Involvement of Gelsolin in Cadmium-Induced Disruption of the Mesangial Cell Cytoskeleton

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Cadmium (Cd2+) is known to cause a selective disruption of the filamentous actin cytoskeleton in the smooth muscle-like renal mesangial cell. We examined the effect of Cd2+ on the distribution of the actin-severing protein, gelsolin. Over 8 h, CdCl₂ (10 μM) caused a progressive shift of gelsolin from a diffuse perinuclear and cytoplasmic distribution to a pattern decorating F-actin filaments. Over this time filaments were decreased in number in many cells, and membrane ruffling was initiated. Western blotting and 125I-F-actin gel overlays demonstrated an increase in actin-binding gelsolin activity in the cytoskeletal fraction of cell extracts following Cd2+ treatment. In in vitro polymerization assays, gelsolin acted as a nucleating factor and increased the rate of polymerization. Cytosolic extracts also increased the polymerization rate. Addition of Cd2+ together with gelsolin further increased the rate of polymerization. Gelsolin enhanced depolymerization of purified actin, and Cd2+ partially suppressed this effect. However, cytoskeletal extracts from Cd2+-treated cells also markedly increased depolymerization, suggesting further that Cd2+ may activate cellular component(s) such as gelsolin for actin binding. We conclude that a major effect of Cd2+ on the mesangial cell cytoskeleton is manifest through activating the association of gelsolin with actin, with gelsolin’s severing properties predominating under conditions found in Cd2+-treated cells.

Key Words: F-actin; actin depolymerization; cadmium toxicity.

Cadmium (Cd2+) is a divalent metal ion that exhibits potent toxicity to most cells and organisms (Bhattacharyya et al., 2000; Goering et al., 1995; Nordberg et al., 1992). While the proximal tubule is a major target of Cd2+ toxicity, damage to the glomerulus is also significant (Järup, 2002). Workers exposed to Cd2+ show urinary markers characteristic of glomerular injury (Fels et al., 1994; Roels et al., 1993). Occupational exposure to Cd2+ is associated with glomerular injury and decreased glomerular filtration rate (Järup et al., 1993, 1995). The mesangial cell is a contractile smooth muscle-like cell of the glomerulus involved in regulating the glomerular filtration rate. Cadmium causes rearrangement of the actin cytoskeleton in cultured mesangial cells (Wang et al., 1996) and stimulates them to contract (Barrouillet et al., 1999a,b; L’Azou et al., 2002a,b). It has been suggested that Cd2+-induced mesangial cell contraction underlies the changes in intrarenal hemodynamics and glomerular filtration rate that precede proteinuria (Barrouillet et al., 1999b; Järup et al., 1993, 1995; Roels et al., 1993). The contribution of cytoskeletal disruption has not been studied in detail.

Previously we showed that exposure of mesangial cells to Cd2+ was accompanied by a loss of filamentous (F-) actin structure and organization (Wang et al., 1996; Wang and Templeton, 1996). Exposure of mesangial cells to Cd2+ resulted in F-actin depolymerization without a compensatory increase in cellular G-actin levels (Wang et al., 1996). These smooth muscle-like cells showed some specificity for this effect of Cd2+, a number of other divalent metals having no effect on F-actin at the same concentration as Cd2+. The cytoskeleton of smooth muscle cells in general seems to be particularly susceptible to disruption by Cd2+ (Templeton, 2000). The polymerization of F-actin from monomeric, globular (G-) actin subunits is regulated by a myriad of proteins, many of them Ca2+-dependent (Ayscough, 1998; Carpenter, 2000; Vandekerchove, 1993; Welch and Mullins, 2002). Further, the redox status of the cell, reflected in part in the thiol content of its F-actin cytoskeleton, is another determinant of cytoskeletal structure (Stournaras, 1990; Valentin-Ranc and Carlier, 1991).

