Minireview

The gelsolin family of actin regulatory proteins: modular structures, versatile functions

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Abstract This issue of *FEBS Letters* includes two manuscripts describing structural studies of gelsolin, the best-characterized member of a superfamily of actin binding proteins that sever, cap, and in some cases nucleate and bundle actin filaments. The manuscripts by Narayan et al. and Irobi et al. provide snapshots of gelsolin domains activated by calcium and in complex with the actin monomer, revealing new insights into the remarkable actin regulatory activities of this versatile protein. These studies build upon nearly a quarter of a century of research on gelsolin's effects on actin dynamics and its role in normal and diseased cells. In the following minireview, we summarize the structural studies that have provided insights into gelsolin's severing and capping activities and look to the future of work on this remarkable molecule.

Key words: Actin; Actin binding protein; Cytoskeleton; Gelsolin; Protein structure

1. Gelsolin has multiple actin regulatory activities

Gelsolin was discovered in 1979 by Yin and Stossel based on its ability to activate the gel–sol transformation of actin filaments in a calcium-dependent manner [3]. It occurs in a wide range of vertebrate, lower eukaryotic and plant cells, and its function is as varied as its biochemical activities. Indeed, gelsolin has been implicated in the regulation of cell motility, in the transduction of signals into dynamic rearrangements of the cytoskeletal architecture, and even in stimulating programmed cell death in certain vertebrate cells [4,5].

In the presence of micromolar calcium, gelsolin severs preexisting actin filaments and caps them, thereby preventing monomer addition to their fast-growing ends. The barbed end cap is highly stable, even in the absence of calcium, unless displaced by interactions with regulatory phospholipids such as phosphatidylinositol-4,5-bisphosphate (PIP_2) [6,7]. In the presence of a large pool of profilin, or under depolymerizing conditions, these gelsolin-capped ends allow the disassembly of populations of actin filaments by subunit loss from the pointed ends [8,9]. Paradoxically, under polymerizing conditions, gelsolin exhibits calcium-dependent actin nucleating activity and stimulates actin filament formation from monomers [10,11]. However, the relevance of the nucleation activity of gelsolin to its physiological function within cells, whether de novo, or as the result of uncapping of severed filaments, or as a component of the triggering mechanism for Arp2/3 branching, remains controversial [12].

2. Gelsolin is a member of a large superfamily of actin binding proteins

The diverse activities exhibited by gelsolin and its relatives derive from coordinated movements of homologous, 100–125 amino acid modules that are folded into compact domains. Gelsolin is composed of six of these domains, termed G1–6 [13], that are connected by linker regions of varying lengths (Fig. 1A). Early studies of proteolytic fragments of gelsolin identified three actin binding regions: a calcium-independent strong monomer binding fragment (G1), a calcium-independent filament binding fragment (G2–3), and a calcium-dependent monomer binding fragment (G4–6) [14]. Subsequent work established that domains G1 and G4 bind actin monomers, whereas G2 contains filament binding activity. Sequence analysis reveals that G1 is most closely related to G4, G2 to G5, and G3 to G6. In addition to cytoplasmic gelsolin, a secreted form of the protein containing a 25 amino acid long amino-terminal extension is found in blood plasma where it appears to play a role in the actin-scavenging system [15].

Other members of the family such as severin contain three domains rather than six, yet they retain filament severing, capping, and nucleating activities, although the latter is weaker than that exhibited by full-length gelsolin [16–18]. In addition to size, severin differs from gelsolin in that it contains a second filamentous actin (F-actin) binding site in domain 3 [18]. The fact that these shorter forms retain both calcium sensitivity and nucleating activity, two functions that appear to derive from the C-terminal half of gelsolin, raises questions about the role of domains G4–6. Selden and colleagues have reported that the severing efficiency of gelsolin G1–3 is concentration dependent, suggesting that cooperativity between the N- and C-terminal halves of gelsolin is required for full efficiency [19]. Interestingly, the other well-studied class of

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Abbreviations: F-actin, filamentous actin; EGTA, ethylene glycol-bis[β-aminoethyl ether]-*N*,*N*,*N*′,*N*′-tetraacetic acid; NMR, nuclear magnetic resonance; PPI, polyphosphoinositide; PIP_2, phosphatidylinositol-4,5-bisphosphate

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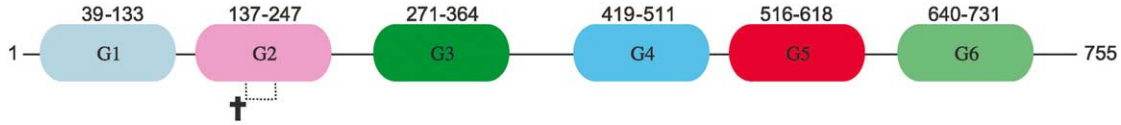
A.



