Identification of Critical Functional and Regulatory Domains in Gelsolin

David J. Kwiatkowski, Paul A. Janmey, and Helen L. Yin

Hematology-Oncology Unit, Massachusetts General Hospital and
the Department of Medicine, Harvard Medical School, Boston, Massachusetts 02114

Abstract. Gelsolin can sever actin filaments, nucleate actin filament assembly, and cap the fast-growing end of actin filaments. These functions are activated by Ca^{2+} and inhibited by polyphosphoinositides (PPI). We report here studies designed to delineate critical domains within gelsolin by deletional mutagenesis, using COS cells to secrete truncated plasma gelsolin after DNA transfection. Deletion of 11% of gelsolin from the COOH terminus resulted in a major loss of its ability to promote the nucleation step in actin filament assembly, suggesting that a COOH-terminal domain is important in this function. In contrast, derivatives with deletion of 79% of the gelsolin sequence exhibited normal PPI-regulated actin filament–severing activity. Combined with previous results using proteolytic fragments, we deduce that an 11–amino acid sequence in the COOH terminus of the smallest severing gelsolin derivative identified here mediates PPI-regulated bind-

ing of gelsolin to the sides of actin filaments before severing. Deletion of only 3% of gelsolin at the COOH terminus, including a dicarboxylic acid sequence similar to that found on the NH_2 terminus of actin, resulted in a loss of Ca^{2+}-requirement for filament severing and monomer binding. Since these residues in actin have been implicated as potential binding sites for gelsolin, our results raise the possibility that the analogous sequence at the COOH terminus of gelsolin may act as a Ca^{2+}-regulated pseudosubstrate. However, derivatives with deletion of 69–79% of the COOH-terminal residues of gelsolin exhibited normal Ca^{2+} regulation of severing activity, establishing the intrinsic Ca^{2+} regulation of the NH_2-terminal region. One or both mechanisms of Ca^{2+} regulation may occur in members of the gelsolin family of actin-severing proteins.

GELSOLIN is a multifunctional actin binding protein found in vertebrate cytoplasm (Yin and Stossel, 1979) and in blood plasma as a secreted protein (Yin et al., 1984; Kwiatkowski et al., 1988). It interacts with actin in at least three ways (Yin and Stossel, 1980; Kurth and Bryan, 1984; Janmey et al., 1985). First, it promotes the growth of actin filaments by binding two actin monomers and nucleating actin assembly. Second, it blocks the "barbed" end of actin filaments. Third, it severs actin filaments by breaking the noncovalent bond between adjacent actin subunits. In vitro, severing is activated by micromolar Ca^{2+} and inhibited by the polyphosphoinositides, phosphatidyl inositol 4,5 bisphosphate (PIP$_2$),[1] and phosphatidyl inositol 4-mono-phosphate (Janmey and Stossel, 1987; Janmey et al., 1987). Gelsolin/actin complexes are also formed reversibly in vivo (Chaponnier et al., 1987; Hinssen et al., 1987; Lind et al., 1987), suggesting that intracellular gelsolin may have a critical role in mediating reorganization of the cytoskeleton in response to changes in Ca^{2+} and polyphosphoinositides concentrations that occur after agonist stimulation of cells (Yin, 1988).

We and others have sought to understand the structural basis for the interaction between gelsolin and actin and its regulation by Ca^{2+} and polyphosphoinositides. One approach has been to examine the properties of proteolytically derived fragments of gelsolin (Kwiatkowski et al., 1985; Chaponnier et al., 1986; Bryan and Hwo, 1986; see Fig. 8 A). Based on these studies, it has been suggested that Ca^{2+} binding to the COOH-terminal half of gelsolin (located within a 38-kD chymotryptic fragment of gelsolin, CT38C, human plasma gelsolin residues 407–755) leads to a conformational change (Kwiatkowski et al., 1986; Hwo and Bryan, 1986; Kilhoffer and Gerard, 1985) which permits the NH_2-terminal half of gelsolin (located within the chymotryptic fragment CT45N, residues 1–406) to sever actin filaments. Severing by CT45N is Ca^{2+} independent, and requires the participation of two actin binding domains located within the chymotryptic fragments CT17N (residues 1–149) and CT28N (residues 150–406). The former peptide binds actin monomers and filament ends with high affinity (Kwiatkowski et al., 1985; Bryan, 1988), while the latter binds to the sides of actin filaments in a PIP$_2$-sensitive manner (Yin et al., 1988; Bryan, 1988).

We report here studies designed to define critical functional and regulatory sequences within gelsolin by COOH-

---

1. *Abbreviation used in this paper:* PIP$_2$, phosphatidyl inositol 4,5 bisphosphate.

terminal deletional mutagenesis. We have capitalized on the fact that a variant of gelsolin is secreted (Yin et al., 1984) to design an eukaryotic expression system for the truncated gelsolins. COS cells were transfected with human plasma gelsolin expression constructs resulting in secretion of plasma gelsolin at high levels and free of cytoplasmic components, so that the culture media could be directly assayed for interactions with actin. We have identified critical sequences required for nucleation and severing, as well as regulation by Ca^{2+} and PIP_2. We have also uncovered an additional cryptic Ca^{2+} regulatory domain in the first 160 residues of gelsolin which may be important in the Ca^{2+} regulation of related filament-severing proteins. The approach described here, secretion by COS cells, is potentially useful for the structure-function characterization of other structural proteins.

Materials and Methods

DNA Methods, Gelsolin Deletion Derivatives, and Expression Constructs

A full-length plasma gelsolin cDNA (Kwiatkowski et al., 1986) was cloned into the Eco RI site of Bluescript KS+ (Stratagene, Inc., La Jolla, CA; BS-PLG, see Fig. 1 A), digested with Hind III/Xba I, and ligated to Hind III/Xba I digested CDM8 (Seed, 1987), to yield the plasma gelsolin expression vector pPLGE (Fig. 1 B).

Site-specific 3' deletion mutants: BS-PLG was digested with a restriction endonuclease, blunt-ended with the Klenow fragment of DNA polymerase I, and ligated to terminator oligonucleotides (TAGCCTGATCTAG, ATCAGGCTA) to generate cohesive Xba I ends. The DNA was digested with Xba I to trim away excess linkers, gel purified, and self-ligated. Subsequently, the Hind III/Xba I insert of the gelsolin 3'-truncated plasmid was subcloned into CDM8. 3' deletion mutants of gelsolin contained in CDM8 are denoted pPGn, where n indicates the last amino acid residue of human plasma gelsolin contained in the derivative.

Exonuclease III-derived 3' deletion mutants: BS-PLG was linearized with Nde I or Eco RV, near the middle or beyond the 3' end of the gelsolin cDNA insert, respectively. Variable length 3' deletions were generated by timed Exo III digestion and blunt ended with S1 nuclease. The resulting gelsolin cDNA fragments were released from the Bluescript KS+ vector with Bam HI, and blunt-ended with the Klenow fragment. Termination linkers (GTAGCTGACTAGCACA, CTAGTCAGCTAC) were ligated, to generate a four-base overhang (CACA) complementary to that produced by Bst XI digestion of CDM8 (Seed, 1987). The insert was gel purified and ligated to Bst XI-digested CDM8. A second cycle of 3' truncation was accomplished by subcloning the Hind III/Xba I insert of gelsolin derivative pPG260 into Bluescript KS+. The resulting plasmid DNA was linearized by Xba I digestion, trimmed with Exo III and S1 nuclease, and ligated to Bst XI termination linkers. The gelsolin insert was released by digestion at the 5' end with Hind III, gel purified and subcloned into Hind III/Bst XI digested CDM8. A third cycle of 3' truncation was accomplished by Exo III treatment of pPG209, after digestion at 3' downstream sites with Bam HI and Xba I. The resultant DNA was blunt ended, ligated to Bst XI termination linkers, digested with Nhe I, gel-purified, and ligated to the large Nhe I/Bst I fragment of CDM8.

The 3' sequence of all Exo III-derived deletion constructs was determined by subcloning into M13mp18 and/or direct double-stranded sequencing using CDM8 and/or gelsolin cDNA-derived oligonucleotide primers.

