Deconstructing gelsolin: identifying sites that mimic or alter binding to actin and phosphoinositides

Paul A Janmey, Thomas P Stossel and Philip G Allen

Gelsolin is involved in cytoskeletal remodeling as it can fragment and guide reassembly of actin networks. Recent advances in defining the structure of gelsolin identified functionally important sites. These structural insights could lead to the design of small molecule analogs to enhance, inhibit or mimic the functions of gelsolin.

Address: Experimental Medicine Division, Brigham and Women's Hospital, 221 Longwood Avenue, Boston MA 02115, USA.

Correspondence: Paul A Janmey
E-mail: janmey@calvin.bwh.harvard.edu

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Introduction

Mutational analyses and studies of cell function have placed the actin-binding protein gelsolin in several clinically important settings, and the recently elucidated gelsolin structure [1,2] indicates sites on the protein that are potential targets for designing therapeutic agents. Gelsolin is an 84–87 kDa single polypeptide closely related to actin-binding proteins expressed in a wide variety of organisms (Figure 1). Mammalian gelsolin is expressed as both an intracellular (84 kDa) and secreted (87 kDa) protein by alternative splicing of a single gene [3]. *In vitro*, gelsolin has multiple effects on monomeric and polymeric actin that suggest gelsolin functions in the cell to fluidize the cortical actin cytoskeleton, and to allow selective growth of filaments at sites where gelsolin is inactivated [4].

The functions of gelsolin in modulating the actin cytoskeleton and some of its potential regulatory elements are shown in Figure 2. Gelsolin can bind two actin monomers to form nuclei to seed polymer growth. It also binds tightly to the fast growing end of actin filaments (F-actin) to prevent their growth and can bind at random to the actin filament and sever it. Many of these functions are activated *in vitro* by greater than micromolar concentrations of Ca^{2+} or pH < 6, and inactivated by inositol phospholipids. Actin, calcium ions and phosphoinositides are the most extensively characterized cellular ligands for gelsolin, although binding to tropomyosin, phospholipase C-γ (PLC-γ), phosphatidylinositol 3-kinase (PI 3-kinase) and phospholipase D (PLD) [5] have also been reported. In the cytoplasm, gelsolin may function by regulating the actin-based cytoskeleton [6] or by altering phosphoinositide turnover [7]. Ligands and regulators of gelsolin are summarized in Table 1.

The viability of gelsolin-null animals shows that any essential function of gelsolin can be performed by other factors, although the animals do have prolonged bleeding times, caused by altered platelet activation, and an impaired inflammatory response [8]. The sluggish cell activation *in vivo* is consistent with a relationship between

Figure 1

Gelsolin family members are widely expressed in eukaryotes. Individual species can express several gelsolin family members that can be restricted to specific cell types and developmental stages or co-expressed in the same cell type. The presence of gelsolin family members in plants, animals and unicellular organisms, indicated that the gelsolin family probably originated with the earliest eukaryotes. The names of gelsolin-related proteins are highlighted in red, and the organisms in which they are found are in black.