B.

Domains and Residues	Structural/Functional Relevance	Technique	Reference	PDB ID
G1, G2, G3, G4, G5, G6: 27-755 linker: 365 -418, C-terminal helix: 745-754	Structure of inactive plasma gelsolin G3-G4 linker, inactive form	X-ray	[26]	1D0N
G4, G5, G6: 414-453, 460-742	G4-G6 (+Calcium/-Actin), 2 Ca^{2+} ions	X-ray	[31]	1NPH
G4, G5, G6: 414–455, 460-741	C-terminal half and Ca^{2+}, activated G4-6	X-ray	[1]	1P8X
G4, G5, G6: 412-455, 460-742	G4-G6:actin co-crystal, 2 Type 2 Ca^{2+} sites	X-ray	[33]	1H1V
G1: 26-150	F-actin trimer disrupted by G1(x-actin)	X-ray	[41]	1MDU
G1:26-150	Human G1:actin complex Calcium binding sites	X-ray	[42]	1EQY
G1+: 26-156	G1+:actin co-crystal, orientation of G1-G2 linker	X-ray	[2]	1P8Z
G2: 158-261	D187 as part of Ca^{2+} site (lost in FAF gelsolin)	X-ray	[64]	1KCQ
G1, G2, G4, Y68, K71, Y133, F163, Y453, P726, P727, P745, C-terminal helix	Exposed upon activation by Ca^{2+}	X-ray Radiolysis and MS	[34]	---
G2, G3, G4, G5, G6	G2-G6:F-actin interaction, F-actin binding site, orientation of G4-6 in activated fragment	Cryo-EM	[36]	---
G2	F-actin binding site (residues 198-227)	Fluorometric Measurements	[70]	---
G1, G2: 135-169 (P1 and P2 PPIs sites), G5: 530-545	Response of C and N-terminal halves to PPIs	CD, molecular modeling	[68]	---
G1: 1-149	PIP_2 binding site: 135-149	Mutagenesis	[71]	---
G2: 150-169	PPI binding site	Fluorescent measurements	[72]	
G2, G3, G4, G5, G6	Disruption of C188-C201† in FAF gelsolin	Mutagenesis	[73]	---

C.

Protein and Region investigated	Structural/Functional Relevance	Technique	Reference	PDB ID
Severin, domain 2	F-actin binding domain	NMR	[48]	1SVR
Villin, C-terminal Headpiece (735-826)	F-actin binding headpiece	NMR	[75]	1VII
Villin, 14T (amino terminal domain)	Conserved fold of actin-severing proteins	NMR	[76]	2VIL
CapG, 84-90, 124-129	Residues crucial for severing activity	Mutagenesis	[24]	---

Fig. 1. Domain organization with references to structures determined. A: Residues have been numbered according to domains of human plasma gelsolin as described in [26]. † indicates the location of the C188–C201 disulfide bond of plasma gelsolin [74]. B: Quick reference for structural data on gelsolin. Residues listed are those visible in the respective X-ray or NMR models numbered according to plasma gelsolin. Data are from [1,2,26,31,33,34,36,41,42,64,68,70–73]. C: References for other key members of the gelsolin family of proteins. Abbreviations: MS, mass spectroscopy; CD, circular dichroism; NMR, nuclear magnetic resonance; and FAF, familial amyloidosis-Finnish type. Data are from [24,48,75,76].

actin filament severing proteins, the actin depolymerizing factor (ADF)/cofilins, also bind cooperatively and require multiple binding events for severing to occur, although in other respects these proteins differ quite dramatically from gelsolin [20,21].