The biological interactions of Cd2+ are dominated by two overriding chemical features. First, its strong propensity for sulfur as a ligand disrupts the structure and function of a number of proteins and enzymes (Beyersmann and Hechtenberg, 1997; Bhattacharyya et al., 2000; Goering et al., 1995; Waalkes et al., 1992) by poisoning protein thiol groups. Second, it has an ionic radius (0.97 Å) very close to that of Ca2+ (0.99 Å), and so potentially can interfere with Ca2+-dependent signaling processes; it can substitute for Ca2+ in vitro, activating Ca2+-dependent forms of protein kinase C and supporting calmodulin signaling (Mazzei et al., 1984; Suzuki et al., 1985). Thus, because of its interactions with thiol groups and substitution...
for Ca$^{2+}$, it is not unexpected that Cd$^{2+}$ will influence cytoskeletal integrity. The depolymerizing effects of Cd$^{2+}$ on F-actin were, though, found to be independent of its effects on cytosolic [Ca$^{2+}$], and independent of direct effects on Ca$^{2+}$-binding proteins (Wang et al., 1996). In fact, Cd$^{2+}$ stimulated actin polymerization in a reconstituted in vitro assay system. However, extracts made from cells that had been treated with Cd$^{2+}$ mimicked the depolymerizing effects of Cd$^{2+}$ on actin in cultured cells (Wang and Templeton, 1996). And, exposing mesangial cells to Cd$^{2+}$ influenced actin-binding activity of protein(s) with molecular weights of approximately 90 kDa and 45 kDa, differentially in cytosolic and cytoskeletal-associated fractions. The indication is that Cd$^{2+}$ does not act directly on the cytoskeleton, but affects the expression and (or) activity of proteins that may regulate the status of F-actin.

Gelsolin represents a family of ca. 90 kDa monomeric actin-binding proteins present in most animal cells, that sever F-actin and cap the quickly growing barbed end, thus favouring depolymerization from the pointed end (Ayscough, 1998; Sun et al., 1999; Yin and Stossel, 1979). Cardiac myocytes from gelsolin-/− mice show increased F-actin bundling of stress fibers in culture (Lader et al., 1999), whereas overexpression of gelsolin enhances cytoskeletal reorganization and fibroblast motility (Cunningham et al., 1991). Gelsolin binding to actin is regulated by Ca$^{2+}$ and polyphosphoinositide-4,5-bisphosphate (Gremm and Wegner, 2000; Kwiatkowski, 1999; Sun et al., 1999). At sub-micromolar Ca$^{2+}$ concentrations, Ca$^{2+}$-regulated capping and severing activities can occur independently of one another (Bryan and Coluccio, 1985; Gremm and Wegner, 2000). Gelsolin can also bind to G-actin monomers (Gremm and Wegner, 1999, 2000) and may facilitate nucleation in some circumstances.

We have pursued effects of Cd$^{2+}$ on the mesangial cell cytoskeleton in order both to elucidate potential mechanisms of cadmium toxicity and to gain further insight into features controlling the F-actin–G-actin equilibrium in these cells. The present study was undertaken to evaluate the effects of Cd$^{2+}$ on gelsolin expression and binding in mesangial cells, and in particular to evaluate gelsolin’s involvement in Cd$^{2+}$-induced disruption of cellular F-actin content.

**MATERIALS AND METHODS**

**Cell culture conditions and treatment.** Rat mesangial cells were isolated as described previously (Wang et al., 1994) and were grown in RPMI 1640 medium without antibiotics, supplemented with 10% (v/v) fetal bovine serum, in a humidified atmosphere of 5% CO$_2$ at 37°C. Cells growing on 10-cm dishes at 70% confluence, or on cover slips, were made quiescent by serum deprivation (0.2% serum for 48 h). Quiescent cells were treated with 10 μM CdCl$_2$ in RPMI 1640 for up to 8 h. All experiments were performed with cells between passages 5 and 20. Cytosolic and cytoskeletal-enriched fractions were prepared by lysis of cells in actin polymerization buffer (see below) containing 0.2% Triton X-100 as described previously (Wang and Templeton, 1996).