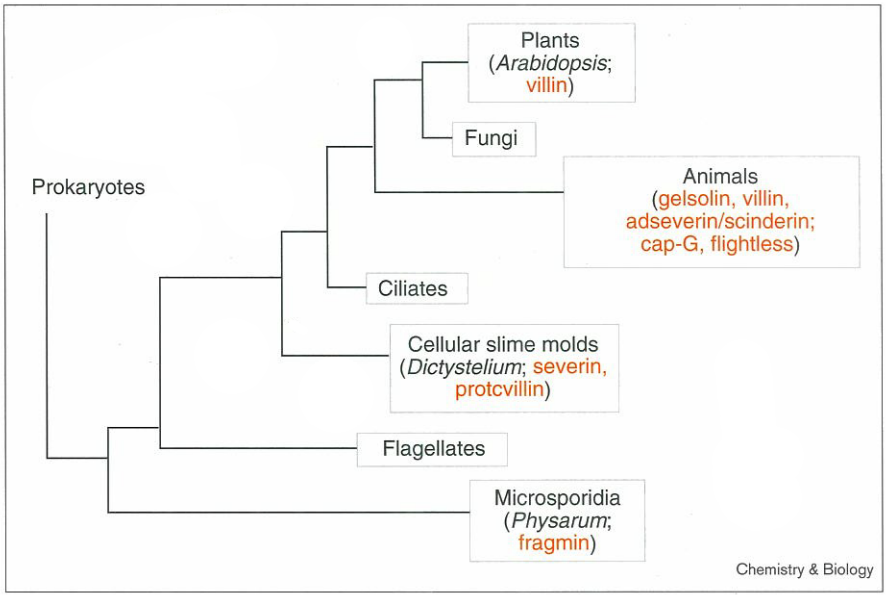
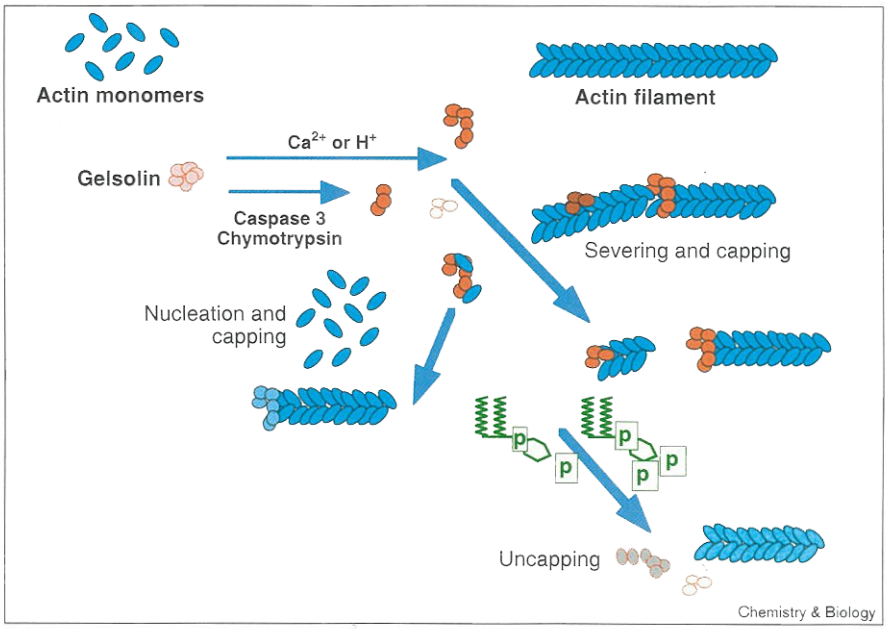


Figure 2



Actin binding and regulatory events during gelsolin-mediated cytoskeletal remodeling. Gelsolin (light red) is activated either by Ca^{2+} or H^+ (pH < 7) to reveal actin-binding sites, or by proteases that cleave inhibitory domains to release active protein fragments (solid red) that interact with both monomeric or polymeric actin (blue). Polyphosphoinositides (green) phosphorylated either at the 3′ or 4′ position of the inositol ring inactivate gelsolin and expose the barbed end of the actin filament.

experimentally altered gelsolin expression and cell motility *in vitro* [6,9] and the placement of gelsolin downstream of rac in signaling for cytoskeletal changes [10].

Gelsolin and disease

Extracellular gelsolin is thought to function as part of a scavenging system involved in clearing actin released from cells into the blood upon injury or trauma [11]. The exogenous administration of gelsolin could, potentially, soften pathologically stiff materials such as cystic fibrosis sputum or actin-containing blood clots. An amyloid disease, Finnish familial amyloidosis (FAF), characterized by cranial neuropathy and corneal lattice dystrophy, is caused by a point mutation in gelsolin. The mutation of Asp187→Asn allows a single intracellular proteolytic event as the protein is targeted for secretion, allowing a further cut in the blood plasma to form an amyloidogenic fragment (see [12] for a recent reference).

Intracellular gelsolin is a prominent target of caspase 3, suggesting gelsolin is involved in apoptosis, although in different contexts gelsolin can either promote [13] or inhibit [14] the onset of cell death. Neurons from mice lacking gelsolin are also more susceptible to excitotoxic cell death caused by increased influx of calcium through *N*-methyl D-aspartate (NMDA) receptors and voltage-dependent calcium channels [15].

In some types of human cancers decreased gelsolin expression correlates with cell transformation. A relationship between gelsolin expression and human cancers was proposed by Chaponnier and Gabbiani [16] on the basis of the absence of detectable gelsolin immunoreactivity in epithelial cells of breast carcinoma. Gelsolin is among the most prominently downregulated proteins in transformed

Table 1

Ligands and modulators of gelsolin.