In addition to the array of gelsolin-related severing proteins, variations of the basic gelsolin six- or three-repeat structure are found in molecules that possess somewhat different sets of actin regulatory activities. Addition of a small C-terminal F-actin binding 'headpiece' domain converts a gelsolin-like molecule into a bundling protein, termed 'villin' based on its localization in microvilli [22,23]. And, substitution of a handful of residues in domain G1 appears to be key for distinguishing an actin filament capping factor, CapG, from a severing protein [24]. Further variations of gelsolin and related proteins continue to be identified. In most cases, these molecules possess additional domains that tailor their expression, localization, and regulation to the appropriate regions of motile cells (reviewed in [25]). Taken together, these diverse activities make the gelsolin superfamily one of the most versatile regulators of actin's function in cells.

3. Molecular snapshots of severing

The holy grail of the gelsolin field is a molecular movie of this dynamic molecule as it undergoes large-scale conformational changes during calcium activation, binding, severing, capping, and uncapping. The difficulties in achieving this goal reside in the speed with which gelsolin severs, the multiplicity of steps in the reaction, the relatively small size of gelsolin, the number of actin subunits involved, and the inherently dynamic nature of the actin filament itself. Nevertheless, significant progress in developing such a movie has been made over the past 15 years. Some of the key structural

snapshots that have come into focus in recent years are described below (summarized in Fig. 1B,C).

3.1. Crystal structure of full-length gelsolin in EGTA: inactive gelsolin is 'latched' shut

A major leap forward was achieved with the determination of the structure of the full-length molecule by X-ray crystallography [26]. In addition to confirming the structural similarity of the six domains of gelsolin, this structure suggests how the arrangement of these domains in the absence of calcium leads to inactivation. One striking feature of the model is the appearance of a so-called 'latch helix' at the C-terminus that blocks the putative actin binding site in domain 2. The importance of this helix in calcium regulation was revealed, long before its existence was known, by deletion studies in which removal of the ~20 C-terminal residues in gelsolin eliminated calcium regulation [4,27]. Also significant is the presence of a large 53-residue linker between the N- and C-terminal halves of the molecule that is presumably necessary for gelsolin's C-terminal half to reach its binding site when the N-terminal half is bound to the filament.

3.2. X-ray structures of G4–6, radiolytic footprinting of active gelsolin: calcium activation triggers large domain movements

Thus far, the high-resolution structure of calcium-activated gelsolin has remained elusive in part because the large-scale conformational changes that occur during activation would disrupt gelsolin crystals obtained in EGTA if soaked in calcium [28–30]. Nevertheless, crystal structures of G4–6 activated by calcium both on its own [1,31] and bound to actin [32,33] reveal large conformational changes in the C-terminal half of the molecule and suggest, by analogy, that similar changes might occur in G1–3.

Additional insights into the conformational changes that accompany calcium activation have derived from recent radiolytic protein footprinting analysis (a method in which synchrotron irradiation is used to modify solvent-accessible regions of proteins) [34]. These studies point to residues that are buried in the EGTA form of gelsolin [26], but become exposed upon binding calcium. The exposed residues are distributed throughout five peptides: 49–72 (G1), 121–135 (G1), 162–166 (G2), 431–454 (G4), and 722–748 (C-terminal 'latch' helix). Other peptides in domains G3 (276–300) and G6 (652–686) become less accessible as the domains move relative to one another. The dose responsiveness of gelsolin to calcium suggests a three-state transition, with major activation occurring at concentrations of calcium below 100 nM, and followed by actin binding at micromolar levels [34,35]. This means that it is doubtful that a single crystal structure of calcium-activated gelsolin will be sufficient to address all aspects of the severing mechanism.

3.3. Electron cryomicroscopy of G2–6:F-actin in calcium: G2 binds two actin subunits when targeting filaments during severing

Although no atomic structure yet exists of gelsolin targeting F-actin, a low-resolution 'snapshot' of the third step in severing was obtained by electron cryomicroscopy [36]. In these studies, a truncated form of gelsolin with calcium-activated severing activity, G2–6, was imaged in association with F-actin and a three-dimensional reconstruction calculated to ~3.5 nm resolution. Difference mapping revealed that the G2–6 molecule had two major regions that exhibited different structural properties. The F-actin binding region (presumably G2–3) was statistically well determined, consistent with its placement at low radius firmly attached to two longitudinally associated actin subunits on the filament. The second mass at high radius in the structure (presumably G4–6) was relatively weak and poorly determined. One explanation for this is that the G2–6 molecules were trapped at different conformational states during severing resulting in a 'smeared' snapshot of the moving molecules. Time-resolved electron cryomicroscopy [37] of G2–6 molecules frozen at different moments in time following calcium activation should help to resolve this question.