**Actin purification.** Rabbit skeletal muscle actin was prepared according to Selden et al. (2000). Briefly, G-actin was extracted from skeletal muscle actone powder (prepared as described previously; Wang and Templeton, 1996) with buffer G (2 mM Tris-HCl, pH 8.0 at 25°C, with 0.1 mM CaCl$_2$ and 0.5 mM dithiothreitol [DTT]) containing 0.2 mM ATP. The mixture was centrifuged at 10,000 × g for 20 min and the supernatant was further centrifuged at 150,000 × g for 30 min at 4°C. The supernatant was polymerized by addition of 3 M KCl to a final concentration of 0.8 M and left over night at 4°C with very slow stirring. The solution was centrifuged at 150,000 × g for 2 h and the F-actin pellet was soaked in buffer G for 2 h on ice. Pellets were picked up with a glass rod, homogenized carefully in a Dounce homogenizer, and dialysed against 500 ml of buffer G for 48 h. The dialysed solution was centrifuged at 150,000 × g for 2 h at 4°C. The polymerization step was repeated twice and G-actin concentration was determined spectrophotometrically at 290 nm using an absorption coefficient of ε = 26,600 M$^{-1}$ cm$^{-1}$. G-actin was further purified by gel filtration on a 2.6 × 100 cm Sephadex G-150 column pre-equilibrated with buffer G. G-actin (5 mg/ml) was eluted at a flow rate of 1 ml/min. Five-milliliter fractions were collected and the absorption at 280 nm was monitored. Fractions corresponding to G-actin (Mr = 43 kDa) were pooled and stored at 4°C for up to 2 weeks.

**Immunoblotting and immunoprecipitation.** Mesangial cell extracts were prepared by harvesting cells at the indicated times after addition of 10 μM CdCl$_2$ and lysis in buffer A (20 mM Tris-HCl, pH 7.4, with 2 mM MgCl$_2$, 138 mM KCl, 1 mM ATP, and 0.2% [v/v] Triton X-100). The lysates were cleared by centrifugation (10,000 × g, 15 min) and the pellets were resuspended in buffer A without Triton X-100, and either used immediately or stored at −80°C. SDS-PAGE was performed on (4–20%) gradient gels. Proteins were transferred to polyvinylidene difluoride (PVDF) membranes, and probed with polyclonal anti-gelsolin antibody (C-20) and secondary horseradish peroxidase-conjugated anti-goat IgG, both from Santa Cruz Biotechnology (Santa Cruz, CA). Bands were visualized by enhanced chemiluminescence detection kit (ECL, Amersham) according to the manufacturer’s instructions.

For immunoprecipitation, mesangial cells were treated with CdCl$_2$, and rinsed with ice-cold PBS, harvested, and lysed in immunoprecipitation buffer C (buffer A + 2 μg/ml aprotenin, 2 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 10 mM NaF, 1 mM NaVO$_4$). Samples containing 500 μg protein were incubated overnight at 4°C with anti-gelsolin antibody (1 μg/sample). Immune complexes were isolated by the addition of protein G Sepharose (2 h, room temperature) and washed five times with buffer C. Proteins bound to the Sepharose beads were released by boiling in SDS-PAGE sample buffer for 5 min, resolved by gradient SDS-PAGE (4–20%), transferred to PVDF, blocked with Membrane blocking agent (Amersham), and probed with anti-gelsolin antibody or 125I-F-actin.

