF) has recently been established as a regulator of the actin cytoskeleton with a unique role in remodeling actin network architecture. Conserved from yeast to mammals, GMF is one of five members of the ADF-H family of actin regulatory proteins, which includes ADF/Cofilin, Abp1/Drebrin, Twinfilin, and Coactosin. GMF does not bind actin, but does bind the Arp2/3 complex with high affinity. Through this association, GMF catalyzes the debranching of actin filament networks and inhibits actin nucleation by Arp2/3 complex. Here, we discuss GMF's emerging role in controlling actin filament spatial organization and dynamics underlying cell motility, endocytosis, and other biological processes. Further, we attempt to reconcile these functions with its earlier characterization as a cell differentiation factor.

Keywords

actin; ADF-H; Arp2/3 complex; cell motility; lamellipodia

GMF: a small protein with a big reach

Glia Maturation Factor (GMF) is a 17 kDa protein with clear orthologues across species as diverse as yeast, flies, zebrafish, and mammals (Figure 1A). GMF was first identified over 40 years ago as a factor in brain extracts that could induce the differentiation of cultured glioblasts, gliomas, and neuroblastoma cells [1–4]. Thus, GMF was studied for decades as a signaling factor controlling brain cell differentiation and function [1, 2]. Inconsistent with its early assignment as a cell differentiation factor, GMF is conserved from single-celled yeast to mammals. In fungal species and invertebrates, there is typically a single GMF gene/protein. However, vertebrates express two different GMF genes, GMF β and GMF γ . These isoforms are 82% identical in sequence [5] and have similar 3-D structures [6] (Figure 1B & D). GMF β and GMF γ show overlapping yet distinguishable expression patterns in different tissues [7–9]. Although GMF β has been described in some studies as a brain-specific isoform, its expression has also been detected at the protein level in lung, spleen, colon, and thymus [8], and even more ubiquitously as a transcript [5, 7, 10].

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In 2006, pioneering work from Ikeda and colleagues showed that GMF localizes to and regulates the **actin cytoskeleton** (see Glossary), and noted its sequence similarity to ADF/**Cofilin** [11]. In 2009, the structure of GMF [6] was solved, revealing it to be a *bona fide* member of the **ADF-H** (actin depolymerizing factor homology) family, which includes: ADF/Cofilin, Twinfilin, Abp1 (actin binding protein 1)/Drebrin, and Coactosin [6, 12]. All known ADF-H proteins bind to either actin and/or actin related proteins (Arps), and have conserved roles in actin cytoskeleton **remodeling** [12]. Below, we highlight recent work defining GMF's biochemical and cellular roles in binding **Arp2/3 complex** to remodel branched actin filament networks and thereby control endocytosis and cell motility. Finally, we attempt to integrate these new functions with its earlier assignment as a cell signaling and differentiation factor.

GMF branches out as an actin regulator

GMF γ was first established as a component of the actin cytoskeleton when it was shown to be highly expressed in microvascular endothelial cells and found to colocalize with actin at the **leading edge** of migrating cells [11]. It was also established that GMF γ has 39% similarity in sequence to ADF/cofilin, a prominent actin filament **severing** protein, and that GMF γ coimmunoprecipitates with the actin-nucleating Arp2/3 complex. Shortly thereafter, the NMR structures of GMF β and GMF γ were solved, and found to be remarkably similar to ADF/cofilin [6] (Figure 1D-E). These pioneering studies prompted other groups to further investigate the cytoskeletal functions of GMF in other model systems.

