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Gelsolin: The Tail of a Molecular Gymnast

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Gelsolin superfamily members are Ca^{2+}-dependent, multidomain regulators of the actin cytoskeleton. Calcium binding activates gelsolin by inducing molecular gymnastics (large-scale conformational changes) that expose actin interaction surfaces by releasing a series of latches. A specialized tail latch has distinguished gelsolin within the superfamily. Active gelsolin exhibits actin filament severing and capping, and actin monomer sequestering activities. Here, we analyze a combination of sequence, structural, biophysical and biochemical data to assess whether the molecular plasticity, regulation and actin-related properties of gelsolin are also present in other superfamily members. We conclude that all members of the superfamily will be able to transition between a compact conformation and a more open form, and that most of these open forms will interact with actin. Supervillin, which lacks the severing domain 1 and the F-actin binding-site on domain 2, is the clear exception. Eight calcium-binding sites are absolutely conserved in gelsolin, adseverin, advillin and villin, and compromised to increasing degrees in CapG, villin-like protein, supervillin and flightless I. Advillin, villin and supervillin each contain a potential tail latch, which is absent from CapG, adseverin and flightless I, and ambiguous in villin-like protein. Thus, calcium regulation will vary across the superfamily. Potential novel isoforms of the superfamily suggest complex regulation at the gene, transcript and protein levels. We review animal, clinical and cellular data that illuminate how the regulation of molecular flexibility in gelsolin-like proteins permits cells to exploit the force generated from actin polymerization to drive processes such as cell movement in health and disease. © 2013 Wiley Periodicals, Inc.

Key Words: gelsolin; villin; CapG; flightless; adseverin

Introduction

Eukaryotic cells are able to harness the force generated by the polymerization of actin monomers (G-actin) at the rapidly growing ends of pre-existing and nucleated filaments to exert pressure on objects in the direction of filament growth. Once formed, and especially when organized into meshes or fibrils, filamentous actin (F-actin) determines cell shape and organization. The consequence of these two general roles is that actin is crucial to a diversity of processes that involve motility and/or structural rearrangement of or within cells, e.g., cell migration, development, morphogenesis, and metastasis [reviewed in Jiang et al., 2009; Ren et al., 2009a], cell–cell and cell–matrix adhesion, which are determinants of the invasiveness of certain cell types [reviewed in Albiges-Rizo et al., 2009; Ren et al., 2009a] and apoptosis [Gourlay and Ayscough, 2005; Papakonstanti and Stournaras, 2008].

More than 100 actin-binding proteins have established roles in the temporal and spatial modulation of actin filaments, with ~300 binding proteins reported in total [Dos Remedios et al., 2003; Siripala and Welch, 2007a,b; http://www.bms.ed.ac.uk/research/others/smaciver/encyclop/encycloABP.htm]. An individual actin-binding protein may alter the kinetics of actin polymerization by nucleating filaments, by capping filaments at either their pointed or barbed ends, by cross-linking filaments to form bundles and networks, or by disassembling filaments and networks through depolymerization or severing activities. Others may regulate G-actin pool sizes through monomer sequestration or promotion/inhibition of nucleotide exchange. Actin-binding proteins also mediate interactions

Abbreviations used: G1 through G6, gelsolin domains 1 through 6, respectively; PIP_2, phosphatidylinositol 4,5-bisphosphate.

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between actin and other cellular components, such as membranes, microtubules, and other regulatory proteins. While some actin-binding proteins regulate the actin cytoskeleton, others use the binding of actin monomers or filaments to regulate their own activities or direct their cellular location.

This review focuses on gelsolin, the eponymous member of a superfamily of actin-binding proteins that exhibit a variety of actin regulatory functions. Gelsolin (*GSN*) was first identified as an agent that could transform macrophage extracts from viscous gels to fluid sols, subsequently accredited to its calcium-sensitive ability to sever F-actin [Yin and Stossel, 1979; Yin et al., 1981]. It is, in fact, a multifunctional regulator of actin dynamics that, in addition to severing actin filaments, can cap actin filaments, sequester actin monomers and, in vitro, nucleate actin filaments.

The Gelsolin Domain

Gelsolin contains six conserved copies (named G1 through G6 as they progress through the protein sequence from the N- to the C-terminus) of a prototypical domain referred to simply as the gelsolin domain. This domain comprises 97–118 residues folded into a 5- or 6-stranded β-sheet sandwiched between a long helix that is approximately parallel, and a short helix that is approximately perpendicular, to the strands in the sheet (Figs. 1A and 1B) [McLaughlin et al., 1993; Burtnick et al., 1997]. Different gelsolin superfamily proteins contain a variety of multiples of this domain that in some cases are fused to other unrelated domains, which confer additional functions (Fig. 2A).

There are eight known mammalian gelsolin superfamily proteins: CapG, adseverin, gelsolin, flightless I, advillin,

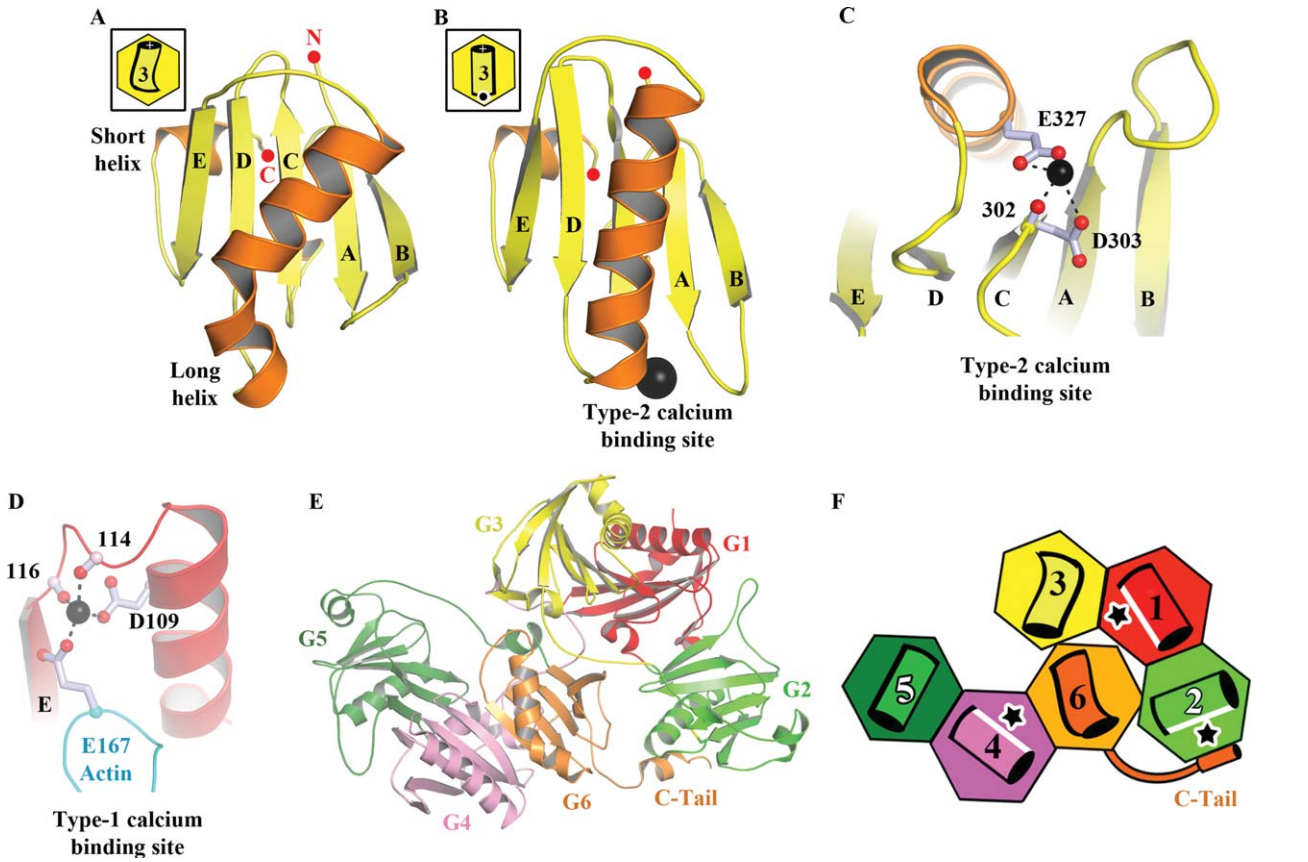

Fig. 1. The gelsolin domain. (**A**) The third domain of gelsolin (G3) is shown as an example of the gelsolin domain fold in its inactive conformation. The fold consists of a central β-sheet sandwiched between two perpendicular helices. The long helix is kinked in the inactive conformations of G3 and G6, but straight in their active conformations and in all other domains. The five strands of the β-sheet are labeled A through E (black) and the N- (red label, N) and C-termini (red label, C) are highlighted (red dots). G2 and G5 are unusual in that they contain six strands in their inactive conformations, one strand of which G2 gives up on activation. Inset: cartoon representation of the domain. A plus sign indicates the C-terminal end of the long helix. (**B**) The active conformation of G3, in which Ca^{2+} binding to the type-2 site results in straightening of the long helix. (**C**) Close up of the conserved type-2 calcium-binding site. The calcium is coordinated by a glutamic acid at the N-terminal turn of the long helix and an aspartic acid and the preceding mainchain carbonyl at the start of strand C. These interactions allow the long helix to move relative to the β-sheet. **(D)** A close up of the G1 (red) type-1 calcium-binding site. Type-1 sites only exist in G1 and G4 and require actin (cyan) residue Glu167 to complete the Ca^{2+} coordination. **(E)** The structure of full-length gelsolin, and **(F)** a cartoon representation arrangement of the domains. Stars and white sides at the long helices of G1, G2 and G4 indicate major actin-binding regions. White and black domain numbers denote that the long helix is behind or in front of the β-sheet, respectively.

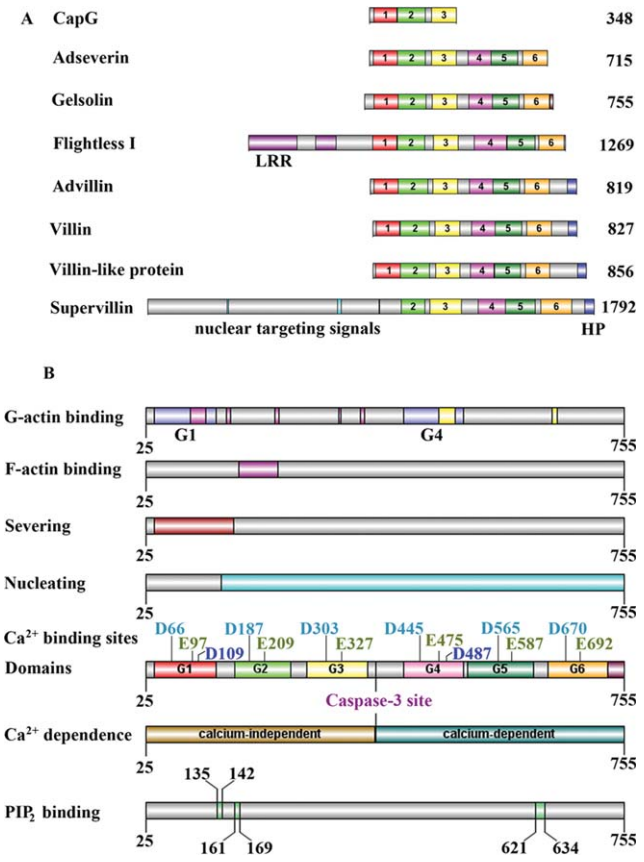

Fig. 2. Gelsolin superfamily proteins and modular distribution of functional motifs (**A**) Gelsolin superfamily proteins from humans. The protein schematic images are drawn to scale based on amino acid sequence length in DOG version 2.0 [Ren et al., 2009b]. For proteins with multiple isoforms, isoform 1 was selected for this figure. The conserved gelsolin domains are labeled 1 through 6, and colored according to Figure 1. The C-terminal tail is shown in brown. Corresponding domains from different proteins share the same color. Villin, advillin, villin-like protein and supervillin have a C-terminal headpiece domain (HP; dark blue). Flightless I straddles two protein families, namely the LRR (conserved domain in purple) superfamily and the gelsolin superfamily. Supervillin has multiple nuclear localization signals in its N-terminal region (cyan). **(B)** The modular distribution of gelsolin functions. The various functional regions are colored into the grey backbone of the protein. The G-actin binding sites determined from biochemical studies are in violet and those determined from X-ray studies are in pink [Burtnick et al., 2004] and yellow [Robinson et al., 1999]. The conserved aspartate (cyan) and glutamate (gold) residues of the type-2, and the aspartate (dark blue) residues of the type-1 calcium-binding sites are labeled. The site at which caspase-3 cleaves gelsolin [Kothakota et al., 1997] to yield the calcium-independent active N-terminal half (gold), and calcium-dependent C-terminal half (green), is labeled. PIP_2 binding (sites shown in green) has key roles in uncapping of filaments.

villin, villin-like protein, and supervillin (Fig. 2A). Amongst them, CapG has three gelsolin-type domains, supervillin has five, and each of the others has six. Other three-domain proteins, severin, fragmin, and fragmin60 [Schleicher et al., 1988; Sklyarova et al., 2002], have been identified in lower eukaryotes. Due to the initial identification of only three- and six-domain gelsolin superfamily proteins, it was proposed that the superfamily evolved from a single domain prototype by gene triplication followed by a duplication event [Kwiatkowski et al., 1986; Way and Weeds, 1988]. However, detailed comparison of CapG, gelsolin, and villin [Mishra et al., 1994], as well as the discovery of invertebrate gelsolin superfamily proteins with novel domain architectures incorporating two, four or five gelsolin-type domains, suggest a significantly more complex evolutionary pathway that remains unmapped [Stocker et al., 1999; Kawamoto et al., 2002; Gloss et al., 2003; Klaavuniemi et al., 2008]. Charting the course of evolution of the gelsolin superfamily proteins will improve our understanding of their functional and regulatory diversity, and possibly highlight future directions for specific manipulation to manage disease states.