Interestingly, electron microscopy (EM) has also shown that filaments are kinked in the presence of activated G2–6, but not when decorated with EGTA-bound G2–6 or by G2–3 [36,38,39]. This observation is consistent with video microscopy images in which filaments are seen to distort and kink during filament severing by gelsolin [40]. Crystal structures of cross-linked actin oligomers bound with gelsolin G1 also show a distorted 'filament' structure leading some to speculate that distortion by twisting might accompany severing [41] in a manner reminiscent of cofilin [20]. Whether or not filament distortion of the flexible actin filament is actively induced during severing or merely exploited by gelsolin (or perhaps required by a truncated form like G2–6 that lacks the strong monomer binding domain) remains to be seen.

3.4. Co-crystals of G1 with actin monomer: G1 binds the 'barbed' end of actin, disrupting filament interactions

There have been a number of crystal and nuclear magnetic resonance (NMR) structures of domain G1, both on its own and in complex with actin. Gelsolin domain G1 has proven to be an effective tool for studying actin monomer structure owing to its high-affinity binding site on actin away from the adenosine triphosphate (ATP) binding loop. In the interest of space, we will discuss only one of the structures here.

Because G1 on its own binds actin monomers and prevents actin polymerization, the G1:actin complex could be crystallized and its structure determined by X-ray crystallography [42]. In addition to providing the first molecular picture of the gelsolin fold, this study shows that domain G1 binds near the barbed end of the actin molecule in a cleft between actin subdomains 1 and 3. This site overlaps, but is distinct from, the binding site for profilin, a monomer binding protein that binds at the barbed end surface of actin [43]. Comparison of G1's binding 'footprint' on the surface of actin to those identified for gelsolin G2 and cofilin by electron cryomicroscopy reveals striking similarities (fig. 5 in [44]). The main differences (aside from those attributable to differences in resolution) are the involvement of a second surface of actin (situated on subdomains 1 and 2) in the F-actin binding proteins' footprints and the involvement of the Glu167-containing 'loop' in actin subdomain 3 in both the gelsolin G1 and profilin binding sites. The latter may be significant since this loop appears to participate in an important actin–actin contact predicted by the Lorenz model of the actin filament [45,46].

3.5. Co-crystals of G4–6 with actin monomer: G4 binding resembles G1

Another important structure that has been determined by

X-ray crystallography is the G4–6:actin complex [32,33]. Although the locations of the strong calcium-independent monomer and the filament binding domains were identified relatively early in gelsolin studies, the identification of the second monomer binding domain remained elusive owing to difficulties in expressing functional G4, G5, and G6 domains. This issue was resolved when the G4–6:actin structure revealed that G4 binds actin in a manner analogous to G1. Comparison of calcium-activated G4–6:actin and G4–6 with each other and with the EGTA form of gelsolin reveals that actin is not required for activation and shows how calcium binding produced rearrangements between domains.

3.6. Combining X-ray, NMR, and EM structures: models of filament capping by gelsolin

It will be challenging to determine the structure of the capped filament directly. Thus far it has proven difficult to obtain crystals of F-actin for X-ray crystallography; and the small size of the capped end (~130–170 kDa for two to three actin subunits plus one G1–3; ~175–215 kDa for two to three actin subunits plus gelsolin) and lack of internal symmetry hinders structure determination by electron cryomicroscopy. Nevertheless, reasonable models of filament capping have been put forth by combining structures obtained with X-ray crystallography, EM, and NMR spectroscopy in the context of biochemical data (Fig. 2).

3.6.1. Positioning domains G1–3 in the capped filament. There is general agreement that when domains G1–3 are bound to filaments following severing they interact with two actin subunits, consistent with binding studies of the isolated domains [47]. Crystallography has shown how G1 interacts with the actin monomer, but for several years there was debate about the placement of the F-actin binding domain, G2, both on the actin filament and relative to G1 [42,47–49].