In 2010, two studies in yeast revealed exciting new functions for the *S. cerevisiae* and *S. pombe* homologs of GMF, both called Gmf1 (Figure 2) [13, 14]. These studies showed that purified Gmf1 lacks appreciable affinity for **G-actin** or **F-actin**, but directly regulates activities of the Arp2/3 complex. The Arp2/3 complex contains two actin-related proteins, Arp2 and Arp3, and upon binding to an activator such as WASP or WAVE, it binds to the side of an existing actin '**mother filament**' and nucleates formation of a '**daughter filament**' branch (see Box 1). In this manner, Arp2/3 complex forms arborized or 'dendritic' actin filament networks, including those found at the leading edge of migrating cells and at sites of endocytosis. Gmf1 was found to inhibit actin nucleation co-stimulated by Arp2/3 complex and the VCA domain of WASP, as demonstrated both in bulk actin assembly assays and single-filament total internal reflection fluorescence (TIRF) microscopy assays [13, 14]. Inhibition of Arp2/3 complex involved competition between Gmf1 and the WASP VCA domain for binding Arp2/3 complex, and strong inhibition required low micromolar concentrations of Gmf1. Gmf1 also catalyzed **debranching**, or 'pruning' of daughter filaments by dissociation at branch sites [13]. Earlier it had been shown that branch junctions generated by Arp2/3 complex *in vitro* are unexpectedly stable, failing to dissociate for tens of minutes in the absence of additional factors [15, 16]. In contrast, branches *in vivo* at the leading edge and at endocytic sites turn over in seconds [17, 18], suggesting the existence of cellular factors that might catalyze debranching. Low nanomolar concentrations of Gmf1 induced rapid 'pruning' of daughter filaments from mother filaments without severing elsewhere along the filaments [13]. The much higher concentration of GMF required for inhibition of nucleation by Arp2/3 complex versus debranching may reflect a requirement

for GMF to occupy two separate binding sites on Arp2/3 complex to block nucleation, one high affinity and one low affinity site (see below).

These observations established GMF as a dedicated debranching factor and suggested that it may be involved in remodeling branched actin networks into unbranched arrays, as has been observed to occur only a short distance from the leading edge [19–21]. Other factors such as ADF/Cofilin and **Coronin** stimulate debranching *in vitro* [22, 23], but additionally promote severing of actin filaments along their lengths [13, 24], and may therefore lead to actin network **disassembly** rather than remodeling, *i.e.* they may promote the dissolution of filaments to yield actin monomers, as opposed to the rearrangement of filaments within a network. Subsequent studies showed that the two biochemical functions of GMF on Arp2/3 complex (inhibition of nucleation and stimulation of debranching) are conserved for the *Drosophila* homolog and both mammalian isoforms of GMF [14, 25–27]. Thus, GMF activities on Arp2/3 complex are conserved across great evolutionary distances, consistent with the high degree of sequence conservation in GMF's two key functional surfaces (Figure 1B).

Genetic observations from these studies suggest that GMF regulates Arp2/3 complex and actin dynamics *in vivo* [13, 14]. Budding yeast Gmf1 localizes to cortical **actin patches**, sites of endocytosis assembled by Arp2/3 [13, 14]. Furthermore, deletion of *GMF1* exacerbates the growth defects of a *cof1-22* mutant [13], and extends the lifetime of actin patches. In contrast, overexpression of Gmf1 reduces both the number of actin patches and the levels of Arp2/3 complex at actin patches, and rescues specific mutant alleles of Arp2/3 complex [14]. In addition, *gmf1* mutants show synthetic defects in actin organization and cell growth with other actin turnover mutants [28]. Collectively, these genetic and biochemical observations provide strong evidence that Gmf1 promotes the remodeling and/or turnover of branched actin arrays *in vivo*. It is also noteworthy that *gmf1* cells exhibit disorganized actin cables (actin structures polymerized by formins) [28], and defects in mitochondrial distribution likely caused by disorganized cables, as suggested by its alias name, *Aim7*, Altered inheritance of mitochondria 7 [29]. Importantly, the cable defects of *gmf1* mutants may arise from reduced actin patch turnover, leading to an imbalance in the homeostatic distribution of actin subunits between patches and cables [30].

Mechanism of GMF-induced debranching

Mechanistic work has revealed that GMF debranches actin filaments by a mechanism related to cofilin severing of actin filaments, except that it is specialized for Arp2/3-actin junctions [13, 26, 31]. It has been reported that there are two binding sites for Gmf1 on Arp2/3 complex, one high affinity and one low affinity (with $K_d = 10$ nM and 1 μ M, respectively) [26]. Furthermore, single particle electron microscopy showed that Gmf1 induces an open/inactive conformation in the Arp2/3 complex [41], related to Coronin's effects on Arp2/3 structure [32, 33]. Employing fluorescence anisotropy, mutagenesis, and chemical cross-linking, it was found that Arp2/3 complex binding is mediated by a broad surface on Gmf1 ("Site 1"), analogous to the G/F (globular/filamentous) actin-binding surface on cofilin (Figure 1B-C) [26], and suggests that Site 2 (analogous to the F-site on cofilin) interacts with the first conventional actin subunit in the daughter filament. Both Site 1 and Site 2 were