**Indirect immunofluorescence and confocal microscopy.** Mesangial cells treated as described above were washed twice in PBS and fixed with 3.8% paraformaldehyde (4°C, 40 min). After two washes with PBS, cells were permeabilized with 0.2% Triton X-100 in PBS and blocked in PBS containing 5% bovine serum albumin for 1 h at room temperature, followed by a further 1 h incubation with anti-gelsolin antibody. After three 10-min washes with PBS, cells were exposed for 1 h to secondary antibody conjugated to fluorescein isothiocyanate (FITC-5000; Vector Laboratories, Burlingame, CA). Cells were rinsed three times with PBS and incubated with Alexa Fluor 568-labeled phallloidin (Molecular Probes; Eugene, OR), following the manufacturer’s instructions. Following six washes in PBS samples were mounted in 4’,6-diamidino-2-phenylindol dihydrochloride (DAPI) containing Vectashield mount medium (Vector Laboratories). Photomicrographs were obtained with a Nikon Eclipse E-600 microscope equipped with a Hamamatsu digital camera (Hamamatsu Inc., Japan), using Simple PCI software (Compix Inc., PA). Confocal microscopy was performed with a Zeiss LSM 410 inverted laser scanning microscope equipped with fluorescein and rhodamine filters and a Zeiss 63 × 1.4 Plan-Apochromat oil immersion lens. Image analysis was performed with ImageJ 1.24t software (National Institutes of Health, U.S.). The results are representative of at least three independent experiments conducted in triplicate.

**125I-F-actin preparation.** G-actin (1 mg/ml) prepared as described above was labeled with 250 μCi 125I (carrier-free NaI; ICN Pharmaceuticals,
the mixture was chromatographed on a PD-10 Sephadex column and 500
fractions were collected after elution with buffer G. The concentration of
dialysed against buffer G for 48 h at 4
overnight with continuous stirring at 4
sulfoxide and added to G-actin at a 10:1 molar ratio. The mixture was left
104 M
depolymerization pyrene-F-actin was initially diluted to 200 nM in the same
138 mM KCl, and 1 mM ATP). Pyrene-G-actin (2
in polymerization buffer (20 mM Tris-HCl, pH 7.4, containing 2 mM MgCl2,
and gelsolin, pyrene-
þ
1i and 1j) confirmed a shift of gelsolin from a diffuse distribution before Cd2+
before Cd2+
þ
exposure to Cd2+
þ
exposure in cells
8 h of Cd2+
þ
exposure, cells showed prominent staining for
gelsolin in the cytoplasm and evidence of some filamentous
arrangement (Fig. 1d). After 8 h of Cd2+
þ
treatment, gelsolin was predominately localized along any remaining F-actin filaments
(Fig. 1e). In cells in which the F-actin filaments remained more or less intact after 8 h of Cd2+
þ,
strong colocalization of gelsolin with actin was observed (Figs. 1f–1h).
Scanning across magnified fields of confocal images of cytosolic regions (Figs. 1i and 1j)
þ
treatment, to take on a periodicity matching that of
F-actin at 8 h. Images and analyses in Figures 1c–1j are re-
representative of multiple fields from at least 10 different experiments.
(Not that panels i and j are produced from the high resolution confocal images in panels c and e,
and are magnified to allow visualization of the pixelated images.) The percentage of the cells showing localization of gelsolin to F-actin fibers steadily increased with the time of Cd2+
þ
treatment (not shown).
These observations suggest that gelsolin redistribution from the nucleus and cytoplasm to the actin cytoskeleton can be triggered by Cd2+
þ treatment. In addition, Cd2+
þ treatment and accompanying stress fiber reorganization induces areas of membrane ruffling, or lamellipodia, rich in gelsolin (Fig. 2). These are seen rarely in control cells (an example is shown in Fig. 2a), but are frequent in Cd2+
þ-treated cells (Fig. 2b). Gelsolin localization to the membrane ruffles was detected in over 60% of the cells after 8 h of Cd2+
þ exposure, where it colocalized with actin filaments (Fig. 2c).