Ligand	Function
G-actin	Binds to domains 1 and 4 to nucleate actin assembly
F-actin	Severs filaments and blocks the barbed end
Ca^{2+}	Binds at multiple sites to stimulate activity
Inositol lipids	Bind to domains 1 and 2 to prevent actin binding; preferential binding to PI(3,4)P2; gelsolin–PIP3 complex isolated from osteoclasts
pH	Activates in absence of Ca^{2+}
Caspase 3	Cleaves to activate severing
Fibrin/fibronectin	Possible concentration in clots
ATP	ADP–actin binds faster to gelsolin than ATP–actin; possible direct binding to gelsolin
Tropomyosin	Direct binding *in vitro*; in conjunction with caldesmon inhibits severing and allows reannealing of filaments
Phospholipase C-γ	Inhibits activity; thrombin activation dissociates gelsolin–PLC complex in platelets
Phospholipase C-β	Gelsolin-derived peptide stimulates activity
Phospholipase D	Stimulates activity
PI 3-kinase	Stimulates activity
c-Src	Phosphorylates gelsolin/PIP2
rac	Dissociates gelsolin/actin in cell extracts
DNase	Activates DNase/actin

human fibroblasts and epithelial cells, breast cancers in several species, bladder carcinoma and in late-stage, non-small cell lung cancers (see [17] for a recent reference). Reversion of the transformed phenotype of ras-transformed cells is accomplished by the overexpression of a His321 mutant form of gelsolin that has different effects to wild-type gelsolin on both actin and phospholipase function *in vitro* and appears to have tumor-suppressive activity [18]. Reversion to a more spread morphology with enhanced stress fiber formation by the Src-like kinase inhibitor radicicol was also accompanied by strong expression of gelsolin [19], a finding that may relate to the observation that gelsolin is phosphorylated by c-Src in a phosphatidylinositol 4,5-bisphosphate (PIP2)-dependent manner *in vitro* [20].

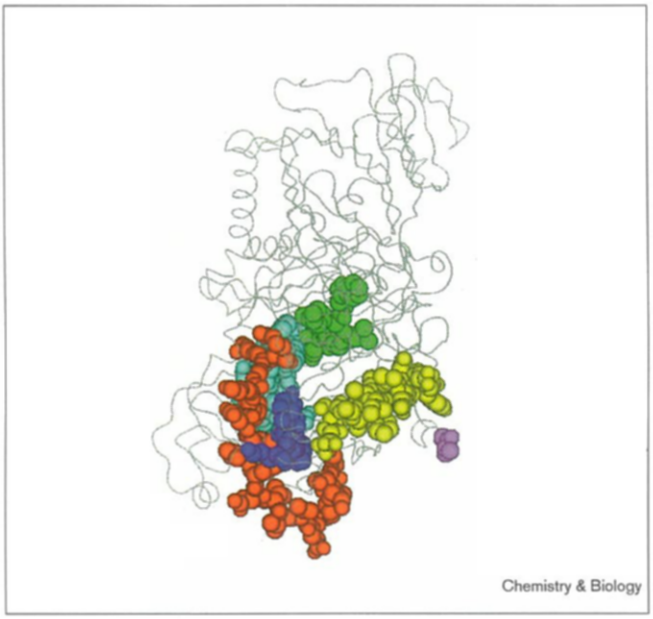
Gelsolin structure

Recent elucidation of the tertiary structure of gelsolin by Burtnick *et al.* [2] and of a complex of actin with a gelsolin fragment by McLaughlin *et al.* [1] provides insight into how this multifunctional protein interacts with actin and suggests sites where competing ligands may be targeted to alter selectively some of these functions for possible therapeutic benefits in thrombolytic or inflammatory disorders. Sequence analysis suggests that gelsolin consists of six similar domains, and the domains are evident in the crystal structure. Biochemical analyses of proteolytic fragments and expressed protein truncates demonstrate that domains 1 and 4 can bind actin monomers with high affinity, domain 2 is necessary for binding to the sides of filaments, and truncation of the carboxy-terminal residues of domain 6 causes a loss of calcium sensitivity (see [21] for recent references). Domains 1 and 2 contain the minimal residues necessary for actin filament severing and regions within domains 2–6 are necessary for nucleation activity. Localization of domains necessary for the generation of a high affinity actin filament cap is more complicated. Constructs containing domain 2 of gelsolin strongly inhibit depolymerization but not elongation from the barbed end of the actin filament, whereas both amino-terminal and carboxy-terminal truncates prevent monomer addition to filaments.