found to be important for Gmf1 functions in yeast cells, suggesting that debranching is critical for Gmf1 function *in vivo*. In parallel, when the crystal structure of GMF γ bound to mammalian Arp2/3 complex was solved [31], it validated one of the two proposed binding sites of GMF on the Arp2/3 complex, and provided an atomic level detail of the interaction. This structure reveals that GMF directly contacts the Arp2 and p40/ARPC1 subunits, which is presumed to represent GMF's high affinity binding site on Arp2/3 complex, and suggests that surfaces on Arp2 may have co-evolved with GMF to maintain GMF interactions while excluding cofilin interactions [31]. A recent study using molecular dynamics simulations provides additional support for the existence of a second, lower affinity binding site for GMF on Arp2/3 complex, that involves interactions with the Arp3 subunit [34].

The above-mentioned studies also established that GMF catalyzes branch dissociation using its two distinct functional surfaces, with Site 1 binding to the Arp2 and p40/ARPC1 subunits and Site 2 binding to the first actin subunit in the daughter filament. Site 1 on GMF competes for binding with the nucleation promoting factor (NPF) WASP-VCA domain, explaining how GMF attenuates VCA-induced Arp2/3-mediated nucleation [31] (Figure 2C). Because GMF and cofilin are structural homologues with similarly-positioned functional surfaces/sites (Figure 1B), GMF may destabilize Arp2/3-actin branch junctions by a mechanism related to how cofilin destabilizes actin filaments to induce fragmentation. This argument is strengthened by GMF and cofilin both preferring the ADP-bound state of their ligands, Arp2/3 complex or actin, respectively. Given that cofilin induces a twist in F-actin conformation and alters actin-actin contacts [35, 36], it is possible that GMF exerts its debranching effects by similarly inducing conformational changes at Arp2/3-actin junctions.

GMF remodels actin networks at the leading edge

How are the conserved activities of GMF used *in vivo* to regulate branched actin networks, e.g., at sites of endocytosis and at the leading edge (Figure 2A-B)? In animal cells, GMF's regulatory effects on actin networks appear to govern lamellipodial dynamics and cell motility (Box 1). In IA32 mouse embryonic fibroblasts, GMF β localizes to mature rather than nascent lamellipodia, and overexpression of GMF β demonstrates that it promotes lamellipodial retraction and ruffling, while depletion of GMF β dramatically alters leading edge dynamics [10]. Consistent with observations made at yeast cortical actin patches [14], knockdown of GMF β leads to increased Arp2/3 levels in lamellipodia. Conversely, GMF β overexpression reduces Arp2/3 levels, suggesting that GMF β catalyzes Arp2/3 turnover in actin networks [10]. Further, a point mutant was introduced into GMF β to impair debranching, but not nucleation inhibition (designed based on the 'site 2' mutant in yeast Gmf1; [26]). Experiments using this mutant and a small molecule inhibitor of Arp2/3 complex demonstrated that GMF β debranching activity is critical for proper leading edge dynamics [10]. Thus, GMF debranching activity is essential for its *in vivo* functions in promoting actin network remodeling and turnover. On the other hand, no tool has yet been generated to disrupt GMF inhibition of Arp2/3-mediated nucleation without also disrupting debranching, making it more difficult to assess the importance of GMF's nucleation inhibition activity *in vivo*.

Additional support for GMF regulating lamellipodial dynamics comes from studies in *Drosophila* S2 cells and embryonic cells undergoing collective migration [25]. Here, GMF is enriched in lamellipodia undergoing retraction, a phase in which branched actin networks are rapidly disassembled and/or remodeled (debranched) into parallel bundles called ‘arcs’ (Figure 2B) [37]. GMF mutant flies also have reduced rates of border cell migration [25], demonstrating the importance of GMF function for directed cell migration in the physiological context of an intact animal. Similar roles may also explain the functions of the GMF γ isoform, which is highly expressed in immune tissues [8, 38]. GMF γ has important roles in hematopoietic lineage differentiation [9, 39–41], lamellipodial dynamics and chemotaxis in neutrophils and T-cells [42, 43] and vascular development in zebrafish [7].