Western Blotting and Gel Overlays
To confirm the Cd2+
þ-dependent redistribution of gelsolin binding activity, cytosolic and cytoskeletal fractions from
control and Cd-treated cells were subjected to Western blotting. Two actin-binding protein bands with Mr values of about 90 and 40 kDa were detected (Fig. 3). The band with Mr = 90 kDa corresponds to full-length gelsolin and the 40 kDa band may be attributed to a cleaved gelsolin peptide (see Discussion) recognized by the antibody against a C-terminal peptide; this lower band is also present in a purified gelsolin standard. Although no change in total gelsolin mRNA in whole cell extracts was found (data not shown), the intensity of both gelsolin protein bands increased in the cytoskeletal fraction in

RESULTS
Immunohistochemical Localization of Gelsolin
Cells were seeded on a glass surface, starved for 48 h in 0.2% serum, and then treated with 10 µM CdCl2 for up to 8 h. Longer exposures and higher concentrations result in some cell death with loss of filaments in all cells (not shown; see Wang et al., 1996), whereas shorter exposures resulted in less cytoskeletal disruption. Fluorescence photomicrographs of phalloidin-stained cells (Figs. 1a and 1b) show a disruption of actin filaments in many cells after Cd2+
þ treatment, confirming earlier observations (Wang et al., 1996). The response is heteroge-
a time-dependent manner following Cd\textsuperscript{2+} treatment, consistent with the results of immunofluorescence. The putative cleaved fragment at 40 kDa was absent from cytosol before Cd\textsuperscript{2+} treatment but was apparent in this fraction at and after 4 h.

To demonstrate that these bands represented actin-binding activity, cell fractions were electrophoresed and blotted with \[^{125}\text{I}]-F\text{-actin. Despite the high background inherent in this technique, at least five actin-binding bands were identified.\)
Two of these (bands 2 and 5) were prominent in the gelsolin standard, representing intact gelsolin (band 2) and probably a cleaved fragment containing the C-terminal epitope (band 5). The nature of the other bands was not pursued in this study, including the very prominent band 4 which is restricted to the cytoskeletal fraction and increases markedly with Cd\textsuperscript{2+} treatment. Immunoprecipitates with anti-gelsolin antibody were prepared to confirm the identity of bands 2 and 5. \textsuperscript{[125I]}-F-actin overlays of gelsolin immunoprecipitates showed only bands 2 and 5 (Fig. 4c). These bands show little or no change in binding activity in the cytosolic fraction after 8 h of treatment with Cd\textsuperscript{2+}. However, actin-binding activity of gelsolin, especially of the 40 kDa fragment, increases in the cytoskeletal fraction after Cd\textsuperscript{2+} exposure, consistent with the results of immunofluorescence and Western blotting.

Polymerization/Depolymerization Studies

We previously showed that Cd\textsuperscript{2+} both facilitates polymerization and suppresses depolymerization of actin in an \textit{in vitro} assay (Wang and Templeton, 1996), both counter to the loss of actin filaments from the cell. Therefore, direct interaction of Cd\textsuperscript{2+} with F-actin/G-actin is unlikely to account for Cd\textsuperscript{2+}-induced loss of filaments \textit{in vivo}. However, the above results raise the possibility that Cd\textsuperscript{2+} may have different effects when gelsolin is present, and we therefore studied the polymerization and depolymerization of purified actin in the presence of Cd\textsuperscript{2+} and gelsolin together. Pyrene-labeled G-actin was dissolved in polymerization buffer at 2 \textmu M and polymerization was initiated by the addition of ATP, resulting in an apparent first-order rate of increase in fluorescence intensity (Fig. 5a). When gelsolin was included in the reaction solution, a sigmoidal polymerization curve was observed, representing a lag phase indicative of a nucleation process followed by a linear phase of polymerization at a markedly increased rate. We confirmed our previous observation (Wang \textit{et al.}, 1996) that inclusion of Cd\textsuperscript{2+} in the polymerization mixture increased the rate of polymerization at concentrations in the 200 \textmu M range, though concentrations of 10–100 nM were without significant effect in the absence of gelsolin (not shown). When low concentrations of Cd\textsuperscript{2+} (20–80 nM) were added to a polymerization mixture containing 5 nM gelsolin, there was an indication that the lag phase was decreased and the rate of polymerization was increased.
unaffected or increased (Fig. 5a), although not in any apparent concentration-dependent manner. These results are presented to demonstrate that neither Cd2\textsuperscript{+} nor gelsolin, alone or in combination, drive depolymerization of the filaments.