The most widely agreed-upon model places G2 on the outer domain of actin bridging actin subunit 1, the same subunit to which G1 is bound, and subunit 3, the next subunit running towards the pointed end along the long-pitch helix of actin (see Fig. 2, both panels) [36,39,47,49]. This binding model for G2 explains why the F-actin binding domain of alpha-actinin, αA1-2, can substitute for G2–3 in a hybrid molecule that possesses filament severing activity [50,51].

Additional insights into the structure of the G1–3 cap are provided in this issue of FEBS Letters. Irobi and colleagues extended the analysis of G1:actin interactions by co-crystallizing actin with G1+, a gelsolin construct containing G1–G2 linker residues [2]. In previous biochemical studies these linker residues conferred severing activity on domain G1 suggesting they possess actin binding activity [2,4]. The structure by Irobi and colleagues shows the placement of the G1–G2 linker on the surface of actin and provides support for the models of G1–2:F-actin interactions in which G1 and G2 are bound to adjacent subunits on the same long-pitch helix of the filament [36,39,47,49,50,52].

3.6.2. Positioning domains G4–6 in the capped filament. The placement of the C-terminal half of gelsolin in the capped filament is less well understood. First, there is debate concerning how many actin subunits are involved in the capped end with some studies suggesting two [11,47] and others suggesting three actin subunits are bound to gelsolin in the complex [53]. For simplicity's sake, we will present models of filaments capped by gelsolin assuming that all three actin

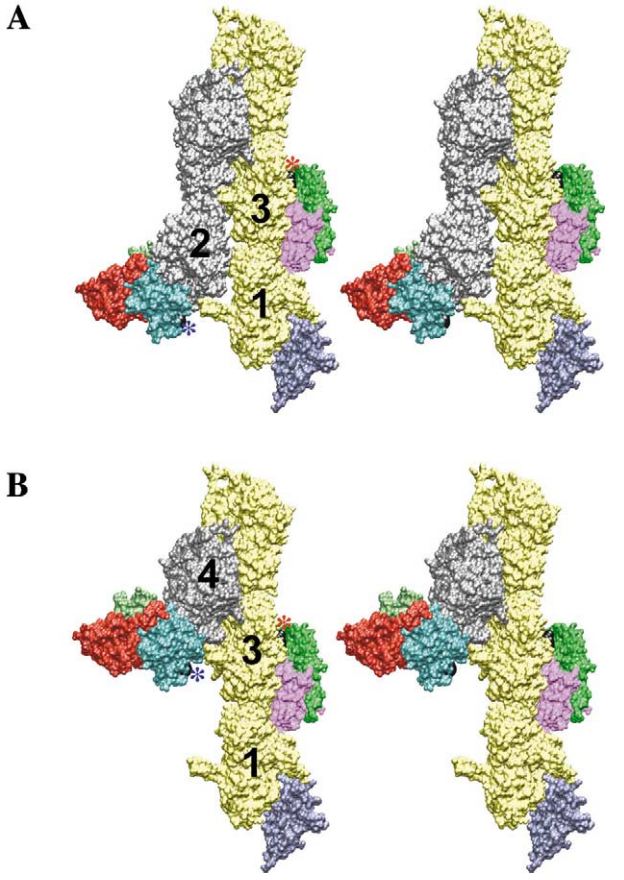


Fig. 2. Two possible modes of filament capping. In both models G1 (blue-violet domain; PDB 1EQY) is docked onto the terminal actin (subunit 1, yellow strand) according to the co-crystal structure of McLaughlin et al. [42]. G2 (pink domain; PDB 1D0N) was aligned to the gelsolin portion of the G1:actin structure on subunit 3 using the same strategy as Puius et al. [52] and consistent with the electron cryomicroscopy data of McGough et al. [36]. The adjacent domain G3 (green) was displayed following alignment based on the structure of full-length EGTA-bound gelsolin. Note: aligning G2–3 in this way only provides an approximation of this domain's position on F-actin as it leads to steric clashes with the lower actin subunit. A: Placing the G4–6:actin structure (PDB 1H1V) on subunit 2 of the filament requires that the G3–G4 linker spans a distance of ~100 Å. B: In the alternative model, G4 binding to actin subunit 4 requires the linker to span a distance of ~75 Å. (Note: in both instances the shortest route bisects actin subunits.) The C-terminus of domain G3 is indicated with a red asterisk. The N-terminus of domain G4 is indicated with a blue asterisk. Actin filaments are represented by space-filling models oriented with the minus or slow-growing end up. Actin subunits from one long-pitch 'strand' are colored yellow and those from the other are colored gray. Gelsolin domains are colored according to the scheme used in Fig. 1 as follows: G1 = blue-violet, G2 = pink, G3 = green, G4 = aqua, G5 = red, and G6 = lime green. Both models are shown in side-by-side stereo. Molecular models were generated with the program O [69] and rendered with the program VMD [70].