COS Cell Expression

COS M6 cells (Gluzman, 1981) were grown in DME with 5% FCS, 10 mM Hepes, 2 mM L-glutamine, 110 μg/ml sodium pyruvate, 50 U/ml penicillin, and 50 μg/ml streptomycin, in 5% CO_2. For transfection, 5–10 μg plasmid DNA in 100 μl DEAE–Dextran (10 mg/ml in PBS, pH 7.4) was added dropwise to a 10-cm dish of cells (30–50% confluence) in 4 ml DME with 10% Nu-serum (Collaborative Research, Inc., Waltham, MA). Chloroquine sulfate was then added dropwise to 100 μM. After 3–4 h at 37°C, the cells were treated with 10% DMSO in Hank's Balanced Salt Solution (HBSS) for 1 min, rinsed with HBSS, and placed in DME with 5% FCS. After 1–2 d,

the cells were rinsed with HBSS and placed in a 50:50 mix of DME–Ham's nutrient mix F12, supplemented with transferrin (5 μg/ml), insulin (5 μg/ml), and sodium selenite (5 ng/ml) (Sigma Chemical Co., St. Louis, MO). The culture medium was collected after 2–3 d, centrifuged at 2,000 g for 5 min, and the pH of the supernatant was corrected to 7.4 with 2 M Tris-HCl, pH 8.8.

Functional Characterization of Gelsolin Mutants

Quantitation of gelsolin expression: COS cell media were analyzed on 5–15% or 5–20% gradient SDS polyacrylamide gels, and either stained directly with Coomassie blue or detected by immunoblotting by the method of Towbin et al. (1979). The blotting membrane used was either nitrocellulose BA85 (Schleicher & Schull, Keene, NH) or Immobilon (Millipore Corp., Bedford, MA). The primary antibody used was a rabbit antiserum (1:50 dilution) directed against a 15-residue synthetic peptide derived from the amino terminus of human plasma gelsolin (Chaponnier et al., 1986), denoted anti-N antibody. The secondary antibody was an ^{125}I-labeled goat anti–rabbit IgG (10 μCi in 10 ml; New England Nuclear, Boston, MA; DuPont Co., Wilmington, DE). After autoradiography, signals were quantified by densitometry in the linear range of absorbance with a Zeineh laser scanning densitometer (Biomed Instruments, Inc., Fullerton, CA). The amount of gelsolin expressed was determined by comparison with the staining intensity of purified human gelsolin, the concentration of which was estimated from its extinction coefficient ($E^M_{280} = 117,580$ M^{-1} cm^{-1}; Yin et al., 1988).

Binding to actin-Sepharose beads: The COS cell media were dialyzed against two changes of a solution containing 2 mM $MgCl_2$, 0.5 mM ATP, 1 mM EGTA, 0.5 mM β-mercaptoethanol, 150 mM KCl, 20 mM Tris-HCl, pH 7.5 (buffer B), with 1 mM NaN_3. 25 μl actin-Sepharose, prepared as described (Kwiatkowski, et al., 1985) was incubated with 0.5–1.5 ml of dialyzed COS cell media at 4°C for 1–2 h. For binding in Ca^{2+}, $CaCl_2$ was added so the solution contained 1.1 mM $CaCl_2$/1 mM EGTA. The actin-Sepharose was then washed three times with 0.15 M NaCl, 10 mM Tris-HCl, pH 7.4 (TBS), containing either 1 mM EGTA or 0.2 mM $CaCl_2$. Adsorbed proteins were released by boiling in gel sample buffer containing 1% SDS and 1% β-mercaptoethanol, and analyzed by electrophoresis on 5–15% or 5–20% gradient SDS polyacrylamide gels, followed by Coomassie blue staining.

Immunoprecipitation: Monoclonal antigelsolin IgG (clone 2C4) was covalently bound to CNBr-activated Sepharose as described (Chaponnier et al., 1987). To immunoprecipitate gelsolin and derivatives, 25 μl of the mAb-Sepharose was incubated with COS cell medium for 1 h at 4°C, washed three times in TBS-EGTA, and analyzed by SDS-PAGE.

Interaction with pyrene-labeled actin: G-actin was labeled with N-(1-pyrenyl)iodoacetamide by the method of Kouyama and Mihashi (1981). Actin nucleation and severing activities were measured by following the rate of polymerization and depolymerization of pyrene-labeled actin, respectively, as described in Janmey et al. (1985). For severing assays, 30–300 μl of the dialyzed media were placed in cuvettes and the total volume adjusted to 300 μl with buffer B. 5 μl of pyrene-labeled F-actin, polymerized with 0.15 M KCl and 2 mM $MgCl_2$, was added to a final concentration of 2×10^{-7} M, and the fluorescence was monitored in a Perkin-Elmer LS-5 or an I.S.S. Greg PC fluorescence spectrophotometer. Severing in the presence of Ca^{2+} was performed in a solution containing 1.1 mM $CaCl_2$/1 mM EGTA. For nucleating assays, pyrene-labeled G-actin was added to 300-μl dialyzed COS cell media/buffer to a final concentration of 1–2 × 10^{-6} M, with subsequent fluorescence monitoring. PIP_2 (Sigma Chemical Co.) was resuspended in water by sonication with an apparatus (model W185; Heat Systems-Ultrasonics, Farmingdale, NY) operating at maximum intensity until an optically clear solution was formed.

Rheologic measurements: The dynamic shear modulus G' and the static shear compliance J were determined for F-actin in the presence and absence of PG160, using a torsion pendulum. The principle of the method and the experimental design are described elsewhere (Ferry, 1980; Janmey et al., 1988). Briefly, actin was polymerized in solutions with or without PG160 between the parallel circular plates of the pendulum. After 6 min of polymerization, when pyrene fluorescence confirmed that most of the actin was polymeric, a constant shear stress was applied to the upper face of the sample. The subsequent deformation is quantified as the shear compliance, J, the ratio of shear strain to the imposed stress. Previous studies have shown that the shear compliance depends strongly on the average actin filament length (Janmey et al., 1988). The dynamic shear modulus G' was determined by applying a momentary displacement to the arm attached to the upper sample plate and measuring the frequency and damping of the resulting
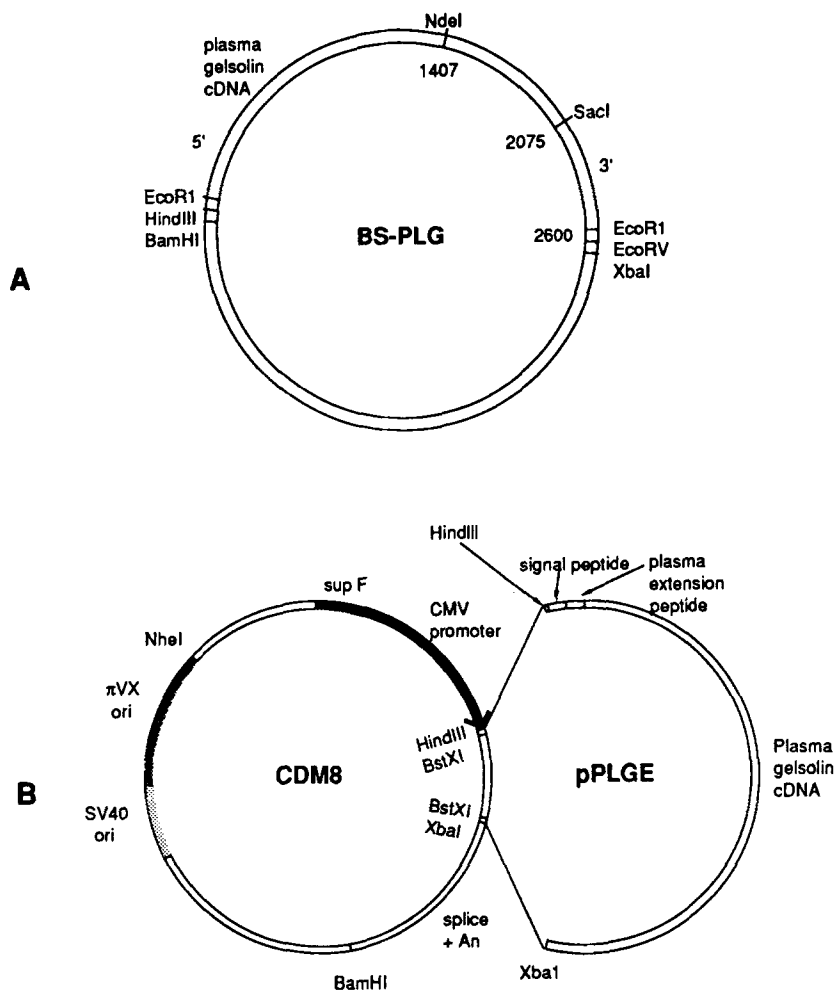
Figure 1. DNA constructs. (*A*) BS-PLG, a derivative of Bluescript KS+, containing the full-length plasma gelsolin cDNA. (*B*) CDM8, a mammalian expression vector (Seed, 1987). The cytomegalovirus (CMV) promoter, sup F tRNA gene, π VX origin of replication, SV-40 origin of replication, splice and polyadenylation sequences, and relevant restriction enzyme sites are indicated. *pPLGE*, a plasma gelsolin expression vector derived from BS-PLG and CDM8.

free oscillations. The shear modulus G' is approximately equal to $\omega^2 I/b$ where ω is the angular frequency, I is the moment of inertia of the arm, and b is a geometric factor determined by the sample dimensions. G' is a measure of the elasticity of a material and for actin-containing samples, this quantity also depends on the average filament length (Zaner and Hartwig, 1988; Janmey et al., 1988).