The discovery that GMF drives lamellipodial retraction rather than protrusion lends important insights into how GMF promotes cell motility. Specifically, GMF may help remodel branched actin networks into unbranched structures such as actin arcs [37], which form parallel to the leading edge and treadmill rearwards [43]. Additionally, GMF may promote motility by governing the density of branches in actin networks at the leading edge. A number of studies, reviewed in more detail elsewhere [44], have explored the link between branch density, lamellipodial dynamics, and cell migration by altering expression levels of actin elongation factors (e.g., ENA/VASP, formins, and CARMIL), inhibitors of elongation (e.g., capping protein), branch stabilizers (e.g., cortactin), and branch destabilizers (e.g., coronin). Together, these studies suggest that densely branched networks comprised of shorter filaments lead to slow and steady lamellipodial protrusion, and there is an inverse correlation between protrusion rate and persistence [44]. Consistent with this view, depleting GMF, which should increase branch density, increases protrusion persistence [10]. In addition to these roles, GMF debranching is predicted to expose minus ends of filaments, thereby increasing the depolymerization flux and recycling of Arp2/3 complex, which in turn allows for continued network assembly at the leading edge [45].

Persistently protruding lamellipodia drive more continual *directional* cell migration [44, 46], and studies on GMF support this [10]. However, there are conflicting reports about the relationship between lamellipodial protrusion rates and cell speed. In one study, slow and steady protrusion correlated with faster cell speed [47], while in a different study, rapid protrusion correlated with faster cell speed [10]. It is possible that other parameters, such as the frequency and extent of lamellipodial retraction events, rather than protrusion rate, are the more relevant determinants of cell speed [46]. Finally, GMF may suppress unproductive lateral cell protrusions, leaving more Arp2/3 complex and actin monomers available for advancement of the leading edge of the cell [10, 25, 42]. This idea is similar to the proposed mechanism for Arpin, another direct inhibitor of the Arp2/3 complex that steers cell motility [48]. Indeed, in some cell types the Arp2/3 complex is dispensable for cell migration, yet required for steering cells toward haptotactic [49] or chemotactic signals [50].

Roles for GMF in endocytic traffic

In addition to its roles in cell migration, GMF regulates actin-dependent endocytosis in yeast and mammals [13, 14, 51], and promotes the turnover of focal adhesions in immune cells [43, 52]. Vinculin, a central component of focal adhesions that anchors cells to their

substrate, binds to the Arp2/3 complex at focal adhesions [53]. This raises the possibility that GMF helps stimulate the turnover and recycling of Vinculin-Arp complexes, or possibly facilitates other Arp2/3-dependent steps in focal adhesion dynamics. GMF γ also has been shown to localize to early and late endosomes in macrophages, where it is required for proper internalization and trafficking of the TLR4 receptor [51]. Together, these studies suggest that GMF regulates Arp2/3 complex-dependent functions in endocytic trafficking.

Open questions about GMF's activity and mechanism

To gain a more complete understanding of GMF's regulatory effects on Arp2/3 complex, further studies are needed to examine GMF's precise biochemical role in promoting debranching, and its activities in the context of other actin-associated proteins. GMF's debranching activity is likely influenced by whether the Arp2 and Arp3 subunits of Arp2/3 complex are bound to ATP or ADP nucleotides. ATP hydrolysis and phosphate (P_i) release on Arp2 are thought to precede debranching [54], possibly leading to GMF targeting older branches while leaving younger branches intact. Consistent with this model, GMF γ preferentially binds ADP-Arp2/3 complex over ATP-Arp2/3 complex in solution [55]. GMF binding to Arp2/3 complex may also catalyze P_i release on Arp2 and/or Arp3, similar to cofilin's stimulatory effects on P_i release from F-actin [56].