binding domains (G1, G2, and G4) remain bound to actin in the cap. Second, although crystallographic studies have shown how the C-terminal half of gelsolin (G4–6) binds actin monomers, it is still not clear to which actin subunit in the filament G4 is bound.

There appears to be general consensus that gelsolin reaches across the filament axis, placing G4 on the adjacent long-pitch strand (gray strand in Fig. 2). Most models place G4 on sub-

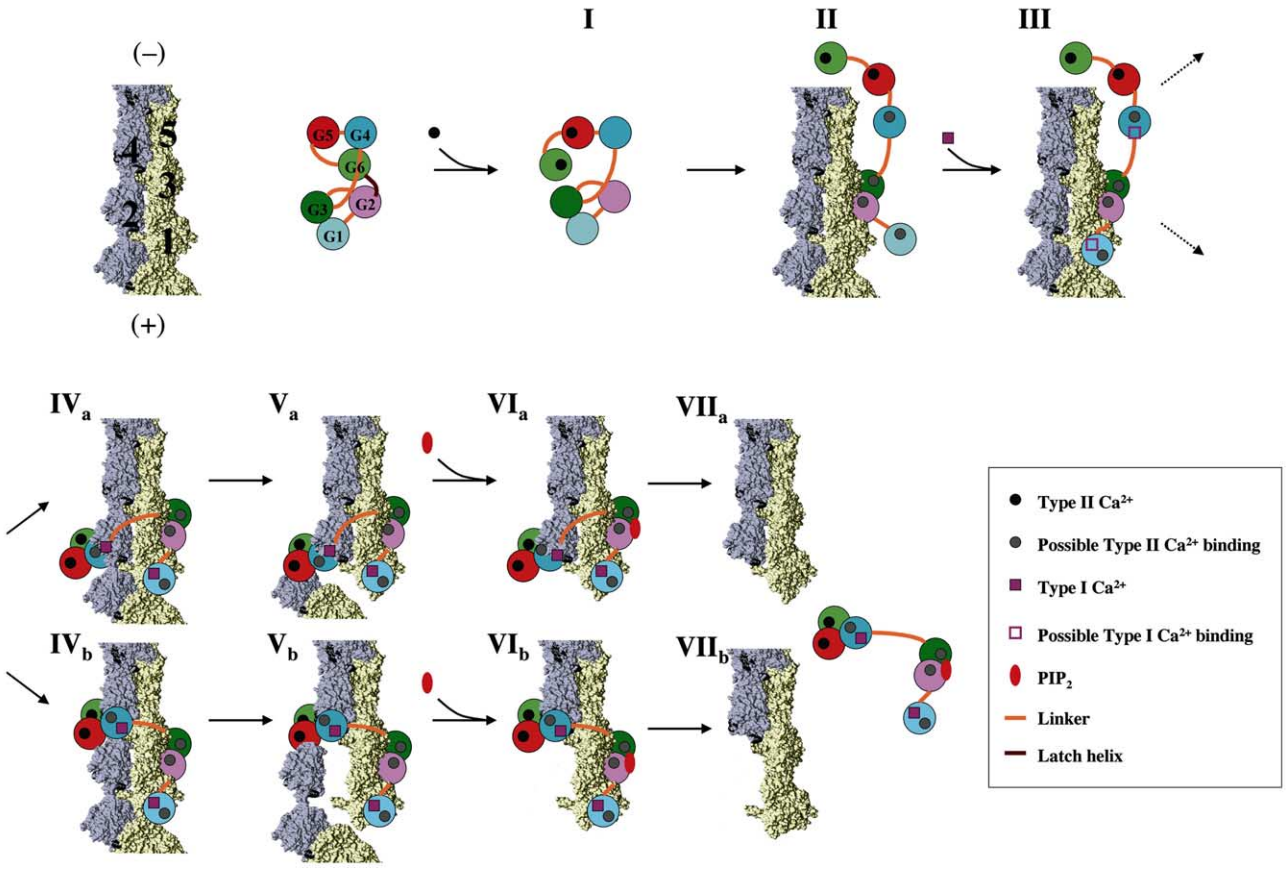


Fig. 3. Schematic of gelsolin severing mechanism. In this schematic the actin filament is represented by a space-filling model oriented with its slow-growing or minus end up. Actin subunits from one long-pitch 'strand' are colored yellow (subunits 1, 3, 5) and those from the other are colored gray (subunits 2, 4, 6). The actin filament can also be described by the so-called 'genetic helix' which runs in a left-handed sense from the plus towards the minus end of the filament through subunits 1, 2, 3, 4, 5, etc. Gelsolin is represented schematically as six domains (circles, G1–G6) connected by linkers of varying lengths. In EGTA, gelsolin is a compact structure with potential actin binding sites masked by interdomain associations and the C-terminal 'latch helix'. I: According to the scheme of Kolappan et al. ([31]) upon binding of calcium to G6, salt bridges between G2 and G6 are broken and the C-terminal latch helix is released, exposing the F-actin binding site of G2. Binding of calcium to G5 stimulates further movements that complete activation. The conformation of G1–3 during the activation is unknown. II: The activated gelsolin undergoes a conformational change, allowing G2 to bind to two longitudinally associated actin subunits (labeled 1 and 3 on this diagram) which fall on one of the two long-pitch helical strands. Possible type-2 calcium binding sites are shown (solid gray circles); however, the order of calcium binding is not known. III: After binding of G2 to F-actin, G1 is directed to its actin binding site. Type-1 calcium is known to exist at the G1 and G4 actin interfaces (open pink boxes) and binds to G1 and G4 in the presence of actin (solid pink boxes). IV–V: Severing and capping proceeds through binding of G4 to an actin subunit on the adjacent long-pitched strand, most likely either actin 2 (V_a) or 4 (V_b). (Actin residues that could