Ca^{2+} titration: A series of Ca^{2+}-buffered solutions were prepared containing 0.82 mM EGTA and 0–1.0 mM $CaCl_2$ in buffer B. PLGE and PG160 were dialyzed against two changes of buffer B without EGTA, prepared from distilled water passed over a milli Q column (Millipore Corporation, Bedford, MA). After dialysis, 90-μl samples were added to 210 μl Ca^{2+}/EGTA buffered solutions. Severing activity was assessed after addition of 5-μl F-pyrene actin to a final actin concentration of 2 × 10^{-7} M, as above. Free Ca^{2+} concentrations were calculated as described in Perrin and Sayce (1967).

Results

Expression of Plasma Gelsolin in COS Cells

A plasmid construct containing the full-length plasma gelsolin cDNA (Fig. 1 *A*), which includes the initiator methionine and sequences encoding the presumed signal peptide and plasma extension peptide of human plasma gelsolin (Kwiatkowski et al., 1986), was subcloned into the expression vector CDM8 (Fig. 1 *B*; Seed, 1987) to yield the plasmid pPLGE. Fig. 2 *A* shows that recombinant gelsolin (*PLGE*) was immunoprecipitated from the medium of pPLGE-

transfected cells. The heavy and light chains (*HC* and *LC*, respectively) of IgG were eluted from the Sepharose by β-mercaptoethanol. No gelsolin was immunoprecipitated from cells which were mock transfected with CDM8 lacking the gelsolin insert, although the mAb against human gelsolin used here cross reacts with endogenous monkey COS cell gelsolin (data not shown). These results show that PLGE is specifically secreted by COS cells after transfection of the pPLGE construct, and establish that the cDNA construct contains a functional signal peptide.

PLGE was quantitated by immunoblotting with the anti–NH_2-terminal peptide (anti–N) antibody (Chaponnier et al., 1986) in comparison with known amounts of purified gelsolin. Fig. 2 *B* shows that there was a linear increase in the autoradiographic signal as increasing amounts of purified plasma gelsolin were loaded on the gel, and the staining intensity of PLGE in 10 μl of transfected COS cell medium is comparable to that of 3.6 pmol of gelsolin. Therefore, in this transfection, PLGE was present at 3.6 × 10^{-7} M (30 μg/ ml). Based on results from multiple independent pPLGE transfections, the PLGE level in the media varied between 5 and 30 μg/ml.

PLGE bound to actin–Sepharose (Fig. 2 *C*, lanes *1* and *2*) and severed actin filaments, as assessed by interactions with pyrene F-actin (Fig. 3, *top left*) in a Ca^{2+}-dependent manner similar to naturally occurring human plasma gelsolin. The
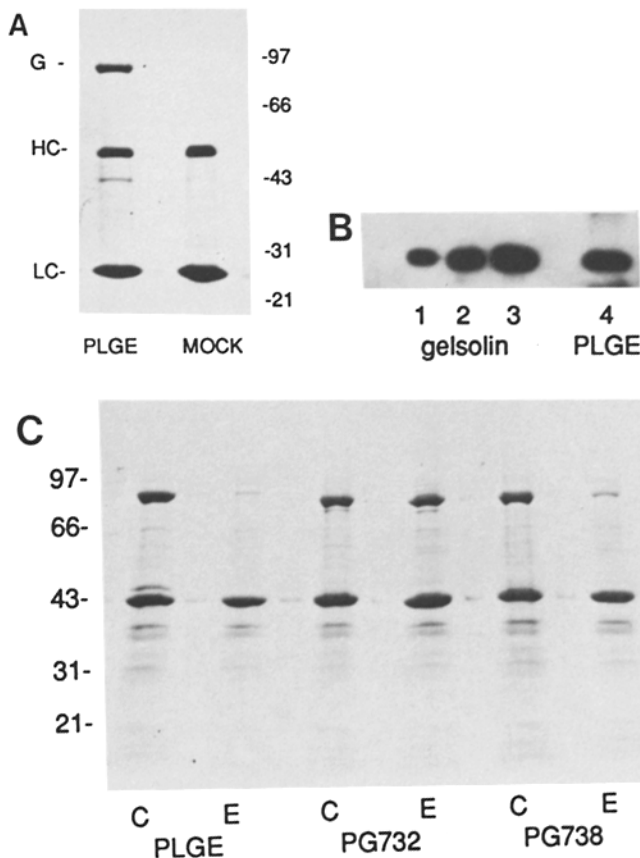
Figure 2. Expression of secreted gelsolin and derivatives by COS cells. (A) SDS-PAGE analysis of immunoprecipitated COS cell media. 750 μl of media from transfected COS cells was incubated with 25 μl of mAb antigelsolin IgG-Sepharose. Adsorbed proteins were separated on a 5-15% polyacrylamide gel and stained with Coomassie blue dye. pPLGE-transfected and mock- (CDM8) transfected cell media are indicated. Molecular mass standards are indicated on right in kilodaltons. G, gelsolin; HC and LC, heavy and light chains of IgG, respectively. (B) Autoradiograph of immunoblotted gelsolin and PLGE, using the anti-N antibody and ^{125}I-labeled secondary antibody. 1.8, 3.6, and 7.2 pmol purified human plasma gelsolin (lanes 1-3) and 10 μl of PLGE-transfected COS cell medium (lane 4) were analyzed. (C) Effect of Ca^{2+} on actin-Sepharose binding by PLGE, PG738, and PG732. 25 μl actin-Sepharose was incubated with 750 μl of dialyzed COS cell media in the presence of 1.1 mM $CaCl_2$/1 mM EGTA (C) or 1 mM EGTA (E) and analyzed by SDS-PAGE and Coomassie blue staining. Molecular mass standards are indicated on left in kilodaltons.

specific activity of severing of PLGE was comparable to that of plasma gelsolin (data not shown). Mock-transfected COS cell media had no severing activity.

Minimal COOH-Terminal Deletions Cause a Loss of Ca^{2+} Regulation

The COOH-terminal half of gelsolin is thought to mediate the Ca^{2+} regulation of gelsolin-actin interactions (Bryan and Hwo, 1986; Chaponnier et al., 1986). To identify sequences critical for Ca^{2+} regulation, minimal COOH-terminal deletions in the gelsolin cDNA were generated by progressive Exo III digestion or digestion by restriction endonucleases. The extent of deletion, determined by DNA sequencing, is

shown in Table I. Due to the method of construction and the termination linkers used, two to three additional amino acid residues were introduced at the COOH-terminus of some of the gelsolin derivatives. These 3'-truncated pPLGE clones are denoted pPGn, where n indicates the last plasma gelsolin amino acid residue retained, and the corresponding expressed gelsolin proteins are referred to as PGn.

Fig. 2 C shows that PLGE and PG738 bound actin-Sepharose in the presence of 0.1 mM free Ca^{2+} but not in EGTA. In contrast, PG732, which migrated slightly ahead of PG738 and has lost six more amino acids, bound to actin-Sepharose both in Ca^{2+} and EGTA.

The loss of a Ca^{2+} requirement was also observed for actin severing during the transition from PG738 to PG732. Fig. 3 shows that PG732 did not require Ca^{2+} to sever actin filaments, while PG738 and PLGE did. Similarly, PG660 and PG260 were active in the absence of Ca^{2+}. Therefore, we conclude that the sequence between residues 739 and 755 is not required for Ca^{2+} regulation, while that between residues 732 and 738 is potentially important for this regulation.

The amounts of gelsolin derivatives used in these assays were determined by quantitative immunoblotting (data not shown). This analysis indicated that PLGE, PG738, PG732, and PG660 had similar severing activities on a molar basis (100:84:92:110, respectively).