Single domain gelsolin superfamily proteins have not been identified, although the gelsolin domain fold resembles that found in ADF/cofilin family members [Hatanaka et al., 1996], which exist most commonly as single domain proteins. The gelsolin domain is distinguished from the cofilin domain in containing a calcium ion-binding site (termed type-2) comprising a glutamic acid at the beginning of the long helix and an aspartic acid and mainchain carbonyl from the N-terminal end of the C strand of the β-sheet (Figs. 1C and 2B). An unrelated calcium ion-binding site (type-1) exists at each of the actin interfaces with G1 and G4 (Fig. 1D). The type-2 site is conserved in each of the six domains of gelsolin and in many other domains of gelsolin superfamily members [Choe et al., 2002]. Sequence analysis reveals that CapG, adseverin, advillin, and villin have the full complement of type-2 sites, albeit adseverin has a homologous change in domain 3 (Asp281Glu, Fig. 3). However, flightless I lacks four of the six type-2 sites (domain 1–Glu557Lys, domain 2–Asp646Phe, Glu668Ser, domain 3–Asp774Cys, Glu796Val, domain 4–Glu996Gly). Domains 5 and 6 have complete type-2 sites and are expected to bind calcium although domain 5 has a homologous substitution (Asp1083Glu) similar to adseverin domain 3. Villin-like protein lacks the domain 1 type-2 site (Asp39His, Glu72Ala) while those in domains 4 (Asp418Asn) and 5 (Glu559Gln) may be compromised. Supervillin lacks the type-2 sites in domain 5 (Asp1484Thr, Glu1508Thr) and domain 6 (Asp1616Pro, Glu1641Lys), while that in domain 3 (Asp1167Glu, Glu1189Gln) may be compromised. These substitutions suggest that flightless I, villin-like protein and supervillin will respond to calcium in a manner distinct from that observed for gelsolin. Asp to Asn and Glu to Gln mutations in gelsolin partially mimic occupancy of a calcium-binding site [Nag et al., 2009]. These mutations destabilize both the resting, and the final activated, conformations of gelsolin and the mutants are likely to bind calcium ions less tightly.

Calcium binding to type-2 sites induces small-scale local structural changes such as the exact positioning of the long

helix with respect to the β-sheet, and the straightening of the long helices of domains 3 and 6 (Fig. 1B) [Wang et al., 2009]. The long helix acts as a calcium sensor that transmits local changes at the calcium-binding site to other areas of the parent domain to trigger large-scale rearrangements of domain positions relative to each other, while maintaining the structural integrity of the individual domains [Choe et al., 2002; Kazmirski et al., 2002; Burtnick et al., 2004]. Reported calcium-binding affinities for the type-2 sites of gelsolin range from 0.2 to 600 μM, for example, K_d = 600 μM in G1 [Zapun et al., 2000], K_d = 0.7 μM in G2 [Chen et al., 2001], unknown K_d for G3, K_d = 1.8 μM in G4 [Pope et al., 1995], K_d = 100 μM in G5 [Khaitlina et al., 2004], and K_d = 0.2 μM in G6 [Pope et al., 1995], suggesting that the domains may bind calcium sequentially with increasing calcium concentrations, thus widening the range of calcium concentration to which the protein can respond and fine-tune its activity. However, one must remember that the local environment modulates the response of this calcium-sensing domain as evidenced by the variation in the measured K_d values depending on whether a given domain is studied in isolation, or in the context of other domains, activators, or in the presence of the target protein, actin [Pope et al., 1995; Zapun et al., 2000; Chen et al., 2001; Chumnarnsilpa et al., 2006; Roustan et al., 2007]. Therefore, subtleties of the calcium-binding process, such as cooperativity or dependence on binding partners, may be obscured in these studies.

Gelsolin Tertiary Structure and Inhibitory Latches

Gelsolin is present in the cytoplasm at ~5 μM [Dos Remedios et al., 2003], and must be maintained in an inactive state until required for actin severing. Here we consider the mechanism through which the inherent structural features of dormant gelsolin inhibit binding to actin. Inactive gelsolin folds into a compact globule with G6 as the centerpiece, around which the other five domains are arranged in a manner such that all actin-binding surfaces are hindered (Figs. 1E and 1F) [Burtnick et al., 1997]. Although the six gelsolin domains are highly conserved [Kwiatkowski et al., 1986], domains G1, G2, and G3 exhibit the highest sequence and structural similarity to domains G4, G5, and G6, respectively, effectively dividing the protein into two halves that adopt pseudosymmetric structures in the inactive state (Figs. 4B and 4C, left). Three latches hold gelsolin in its inactive state [Way et al., 1989; Burtnick et al., 1997; Choe et al., 2002; Burtnick et al., 2004; Ashish et al., 2007]: the C-terminal tail latch, formed by the interaction of the C-terminal helix with the long helix of G2 (Fig. 4A, left); the G1–G3 latch, formed by abutment of the core β-sheets of G1 and G3, and stabilized by interactions between their E strands (Fig. 4B, left); and the G4–G6 latch, formed by the interactions between the E strands of the core β-sheets of G4 and G6 (Fig. 4C, left). The calcium-sensing long helices of G3 and G6 are kinked (Fig. 1A) to avoid steric clashes with the long helices of G1 and G4, respectively, and their straightening is key to the release of their respective latches [Robinson et al., 1999; Wang et al., 2009].

Homology of residues involved in the E strand:E strand interaction of the G1:G3 and G4:G6 interfaces suggests that G1:G3-like and G4:G6-like latch interactions may be present in all human gelsolin superfamily members (Fig. 3). However, CapG, which lacks domains 4–6, can maximally only have the G1:G3-like latch, and supervillin only the G4:G6-like latch, since it lacks a domain 1. The residues at the kink point of the long helices of G3 (Leu332) and G6 (Leu697), and the interacting residue of the former in the D strand of G1 (Tyr87), are also conserved throughout the human gelsolin superfamily members (Fig. 3). Homologous residues within the G2:G6 interface in the longer human gelsolin superfamily members suggest that these proteins may also adopt the tight packing observed between these domains in gelsolin. The presence of an amphipathic helix immediately following the sixth domain suggests that advillin (residues 717–730), villin (residues 720–734) and supervillin (residues 1702–1714) may contain a gelsolin-like tail latch preceding their headpiece domains (Fig. 5A). Adseverin and flightless I terminate immediately after their sixth domains and hence do not contain the tail latch, and the presence of the tail latch in villin-like protein is difficult to assess due to this region being expanded relative to the other villin family members. In summary, domain:domain and tail:domain interactions lock gelsolin in an inert conformation, and many of these features are likely to be reproduced in the other superfamily members.

Gelsolin Activation

The binding of calcium ions activates gelsolin by opening the three latches that stabilize the inactive structure (Fig. 4). Early in this process, the tail latch must be released, exposing the F-actin side-binding site on G2 and allowing the G3–G4 linker to adopt an extended conformation that enables the two halves of gelsolin to separate from each other (Fig. 4A). Subsequent opening of the G1–G3 and G4–G6 latches entails dramatic rearrangement of the relative positions of the domains (Figs. 4B and 4C) [Robinson et al., 1999; Choe et al., 2002; Burtnick et al., 2004]. Relative to its starting position adjacent to G4 in inactive gelsolin, G6 moves ~40 Å and rotates through ~90° to expose the actin-binding surface on the long helix of G4. The interaction of G4 with G5 is maintained while G6 forms a new interface with G5, yielding the roughly L-shaped structure with G5 at the vertex between G4 and G6 (Fig. 4C, right). The calcium-activated C-terminal half of gelsolin is able to adopt this conformation independently of the presence of

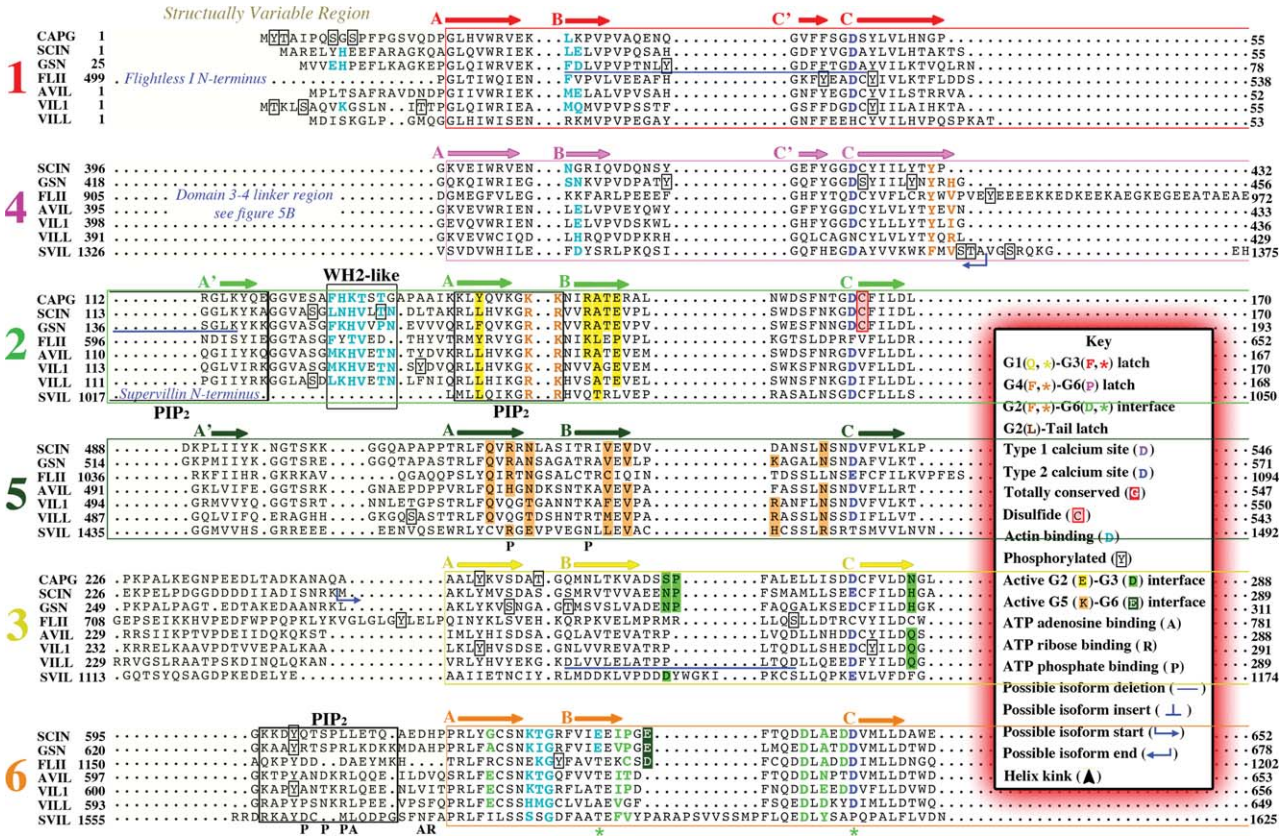

Fig. 3. Gelsolin domain sequence alignment. Sequences of gelsolin domains aligned for all human members of the superfamily. Conservation of important gelsolin residues is highlighted according to the key panel. The alignment was assembled using elements from MULTALIN [Corpet, 1988], JALVIEW [Waterhouse et al., 2009] and ESPript [Gouet et al., 1999] based on the alignment of gelsolin domains [Choe et al., 2002]. Phosphorylation sites were taken from PhosphoSitePlus [Hornbeck et al., 2012] and PHOSIDA [Gnad et al., 2011].

actin [Kolappan et al., 2003; Narayan et al., 2003; Robinson et al., 1999].