Additionally, it will be important to determine GMF's functions in the presence of cofilin and coronin, both of which have been implicated in debranching. Cofilin has been observed to stimulate debranching in TIRF microscopy assays [23], yet recent structural work indicates that GMF-Arp2 contacts are evolutionarily divergent from cofilin-actin contacts [31], and there is minimal evidence to suggest cofilin directly binds Arp2/3 complex. Instead, binding of cofilin to actin at or near branch junctions may alter F-actin conformation [35] to weaken branch sites and catalyze daughter dissociation. Alternatively, cofilin debranching, as seen in TIRFM assays, may not reflect actual dissociation of the branch sites, but instead severing events located near branch junctions. Coronin also appears to have functions in debranching. Like GMF, coronin binds with high affinity to Arp2/3 complex and alters Arp2/3 conformation and activities [32, 33, 57–60]. Further, coronin was recently shown to synergize with GMF in inhibiting Arp2/3-mediated actin nucleation [33]. Coronin also co-purifies with Arp2/3 complex on GMF affinity columns [13], and recent structural evidence suggests that coronin and GMF have non-overlapping binding sites on Arp2/3 complex [33]. Therefore, coronin and GMF may be capable of binding simultaneously to Arp2/3 complex, and it will be important to test how they affect debranching in combination. Some genetic evidence has supported a collaboration between GMF and coronin, *e.g.*, yeast *gmf1 crn1* double mutants having synthetic growth defects [28]. On the other hand, RNAi depletion of mammalian coronin-1B, which weakly stimulates debranching *in vitro* [22], shows opposite effects to GMF β depletion [10], reducing instead of increasing lamellipodial persistence [22, 60]. This might be explained by GMF and coronin having distinct, and only partially overlapping mechanistic roles in regulating actin networks, although the interpretation of these results could also be confounded by coronin's ability to promote cofilin-mediated severing [24, 61]. Last, it will be important to determine how the activities of GMF, cofilin, and coronin on actin network

remodeling and turnover are affected by other binding partners of Arp2/3 complex, particularly cortactin and Abp1, which are suggested to stabilize branches.

Spatial and temporal control of GMF function *in vivo*

There is evidence to suggest that GMF activities are regulated *in vivo* by post-translational modification. indeed, there is strong precedent for ADF-homology family proteins being regulated in this manner. Cofilin is inhibited by **phosphorylation** of a serine residue at its N-terminus. This residue lies within the G/F-actin binding site of cofilin, and its phosphorylation by LIM kinase weakens F-actin binding and increases cofilin's cooperativity of binding, leading to reduced mechanical discontinuities and reduced severing [62, 63]. Phosphorylation of GMF γ at its analogous Ser2 also has a regulatory role. Purified phosphomimetic GMF γ (S2E) has weakened affinity for Arp2/3 complex [55], and phosphorylated GMF γ *in vivo* accumulates more strongly on actin networks at the leading edge, possibly the result of failed network debranching [11]. Furthermore, signaling from GTPases Rac1 and Cdc42 promotes the phosphorylation of GMF γ at Ser2, as well as increased accumulation of GMF γ and F-actin at the leading edge and increased lamellipodial protrusion. Thus, it is possible that Ser2 phosphorylation of GMF inactivates its debranching activity to disrupt lamellipodial retraction and promote protrusion [10]. Although the kinase that phosphorylates Ser2 on GMF has not yet been identified, LIM kinase is an intriguing candidate, as this would provide a mechanism for coordinately regulating cofilin and GMF.

There are a number of additional putative phosphorylation sites on GMF (Figure 3). In GMF β , four different Ser or Thr residues are predicted to be phosphorylated by one or more of the following kinases: PKA, PKC, CKII, and S6K [64]. These residues are conserved in GMF γ , which has two additional PKC and CKII kinase consensus sites not found in GMF β [5]. Some of these phosphorylation sites have been implicated in GMF's suggested role in MAP kinase signaling [65], as discussed below. Interestingly, all four sites shared by GMF β and GMF γ reside in or near functional 'Site 2' (Figure 3; [26]), where GMF contacts the first actin subunit in the daughter filament, and therefore could regulate debranching. Additionally, phosphorylation on Tyr104 by c-abl kinase weakens GMF γ interactions with Arp2/3 complex in smooth muscle cells and alters F-actin levels [66]. Tyr104 is located near GMF γ "Site 1" (Figure 3), which contacts Arp2/3 complex, consistent with its effects on GMF γ -Arp2/3 interactions. However, the side chain of Tyr104 is not surface exposed on available structures of GMF, so it is not yet clear how c-abl gains access to this site. Continued investigation into how phosphorylation at each of these sites influences GMF activities and cellular functions is likely to reveal new mechanisms by which cells spatially and temporally tune actin network remodeling and turnover.