Regulatory sites for Ca^{2+} and inositol lipids

Gelsolin requires micromolar concentrations of calcium to interact with actin. Analysis of the calcium sensitivity of gelsolin truncates demonstrates that domain 6 is necessary for normal calcium regulation. As illustrated in Figure 3, a carboxy-terminal truncate lacking the residues beyond amino acid 732, (green in Figure 3) is no longer Ca^{2+} dependent, but retains all actin-binding functions present in the intact protein. Part of the carboxyl terminus of domain 6 (residues beyond 738) forms a helix that contacts the actin-binding helix in domain 2 (blue), but the carboxy-terminal helix can be removed without loss of Ca^{2+} sensitivity. This result demonstrates that although the carboxy-terminal helix may be involved in calcium regulation of gelsolin function, it is not functioning alone. This finding is consistent with many studies indicating that gelsolin has two or possibly more calcium-binding sites involved in regulating activity [22].

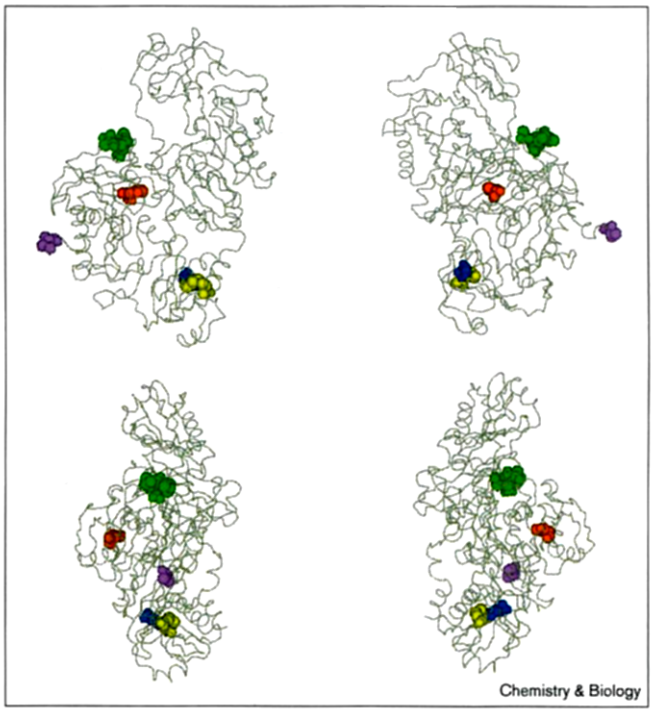
Figure 3



Structure of gelsolin with functional and regulatory domains. The amino-terminal amino acid is highlighted in purple. The actin-binding helix of domain 1 is yellow, the actin-binding helix of domain 2 is cyan, the PPI-binding peptides are identified in dark blue (amino acids 135–142) and red (amino acids 150–169), and the carboxy-terminal sequences necessary for calcium regulation are green. The space-filling model shows the region required for calcium regulation of severing.

Polyphosphoinositides (PPI), especially products of the PI 3-kinase pathway, are potent inhibitors of gelsolin activity, but gelsolin also alters phosphoinositide turnover *in vivo* and selectively affects inositol lipid remodeling *in vitro*, activating PI 3-kinase and PLD, and inhibiting PLC-γ and the erythrocyte PIP kinase. Gelsolin could modulate PPI metabolism either by competing with the enzymes of PPI synthesis and degradation, or by generating specific domains of lipids necessary for normal enzyme function. Two sequences implicated in phosphoinositide binding are at the junction between domains 1 and 2 (Figure 3). Residues 135–142 at the end of domain 1 and the residues 160–169 at the beginning of domain 2 of gelsolin have been identified as key components of gelsolin–phosphoinositide interface. The primary structures of these two PIP2-binding site are similar to sites in other proteins such as PLC-β, PLC-γ, and β-adrenergic receptor kinase [23]. As shown in Figure 3, these two domains are closely aligned, extended structures in the gelsolin crystal. In solution, PPIs induce a coil–helix transition in a peptide encompassing

Figure 4



Regions of interest in the gelsolin structure. These four orientations highlight various alterations in gelsolin structure or sequence, and differ in orientation by a 90° rotation around the Z-axis. For reference, the amino-terminal amino acid is purple. The region of proteolysis by caspase 3 is green. The cysteine residues involved in the disulfide bond are yellow and Asp187, which is mutated in Finnish type amyloidosis, is blue. Red identifies the histidine mutated to proline in revertants of ras-transformed cells.

the PPI-binding sequence in domain 2 [24], suggesting that the effects of PPIs on gelsolin may involve a similar change in secondary structure that would alter or obscure nearby actin-binding sites.