potentially bind G4 are indicated with dark balls on actin subunits from the gray strand.) Gelsolin remains bound after severing actin, functioning as cap and preventing actin monomers from being added to the filament. Most models of filament severing and capping place G4 on actin subunit 2 (VI_a), but the binding site on actin subunit 4 (VI_b) is also close enough to permit G4 binding owing to the helical symmetry of the filament. VI–VII: Binding of PIP_2 to the region between G1 and G2 releases gelsolin from actin, freeing the barbed end for polymerization. The details of uncapping are not known but may involve conformational changes in the linker between domains G1 and G2. Actin molecules were rendered with the program VMD [70].

unit 2, adjacent to subunit 1 along the short-pitched helix (Fig. 2A). Assuming G2 binds actin subunit 2 in a similar position as G1 on subunit 1 [36,52] and that there is no large conformational change in G2–3 when activated by calcium, the distance between the C-terminus of domain G3 and the N-terminus of G4 on subunit 2 is about 100 Å. Alternatively, if G4 binds actin subunit 4, the distance that must be spanned by the 53-residue G3–G4 linker is much shorter (~75 Å; Fig. 2B). It is important to bear in mind, however, that it is possible that actin's flexibility is exploited in the formation of the cap [54–57], in which case the distance between domains G3 and G4 could be very different in the capped filament.

The only direct evidence concerning the positioning of G4–6 during severing suggests that the C-terminal half reaches across the 'front' of the actin filament (closest to the viewer in Fig. 2), rather than across the back [36]. As a result of actin's helical symmetry, subunit 4 would then be rotated towards the C-terminal half (relative to the orientation of subunit 2). According to this scheme, the actin filament capped by gelsolin would have a 'jagged' barbed end owing to the absence of subunit 2. It is not known if actin can adopt this rather unusual configuration of actin subunits at its end. However, a number of EM studies have shown what appear to be independently stable strands of actin at filament ends under a variety of conditions suggesting that a 'jagged' barbed end is possible, particularly if stabilized by multiple contacts with an actin binding protein as has been shown for cofilin [58–61].