Reconciling GMF's actin functions with its roles in signaling and disease

GMF was initially identified as a factor present in brain extracts that induces differentiation of glioblast, glioma, and neuroblastoma cells in culture [1–4]. Subsequent studies indicated that GMF β elicits these effects via (i) an extracellular route, without being internalized by the recipient cells, suggesting GMF β is a secreted protein [67], and/or (ii) an intracellular

signaling pathway [68]. Since GMF β lacks a clear signal sequence for classical targeting to the secretory pathway, its release from cells would likely occur through non-classical “leaderless” secretion [69, 70]). In astrocytes, GMF β is detected both in the cytosol and on the cell surface [68]. However, GMF β activity has not been detected in astrocyte-conditioned media [71], suggesting healthy cells may not secrete GMF β . GMF β also has been identified as a surface-expressed protein on thymic epithelial cells that mature into CD4⁺ (helper) T-cells [72]; however, this may be due to autoimmune regulator (AIRE)-induced expression of non-thymic proteins used to educate developing T cells and prevent autoimmunity [73]. Thus, while it is intriguing to consider roles for GMF in functioning outside of the cell, this model requires further investigation. One possibility that warrants exploration is that GMF β might be released specifically from sick or dying cells.

GMF β was later localized to the cytosol of primary astrocytes and glioma cell lines [68], prompting investigations into its involvement in intracellular signaling. It was found that PKA phosphorylation of GMF β inhibits the ERK1/2 branch of the MAP kinase pathway [74], yet activates the stress-responsive p38 pathway [65]. GMF β -stimulated p38 signaling in turn induced NF- κ B-mediated transcription, leading to secretion of: (i) growth factors such as BDNF and NGF to promote neurite outgrowth and neuroprotection, [75–78], and (ii) proinflammatory cytokines such as TNF- α and IFN- γ [79, 80]. These observations, combined with studies on GMF $\beta^{-/-}$ knockout mice have suggested that GMF β has neuroprotective roles in Alzheimer’s disease and Parkinson’s disease (see Box 2).

How can we reconcile these observations for GMF with its well-defined biochemical and cellular functions in actin network remodeling? One possibility is that GMF signaling and cytoskeletal functions are mediated by distinct isoforms, GMF β and GMF γ , respectively. However, the striking similarity in structure between GMF β and GMF γ , (Figure 1) argues against this, as do studies showing that GMF β and GMF γ both regulate actin [10, 11, 14, 25, 27, 81]. Further, at least one study has indicated that GMF γ , like GMF β , functions in MAP kinase signaling [42]. Therefore, there is little evidence to support a ‘separate isoforms’ model. Alternatively, signaling and cytoskeletal functions of GMF could be integrated, such that association of GMF with actin networks spatially regulates MAP kinase signaling. This integration could lead to GMF availability for signaling being dependent on the local state of actin turnover, making MAP kinase signaling responsive to actin dynamics. Reciprocally, GMF modification by signaling pathways could affect its availability for actin remodeling. In fact, GMF inhibits ERK signaling [74], and ERK phosphorylates/activates components of the WAVE regulatory complex to promote Arp2/3-mediated actin assembly at the leading edge [82]. Therefore, GMF enrichment in retracting lamellipodia could not only promote debranching and direct Arp2/3 inhibition, but also indirectly block new actin assembly by inhibiting ERK signaling to WAVE (Figure 2).

Concluding Remarks and Future Directions

Over the past 40 years, our view of GMF has evolved from a secreted factor that promotes cellular differentiation, to an intracellular signaling protein, to a *bona fide* component of cellular actin networks with highly specific roles in controlling Arp2/3 complex activities and actin filament remodeling. This leaves it uncertain why GMF was initially identified in

the extracellular space, or how it was able to induce cell differentiation, and whether this involved its internalization into cells. Future work is also needed to investigate the cross-relationships and interdependence of GMF functions in actin regulation and signaling. In addition, there are a number of questions yet to be answered about GMF mechanism and function in governing Arp2/3 complex activity, alone and in combination with other proteins (see Outstanding Questions). These mysteries deserve new attention using modern cell biological techniques, and taking advantage of separation-of-function mutations now available, to rigorously test the *in vivo* importance of these suggested functions. This will also be a crucial step in understanding which functions of GMF underlie its genetically demonstrated neuroprotective and anti-inflammatory functions.