To study depolymerization, pyrene-labeled F-actin was diluted to 200 nM (based on the G-actin subunit) by the addition of buffer. This is below the critical concentration for polymerization, and spontaneous depolymerization was observed by fluorescence quenching. Depolymerization was enhanced by inclusion of gelsolin in the dilution buffer (Fig. 5b), and increased with increasing gelsolin:actin ratio (not shown), consistent with well documented capping at the barbed end and dissociation from the pointed end. Inclusion of Cd2\textsuperscript{+} in the gelsolin mixture prior to addition of actin slowed the rate of depolymerization in a concentration-dependent manner, though even at a ratio of Cd2\textsuperscript{+}:gelsolin of 10:1 depolymerization was still more rapid than in the absence of gelsolin.

Because Cd2\textsuperscript{+} exposure facilitated a localization of gelsolin with the cytoskeleton in cultured cells, cytosolic and cytoskeletal cell extracts from control and Cd2\textsuperscript{+}-treated cells were examined for their ability to affect depolymerization of purified F-actin. Cytosolic extracts from control cells did not affect the spontaneous depolymerization of actin below its critical concentration (Fig. 6a). Cytoskeletal extracts from control cells increased the rate of depolymerization (Fig. 6b), consistent with the presence of protein(s) in this fraction that facilitate depolymerization. When obtained from Cd2\textsuperscript{+}-treated cells, both cytosolic and cytoskeletal fractions increased the rate of depolymerization.

**DISCUSSION**

Rat mesangial cells have previously been shown to undergo disruption of F-actin after treatment with Cd2\textsuperscript{+} (Barrouillet et al., 1999b; L’Azou et al., 2002b; Wang et al., 1996; Wang and Templeton, 1996). The present study corroborates these findings and shows that Cd2\textsuperscript{+} treatment results in F-actin-gelsolin interactions that accompany changes in F-actin stress fiber organization and distribution, cell-cell contacts, and organization of membrane ruffles. A redistribution of gelsolin into the detergent-insoluble cytoskeletal fraction on Western blots and immunolocalization of gelsolin to the actin cytoskeleton in those cells where actin filaments remained, indicates that Cd2\textsuperscript{+} treatment favors association of gelsolin with actin filaments. This association is seen as early as 2 h after exposure to Cd2\textsuperscript{+} and precedes gross actin disruption; gelsolin remains associated with residual shortened actin filaments throughout the process, leading to the eventual disappearance of many

![FIG. 3](image_url)  
FIG. 3. Gelsolin protein expression following Cd2\textsuperscript{+} treatment of mesangial cells. Cells were serum-starved for 48 h and then exposed to 10 μM CdCl\textsubscript{2} for the times indicated. Cytosolic (a) and cytoskeletal (b) extracts were then electrophoresed (50 μg protein/lane) on SDS-polyacrylamide gradient gels (4–20%), transferred to PVDF membranes, and probed with an anti-gelsolin antibody. Positions of molecular weight markers are indicated to the left. Blots are representative of three independent experiments.