COOH-Terminal Deletions Result in a Loss of Nucleating Activity

Fig. 4 shows the severing and nucleating activities of PG738 and PG660 in comparison with purified gelsolin. The two derivatives sever actin filaments equivalently (Fig. 4 A). However, PG738 increased the rate of polymerization of pyrene actin to an extent similar to that observed with plasma gelsolin, while PG660 did not (Fig. 4 B). Although the rate of actin polymerization reflects contributions of severing activity, end capping, and nuclei formation, the difference shown here is attributable to a difference in nucleation because the derivatives have nearly identical severing activities. Minimal nucleating activity was observed in all gelsolin derivatives shorter than PG660 (Table I). Therefore, we conclude that the sequence between residues 738 and 660 is important for nucleation. This is consistent with the previous observation that CT45N has only 5-10% of the nucleating activity of intact gelsolin (Kwiatkowski et al., 1985).

Identification of the Smallest Gelsolin Derivative Capable of Actin Filament Severing

To define the minimal gelsolin sequence required for severing, we generated a series of derivatives from the Nde I site of BS-PLG by progressive Exo III deletions, as shown in Table II. Fig. 5 A shows the severing activity of equal aliquots of COS cell media containing PLGE, PG160, PG149, and PG139, as assessed by the dilutional F-pyrene-actin fluorescence assay. The amount of each derivative in the media was quantified by densitometric scanning of Coomassie blue-stained bands in SDS polyacrylamide gels (Fig. 5 B). As shown in Table III, the molar amounts expressed, and hence that used in the severing assay, were comparable, except in the case of PG149 which was expressed at very high levels. Table III shows that PG160 severed actin filaments roughly as well as native gelsolin, PG149 had very little severing ac-
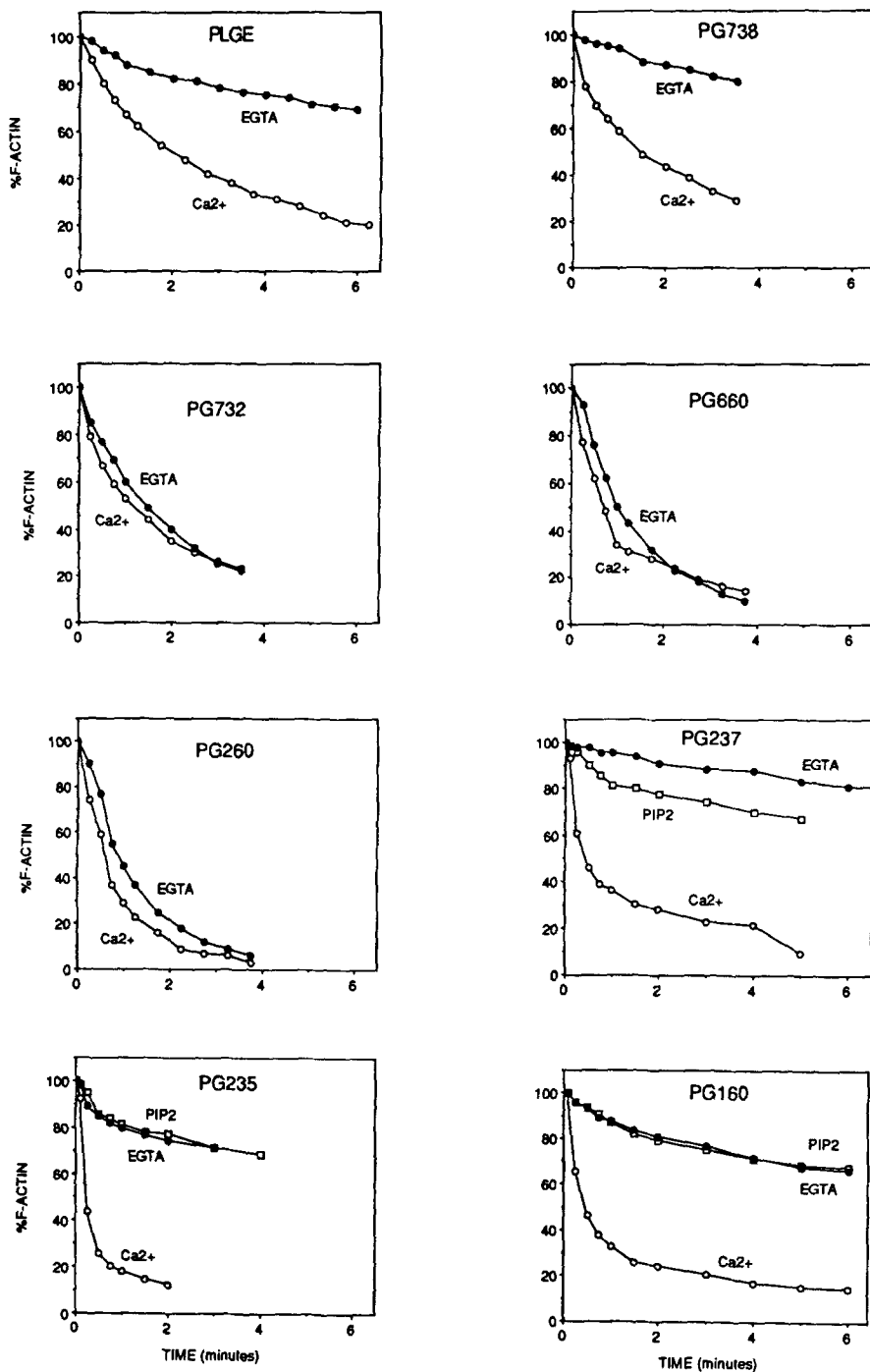
PLGE

PG738

PG732

PG660

PG260

PG237

PG235

PG160

TIME (minutes)

TIME (minutes)

Figure 3. Fluorimetric analysis of the severing activity of gelsolin derivatives. F-pyrenyl actin was added to 90–300 μl dialyzed COS cell media to a final concentration of 2×10^{-7} M. The change in fluorescence was monitored and used to calculate the percent F-actin as a function of time in minutes. Ca^{2+} indicates the presence of 1.1 mM $CaCl_2$/1 mM EGTA; *EGTA* indicates the presence of 1 mM EGTA (less than micromolar Ca^{2+}); PIP_2 indicates the presence of Ca^{2+} and 100 μg/ml PIP_2. The concentrations of the large gelsolin derivatives were determined by immunoblotting with anti-N antibody, as described in the text. The final concentrations of PLGE, PG738, PG732, and PG660 were 4.9, 7.4, 8.3, and 8.3×10^{-8} M, respectively.

tivity, and PG139 had none. The PG160, PG149, and PG139 content determined by Coomassie blue staining also agrees with measurements of their relative molar amounts by immunoblotting with the anti-N antibody (Fig. 5 *C*), confirming the conclusion that there is a significant loss of severing during the transition from PG160 to PG149, and complete loss of severing after truncation to PG139. These data suggest that the severing domain of gelsolin resides within its first 160 residues, with a critical dependence on the sequence between 150 and 160.

A direct measurement of the effects of PG160 on the vis-

coelastic properties of F-actin solutions was made using a torsion pendulum. Fig. 6, *A* and *B* show the movements of a torsion pendulum during free oscillation after a momentary displacement. The pendulum plates contained F-actin in the absence (Fig. 6 *A*) and presence (*B*) of PG160. PG160 increases the period of oscillation and decreases G' by a factor of four compared to the F-actin control. Fig. 6 *C* shows the compliance of F-actin solutions during creep under constant shear stress. The sample containing PG160 exhibits much higher initial compliance and viscous flow at long times, in contrast to the F-actin control which has a lower compliance

Table I. Gelsolin COOH-Terminal Deletion Derivatives

Gelsolin derivative*	Added COOH-terminal residues	Ca²⁺ regulation‡	Nucleation activity§
PLGE	–	+	+
PG753	val-ala-asp	+	+
PG738	val-ala-asp	+	+
PG732	cys-ser	–	+
PG669	val-ala-asp	–	–
PG660 (Sac I)	ND	–	–
PG549 (Sma I)	ND	–	–
PG484 (Esp I)	ND	–	–

* *PLGE*, full-length recombinant gelsolin; *PGn*, COOH-terminal truncated derivatives, with *n* denoting the last residue of plasma gelsolin retained in the protein. The restriction enzymes used to generate the truncated derivatives are indicated in parentheses.