While the conformation of the N-terminal half of inactive gelsolin closely matches that of the C-terminal half, the activated structures of the two halves differ dramatically. G1 becomes separated from G2 and G3, which form a new interface (Fig. 4B, right). The distinctive domain interaction patterns of G2 with G3, relative to G5 with G6, highlight how subtle divergences in individual domain sequences have had major consequences for interactions and function. The active G2:G3 and G5:G6 interaction interfaces show homology across the human gelsolin superfamily protein sequences, suggesting that these proteins may form similar interfaces when activated. Hence, interdomain rearrangements contort symmetric inactive gelsolin into an asymmetric active structure with all the actin-binding sites exposed and available.

The Role of Calcium in Activation

Calcium, a pivotal second messenger, is the best-studied regulator of gelsolin activity. It triggers large conformational rearrangements in gelsolin during activation. Calcium binding to G6 straightens its kinked long helix and induces steric clashes that assist in releasing the G4–G6 latch and pushing the two halves of gelsolin apart (Fig. 4A) [Wang et al., 2009]. However, additional calcium binding events are necessary for the C-terminal half to adopt the active conformation required for binding actin [Pope et al., 1995; Khaitlina et al., 2004]. Release of the constraints imposed by interactions between the two halves of gelsolin may encourage straightening of the G3 long helix, and disruption of the G1–G3 latch (Figs. 1A, 1B and 4B), resulting in activation of the N-terminal half of gelsolin without the requirement for binding additional calcium ions. The isolated N-terminal half of gelsolin severs actin filaments in the absence of calcium ions, although addition of calcium improves severing efficiency, indicating that some of the activating conformational changes in gelsolin are driven through contact with actin [Bryan and Hwo, 1986; Kwiatkowski and Yin, 1987; Hellweg et al., 1993; Allen and Janmey, 1994; Selden et al., 1998].

While comparison of the structures of the activated halves of gelsolin with those in inactive gelsolin defines the beginning and end points of the activation process, its mechanism remains largely speculative. Our understanding

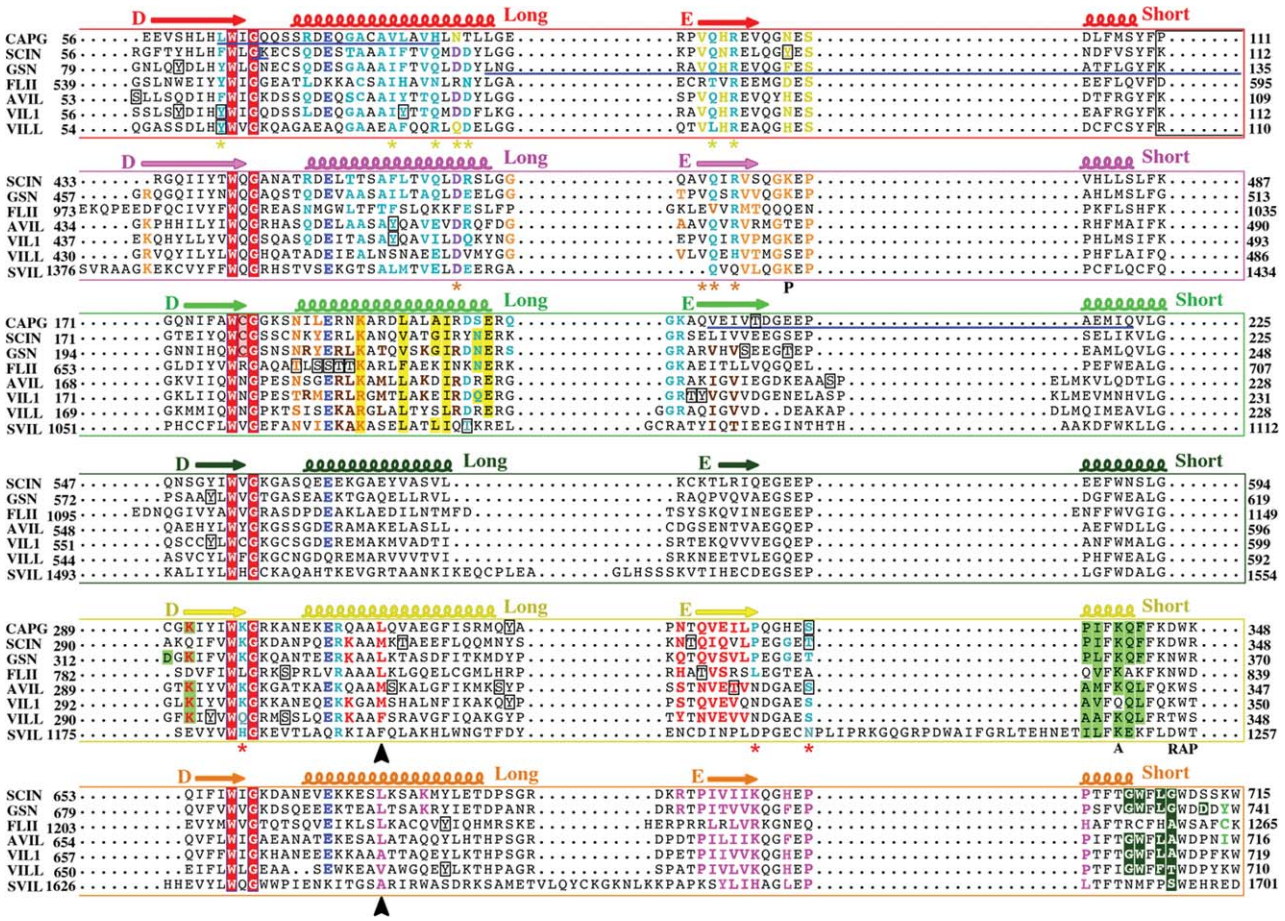

Fig. 3. Continued

of the intermediate stages of the activation pathway depends on sometimes conflicting data from bulk assays of calcium-dependent conformational changes. The multidomain nature of gelsolin presents challenges in ascribing specific calcium-binding events to particular conformational changes. Calcium binding affects both the affinity [Way et al., 1992a,b] and the rate of binding [Kinosian et al., 1996; Selden et al., 1998] of gelsolin to actin. Not surprisingly, the kinetics of gelsolin activities also depend on protein concentration [Gremm and Wegner, 2000]. Reports disagree about the number of calcium-binding events required to activate gelsolin, the calcium concentrations at which different transitions occur, and the roles of different calcium-binding sites in the activation process. Inactive gelsolin undergoes either two or three conformational changes as calcium concentrations rise from 10 nM to ~5 μM, as a result of binding of two or three calcium ions with K_d values estimated to be 0.1 and/or 0.3 μM and 6.4 μM [Kinosian et al., 1998; Lin et al., 2000]. These events may reflect the release of the tail latch [Kwiatkowski et al., 1989; Pope et al., 1997; Kinosian et al., 1998; Lin et al., 2000; Lueck et al., 2000; Choe et al., 2002; Kolappan et al., 2003; Lagarrigue et al., 2003a,b], for which recent evidence [Nag et al., 2009] implicates a cooperative pair of calcium-binding events at the type-2 sites in G2 and G6. Indeed, sequence analysis reveals that human gelsolin superfamily proteins that lack a full complement of six type-2 sites still retain a type-2 site in domain 2 (supervillin) or domain 6 (flightless I), testifying to the importance of breaking the domain 2:domain 6 interactions during activation (Fig. 3).

Additional binding of three to six calcium ions is reported to occur in the calcium concentration range between 0.2 and 1 mM, and complete the activation process [Ditsch and Wegner, 1995; Kinosian et al., 1998; Lin et al., 2000; Kiselar et al., 2003a,b; Ashish et al., 2007]. However, such levels of calcium ions, while present in extracellular fluids, are not achievable within cells. To explain the anomaly, we must consider that actin is known to alter the affinity of gelsolin for calcium, and to elicit activating conformational changes [Weeds et al., 1995; Pope et al., 1997]. Therefore, actin binding will stabilize the active conformation of gelsolin, and will facilitate the sequestration of calcium by gelsolin [Allen and Janmey, 1994; Weeds et al., 1995; Kinosian et al., 1998; Burtnick et al., 2004].

Low pH can also reduce the calcium levels required to maximally activate gelsolin (severing activity at pH 5 and

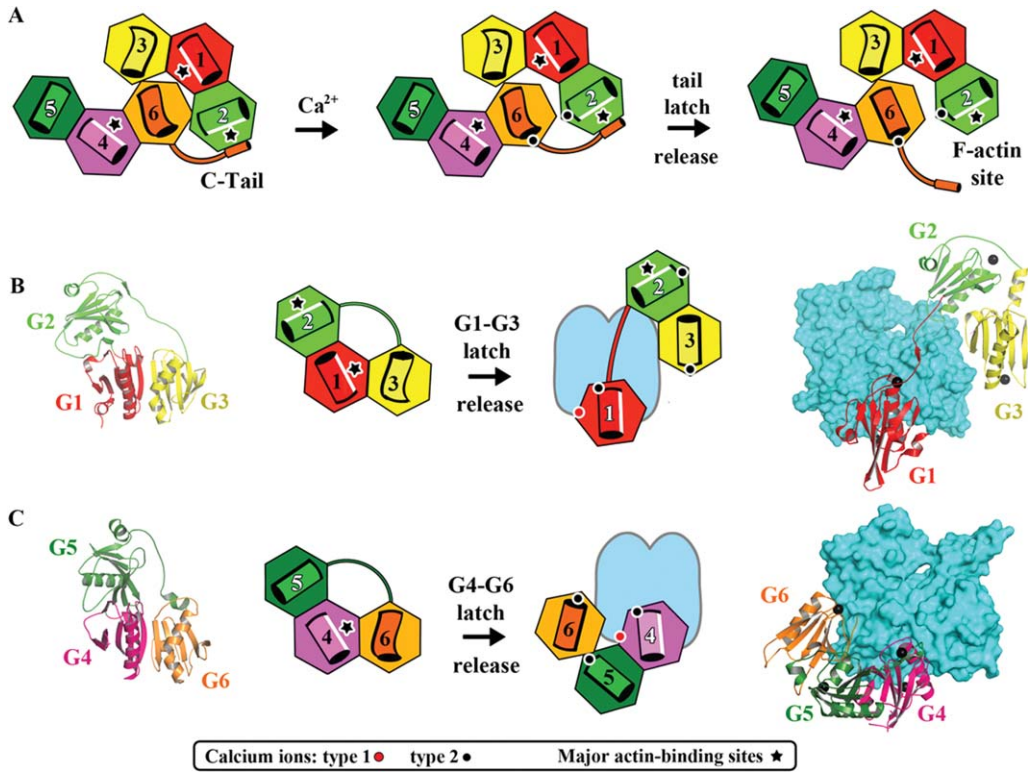

Fig. 4. Gelsolin conformational gymnastics. Gelsolin activation proceeds through the opening of three inhibitory latches to expose actin-binding sites. (**A**) The C-terminal tail latch. (**B**) The G1-G3 latch. (**C**) The G4-G6 latch. Cartoons are derived from Figure 1F and have identical markers. In particular, white sides to the long helices of G1, G2 and G4 indicate actin-binding regions. Calcium ions are shown as spheres that bind to the type-1 (red) and type-2 (black) sites. The crystal structures that are the basis of the cartoon representations in (B) and (C) are shown distal to the cartoons.