4. Gelsolin undergoes coordinated movements of homologous domains during severing

The structural studies summarized above, coupled with a wealth of biochemical and biophysical data, have led to a generally agreed-upon scheme for the gelsolin severing mechanism depicted schematically in Fig. 3. Although this scheme is far from complete, it serves as a useful discussion point for interpreting new data and guiding future experiments.

4.1. Calcium activation

The first step in severing is activation by calcium (Fig. 3, step I). Recent evidence suggests that this is a three-state process involving two types of calcium ions, type-1 and type-2. (Type-1 calcium sites involve coordination between gelsolin and actin residues, whereas type-2 calcium sites consist solely of gelsolin residues [33].) At submicromolar concentrations, calcium occupation of type-2 sites of domains G5 and G6 leads to the release of a so-called 'latch' helix at state 2, which masks the F-actin binding domain G2 in the absence of calcium [26,34]. In order for severing to occur, higher concentrations of the ion are required (10 μM for half-maximal activity in vitro [62]). Additional calcium binding, most likely to domains G1, G2 and G4, provides full activation through saturation of the molecule, thus allowing actin binding and severing to take place. In total there appear to be eight potential calcium binding sites on gelsolin, two type-1 and six type-2, although it is not clear that all are ever occupied [33].

4.2. Filament binding

Following activation and release from the latch, the F-actin binding domain (G2) is exposed, permitting filament targeting (Fig. 3, step II). In so doing G2 brings G1, the strong calcium-independent monomer binding domain, in proximity to its binding site on the actin filament. Recent evidence suggests that there may be some additional structural changes in G2 upon calcium activation [63,64], although G2 on its own (or as part of G2–3 or G2–6) can bind F-actin in the absence of calcium [14,38,51].

4.3. Filament severing

After binding, gelsolin rapidly severs F-actin. To do this G1 must insert itself between two longitudinally associated actin subunits along one of the long-pitch strands in the filament (Fig. 3, step III). Interestingly, both severin and gelsolin G1–3 can sever about as efficiently as full-length gelsolin [4,17,38,65]. Thus, it appears that disruption of the longitudinal actin–actin bonds along one filament strand is sufficient to permit severing even though there are additional actin–actin bonds on the adjacent F-actin strand that would appear to be undisrupted when G1 binds. Interestingly, filaments decorated by the actin severing protein cofilin show single-stranded breaks along their lengths [61], although more work is needed to determine if these breaks represent intermediate steps in cofilin's severing mechanism.

4.4. Filament capping and uncapping

Presumably the actin filament capped by full-length gelsolin would also involve interactions of domain G4 of gelsolin and a barbed end subunit on the actin filament, although it is still not known which actin subunit is bound by G4 (Fig. 3, steps IV–V; compare pathways a and b). It has been suggested that each gelsolin contacts three actin subunits of F-actin during severing but only two of those remain bound in the capped filament [47]. This cap can be removed by specific interactions between the N-terminal half of gelsolin and polyphosphoinositides (PPIs) at sites near the G1–G2 interface (Fig. 3, steps VI–VII). Two formal possibilities exist for the disruption of the gelsolin cap by PPI binding [66]. One involves competition for binding sites much as is thought to occur with other PPI-regulated actin binding proteins. Alternatively, PPIs may alter the conformation of the G1–G2 linker, destabilizing the cap in the process [39,67,68].

5. Concluding remarks

Great strides have been made in understanding the molecular mechanism that gelsolin uses to bind, sever, and cap filaments using a combination of structural biology, biophysics, biochemistry, genetics and molecular modeling since its discovery nearly a quarter of a century ago. X-ray crystallography has provided the most detailed pictures of gelsolin's inter- and intramolecular interactions; whereas NMR spectroscopy, X-ray footprinting, electron cryomicroscopy, and molecular modeling give insights into the dynamic changes gelsolin undergoes during activation, severing, and uncapping. Thus, it seems likely that producing a molecular movie of filament severing by gelsolin will require a combined approach in which high-resolution snapshots determined by X-ray crystallography are fit together in space and time using other biophysical methods, much as has proven to be the case for the study of the molecular motor myosin.

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