‡ Determined by comparing pyrene actin–severing and actin–Sepharose-binding activity in the presence of 0.1 mM CaCl₂ or excess EGTA (less than micromolar Ca²⁺). + indicates >80% difference in severing activity in Ca²⁺ vs. EGTA. – indicates <20% difference.

§ + indicates ability to increase rate of actin polymerization.

and deforms very little thereafter. Both of these sets of observations are consistent with the presence of shorter filaments in the sample containing PG160, and confirm its actin filament shortening activity.

Fig. 3 shows that the severing activity of PG237, PG235, and PG160 was inhibited by PIP₂, as has been shown previously for full-length gelsolin and the NH₂-terminal half fragment of gelsolin (Janmey and Stossel, 1987). PG160, the smallest derivative with active severing ability, is inhibited by PIP₂ with a concentration dependence similar to that of native plasma gelsolin (Fig. 5 D). The effect of PIP₂ on PG149 could not be determined because of its already low severing activity. These data suggest that PIP₂-regulation of gelsolin's severing activity also resides within its first 160 residues.

Unmasking of Cryptic Ca²⁺-Regulation in the NH₂-Terminal Half of Gelsolin

Fig. 3 shows that PG732, PG660, and PG260 actively severed actin filaments in the presence of EGTA. However, further truncated derivatives PG237 and PG160 required Ca²⁺ for severing. A survey summarized in Tables I and II shows that each of the derivatives containing between 732 and 260 residues severed actin filaments in the presence of 1 mM EGTA, whereas those containing 237–160 residues required Ca²⁺ for significant severing activity. Fig. 7 A compares the effect of increasing Ca²⁺ concentration on the severing activity of PG160 and PLGE. Both proteins were activated by Ca²⁺ with half-maximal activation observed at ~3 μM. Although the small gelsolin derivatives required Ca²⁺ for actin severing, they did not require Ca²⁺ to bind actin-Sepharose (Fig. 7 B). Fig. 7 B also shows that PG139, which is 10 residues shorter than CT17N at its COOH terminus, bound actin-Sepharose. We were unable to obtain significant levels of expression of even shorter derivatives, and therefore have not defined the minimal gelsolin NH₂-terminal sequence required for actin-Sepharose binding activity.

Discussion

Gelsolin is representative of a family of Ca²⁺-activated actin filament severing proteins found throughout the animal kingdom (Stossel et al., 1985; Hasegawa et al., 1980; Yamamoto et al., 1982; Glenney et al., 1980). Members of this family have highly conserved primary sequences (Ampe and Vandekerckhove, 1987; Andre et al., 1988; Bazari et al., 1988; Arpin et al., 1988; Ankenbauer et al., 1988; Way and Weeds, 1989) which contain repetitive structural motifs (Kwiatkowski et al., 1986). Gelsolin and villin, found in vertebrates, contain six repeated domains, while severin and fragmin, found in lower eukaryotes, are half as large and contain a threefold repeat. Fig. 8 A shows the proteolytic cleavage pattern of gelsolin by chymotrypsin and its alignment with the six repeated domains (Kwiatkowski et al.,
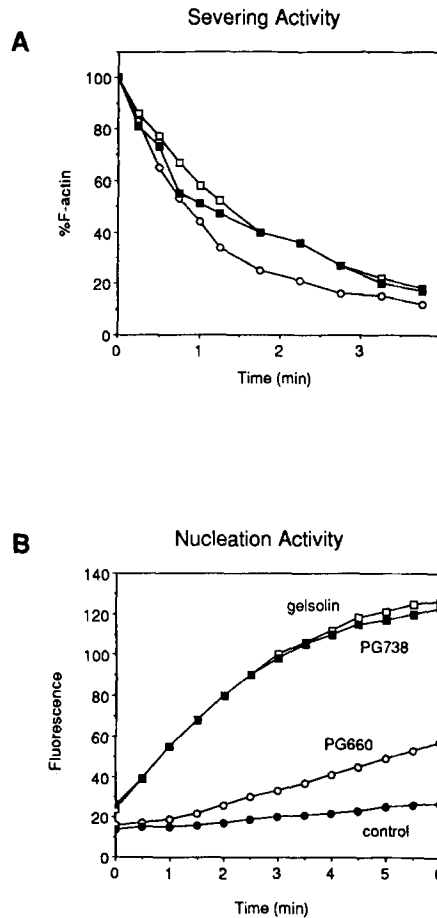

Figure 4. Comparison of severing and nucleation activity of gelsolin, PG738, and PG660. (A) Severing activity, assayed by the pyrene-F-actin depolymerization method. After dialysis, aliquots containing purified plasma gelsolin, PG738, and PG660 (in COS cell media), respectively, were added to buffer containing 1.1 mM CaCl₂/1 mM EGTA, to final gelsolin concentrations of 4.0, 7.4, and 8.3 × 10⁻⁸ M, respectively. ■, PG738; □, gelsolin; o, PG660. (B) Nucleating activity. The same amounts of protein were incubated with 2 × 10⁻⁶ M pyrene-actin, 0.94 mM CaCl₂, 0.86 mM EGTA, 2 mM MgCl₂, 150 mM KCl, 0.2 mM ATP, 0.2 mM β-mercaptoethanol, pH 7.8, and the fluorescence determined.

Table II. Severing Activity and Ca²⁺ Sensitivity of Small Gelsolin Derivatives

Gelsolin derivative	Added COOH-terminal residues	Severing activity	Ca²⁺ dependence
PG437 (Nde I)	ND	+	−
PG421	met	+	−
PG364	cys-ser	+	−
PG260	val-ala-asp	+	−
PG237	ser-ser	+	+
PG235	val-ala-asp	+	+
PG209	ser-ser	+	+
PG205	arg	+	+
PG160	val-ala-asp	+	+
PG149	ser-ser	+/−	+
PG142	none	−	
PG139	val-ala-asp	−	

1986; Matsudaira and Janmey, 1988). Several proteases generate similar peptides in gelsolin and villin (Chaponnier et al., 1986; Bazari et al., 1988), suggesting that there are "hinge" sites joining structural/functional domains of the proteins.

We found that there is a sharp drop in the ability of gelsolin derivatives to nucleate actin filament growth when residues 661–738 are deleted (Fig. 8 B). Because the COOH-terminal half of gelsolin can bind actin but does not nucleate actin assembly by itself (Kwiatkowski et al., 1985), nucleation most likely requires occupation of actin binding sites on both halves of the molecule. A most straightforward interpretation of our result is that the COOH-terminal actin binding site is located between residues 661 and 738, although in the absence of information on the three-dimensional structure of gelsolin, nonspecific conformational disruption has not been ruled out. The fact that severin, which may be considered a gelsolin NH₂-terminal half equivalent, also nucleates poorly (Yamamoto et al., 1982) suggests that gelsolin/villin may have acquired the ability to nucleate actin filament assembly through gene duplication of severin/fragmin.