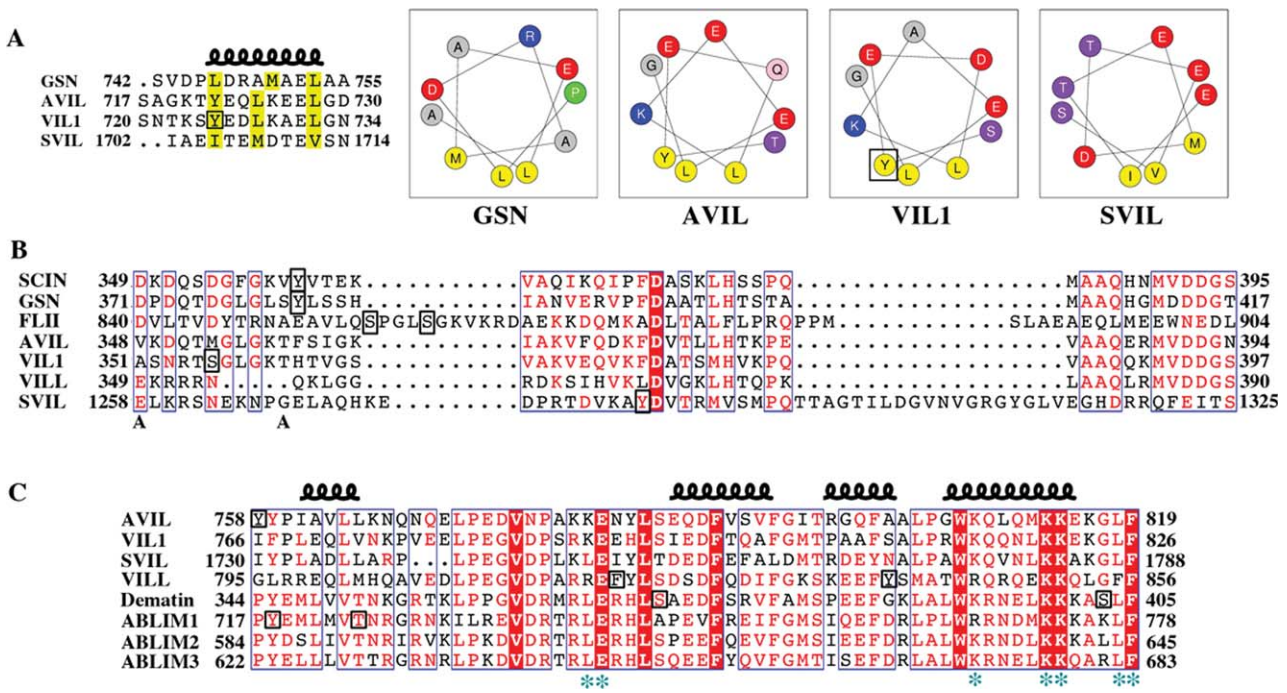

Fig. 5. Non-gelsolin domain sequence alignments. (**A**) Tail-latch alignment and helical wheel representations showing the predicted amphipathic helices. Boxed residues indicate phosphorylation sites as in Fig. 3. The helical wheels were generated in HELIQUEST [Gautier et al., 2008]. (**B**) G3-G4 linker alignment demonstrating a shared length and homology between some members of the superfamily. Red indicates conservation. (**C**) Villin-like headpiece alignment. Homologous domains from dematin and the ABLIMs are included for completeness. Actin-binding residues from villin are indicated by cyan stars [Doering and Matsudaira, 1996].

40 nM Ca^{2+} is equivalent to that at 1 mM Ca^{2+} at pH 8), and partially activate gelsolin in the complete absence of Ca^{2+} [Lamb et al., 1993; Lueck et al., 2000; Garg et al., 2011]. Despite their established interdependence, activation by pH and by Ca^{2+} have been suggested to occur through different conformational pathways, and these mechanisms remain poorly understood [Lagarrigue et al., 2003b; Garg et al., 2011]. Thus, calcium binding destabilizes domain: domain interactions specific to inactive gelsolin and stabilizes those specific to active gelsolin in a cooperative manner that is also dependent on H^+ and actin binding, both of which are expected to destabilize the inactive and stabilize the active conformations of gelsolin, respectively.

A tragic consequence of disruption of the effects of calcium on the structure of normal gelsolin is manifested in the heritable disease, familial amyloidosis [recently reviewed in Solomon et al., 2012] in which a mutation in the type-2 calcium-binding site of domain G2 (D187N or D187Y) renders gelsolin susceptible to cleavage by furin during transport through the Golgi [Chen et al., 2001]. The larger of the truncated products undergoes further proteolysis in the extracellular space to yield 5 and 8 kDa peptides that self-assemble into amyloid fibrils that result in proteotoxicity and cytotoxicity. Amyloid deposition results in malfunction of certain tissues and organs, including eyes, nerves, skin, and kidneys. The loss of calcium binding by G2 slightly destabilizes the domain in isolation, potentially contributing to the disease [Kazmirski et al., 2002; Ratnaswamy et al., 2001; Robinson et al., 2001]. However, within the intact protein, loss of calcium binding by G2 most likely impairs gelsolin activation, stranding it in intermediate states in which the furin cleavage site is exposed and primed to initiate the first step in the amyloidogenic pathway [Burtnick et al., 2004; Nag et al. 2009].

Actin Binding by Gelsolin

The net result of these conformational gymnastics is to reveal the actin-binding sites described here. The regulatory functions of gelsolin are distributed in discrete regions along its sequence (Fig. 2B). Three major actin-binding sites on G1, G2, and G4, respectively, are able to interact with actin using a stretch of homologous residues in their conserved long helices (Figs. 1B, 2B and 3) [Pope et al., 1991, 1995; McLaughlin et al., 1993; Van Troys et al., 1996; Robinson et al., 1999; Choe et al., 2002; Burtnick et al., 2004]. The actin-binding sites on G1, on G2 and on G4 are conserved across all human gelsolin superfamily proteins that contain these domains, with the exception of supervillin, which appears to have incompatible residues on domain 2 (Fig. 3). Additional actin-binding sites have been identified on G3 [Burtnick et al., 2004] and G6 [Robinson et al., 1999], in the G1-G2 linker peptide [Irobi et al., 2003], and at a second patch on the surface of G2 [Burtnick et al., 2004]. The G3 actin-binding site is potentially conserved in all gelsolin superfamily proteins in humans, while that on the WH2-like G1-G2 linker may be present in all gelsolin superfamily proteins in humans with the exception of supervillin (Fig. 3). It is also likely that the G3–G4 linker contacts actin. This region displays conservation between gelsolin, adseverin, advillin, villin, and villin-like protein, and rather weaker similarity in supervillin and flightless I (Fig. 5B).

Comparison of the structure of G1–G3 bound to actin with that of G4–G6 reveals that G4 binds to actin analogously to G1, in the groove between actin subdomains 1 and 3 (Figs. 4B and 4C). G5 acts as a bridge to allow G6 to contact subdomain 3 of actin, without itself contacting actin, whereas the G1-G2 linker peptide extends along the surface of actin to reach G2, bound to subdomain 2 of actin. G3 in this activated complex nestles against both G2 and actin, near the junction of actin subdomains 1 and 2.

Besides binding actin, gelsolin domains G1 and G4 each bind an additional calcium ion. These conserved type-1 calcium-binding sites require interaction with actin residue Glu167 to complete the coordination sphere of the bound metal ion (Fig. 1D) [McLaughlin et al., 1993; Robinson et al., 1999; Choe et al., 2002]. Thus, calcium bridges between actin and gelsolin, stabilizing interprotein interactions and regulating the affinity between the two proteins. The type-1 site in domain 4 is maintained in all human gelsolin superfamily members, with the exception of flightless I (Asp to Phe). In domain 1 the site is conserved in gelsolin, adseverin, advillin, and villin, compromised in CapG (Asp to Asn) and villin-like protein (Asp to Gln), and changed to arginine in flightless I, where the arginine may mimic aspartic acid bound to calcium. Maximal activation of gelsolin results from eight calcium ions being bound by gelsolin in its type-1 and type-2 sites, however, submaximal levels of calcium binding produce varying degrees of activity [Choe et al., 2002; Nag et al., 2009]. In summary, calcium-activated gelsolin presents actin-binding surfaces on the extended interdomain linker regions and on each of the domains, with the exception of G5.

Actin Severing by Gelsolin

Based on this understanding of how gelsolin interacts with actin we now focus on the mechanism of actin filament severing. Gelsolin's potent severing activity allows it to modulate actin dynamics by dismantling existing polymers to produce a pool of capped shorter oligomers. These are primed to either nucleate rapid and directed filament growth upon dissociation of gelsolin by an uncapping agent, or to completely depolymerize and supplement the cytoplasmic pool of actin monomers. Gelsolin fragments G1 and G4-G6 are effective filament cappers that can each sequester one actin monomer (Figs. 4B and 4C). They compete for the same site on actin [Pope et al., 1991], located in the groove between actin subdomains 1 and 3

[McLaughlin et al., 1993; Robinson et al., 1999]. Although severing activity requires a minimal fragment of gelsolin, comprising only G1 plus the G1-G2 linker [Kwiatkowski et al., 1989; Way et al., 1992a], in intact gelsolin the interaction between G2 and F-actin is most likely the first step of severing. The G1-G2 linker subsequently extends across the surface of actin to position G1 near its binding site on actin [Irobi et al., 2003; Burtnick et al., 2004].

Thus, gelsolin is initially targeted to the side of a filament, where the G2G3 unit binds to subdomain 2 of an actin unit as observed in known G1-G3/actin structures [Burtnick et al., 2004; Nag et al., 2009] (actin protomer III in Figs. 6A–6C and 7), and to subdomain 1 of the longitudinally adjacent actin unit (protomer V). G3 nestles up against G2 and aids it in anchoring gelsolin to the filament at protomer III. The extended G1-G2 linker interacts with the surface of actin protomer III, positioning G1 to bind to that actin unit between its subdomains 1 and 3, and introduce steric clashes with protomer I (Figs. 6A, 6D, and 7). Analogously, the G3-G4 linker extends across the filament to position G4 of the activated C-terminal half of gelsolin near the groove between subdomains 1 and 3 of protomer IV, which introduces steric clashes with protomer II (Figs. 6B, 6E, and 7). As presented, the model suggests that the gelsolin domains bind actin cooperatively, and severing is the consequence of a pincer movement resulting from simultaneous competitive events in which G1 and protomer I vie for binding to protomer III, while G4 and protomer II vie for binding to protomer IV. We speculate that this mechanism will apply to all human gelsolin superfamily members with the exceptions of CapG and supervillin. Three-domain CapG reportedly caps but does not sever actin filaments [Southwick, 1995], and supervillin lacks many of the actin-binding surfaces needed for severing (Fig. 3).

Severing by gelsolin is probably an opportunistic event in which the filament-targeted gelsolin relies on fluctuations in the structure of the actin filament in order to access the G1 and G4 binding sites. However, the initial binding of the G2G3 unit to actin may promote changes in the twist of the filament that would weaken longitudinal interactions between adjacent protomers and tip the scales in favor of severing [Bearer, 1991; Prochniewicz et al., 1995; McGough et al., 1998; reviewed in Hild et al., 2010].

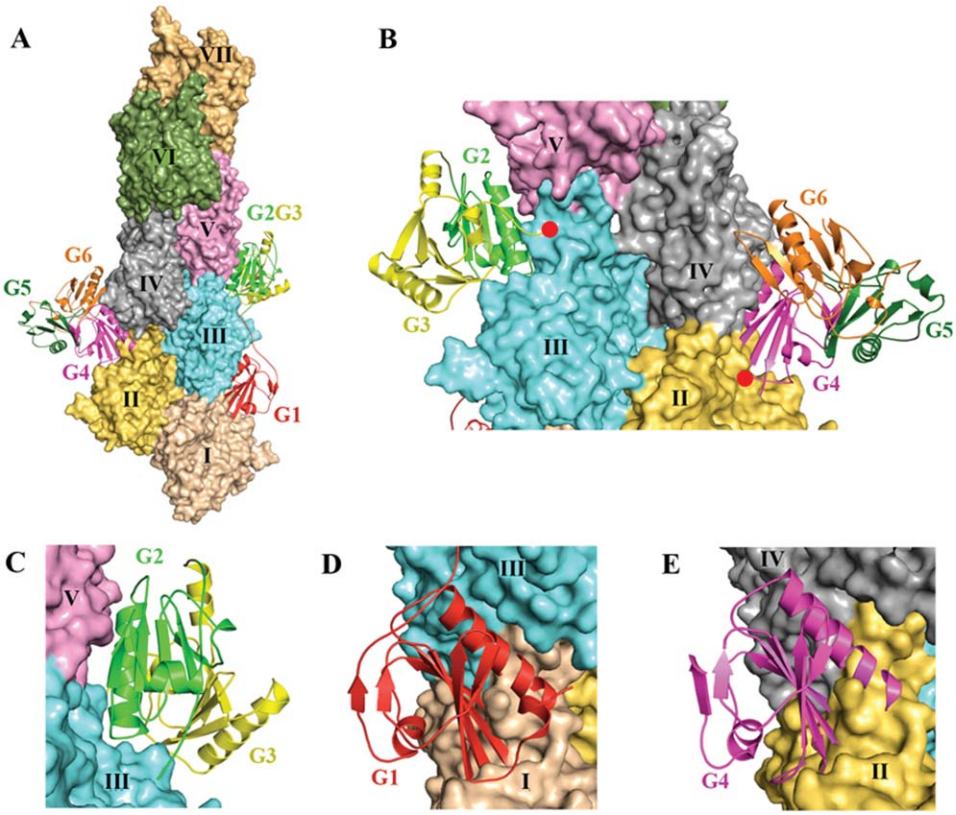

Fig. 6. Structural basis for the actin filament severing model. (**A**) Structures of the activated N-terminal half (G1-G3) and C-terminal half (G4-G6) of gelsolin are shown docked onto a model of F-actin [Oda et al., 2009] by superimposing the actin monomers bound to G1-G3 (PDB 1RGI) and G4-G6 (PDB 1H1V) onto protomers III and IV of the filament, respectively. (**B**) The filament is shown rotated by 180° relative to **A**. Red dots mark the position of the terminal residues of the G3-G4 linker that are visible in the X-ray structures. (**C**) Interface of the F-actin targeting G2G3 unit with the filament. G2 forms interactions with two vertically adjacent protomers. (**D**) G1 bound to protomer III clashes extensively with protomer I. (**E**) G4 bound to protomer IV clashes extensively with protomer II. These clashes suggest that G1 and G4 sever a filament by displacing the protomers below the ones to which they bind.