![FIG. 4](image_url)  
FIG. 4. Detection of F-actin binding proteins by 125I-labeled F-actin blot overlay. Cytosolic (panel a) and cytoskeletal (panel b) extracts were prepared from rat mesangial cells serum starved for 48 h and treated with 10 μM CdCl\textsubscript{2} for 0 or 8 h. Fifty μg protein/lane were resolved on 4–20% SDS-PAGE and blotted onto PVDF membranes. The blots were probed with 125I-labeled F-actin overnight (upper panels). Numbers to the left side of the figure denote protein bands positively recognized by F-actin binding activity. Standard gelsolin protein was run alone in the two left-most lanes. The lower panels show Coomassie blue stains of the gels, indicating uniform loading. Panel (c) is a blot of a sample of an immunoprecipitate with an anti-gelsolin antibody from extracts of cells treated for 8 h with 10 μM CdCl\textsubscript{2}. Panels (a) and (b) are representative of three independent experiments. The immunoprecipitation (c) was performed once.
fibers by 8 h. Thus, gelsolin is a leading candidate for mediating the effects of Cd\(^{2+}\) on the cytoskeleton.

Disruption of F-actin by enhanced association with gelsolin is consistent with the well-described capping and/or severing properties of gelsolin (Ayscough, 1998; Gremm and Wegner, 2000; Kinosian et al., 1998; Lagarrigue et al., 2003; Sun et al., 1999). Nevertheless, kinetic analysis indicates that complex mechanisms are involved. Thus, gelsolin is also an effective nucleating factor for G-actin polymerization under some circumstances (Burtnick et al., 1997; Ditsch and Wegner, 1994; Feinberg et al., 1997). This effect dominates in the assay with purified actin, e.g., at a gelsolin:G-actin ratio of 1:400.

**FIG. 5.** Polymerization/depolymerization of purified actin. Panel (a), samples for polymerization containing 2 \(\mu\)M pyrene-labeled G-actin were mixed with various amounts of gelsolin and/or Cd\(^{2+}\) in 20 mM Tris-HCl, pH 7.4, with 2 mM MgCl\(_2\), 138 mM KCl, and 1 mM ATP. The polymerization of pyrenyl actin was observed by an increase in fluorescence. ‘Con’ denotes control in which no gelsolin or Cd\(^{2+}\) was added. In other curves, 5 nM gelsolin was included, with 0, 20, 40, or 80 nM CdCl\(_2\) as indicated. Digitized fluorescence data were collected at each second over the indicated time scale. Each plotted point is the average of 5 independent experiments, fixed to a common point at time zero. Panel (b), changes of fluorescence intensity of 200 nM pyrene-labeled F-actin were followed after initiation of depolymerization by dilution below the critical concentration as described in Methods. ‘Con’ denotes spontaneous depolymerization in the absence of added Cd\(^{2+}\) or gelsolin. Other reactions are in the presence of 80 nM gelsolin mixed with 0, 200, 400, or 800 nM CdCl\(_2\) as indicated. Points are averages of 5 values as in panel (a).