In contrast to nucleation, PIP₂-regulated severing requires only a small part of gelsolin. Deletion of 79% of the gelsolin sequence from the COOH terminus generates PG160 which has full severing activity and responds to PIP₂ in the same manner as native gelsolin. Severing activity by PG160 was confirmed by direct measurement of changes in the viscoelastic properties of filament networks. PG160 spans the entire domain 1 (residues 1–149; Fig. 8), and extends 11 residues into domain 2. The domain 2 residues must be critical for severing, because their removal, either by deletional mutagenesis to generate derivative PG149 or by proteolytic digestion to generate CT17N (Kwiatkowski et al., 1985; Bryan, 1988), results in markedly reduced severing activity. Since proteolytic cleavage experiments have shown that CT28N, which consists of domains 2 and 3, binds stoichiometrically to the sides of actin filaments in a PIP₂-inhibited manner (Bryan, 1988; Yin et al., 1988), our current results suggest that the actin filament side binding and PIP₂ binding sites of gelsolin are localized within the first 11 amino acids in domain 2 (residues 150–160). As shown in Fig. 8, this sequence begins with two adjacent
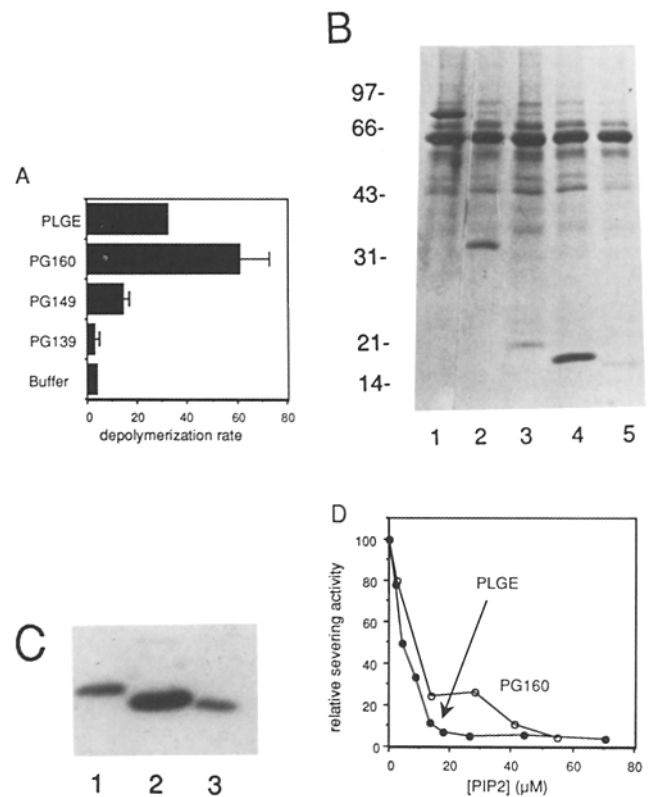
Figure 5. Severing activity and quantitation of expression of small gelsolin derivatives. (A) Relative severing activity of PLGE, PG160, PG149, and PG139 in 1.1 mM CaCl₂/1 mM EGTA. 30 μl of dialyzed COS cell media were mixed with 270 μl of buffer, and pyrene F-actin was added to a final concentration of 2 × 10⁻⁷ M. Severing activity was determined from the initial rate of decrease in fluorescence, and expressed in arbitrary units. The average and the highest severing activity of media from two separate transfection experiments are shown for PG160, PG149, and PG139. (B) SDS-PAGE of transfected COS cell media. 80 μl of transfected COS cell media (lyophilized and resuspended in 25 μl SDS gel sample buffer) were electrophoresed on a 5–20% polyacrylamide gradient gel and stained with Coomassie blue dye. Lanes 1–5 are PLGE, PG260, PG160, PG149, and PG139, respectively. Molecular mass markers are shown in kilodaltons on the left. (C) Autoradiogram of immunoblot. 20 μl of transfected COS cell media were electrophoresed on a 5–20% polyacrylamide gel, transferred to immobilon membranes, and gelsolin derivatives were detected by incubation with rabbit anti-N antibody and ¹²⁵I-goat anti-rabbit IgG. Lanes 1–3 are PG160, PG149, and PG139, respectively. (D) Effect of PIP₂ on the severing activity of PG160 and gelsolin. The pyrene F-actin severing activity of aliquots of PG160 and PLGE media (with equivalent severing activity in the absence of PIP₂) were determined at increasing PIP₂ concentrations and are expressed relative to severing activity in the absence of PIP₂.

residues with basic side groups, which are followed by two sets of three hydrophobic residues. We hypothesize that the two positively charged residues mediate binding to the phosphate groups on PIP₂ (Janmey and Stossel, 1987; Janmey and Matsudaira, 1988) while the hydrophobic residues may be involved in binding to actin and/or the acyl chains of the phospholipid. Since PG149 retains weak severing activity, and PG139 has none, it is possible that residues 139–149 also contribute to severing.

Table III. Relative Severing Activities of Small Gelsolin Derivatives

Gelsolin derivative	Absolute severing activity*	Relative staining intensity‡	Relative molar expression§	Specific severing activity‖
PLGE	18	1.0	1.0	100
PG160	36	0.3	1.5	132
PG149	6	1.7	8.5	4
PG139	0	0.15	0.8	0

* Severing activity of 30 μl media, expressed in arbitrary units and after subtracting the activity of buffer alone, as shown in Fig. 5 *A*.
‡ Determined by densitometric scanning of Coomassie blue–stained bands, as shown in Fig. 5 *B*.
§ Derived from column 2 by adjusting for different masses, assuming that Coomassie blue staining is directly proportional to mass.
‖ Percent severing activity, on a molar basis, relative to PLGE. Determined from the ratio of columns 1 to 3.

PG139 is the smallest gelsolin derivative known to bind actin. Therefore, the actin binding domain in CT17N is located between residues 26–139 (residues 1–25 is the plasma extension not found in cytoplasmic gelsolin; Yin et al., 1984). We were unable to express smaller gelsolin peptides, perhaps due to their susceptibility to proteolysis or aberrant secretion (Lodish, 1988). In contrast, the slightly larger PG149 is expressed at very high levels consistently (Fig. 5, *B* and *C*). Its apparent stability may reflect the fact that it comprises precisely domain 1 (Fig. 8), supporting the concept that hinge regions divide stable structural subunits of the protein.

The present results offer further insight into how Ca^{2+} activates gelsolin and reveal the presence of a cryptic Ca^{2+}-regulated site which may provide an alternative means for the activation of other severing proteins. From previous studies, it has been suggested that the COOH-terminal half of gelsolin imposes Ca^{2+} regulation on the remainder of the molecule, perhaps by obstructing the interaction of the NH_2-terminal domains with actin in the absence of Ca^{2+}. Ca^{2+} induces a conformational change in a COOH-terminal half peptide of gelsolin (Kwiatkowski et al., 1985) and in the intact molecule as well (Kilhoffer and Gerard, 1985; Hwo and Bryan, 1986). Minimal deletion of the COOH-terminal sequence between residues 733 and 738 resulted in a loss of Ca^{2+} requirement, but no change in severing or nucleation activity. Therefore, this deletion has not caused a global disruption of gelsolin conformation, but specifically affected the Ca^{2+} regulation mechanism. The presence of three aspartic acids (residues 737–739) in this critical region suggests that these residues may act as an actin-like pseudosubstrate, resembling the dicarboxylic acids at the extreme NH_2 termini of all actin isoforms (Vandekerckhove and Weber, 1979). Our recent finding that the NH_2-terminal asp/glu of actin can be cross-linked chemically to CT17N as well as CT28N (Sutoh, K., and H. L. Yin, manuscript submitted for publication) supports the importance of this region of actin in its interaction with the NH_2-terminal domains of gelsolin. Therefore, a plausible model is that in the absence of micromolar Ca^{2+}, the COOH-terminal half pseudosubstrate sequence binds to the gelsolin NH_2-terminal actin binding sites. This binding is disrupted either through a Ca^{2+}-induced conformational change in the COOH-half of gelsolin, or in the case of the gelsolin mutants or proteolytic fragments, through a loss of the critical pseudosubstrate sequence.
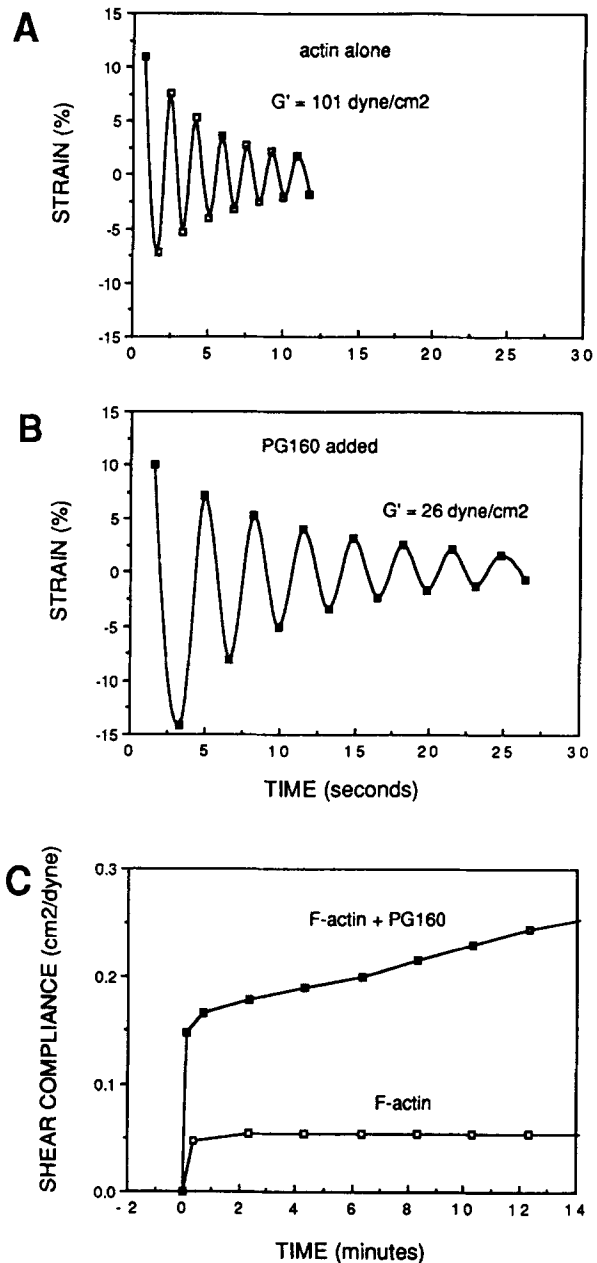
Figure 6. Effects of PG160 on viscoelastic properties of actin filament solutions. (*A* and *B*) 166 μl of 33 μM G-actin was added to 333 μl of either PG160 medium dialyzed against buffer B containing 1.1 mM $CaCl_2$/1 mM EGTA, or dialysis buffer alone, and allowed to polymerize between the plates of a torsion pendulum. The distance between the plates was 1 mm. Shown are typical free oscillations after momentary displacement of F-actin in the absence (*A*) or presence (*B*) of PG160. (*C*) Shear compliance was measured on identically prepared samples by application of a shear stress at time zero, and the subsequent deformation is shown for F-actin in the presence (■) and absence (□) of PG160.