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Glossary

Actin cytoskeleton	A cellular network of thin fibers found in all eukaryotic cells, wherein each filament is a linear polymer of globular actin subunits. The actin cytoskeleton drives many cellular processes, including endocytosis, cytokinesis, and cell crawling
Actin patches	Branched actin filament networks at the cortex of cells, which represent individual sites of endocytosis and have rapid assembly and disassembly dynamics
ADF-H	Actin depolymerizing factor homology, a domain shared by a super family of proteins that includes: ADF/Cofilin, Twinfilin, Abp1/Drebrin, GMF, and Coactosin. All five ADF-H family proteins govern actin cytoskeleton organization and/or dynamics, and bind to actin and/or actin-related proteins
Arp2/3 complex	A conserved complex consisting of seven proteins (Arp2, Arp3, p40/ARPC1, p34/ARPC2, p21/ARPC3, p19/ARPC4, and p15/ARPC5) which nucleates new actin daughter filaments from the sides of existing mother filaments. Arp2/3 complex remains at the branch junction holding the mother and daughter filaments together a debranching event
Cofilin	An essential protein that binds actin filaments and induces severing and depolymerization, and binds actin monomers to block nucleotide exchange (ATP for ADP). Founding member of ADF-H protein family

Coronin	A conserved protein that binds Arp2/3 complex to inhibit actin nucleation and possibly promote debranching, and to actin filaments to enhance Cofilin-dependent severing and disassembly
Daughter filament	An actin filament assembled by Arp2/3 complex off the side of a previously existing mother filament
Debranching	The dissociation of an actin filament branch junction formed by Arp2/3 complex, which holds together a ‘mother’ and a ‘daughter’ filament
Disassembly	The dissociation of actin subunits from filaments within a network
F-actin	Filamentous form of actin; polymers of individual actin monomers.
G-actin	Unpolymerized globular actin monomers, each 42 kDa
Leaderless secretion	Mechanisms of protein secretion that bypass the canonical secretory pathway
Leading edge	The ‘front’ of a polarized, migrating cell, <i>i.e.</i> the side facing the direction of migration. Typically consists of densely branched actin networks (lamellipodia) that generate protrusive force underlying cell crawling
Mother filament	An existing actin filament, to which Arp2/3 complex associates and then nucleates a daughter filament at a 70 degree angle
Phosphorylation	Post-translational modification of a protein by covalent attachment of a phosphoryl group to a specific residue. Phosphorylation is mediated by kinases, and can alter the function and/or conformation of the target protein
Remodeling	The architectural reorganization of individual actin filaments within a larger polymer network
Severing	Fragmentation of an actin filament into shorter pieces. Along with debranching and depolymerization, severing is critical for disassembly and remodeling of cellular actin networks
Turnover	The disassembly of actin filaments into ADP-bound monomers, followed by recycling of monomers to an ATP-bound state, priming them for new rounds of assembly

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Box 1**Arp2/3 complex and the formation of lamellipodia**

Lamellipodia are broad, flat, protrusions that form at the leading edge of cells that are migrating or spreading on a 2D surface. Their shape is determined by the branched actin networks assembled by Arp2/3 complex, and by a variety of other actin regulators that modulate lamellipodial protrusion and retraction dynamics. Lamellipodia form when upstream signals activate Rac1 GTPase, which in turn recruits to the membrane and activates the WAVE family of nucleation promoting factors (NPFs). The C-terminal VCA region of WAVEs induce conformational changes in Arp2/3 complex that increase its affinity for the sides of existing actin filaments and align the Arp2 and Arp3 subunits to mimic the barbed end of an actin filament. WAVEs also recruit the first actin monomers to Arp2/3 complex, and then dissociate, allowing the daughter filament to elongate [83]. Because WAVEs remain associated with the membrane, they are also proposed to continue tracking the growing barbed end of the daughter filament, controlling its growth rate and possibly protecting it from capping protein [84–86]. The branched actin networks found in lamellipodia are organized with the growing barbed ends of filaments oriented toward the membrane, such that network growth is directed toward the membrane and stimulates protrusion. As the network grows, with polymerization occurring near the leading edge to drive protrusion, older portions of the network move inward and are disassembled by cofilin and other proteins to recycle actin and actin-associated proteins. For a recent review on lamellipodia and cell migration, see [44].

Box 2**Studies on GMF^{-/-} mice and suggested roles in health and disease**

The generation of a GMF β knockout mouse in 2004 revealed its importance in normal cognitive functions and that its loss promotes pathological neuroinflammatory disease progression [87]. GMF $\beta^{-/-}$ mice displayed impaired motor skills and learning [87]. They also showed decreased susceptibility to a multiple sclerosis-like disease, which was attributed to a loss of GMF β 's inflammation-inducing activity and might also explain a correlation between GMF β levels and Alzheimer's disease (AD) [80, 88–95].