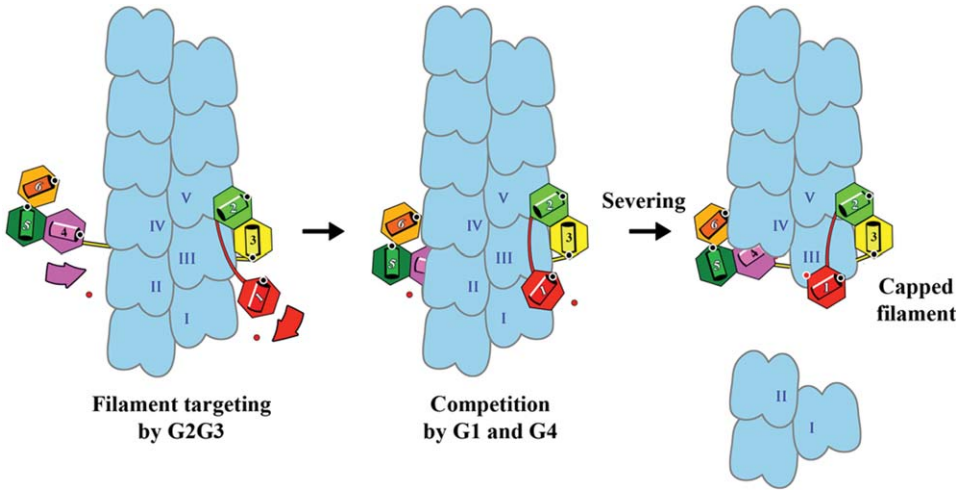

Fig. 7. Cartoon of the model for actin filament severing. The cartoon of actin filament severing by gelsolin is based on the structural data in Fig. 4 and depicted in the schematic representation established in Fig. 3. Red dots indicate calcium ions that will occupy the type-1 sites.

Additional support for this view comes from reports that F-actin behaves as a mechanosensor of environmental changes, responding with alterations in its twist, curvature and flexibility [Hayakawa et al., 2011; Risca et al., 2012]. These introduce protein-specific phenomena. For example, cofilin binding is inhibited by increased tension on a filament, while gelsolin severs more efficiently when a filament is under tension [Hayakawa et al., 2011].

A possible mechanism for G2 induced actin filament changes in twist arises from the predicted interaction of Arg221 from G2 with Glu167 from actin (actin subunit V in Figure 7) [Burtnick et al., 2004]. Actin residue Glu167 forms part of an intersubunit cation-binding site (K^+ or Mg^{2+}) that confers stiffness to the actin filament [Kang et al., 2012]. Thus, binding of G2 may disrupt this cation-binding site and alter the flexibility and twist of the filament. Arg221 is conserved in all human gelsolin superfamily members with the exceptions of flightless I (Asn) and supervillin (Gln), where the substitution for polar residues may stabilize cation binding. Furthermore, actin residue Glu167 completes the coordination sphere of the type-1 calcium-binding sites that are sandwiched between gelsolin domains G1 and G4 with actin subunits III and IV, respectively (Fig. 1D). Thus swapping the K^+ or Mg^{2+} bound to Glu167 in the actin-actin "stiffness" cation-binding site with Ca^{2+} bound to Glu167 at the G1-actin and G4-actin type-1 sites will contribute to the severing process. CapG and villin-like protein are compromised in their domain 1 type-1 sites, containing Asn and Gln, respectively, which may reflect the reported loss of severing in the case of CapG. Flightless I lacks both type-1 sites, containing Arg and Phe in domains 1 and 4 (Fig. 3), respectively. However, the arginine might act to eject the actin-actin "stiffness" cation.

One issue faced by gelsolin on release of the tail latch is to ensure that binding sites on G1 and G4, which become the capping interfaces with actin, are preserved for severing instead of being nonproductively occupied by actin monomers. This is likely achieved through gelsolin being partially activated at cytoplasmic calcium concentrations so that the G-actin-binding/capping interfaces are only revealed subsequent to filament association and F-actin-driven conformational changes. Furthermore, cellular G-actin is sequestered by monomer binding proteins, such as profilin and thymosin-β4, which further reduce monomer binding to gelsolin. Nucleation of actin filaments by gelsolin is prevented in vivo as a consequence of the profilin and thymosin-β4 G-actin bound complexes being sterically precluded from joining the free pointed end of a gelsolin-actin nucleus. Hence, gelsolin is able to wrap itself around the actin filament and direct its capping domains to positions in which they can take advantage of fluctuating actin:actin interactions in order to disrupt the filament.

Phosphatidylinositol 4,5-bisphosphate (PIP_2) Binding and Actin Filament Uncapping

The fraction of severed actin filaments that remains capped at their fast-growing barbed end by gelsolin must be uncapped to nucleate productive, directed filament elongation. Phosphatidylinositides and phosphatidylserines account for a minor fraction of total membrane phospholipids but participate in the crucial transduction of extracellular signals into cytoskeletal rearrangements by modulating the activity of various actin-binding proteins, including gelsolin superfamily proteins [reviewed in Yin and Janmey, 2003]. Phosphatidylinositides also serve as scaffold-targeting molecules that create linkages between the cytoskeleton and the plasma membrane. PIP_2 is the most abundant of the biphosphorylated phosphatidylinositides, and is a potent

regulator of gelsolin that favors filament uncapping and actin polymerization [Doi et al., 1991]. PIP_2 inhibits interactions between free gelsolin and actin [Janmey and Stossel, 1987; Janmey and Matsudaira, 1988; Maekawa and Sakai, 1990; Janmey et al., 1992; Feng et al., 2001] and uncaps actin filaments by disrupting pre-existing interactions with gelsolin [Janmey and Stossel, 1987]. Three PIP_2-binding sites on gelsolin have been identified: one within the G1-G2 linker (residues 135–142); a second that overlaps the G2 F-actin-binding site (residues 161–169); and a third in the G5-G6 linker (residues 621–634) (Fig. 2B) [Tuominen et al., 1999; Feng et al., 2001; Liepina et al., 2003]. The second region is particularly well conserved across the gelsolin superfamily members in humans and suggests similarities in how PIP_2 acts on these proteins (Fig. 3). The third PIP_2-binding site on gelsolin overlaps with the ATP binding site (Fig. 3) [Urosev et al., 2006]. ATP binding stabilizes the inactive gelsolin conformation but has not been shown to bind to other members of the superfamily and its role in uncapping, if any, remains unclear. The mechanism by which uncapping occurs is not fully understood, but PIP_2 may directly compete with actin for binding to gelsolin, or it may alter the conformation of actin-binding site(s) [Janmey et al., 1992; Burtnick et al., 1997; Liepina et al., 2003; Urosev et al., 2006].

There are reports of a correlation between PIP_2 binding to gelsolin with calcium binding, but there is debate as to whether the correlation is positive or negative [Lin et al., 1997; Tuominen et al., 1999; Feng et al., 2001]. A correlation between phosphorylation and PIP_2 binding has also been reported. Actin reorganization effected by direct phosphorylation of gelsolin is required for osteoclastic actin ring formation and the appearance of peripheral podosomes in fibroblasts [Wang et al., 2003]. Gelsolin can be phosphorylated in vitro by c-Src, primarily at Tyr438, and in osteoclasts and fibroblasts by proline-rich tyrosine kinase 2 [De Corte et al., 1997, 1999; Wang et al., 2003]. Phosphorylation is strongly inhibited (~90% decrease) in the presence of bound actin, and enhanced by PIP_2 and lysophosphatidic acid [De Corte et al., 1999]. Conversely, phosphorylation leads to decreased interaction with actin and increased interaction with PIP_2, enhancing filament uncapping and consequent actin polymerization.

There is strong evidence to suggest that local accumulation of PIP_2 in membranes will uncap gelsolin-capped filaments in their immediate vicinity, resulting in rapid, directed filament elongation [Janmey and Stossel, 1987; Hartwig et al., 1995]. Thus, although the mechanism of uncapping remains elusive, cells maintaining a pool of capped actin filaments primed for localized PIP_2-mediated uncapping can efficiently harness the force of actin polymerization to distort membranes and generate movement.

Gelsolin Isoforms

There are three characterized, and a number of potential isoforms of gelsolin. Mammalian gelsolin has two well-established isoforms, best characterized as cytoplasmic and plasma gelsolin, respectively, in addition to a lesser known isoform, gelsolin-3 (Fig. 8A). All gelsolin isoforms originate from the same gene as a result of initiation of transcription at alternative sites and selective mRNA processing [Kwiatkowski et al., 1986; Kwiatkowski et al., 1988; Vouyiouklis and Brophy, 1997; Koepf et al., 1998]. The human gelsolin gene consists of 34 predicted exons located on chromosome 9 (Fig. 9). Preplasma gelsolin is identical in amino acid sequence to mature cytoplasmic gelsolin, except that it contains a 51-residue N-terminal extension, the first 27 residues of which constitute a signal peptide (Fig. 8A). Mature plasma gelsolin, then, is 24 amino acid residues longer at its N-terminus than mature cytoplasmic gelsolin. By convention, amino acid numbering of gelsolin sequences follows that of the mature plasma gelsolin isoform. Gelsolin-3 contains an 11-residue N-terminal extension compared to cytoplasmic gelsolin, and is mainly expressed in oligodendrocytes, lung, and testis [Vouyiouklis and Brophy, 1997].

Databases, such as Ensembl (www.ensembl.org) or the NCBI Evidence Viewer Database (http://projects.insilico.us/SpliceMiner) [Kahn et al., 2007], report several alternatively spliced gelsolin transcripts (Figs. 8B and 9), not only in humans, but also in primates, such as chimpanzee (*Pan troglodytes*), bonobo (*Pan paniscus*), and rhesus macaque (*Macaca mulatta*). In addition, western blot analysis of various tissues has suggested possible isoforms of different sizes [Vouyiouklis and Brophy, 1997; Haverland et al., 2010; Pottiez et al., 2010; http://www.proteinatlas.org]. Alternative splicing is frequently observed in the 5'-untranslated regions, suggesting possible differences in transcription control, associated mRNA binding proteins, and mRNA localization. Alternative splicing in the protein-coding regions is most common within the N-terminal extension, which is proposed to bind loosely to the surface of gelsolin [Fock et al., 2005]. Isoform g lacks the B and C' strands and the start of the C strand from G1 which removes the G1 type-2 calcium binding site and two actin-binding residues (Fig. 3). Isoform h lacks the E strand and the short helix from G1, which may be expected to affect the G1-G3 latch and the actin-binding surface on G1 (Fig. 3). Verification of the existence, and assignation of the roles, of these uncharacterized alternatively spliced isoforms has yet to be performed. However, we can speculate that the variable regions are potentially available for interaction with regulatory proteins, or that they act as localization beacons. Thus, we expect gelsolin regulation to be more complicated at the gene, transcript and protein levels than currently appreciated.