Neither the NH_2-terminal half of gelsolin, CT45N, nor its proteolytically defined subfragments, CT17N and CT28N, require Ca^{2+} for activity. However, small gelsolin derivatives containing the first 160–237 residues regained the Ca^{2+}-activated severing phenotype. Therefore it appears that re-
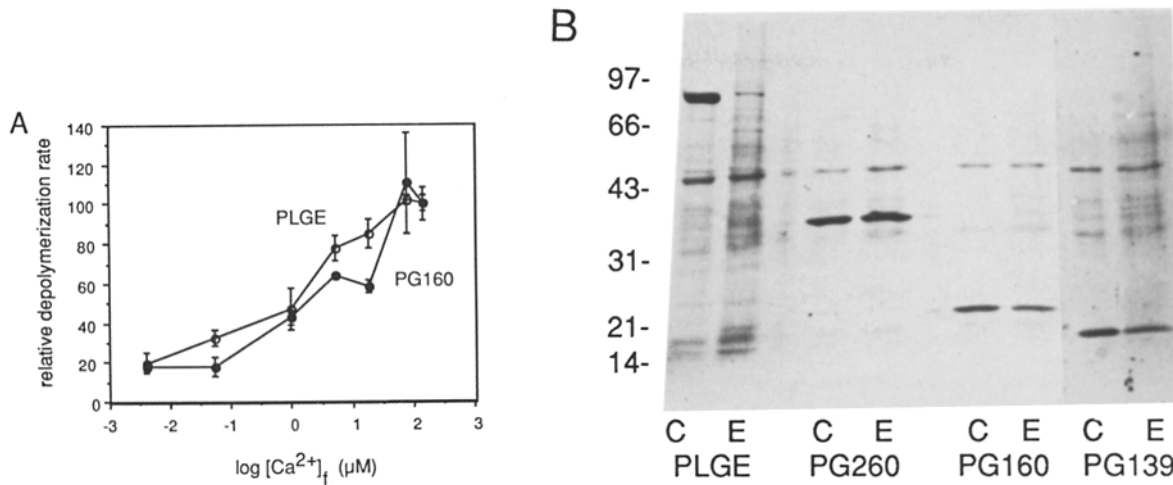
Figure 7. Effects of Ca^{2+} on actin severing and actin–Sepharose binding by small gelsolin derivatives. (A) Ca^{2+} titration of severing activities of PLGE and PG160. Dialyzed media containing PLGE (final concentration of 4.9×10^{-8} M) and PG160 (4.3×10^{-8} M, estimated from Coomassie blue staining intensity) were added to 2×10^{-7} M F-actin in buffers containing 0.58 mM EGTA and increasing amounts of $CaCl_2$. The severing activities of the two samples are equivalent in 1.1 mM $CaCl_2$/1 mM EGTA, and are defined as 100. Severing activity at other Ca^{2+} concentrations was expressed relative to this value. Each data point is the average of duplicate determinations whose values are indicated by the bar. $[Ca^{2+}]_f$, free Ca^{2+} concentration, calculated as described in Perrin and Sayce (1967). (B) Actin–Sepharose binding. 750 μl of dialyzed transfected COS cell media were incubated with 250 μl actin–Sepharose in the presence of 1.1 mM $CaCl_2$/1 mM EGTA (C) or 1 mM EGTA (E). Proteins were resolved by SDS-PAGE (5–20% gradient) and stained with Coomassie blue dye.
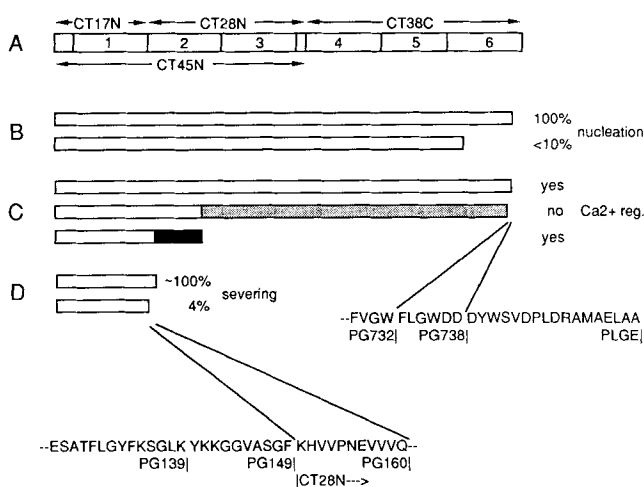

Figure 8. Gelsolin domain structure and critical sequences. A shows the chymotryptic (CT) cleavage pattern of gelsolin and the position of the sixfold repetitive structural motif found in gelsolin and related proteins. The numbers coming after CT denote molecular mass in kilodaltons; N and C denote derivation from the NH_2- or COOH-terminal halves of gelsolin, respectively. B indicates the lengths of gelsolin derivatives with full or markedly reduced nucleation activity. C delineates the lengths of COOH-terminal deletion derivatives that have lost the Ca^{2+} requirement for activation of severing and actin–Sepharose binding (shaded region), and those that have regained Ca^{2+} regulation of severing but not actin–Sepharose binding (dark region). D indicates the severing activity of short gelsolin derivatives, PG160 and PG149 relative to PLGE. At bottom right is shown the COOH-terminal sequence of gelsolin in the single letter amino acid code, with the last residues of PLGE, PG738, and PG732 indicated. At bottom left is shown internal sequence of gelsolin, with the last residues of PG139, PG149, and PG160, and the first residue of CT28N indicated.

moval of sequences in domains 2 and 3 allows a cryptic Ca^{2+}-binding site to become functional, rendering severing Ca^{2+} sensitive. Because we have shown that Ca^{2+} regulation of intact gelsolin appears to be dependent on the COOH-terminal region, the NH_2-terminal Ca^{2+}-regulated site may not be functional in gelsolin. Nonetheless, it may mediate the Ca^{2+} regulation of severing by related proteins such as villin, severin, and fragmin, which lack the actin-like sequence present in the COOH terminus of gelsolin.

In this paper, we have described a novel and convenient approach to the structure–function analysis of gelsolin, capitalizing on the fact that gelsolin, a cytoskeletal protein, also occurs in a variant secreted form so that it can be directly overexpressed and secreted by COS cells after transfection. This method offers several advantages: first, relatively large amounts of protein are generated rapidly by transient expression; second, the protein is expressed in its native form; third, the expressed protein is secreted into the extracellular medium, separated from other intracellular structural components, simplifying the analysis; and fourth, compartmentalization of the protein into secretory vesicles avoids the potential adverse effects of overexpression of the cytoplasmic protein. Secretion of transfected gene products by COS cells may be useful for the analysis of other structural proteins, through the use of heterologous signal peptide sequences, similar to the vectors designed for the bacterial expression and secretion of eukaryotic proteins (Masui, et al., 1983).

References

Ampe, C., J. Vandekerckhove. 1987. The F-actin capping proteins of physarum Polycephalum: cap42(a) is very similar, if not identical, to fragmin and is structurally and functionally very homologous to gelsolin; cap42(b) is Physarum actin. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:4149-4157.

Andre, E., F. Lottspeich, M. Schleicher, and A. Noegel. 1988. Severin, gelsolin and villin share a homologous sequence in regions presumed to contain F-actin severing domains. *J. Biol. Chem.* 263:722-727.