Administration of an anti-GMF β antibody to mice in a multiple sclerosis model greatly reduced the onset and severity of the disease [96]. However, since most cell types do not internalize antibodies, this observation returns us to the enigma of extracellular GMF β (see main text), and the possibility that extracellular GMF β is released by sick or dying cells. In support of this idea, GMF β localizes to extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs) in a mouse model of AD and in post-mortem brains of AD patients [93, 94]. Further, a role for GMF β in the progression of Parkinson's disease has been suggested by the observation that dopaminergic neurons and astrocytes in GMF $\beta^{-/-}$ mice have reduced sensitivity to oxidative stress [97, 98]. Collectively, these observations raise exciting possibilities for the involvement of GMF in disease states of the brain, and for GMF's potential as a therapeutic target. In addition, recent research has suggested a role for GMF γ in promoting metastasis, which may be linked to GMF's actin functions [99]. Overexpression of GMF γ in ovarian cancer cells promotes cell migration and invasion, and alters actin organization, and elevated GMF γ levels correlate with poor patient prognosis. Finally, transgenic mice overexpressing GMF β have shorter lifespans and show accelerated aging particularly in the kidney, suggesting a role for GMF in aging [100].

Highlights

- GMF is structurally related to Cofilin, and is a conserved regulator of the actin cytoskeleton.
- GMF triggers actin filament debranching by targeting Arp2/3 complex at branch sites.
- GMF also inhibits free Arp2/3 complex from nucleating actin assembly.
- GMF localizes to the leading edge, and its debranching activity is critical for lamellipodial retraction, a key step in directed cell migration.

Figure 1.

GMF sequence and structure are evolutionarily conserved.

(A) Alignment of amino acid sequences of GMF from 6 different organisms plus human Cofilin-1. Conserved residues are shaded in the two sites (Sites 1 and 2) in GMF that mediate binding to Arp2/3-actin branch sites and the analogous sites in cofilin that mediate binding to actin.

(B) Heat map showing evolutionary conservation of surface residues on the structure of GMFγ (PDB ID 3L50).

(C) Surface rendered view of the crystal structure of human Cofilin-1 (PDB ID 4BEX).

(D) Overlay of the solution NMR structures of mouse GMFβ (blue) and mouse GMFγ (green) (PDB IDs 1V6F and 1WFS, respectively).

(E) Overlay of the crystal structures of human GMFγ (green) and human cofilin-1 (orange) (PDB IDs 3L50 and 4BEX, respectively). Abbreviations: Arp2/3, actin-related protein 2/3; GMF, glia maturation factor; NMR, nuclear magnetic resonance; PDB, protein data bank.

Figure 2.

Model for GMF function in controlling actin organization and dynamics.

Illustration of a dendritic actin network being assembled by the Arp2/3 complex and driving membrane protrusion (A), and subsequently being debranched and disassembled to promote lamellipodial retraction (B). (C) Model showing steps in Arp2/3 complex cycle at the leading edge, with the roles of GMF highlighted. From the left, WASp VCA domains (green) bound to G-actin associate with Arp2/3 complex (purple) at the plasma membrane and stimulate nucleation of a daughter actin filament, forming a branch. WASp remains associated with the growing barbed end of the daughter filament until it is subsequently capped by Capping Protein (yellow). GMF (orange) binds Arp2/3 complex, possibly at two separate sites, to catalyze debranching, exposing pointed ends. Filaments are further disassembled by other factors (not shown). While GMF remains bound to the released Arp2/3 complex, it blocks nucleation. GMF dissociates from Arp2/3 complex, perhaps catalyzed by other Arp2/3 ligands or GMF phosphorylation, leaving Arp2/3 complex 'primed' for nucleation. Abbreviations: Arp2/3, actin-related protein 2/3; GMF, glia maturation factor; VCA, verprolin/cofilin/acidic domain; WASp, Wiskott-Aldrich syndrome protein.

Figure 3.

Suggested phosphorylation sites on human GMFγ.

Ribbon structure of human GMFγ (green), with labels indicating residues thought to be phosphorylated and the kinases that potentially target those residues based on consensus motifs (see main text for references). Asterisks (*) indicate residues in GMFγ not conserved in GMFβ. Abbreviations: Arp2/3, actin-related protein 2/3; GMF, glia maturation factor; Ser, serine; CKII, casein kinase II; c-Abl, abelson kinase 1; PKA, protein kinase A; PKC, protein kinase C; RSK, ribosomal s6 kinase.