Physiological Functions of Gelsolin

The severing, capping and uncapping activities of gelsolin have an impact on numerous biological functions. Firstly, consider cytoplasmic gelsolin. Actin filaments generally are

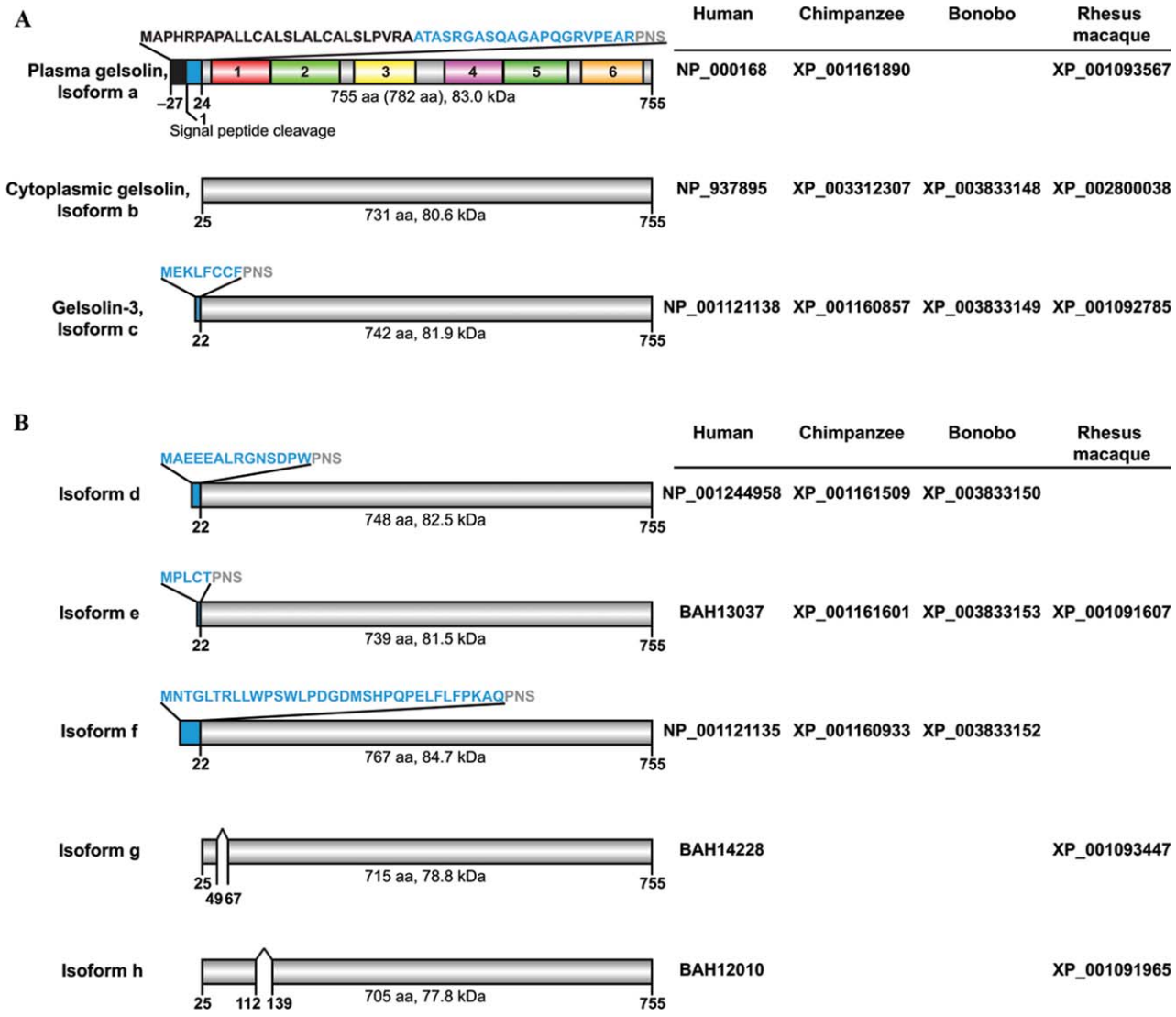

Fig. 8. Gelsolin isoforms. (**A**) The three previously published gelsolin isoforms. (**B**) Possible additional gelsolin isoforms. Plasma gelsolin shows the position of gelsolin domains 1 (red), 2 (green), 3 (yellow), 4 (pink), 5 (dark green) and 6 (orange). Accession numbers of translated cDNA clones of human, chimpanzee, bonobo and rhesus macaque origin are shown beside each isoform. Total number of amino acid residues and the molecular weight are indicated for each isoform. For plasma gelsolin the number of amino acids including the signal peptide (pre-plasma gelsolin) is shown in parenthesis. Numbering refers to residue numbers of plasma gelsolin (isoform a). Gray color refers to overlap in sequence with plasma gelsolin, except for plasma gelsolin, which shows the overlap with cytoplasmic gelsolin. Light blue refers to isoform specific sequences. Sequence of the signal peptide of plasma gelsolin is colored black.

capped in cells, preventing nonproductive polymerization (Fig. 10). On calcium or pH signaling, gelsolin becomes activated and can sever actin filaments. This results in shorter but more numerous filaments. The filaments that contact PIP_2-rich membranes are specifically uncapped, allowing elongation and exertion of force on the membrane resulting in movement. It is worth remembering that PIP_2 is an integral part of the membrane whilst the gelsolin activators, calcium ions and protons are freely diffusing close to the membrane. Hence, severing amplifies the force on the membrane through increasing the number of filament ends that can be uncapped once they encounter the PIP_2-rich membrane. At the same time, the gelsolin-severed filaments are subjected to enhanced turnover since the greater number of pointed ends increases actin depolymerization. Gelsolin-capped filaments are also targeted for dismantling by cofilin, perhaps through changing the twist of the filaments [McGough et al., 1997; Ressad et al., 1999]. The increased turnover allows rapid ADP-actin to ATP-actin monomer recycling and augments polymerization leading to movement. Taken together, gelsolin acts to heighten cell movement by increasing the pools of both ATP-actin monomers and growth-ready nuclei (Fig. 10). Calcium ion concentrations within specific cytoplasmic compartments are subject to variation, typically between about 0.1 and 10 μM. This lies in the correct range for the filament

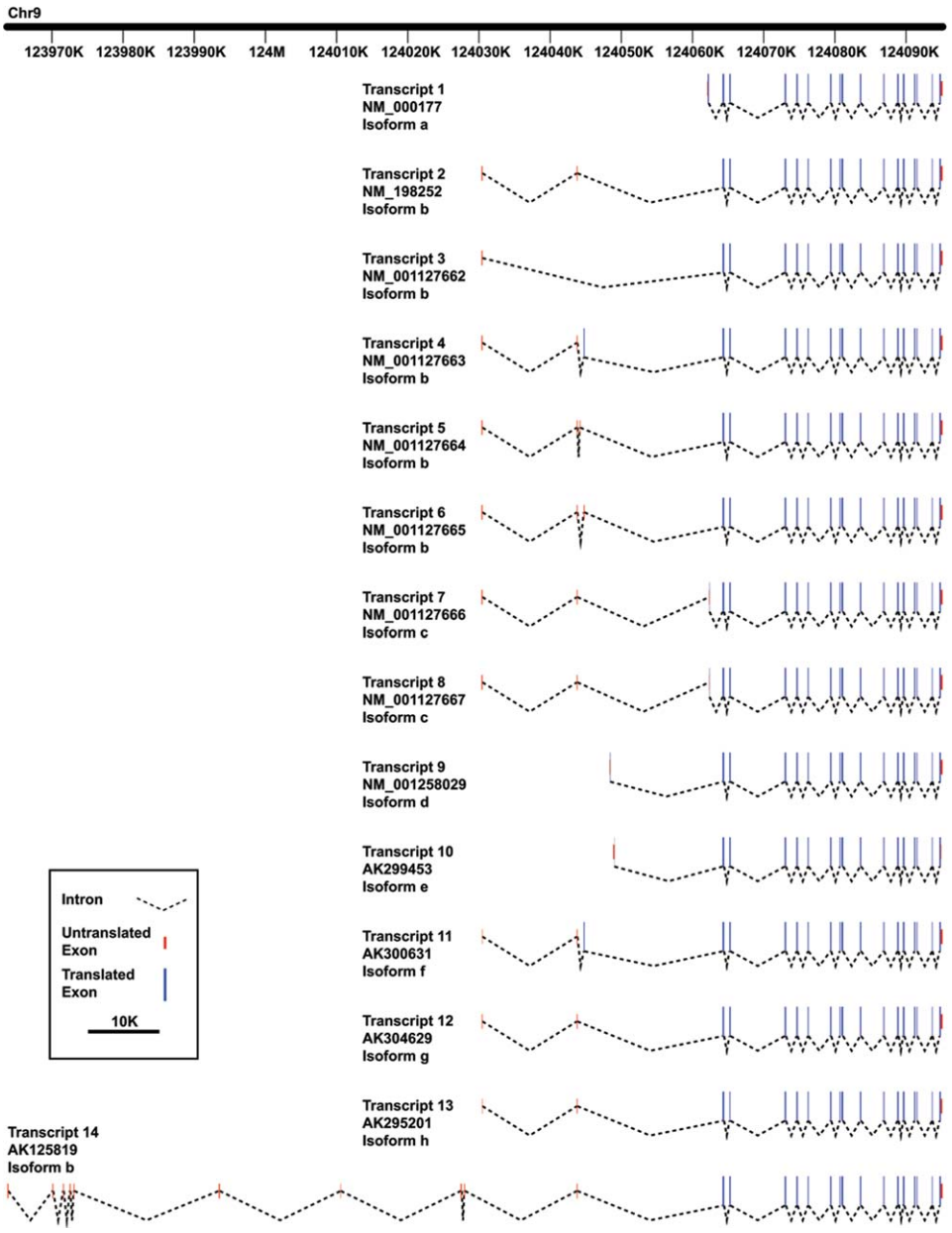

Fig. 9. Gelsolin transcripts. Accession numbers and the protein isoforms (Fig. 8) related to the mRNA transcripts are indicated. Numbering on chromosome 9 refers to Human genome build 37.1. The figure is drawn to scale using FancyGene v1.4 [Rambaldi and Ciccarelli, 2009].

severing activity over which cytoplasmic gelsolin is exquisitely regulated, from being completely inactive to being the most efficient known severer.

The wide range of defects observed in gelsolin-null mice has emphasized the important role of gelsolin in the regulation of actin dynamics in vivo. Gelsolin-null dermal fibroblasts display reduced ruffling response and translational motility, excessive actin stress fibers, and increased contractility, while gelsolin-null mice display greater susceptibility to glutamate toxicity, decreased platelet shape change during clotting and longer bleeding times [Witke et al., 1995; Furukawa et al., 1997; Azuma et al., 1998]. Changes in gelsolin expression have been observed in some invasive cancer types [reviewed in Li et al., 2012]. Several of these effects can be attributed to the severing and capping/uncapping properties of gelsolin.

Secondly, we consider the physiological implications of severing and capping by extracellular gelsolin. Extra- and intracellular concentrations of calcium ions are vastly different. In plasma, millimolar levels of calcium guarantee that plasma gelsolin will always exist in a fully activated state with respect to its actin filament severing and capping activities (Fig. 10). Plasma gelsolin, therefore, is a highly appropriate scavenger of actin filaments that are released into the circulation as a result of lysis of injured or dead cells and tissues, averting pathological blockages of the

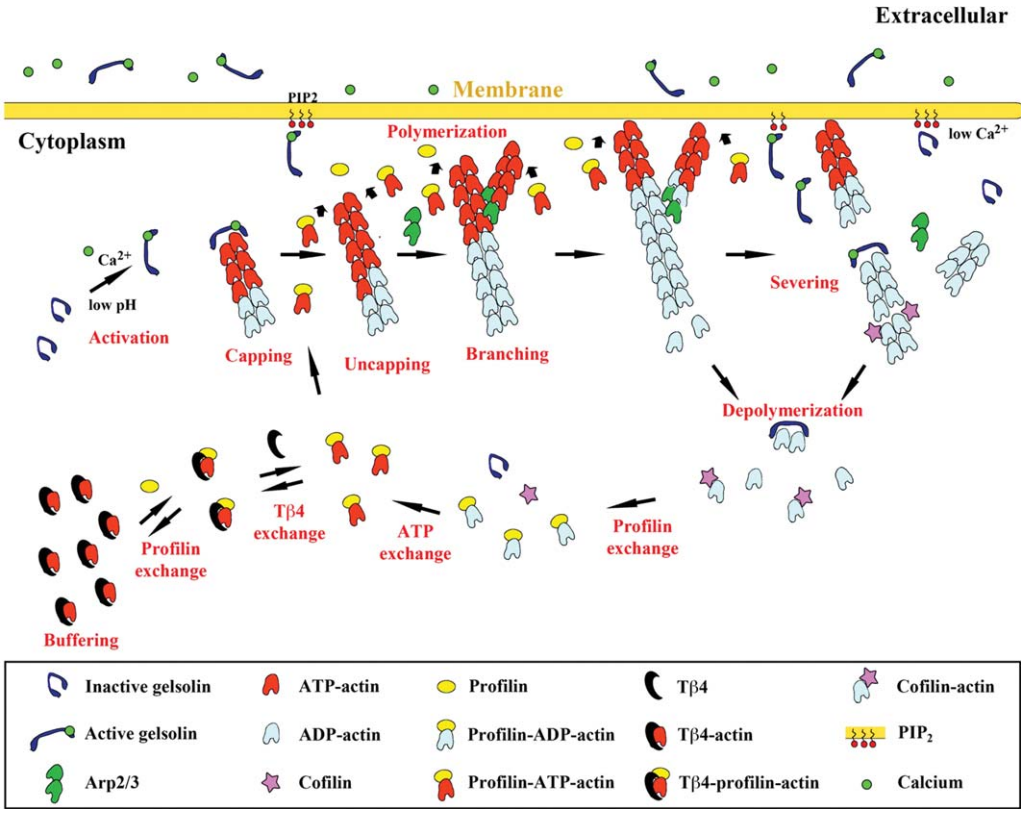

Fig. 10. The cellular and extracellular roles of gelsolin. The cartoon depicts the calcium and pH activation of gelsolin leading to capping and severing of actin filaments in the cytoplasm. Subsequent uncapping, of gelsolin and other capping proteins, leads to directed polymerization of profilin-ATP-actin at the membrane resulting in movement. Arp2/3 complex-dependent nucleation amplifies the force by increasing the number of barbed ends. Gelsolin-capped filaments further away from the membrane are subjected to pointed-end depolymerization and targeted for cofilin severing. Through profilin exchange, the ADP-actin monomers are recycled into profilin-ATP-actin for further rounds of polymerization, or stored as an ATP-actin buffer as thymosin-β4-ATP-actin. Extracellular gelsolin (above the membrane) is fully activated due to the high calcium concentrations in blood plasma. There it acts as a scavenging agent, severing any actin filaments that are released from cell rupture. The figure is based on the dendritic nucleation model of cell movement [Pollard et al., 2000].

microvasculature [Vasconcellos and Lind, 1993]. In the extracellular environment, uncapping and resulting filament growth would be detrimental and are unlikely to occur. Hence, the roles of gelsolin are modulated relative to the location.