Ankenbauer, T., J. A. Kleinschmidt, J. Vandekerckhove, and W. W. Franke. 1988. Proteins regulating actin assembly in oogenesis and early embryogenesis of Xenopus laevis: gelsolin is the major cytoplasmic actin-binding protein. *J. Cell Biol.* 107:1489-1498.

Arpin, M., E. Pringault, J. Finidori, A. Garcia, J. Jeltsch, J. Vandekerckhove, and D. Louvard. 1988. Sequence of human villin: a large duplicated domain homologous with other actin-severing proteins and a unique small carboxy-terminal domain related to villin specificity. *J. Cell Biol.* 107:1759-1766.

Bazari, W. L., P. Matsudaira, M. Wallek, T. Smeal, R. Jakes, and Y. Ahmed. 1988. The villin sequence and peptide map identifies six homologous domains. *Proc. Natl. Acad. Sci. USA.* 85:4986-4990.

Bryan, J. 1988. Gelsolin has three actin binding sites. *J. Cell Biol.* 106:1553-1562.

Bryan, J., and S. Hwo. 1986. Definition of an N-terminal actin-binding domain and a C-terminal Ca^{2+} regulatory domain in human brevin. *J. Cell Biol.* 102:1439-1446.

Chaponnier, C., P. A. Janmey, and H. L. Yin. 1986. The actin filament-severing domain of plasma gelsolin. *J. Cell Biol.* 103:1473-1481.

Chaponnier, C., H. L. Yin, and T. P. Stossel. 1987. Reversibility of gelsolin/actin interaction in macrophages. *J. Exp. Med.* 165:97-106.

Ferry, J. D. 1980. Viscoelastic properties of polymers. 3rd ed. John Wiley & Sons, New York.

Glenney, J. R., A. Bretscher, and K. Weber. 1980. Calcium control of the intestinal microvillus cytoskeleton: its implications for regulation of microfilament organization. *Science (Wash. DC).* 77:6458-6462.

Gluzman, Y. 1981. SV40-transformed simian cells support the replication of early SV40 mutants. *Cell.* 23:175-182.

Hasegawa, T., S. Takahashi, H. Hayashi, and S. Hatano. 1980. Fragmin: a calcium ion sensitive regulatory factor on the formation of actin filaments. *Biochemistry.* 19:2677-83.

Hinssen, H., J. Vandekerckhove, and E. Lazarides. 1987. Gelsolin is expressed in early erythroid progenitor cells and negatively regulated during erythropoiesis. *J. Cell Biol.* 105:1425-1430.

Hwo, S., and J. Bryan. 1986. Immuno-identification of Ca^{2+}-induced conformational changes in human gelsolin and brevin. *J. Cell Biol.* 102:227-236.

Janmey, P. A., and T. P. Stossel. 1987. Modulation of gelsolin function by phosphatidylinositol 4,5-bisphosphate. *Nature (Lond.).* 325:362-364.

Janmey, P. A., and P. Matsudaira. 1988. Functional comparison of villin and gelsolin: effects of Ca^{2+}, KCl, and polyphosphoinositides. *J. Biol. Chem.* 263:16738-16743.

Janmey, P. A., C. Chaponnier, S. E. Lind, K. S. Zaner, T. P. Stossel, and H. L. Yin. 1985. Interactions of gelsolin and gelsolin-actin complexes with actin. Effects of calcium on actin nucleation, filament severing and end blocking. *Biochemistry.* 24:3714-3723.

Janmey, P. A., K. Iida, H. L. Yin, and T. P. Stossel. 1987. Polyphosphoinositide micelles and polyphosphoinositide-containing vesicles dissociate endogenous gelsolin-actin complexes and promote actin assembly from the fast-growing end of actin filaments blocked by gelsolin. *J. Biol. Chem.* 262:12228-12236.

Janmey, P. A., S. Hvidt, J. Peetermans, J. Lamb, J. D. Ferry, and T. P. Stossel. 1988. Viscoelasticity of F-actin and F-actin/gelsolin complexes. *Biochemistry.* 27:8218-8226.

Kilhoffer, M. C., and D. Gerard. 1985. Fluorescence study of brevin, the M_r 92,000 actin-capping and fragmenting protein isolated from serum. Effect of Ca^{2+} on protein conformation. *Biochemistry.* 24:5653-5660.

Kouyama, T., and K. Mihashi. 1981. Fluorimetry study of N-(1-pyrenyl)-

iodoacetamide-labelled F-actin. *Eur. J. Biochem.* 114:33-38.

Kurth, M., and J. Bryan. 1984. Purification and characterization of a gelsolin-actin complex from human platelets. *J. Biol. Chem.* 259:10895-10903.

Kwiatkowski, D. J., P. A. Janmey, J. E. Mole, and H. L. Yin. 1985. Isolation and properties of two actin-binding domains in gelsolin. *J. Biol. Chem.* 260:15232-15238.

Kwiatkowski, D. J., T. P. Stossel, S. H. Orkin, J. E. Mole, H. R. Colten, and H. L. Yin. 1986. Plasma and cytoplasmic gelsolins are encoded by a single gene and contain a duplicated actin-binding domain. *Nature (Lond.).* 323:455-458.

Kwiatkowski, D. J., R. M. Mehl, and H. L. Yin. 1988. Genomic organization and biosynthesis of secreted and cytoplasmic forms of gelsolin. *J. Cell Biol.* 106:375-384.

Lind, S. E., P. A. Janmey, C. Chaponnier, T. J. Herbert, and T. P. Stossel. 1987. Reversible binding of actin to gelsolin and profilin in human platelet extracts. *J. Cell Biol.* 105:833-842.

Lodish, H. F. 1988. Transport of secretory and membrane glycoproteins from the rough endoplasmic reticulum to the Golgi. A rate-limiting step in protein maturation and secretion. *J. Biol. Chem.* 263:2107-2110.

Matsudaira, P., and P. A. Janmey. 1988. Pieces in the actin-severing protein puzzle. *Cell.* 54:139-140.

Masui, Y., J. Coleman, and M. Inouye. 1983. Multipurpose expression cloning vehicles in Escherichia coli. In Experimental Manipulation of Gene Expression. S. L. Berger and A. R. Kimmel, editors. Academic Press, Inc., New York. 15-32.

Perrin, D. D., and I. G. Sayce. 1967. Computer calculation of equilibrium concentrations in mixtures of metal ions and complexing species. *Talanta.* 14:833-846.

Seed, B. 1987. An LFA-3 cDNA encodes a phospholipid-linked membrane protein homologous to its receptor CD2. *Nature (Lond.).* 329:840-842.

Stossel, T. P., C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, and K. S. Zaner. 1985. Nonmuscle actin-binding proteins. *Annu. Rev. Cell Biol.* 1:353-402.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350-4354.

Vandekerckhove, J., and K. Weber. 1979. The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fast skeletal muscle, and rabbit slow skeletal muscle. *Differentiation.* 14:123-133.

Way, M., and A. G. Weeds. 1989. Nucleotide sequence of pig plasma gelsolin: comparison of protein sequence with human gelsolin and other actin severing proteins shows strong homologies and evidence for large internal repeats. *J. Mol. Biol.* 203:1127-1133.

Yamamoto, K., J. D. Pardee, J. Reidler, L. Stryer, and J. A. Spudich. 1982. Mechanism of interaction of Dictyostelium severin with actin filaments. *J. Cell Biol.* 95:711-719.

Yin, H. L. 1988. Gelsolin: a calcium- and polyphosphoinositide-regulated actin-modulating protein. *Bioessays.* 7:176-179.

Yin, H. L., and T. P. Stossel. 1979. Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein. *Nature (Lond.).* 281:581-586.

Yin, H. L., and T. P. Stossel. 1980. Purification and structural properties of gelsolin, a Ca^{2+}-activated regulatory protein of macrophages. *J. Biol. Chem.* 255:9490-9493.

Yin, H. L., D. J. Kwiatkowski, J. E. Mole, and F. S. Cole. 1984. Structure and biosynthesis of cytoplasmic and secreted variants of gelsolin. *J. Biol. Chem.* 259:5271-5276.

Yin, H. L., K. Iida, and P. A. Janmey. 1988. Identification of a polyphosphoinositide-modulated domain in gelsolin which binds to the sides of actin filaments. *J. Cell Biol.* 106:805-812.

Zaner, K. S., and J. H. Hartwig. 1988. The effect of filament shortening on the mechanical properties of gel-filtered actin. *J. Biol. Chem.* 263:4532-4537.