**FIG. 6.** Effects of cytosolic and cytoskeletal-enriched fractions on F-actin depolymerization. Depolymerization of pyrene-labeled F-actin (200 nM) was initiated as in Figure 5b, in the absence (○) or presence (●) of cytosolic [panel (a)] or cytoskeletal [panel (b)] fractions from control cells unexposed to Cd\(^{2+}\). The cytosolic and cytoskeletal fractions were also prepared from cells exposed to Cd\(^{2+}\) (10 \(\mu\)M for 8 h), indicated as (□) in each panel.
While the signal(s) effecting gelsolin redistribution in Cd²⁺-treated cells is (are) unclear, the Ca²⁺ dependence of gelsolin activation is well known. Binding of two Ca²⁺ ions at sites of moderate affinity (K₄ in the μM range) is necessary for activation of actin binding (Weeds et al., 1988), and loss of one site by mutation in domain 2 leads to increased proteolysis and an amyloid-like syndrome (Kazmirski et al., 2002). Calcium binding produces large conformational changes that expose actin-binding sites (Gremm and Wegner, 1999; Hellweg et al., 1993). Gelsolin binds to both G-actin monomer and to actin filaments (Bryan, 1988; Gremm and Wegner, 1999; Pope et al., 1991), perhaps accounting, respectively, for nucleation and severing. Stepwise formation of gelsolin:actin monomer 1:1 and 1:2 complexes is cooperative and Ca²⁺-dependent, and chelation of Ca²⁺ favors dissociation to the 1:1 state (Gremm and Wegner, 1999). Thus, many of the effects of Cd²⁺ on the actin cytoskeleton may arise from effects on Ca²⁺-gelsolin interactions. Cadmium might activate gelsolin for F-actin binding and severing in the cell by substituting directly for Ca²⁺, or alternatively by displacing Ca²⁺ from other cellular sites and making it available to gelsolin. However, when G-actin is the predominant species in localized cell compartments, or as is the case in the in vitro polymerization assay, activation of gelsolin may be balanced by severing nucleation and polymerization. This may account for the persistence of actin filaments, decorated with gelsolin, in many Cd²⁺-treated cells. It is interesting to note that Cd²⁺ can substitute for Ca²⁺ in the gelsolin crystal structure at a Ca²⁺-binding site in domain 2 that is involved in gelsolin stabilization and perhaps activation (Kazmirski et al., 2002).

Our immunoblots and ¹²⁵I-F-actin overlay experiments indicate a lower molecular mass species of gelsolin present in mesangial cells that is also present in the commercial gelsolin standard. This band associates prominently with the cytoskeletal fraction of Cd²⁺-treated cells, and corresponds to the approximately 45 kDa band reported in our earlier study (Wang and Templeton, 1996). Gelsolin cleavage may result from caspase-3 activation as shown by several groups (Azuma et al., 1998; Kothakota et al., 1997; Sun et al., 1999). Kothakota et al. (1997) have demonstrated that caspase-3 cleaves gelsolin within segment 3 at Asp³⁵²/Gly³⁵³ to generate peptides with apparent electrophoretic masses of 40 and 48 kDa. The N-terminal fragment is able to sever F-actin in a Ca²⁺-independent manner. Alternatively, conformational changes induced in the gelsolin molecule by Ca²⁺ unmask relatively specific tryptic cleavage sites that generate fragments of 70, 45, and 30 kDa (Khaitlina and Hinssen, 2002). Ca²⁺-dependant specific cleavage patterns of gelsolin have also been observed with plasmin (Wen et al., 1996). In light of these observations, Robinson et al. (2001) proposed that the fully activated gelsolin molecule is functionally susceptible to proteolysis. Therefore, gelsolin may be bound to the microfilament system as a combination of full-length gelsolin and one or more proteolysed fragments, with disruption of the microfilament system.
dependent upon all forms of gelsolin. Cadmium appears to favor cytoskeletal association of both full-length gelsolin and the 40 kDa peptide.

The intracellular distribution of gelsolin has long been a matter of debate (Carron et al., 1986). In fibroblasts it has been localized to the cell cortex and regions of adhesion. A homogeneous cytoplasmic localization of gelsolin has also been reported (Carron et al., 1986; Cooper et al., 1987), whereas in human gingival fibroblasts the distribution of gelsolin depends on their state of mobility. In migrant cells, it has been found diffusely distributed through the cytoplasm, whereas in non-migrating cells it has been localized along the stress fibers (Arora and McCulloch, 1996). Our results demonstrate that mesangial cell gelsolin alters its intracellular location from nucleus and cytosol in response to Cd²⁺ treatment. The view that gelsolin can actively participate in F-actin disruption and ruffle formation is supported by the demonstration that fibroblasts from gelsolin-null mice exhibit reduced membrane ruffling and an increased amount of F-actin. F-actin disruption and ruffle formation is supported by the demonstration that fibroblasts from gelsolin-null mice exhibit reduced membrane ruffling and an increased amount of F-actin.