The Gelsolin Superfamily

Gelsolin superfamily proteins contain multiple copies of the gelsolin domain (Fig. 2A). Divergent evolution among the constituent domains, along with fusion with nongelsolin-type N- and/or C-terminal domains, has resulted in a variety of actin regulatory functions and has ensured that different gelsolin superfamily proteins respond in different ways to cellular cues. Furthermore, as a consequence of differential expression, gelsolin superfamily proteins have roles in a diversity of cellular processes. Gelsolin is expressed in a range of tissues and particularly strongly in the uterus (Table I).

Adseverin (previously known as scinderin, *SCIN*) is the closest relative of gelsolin, sharing with it 60% sequence identity. Adseverin is strongly expressed in endocrine tissues and skin (Table I) [Sakurai et al., 1990]. First identified in bovine adrenal medulla, adseverin's principal function is to disintegrate the cortical actin network of chromaffin cells in a calcium-dependent manner to allow secretory vesicles to access the plasma membrane, and disgorge their contents by exocytosis [Maekawa and Sakai, 1990; Rodriguez Del Castillo et al., 1990; Dumitrescu Pene et al., 2005]. Adseverin has also been shown to be involved in the regulation of chondrocyte differentiation [Nurminsky et al., 2007], and overexpression of adseverin has been suggested to be the cause of cisplatin-resistance in bladder cancer [Miura et al., 2012]. The actin regulatory functions, domain architecture, and distribution of various functional motifs of adseverin mirror those of gelsolin [Sakurai et al., 1991; Lejen et al., 2002; Chumnarnsilpa et al., 2009], except that adseverin lacks the C-terminal helical tail that plays a significant structural role in calcium regulation of gelsolin (Figs. 3 and 5A). As a consequence, adseverin is referred to as "tailless gelsolin" and since there is no need to release a tail latch, adseverin activation is regulated by only two latches [Lueck et al., 2000]. However, while gelsolin is activated by

Table I. Expression of Gelsolin Superfamily Proteins in Human Tissues.

	Brain	Blood	Liver	Pancreas	GI-tract	Lung	Kidney	Uterus	Placenta	Testis	Skin
CAPG	–	**	–	–	–	***	**	–	–	–	**
SCIN	*	*	*	*	**	–	**	–	**	*	***
GSN	**	**	–	**	**	**	**	***	*	–	**
FLII	**	***	***	**	***	***	***	***	***	***	***
AVIL	*	*	*	*	***	*	*	*	*	*	*
VIL1	*	–	*	–	***	**	***	–	–	–	–
VILL	–	–	–	–	***	*	–	–	–	*	–
SVIL	***	**	**	**	**	***	**	**	**	***	**

	Spleen	Urinary bladder	Smooth muscle	Skeletal muscle	Heart muscle	Gall bladder	Thyroid gland	Adrenal gland	Parathyroid gland
CAPG	*	**	–	–	–	–	**	–	*
SCIN	–	**	–	–	*	**	***	***	**
GSN	**	–	*	–	–	–	–	*	–
FLII	**	***	**	**	**	***	**	***	**
AVIL	n/a	n/a	–	***	–	n/a	–	–	n/a
VIL1	n/a	–	–	–	–	***	–	–	–
VILL	n/a	**	–	*	*	***	–	–	–
SVIL	*	**	**	**	**	**	**	**	*

Data are collated from The Human Protein Atlas (http://www.proteinatlas.org), The UCSC Human Gene Sorter (http://genome.ucsc.edu/), Genatlas (http://genatlas.medecine.univ-paris5.fr/), and Sakurai et al., 1990. Tissue distribution experiments involved, for the most part, immunoblotting, immunohistochemistry, or immunofluorescence detection and may, with the specific antibodies used, falsely exclude certain low expressing tissues or certain protein isoforms. Stars indicate the relative levels of protein expression. n/a, data not available.

the cooperative binding of calcium to the N- and C-terminal halves, the activation of adseverin is dominated by calcium-binding to the N-terminal half [Nag et al., 2009; Chumnarnsilpa et al., 2009]. In addition, while gelsolin function is inhibited by PIP_2 and, to a lesser extent, phosphatidylinositol 4-monophosphate (PIP), adseverin is also inhibited by phosphatidylinositol and phosphatidylserine in addition to PIP_2 and PIP [Janmey et al., 1987; Janmey and Stossel, 1987; Maekawa and Sakai, 1990]. Protein databases report three possible isoforms of adseverin (Fig. 11B). Isoform 2 is missing domains 1 and 2. This isoform is likely to have impaired or nonexistent severing ability. Isoform 3 includes an insertion between the D strand and long helix of domain 1 (Figs. 3 and 11B).

Villin (*VIL1*), a 93 kDa tissue-specific gelsolin superfamily protein, first isolated from microvilli of brush border epithelial cells, shares 50% sequence homology with gelsolin, and has a similar proteolytic cleavage pattern [Janmey and Matsudaira, 1988]. Aside from the gastrointestinal tract, villin also shows high expression levels in the gall bladder and kidney (Table I). Villin has been shown to regulate epithelial cell morphology, actin reorganization, apoptosis, cell motility and wound healing of enterocytes [Tomar et al., 2004, 2006; Athman et al., 2005; Wang et al., 2007, 2008, Ubelmann et al., 2013]. Expression of villin is frequently lost, or down-regulated, in poorly differentiated colon cancer [Arango et al., 2012]. Like gelsolin and adseverin, villin comprises six gelsolin-like domains, but has an additional C-terminal domain called the headpiece (Figs. 2A and 5C), which is capable of binding F-actin [Bretscher and Weber, 1980; Matsudaira et al., 1985; Hesterberg and Weber, 1986; Arpin et al., 1988; Bazari et al., 1988]. The headpiece affords villin the ability to bundle F-actin filaments [George et al., 2007], a function not exhibited by gelsolin. The headpiece and sequence variations within the gelsolin-like domains result in differences in the mechanisms of activation of these two proteins. In particular, a calcium-dependent hinge mechanism exposes the villin headpiece during activation [Hesterberg and Weber, 1983]. This calcium-dependent activity may be in part due to the tail-latch-like helix that resides between domain 6 and the headpiece. Villin has been determined experimentally to contain six internal calcium-binding sites, with two sites located within domain 1 (V1), being responsible for F-actin capping and severing [Northrop et al., 1986; Kumar et al., 2004]. Sequence homology indicates that all eight calcium-binding sites are present and all of the actin-binding surfaces found in gelsolin are present (Fig. 3). However, in contrast to

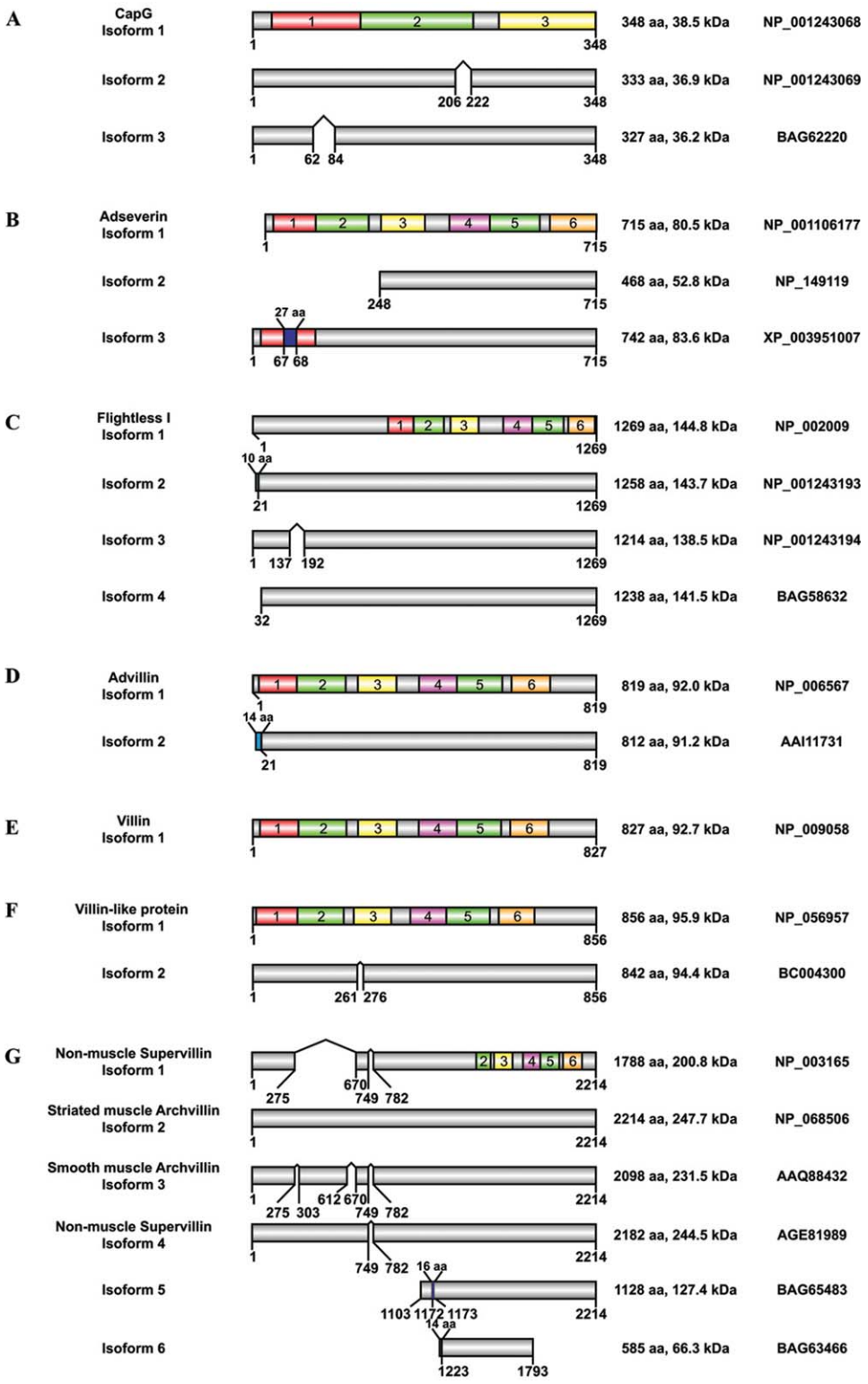

Fig. 11. Known and possible isoforms of gelsolin superfamily proteins. (A) CapG, **(B)** adseverin, **(C)** flightless I, **(D)** advillin, **(E)** villin, **(F)** villin-like protein and **(G)** supervillin. Each isoform 1 shows the position of gelsolin domains 1 (red), 2 (green), 3 (yellow), 4 (pink), 5 (dark green) and 6 (orange). Positions of the splices are shown according to the amino acid number of isoform 1 on each protein, except for supervillin, where the numbering is according to the longer isoform 2. Insertions are shown in dark blue and N-terminal splices in light blue, with the insertion size in number of amino acids above. The length in number of amino acids, molecular weight and accession number of the human protein sequence for all proteins, except for adseverin isoform 3 and supervillin isoform 3, which are of *Pan troglodytes* (chimpanzee) and *Mustela putorius furo* (ferret) [Gangopadhyay et al., 2004] origin, respectively, are shown. Villin-like protein isoform 2 shows the accession number of the human DNA sequence, as the corresponding protein accession number does not show the full protein sequence.

gelsolin, villin requires micromolar to millimolar levels of calcium to function. Tyrosine phosphorylation lowers this threshold to the nanomolar range, suggesting that tyrosine phosphorylation may be the primary mode of regulation of villin activity under physiological conditions [Kumar and Khurana, 2004]. Only one isoform of villin has been reported in protein databases so far (Fig. 11E).