Possibilities for modulating or enhancing gelsolin function

Inhibiting gelsolin

Actin-filament severing is postulated to be a key function of gelsolin for initiating some types of cell protrusion such as membrane ruffling, and this activity requires gelsolin domains 1 and 2. As gelsolin minus platelets and neutrophils are functional but activate more slowly than normal cells, targeting the sites on gelsolin necessary for function could provide a means to reduce inflammation or decrease pathological platelet activation. Many of the contacts present in the complex of gelsolin domain 1 and actin involve the long helix including residues 100–113 of gelsolin [1] (Figure 3, yellow) and include the sequence Leu–Asp–Asp–Tyr–Leu (LDDYL), a sequence found in many actin-binding proteins. Peptide sequences derived from domain 1 of gelsolin alter actin polymerization *in vitro* [25]. Presumably these peptides compete with gelsolin and other actin-binding proteins for actin, and are unlikely to be selective antagonists for gelsolin. Agents that bind to the helix in domain 1 of gelsolin and prevent interactions with actin may be expected to interfere more selectively with gelsolin's severing and monomer-binding activities.

Similarly, the helix within domain 2 is postulated to target gelsolin to the side of the actin filament but the helix is not absolutely necessary for gelsolin's severing activity, as a construct consisting of domain 1 and the first ten amino acids of domain 2 (gelsolin 1–160) can also sever F-actin. The construct has much slower severing kinetics than the full length molecule or truncates including domains 1–3, however. Domain 2 of gelsolin is necessary for both severing and nucleating activities, and agents that interfere with its binding to the actin filament would be expected to be strong inhibitors of gelsolin–actin interactions.

Activating gelsolin

In some contexts, activating rather than inhibiting gelsolin may be physiologically beneficial. Intracellular gelsolin is normally inhibited from severing F-actin until activated locally by Ca^{2+}, or perhaps by low pH. As the actin-binding sites themselves are not directly Ca^{2+}-dependent, however, severing and other functions can be activated by proteolytic cleavage of the inhibitory elements. Gelsolin is a major substrate for the cell-death-promoting protease caspase-3, and cells that lack gelsolin are more resistant to apoptotic stimulation than control cells. Caspase-3 cleaves gelsolin approximately in half to generate a severing competent, but calcium-insensitive fragment that may initiate the cytoskeletal changes accompanying apoptosis. As illustrated in Figure 4, this caspase-sensitive site is part of an unstructured region of the molecule that extends from the rest of the compact crystal and presumably is readily available to the caspase in solution. Similar activation of gelsolin may be achieved by interfering with the domain 6–domain 2 inhibitory interaction and so has the potential for selective destabilization of the cytoskeleton in defective cells.

Altering protein folding

The mutation causing FAF (Asp187→Asn) is adjacent to a cysteine residue forming a disulfide bond between two elements of the β sheet in domain 2 of plasma gelsolin (Figure 4). Expression of the mutant protein causes the inappropriate intracellular proteolysis of plasma gelsolin during its transport. A second proteolytic event in the plasma generates the peptide containing residues 173–243 of plasma gelsolin, that eventually forms the amyloid. Loss of the disulfide bond in recombinant plasma gelsolin also predisposes gelsolin to cleave at a similar site [26]. Stabilizing the β strands near the mutation and disulfide sites long enough to allow secretion may therefore prevent the pair of cleavage steps that form the amyloidogenic peptide.

Phosphoinositide-binding peptides

Agents that target the PPI-binding sites of gelsolin may either enhance or inhibit gelsolin's cellular function, by enhancing the dissociation of gelsolin from the inhibitory PPIs, or by preventing PPI-mediated dissociation of gelsolin from actin. The PPI-binding peptides themselves may also have functions that mimic those of gelsolin. Evidence of the effects of a gelsolin-related PIP2-binding peptide on actin assembly in a cellular context are provided by recent studies of the peptide's ability to suppress PIP2-dependent actin assembly and vesicle motility in a *Xenopus* oocyte extract [27] and its inhibition of platelet contractile function in platelet-rich plasma clots [28].

As the structural changes associated with gelsolin activation begin to emerge, more surfaces where ligands may be designed to interfere with or mimic specific activities will be identified. Already, the elucidation of the site of caspase cleavage on a loop exposed on the gelsolin surface and the location of the FAF mutation very near a disulfide bond critical for stabilizing gelsolin identify regions where cellular gelsolin function can be selectively altered. More structural targets are likely to arise as both the chemistry and cellular function of this versatile protein are explored further.

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