Advillin (*AVIL*), a 92 kDa gelsolin superfamily protein, was first identified from a murine brain cDNA library, and is so named because of its high degree of identity to adseverin and villin, 47 and 59%, respectively. The domain architecture of advillin is identical to that of villin [Marks et al., 1998]. Although these two proteins are least similar within the C-terminal headpiece, the seven residues of the villin headpiece, which are critical for binding and bundling actin (Fig. 5C) [Doering and Matsudaira, 1996; Markus et al., 1997], are conserved in advillin. Advillin contains all eight calcium-binding sites, homologous actin-binding surfaces to gelsolin, and a potential tail-latch (Figs. 3 and 5A). Expressed in neurons, advillin has been implicated in neurite growth and morphogenesis, and in axon regeneration [Shibata et al., 2004; Hasegawa et al., 2007]. High expression levels are present in the gastrointestinal tract and skeletal muscle (Table I). Two possible isoforms of advillin, differing at their N-termini, are reported in protein databases (Fig. 11D).

Villin-like protein (*VILL*; not to be mistaken for villin-2, also known as ezrin or cytovillin, which is a protein belonging to the ezrin, radixin and moesin family) is a gelsolin superfamily protein first identified by DNA sequencing along a region of chromosome 3p22-p21.3 [Ishikawa et al., 1997]. It shares the same domain architecture as villin and advillin [Marks et al., 1998], with 47 and 44% amino acid identity, respectively, and is similarly highly expressed in the gastrointestinal tract and gall bladder (Table I). Northern blot analysis of villin-like protein transcripts reveals two ubiquitously expressed transcripts of different sizes that might correspond to two isoforms reported in protein databases (Fig. 11F) [Ishikawa et al., 1997]. Isoform 2 has a deletion of the B strand in domain 3. Deletion of this region in gelsolin would be expected to affect the interface between G2 and G3 in the activated state and thus have implications for filament recognition (Fig. 3). The villin-like protein gene is one of the genes deleted in mice carrying the oligotriche mutation (*Del(9Ctdspl-Slc22a14)1Pas*), a deletion on murine chromosome 9 leading to male infertility, suggesting the villin-like protein could be involved in spermatogenesis [Runkel et al., 2008]. The human villin-like protein gene is located in the deletion "hot spot" Alu-PCR clone 20 (AP20) region at the telomeric border of the 3p21.3 locus [Protopopov et al., 2003], which has been shown to be deleted in cervical, renal, lung, and breast carcinomas [Ishikawa et al., 1997; Senchenko et al., 2003, 2004]. This gene has recently also been shown to be strongly down-regulated in cervical squamous cell carcinomas [Senchenko et al., In press]. Villin-like protein lacks the calcium-binding sites in domain 1, and may have compromised calcium binding by domains 4 and 5, but sequence alignment suggests that the actin-binding sites are present (Fig. 3). Thus, the mechanism of activation of villin-like protein may be distinct from villin and advillin.

Supervillin (*SVIL*) is an unusual ~200-kDa gelsolin superfamily protein isolated from bovine neutrophil plasma membranes [Pestonjamasp et al., 1997; Wulfkuhle et al., 1999]. Except for the canonical nonmuscle 200 kDa isoform 1 there are also three other known isoforms of supervillin: the striated muscle isoform 2, also named archvillin [Oh et al., 2003], the smooth muscle archvillin isoform 3 [Gangopadhyay et al., 2004], and the larger nonmuscle isoform 4 (Fig. 11G) [Fang and Luna, 2013]. Potential isoform 6 consists mainly of the gelsolin-like domains 2–4 being truncated after the C strand of domain 4. Such a design would ablate any vestigial actin binding by domain 4 and prevent the formation of a gelsolin-like inactive structure (Fig. 3). Supervillin's amino acid sequence indicates the presence of five gelsolin-like domains that correspond to G2–G6 of gelsolin, with a villin-like headpiece at its C-terminus, and a unique intrinsically disordered N-terminal domain [Fedechkin et al., In press] that contains four nuclear localization signals [Wulfkuhle et al., 1999]. Detailed domain analysis suggests that contrary to expectations, the gelsolin-like C-terminal half binds F-actin weakly, that the villin-like headpiece lacks F-actin binding altogether, and that F-actin binding and actin filament bundling activities are localized to the novel disordered N-terminal region [Wulfkuhle et al., 1999; Vardar et al., 2002; Fedechkin et al., In press]. The weak actin binding is consistent with the lack of conservation of actin-binding surfaces present in gelsolin. Only domains 3 and 4 display some semblance of actin-binding motifs (Fig. 3). Supervillin only has the conserved type-2 calcium-binding sites in domains 2 and 4, and a compromised site in domain 3. The type-1 calcium-binding site is present in domain 4, further suggesting that this may be a functional actin binding domain. Supervillin forms links between membranes and the actin cytoskeleton, and participates in myogenesis [Oh et al., 2003], cytokinesis [Smith et al., 2010], cell-substrate adhesion of platelets and tumor cells [Takizawa et al., 2006; Edelstein et al., 2012], cell spreading [Takizawa et al., 2007], motility [Fang et al., 2010], invadosome function in macrophages and tumor cells [Crowley et al., 2009; Bhuwania et al., 2012], transactivation of the androgen receptor [Sampson et al., 2001; Ting et al., 2002] and cytoarchitecture at both the nucleus and plasma membrane [Wulfkuhle et al., 1999; Chen et al., 2003]. Recently, supervillin has also been shown to regulate cell survival through the control of p53 levels [Fang and Luna, 2013]. Supervillin is expressed in a wide variety of tissues (Table I).

Flightless I (*FLII*) was originally identified in a *Drosophila melanogaster* mutant with flight defects [Campbell et al., 1993]. Flightless I comprises six gelsolin-like domains at its

C-terminus, and an N-terminal leucine-rich repeat (LRR) domain. Sequence analysis reveals that flightless I retains the gelsolin-like actin-binding surfaces, but the calcium-binding sites are missing in domains 1–4 (Fig. 3). LRR proteins are involved in protein:protein interactions and the flightless I LRR is speculated to be involved in Ras signal transduction [Claudianos and Campbell, 1995; Silacci et al., 2004]. Flightless I plays an essential role in the development of mice, with deficiency resulting in embryonic lethal mutants [Campbell et al., 2002], and with aberrations in the gene resulting in developmental abnormalities in humans and *Drosophila melanogaster* [Campbell et al., 1993; Chen et al., 1995; Straub et al., 1996]. Flightless I is also a negative regulator of wound healing [Cowin et al., 2007], and has been shown to regulate cell migration through localization to focal adhesions [Mohammad et al., 2010]. Flightless I is a coactivator for nuclear receptor-mediated transcription [Lee et al., 2004; Jeong et al., 2009], interacts with β-catenin to inhibit β-catenin-mediated gene transcription [Lee at al., 2006], and may interact with the oncogene Adenomatous Polyposis Coli [Song et al., 2012], suggesting a regulatory role in the Wnt signaling pathway. In vitro, flightless I binds monomeric actin, but conflicting data exist as to whether flightless I exhibits filament-severing activity [Liu and Yin, 1998; Goshima et al., 1999; Mohammad et al., 2010]. Databases report four possible isoforms of flightless I (Fig. 11C), none of which would likely possess significantly altered gelsolin-like domains. Flightless I is highly expressed in a wide variety of tissues (Table I).

CapG (*CAPG*; also known as macrophage-capping protein), the only three-domain member of the mammalian gelsolin clan, was first isolated from alveolar macrophages and shown to be homologous with the N-terminal half of gelsolin [Southwick and DiNubile, 1986]. However, divergence in the sequence of two short stretches of amino acid sequence of CapG, relative to gelsolin, is reported to essentially eliminate its ability to sever actin filaments, although it is an efficient barbed end capper [Southwick, 1995]. Sequence analysis suggests that CapG may adopt an inactive form similar to gelsolin domains G1–G3, and on activation present similar actin-binding surfaces. However, the type-1 calcium-binding site is missing, which may compromise its ability to sever filaments (Fig. 3). CapG expression is high in tissues in which macrophages are found (Table I). CapG is present in both the nucleus and the cytoplasm [Onoda et al., 1993; Hubert et al., 2008; Hubert et al., 2009] and has roles in cell differentiation, membrane ruffling, phagocytosis and cell motility [Onoda et al., 1993; Parikh et al., 2003; Sun at al., 1995]. CapG has recently also been shown to be involved in cell invasion and metastasis, being highly overexpressed in several metastatic cancers. [De Corte et al., 2004; Renz et al., 2008; Kang et al., 2010; Wu et al., 2011; Morofuji et al., 2012; Davalieva et al., 2012; Kimura et al., 2013; Ha et al., 2013]. Databases report three different isoforms of CapG (Fig. 11A). Isoform 2 has lost the D strand and part of the short helix from domain 2, whereas isoform 3 is missing the end of the D strand and the actin-binding portion of the long helix from domain 1 (Fig. 3).

As demonstrated in the above discussions, gelsolin superfamily proteins exploit the gelsolin fold for a range of biological functions and respond to varied stimuli across a range of cell types.

Gelsolin Superfamily Proteins: Cellular Function and Regulation

Although a comprehensive study of the course of evolution of the gelsolin superfamily proteins has not been reported, and the causes of their diversity are poorly understood, it seems reasonable that diversification of their cellular functions should be based on subtleties in how they modulate cytoskeletal dynamics. Hence, it is not remarkable that the cellular processes in which the gelsolin superfamily proteins participate are as varied as the functions of actin. These include: cell motility, ion-channel regulation, cell signaling, apoptosis, phagocytosis, secretion, platelet activation and modulation of phospholipase C activity [reviewed in Kwiatkowski, 1999; Sun et al., 1999; Trifaro et al., 2002; Silacci et al., 2004]. More recently, nuclear hormone receptor-mediated signaling [reviewed in Archer et al., 2005], wound repair [Kopecki et al., 2009], cell morphology, cell invasion, cell migration, epithelial-to-mesenchymal transition [reviewed in Khurana and George, 2008] and regulation of podosomes and cadherin junctions [reviewed in Chellaiah, 2006; El Sayegh et al., 2007], have made it on to the growing list of cellular functions that involve gelsolin superfamily proteins. Since all of these processes require precise regulation, it is not surprising that altered expression or misregulation of gelsolin superfamily proteins have been associated with an array of diseases and conditions that range from inflammation to cancer [reviewed in Spinardi and Witke, 2007; Bucki et al., 2008; Li et al., 2012].

The participation of gelsolin superfamily proteins in a wide range of cellular processes, and their abilities to influence actin dynamics in multiple and sometimes contradictory ways, require tight regulation. Factors that modulate gelsolin superfamily protein functions beyond calcium concentration, pH, phospholipids, alternative splicing and post-translational modification include the interaction with various proteins [Sun et al., 1999; McGough et al., 2003]. Interactive partners that regulate gelsolin superfamily protein function include actin-binding proteins such as tropomyosin and caldesmon, GTPases such as N-Ras, viral oncoproteins, pathogenic invasion proteins such as SipA, and bacterial endotoxins [Dabrowska et al., 1996; Cossart, 2004; Ramakrishnan et al., 2004; Takiguchi and Matsumura, 2005; Bucki et al., 2005; Keller et al., 2007; Osborn et al., 2007; Gunning et al., 2008; Lindberg et al., 2008].

Thus, in general, gelsolin superfamily proteins are controlled by a number of stimuli within different cell types in order to harness the actin-regulating properties for particular biological processes.

Conclusions

Over the years, much research has been devoted to understanding the calcium-dependent activation of gelsolin and the activities of gelsolin and its superfamily members as they pertain to regulation of actin filament dynamics. However, it is becoming increasingly evident that gelsolin superfamily proteins are subject to control by a number of additional factors that include post-translational modification, transcriptional and epigenetic regulation, and interactions with small molecules and other proteins. In addition, the structure of actin, which itself is subject to modulation by a host of actin-binding proteins, influences the activities of gelsolin and other gelsolin superfamily proteins. To fully understand the function and regulation of gelsolin superfamily proteins in vivo, and their roles in various pathologies, and to take advantage of their potential as biomarkers or therapeutic targets, it is necessary to fill the gaps documented in this review, keeping in mind that gelsolin superfamily members are but small cogs in a complex machine that is subjected to feedback from a myriad of sometimes unexpected sources.

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