Role of Gelsolin in the Formation and Organization of Triton-Soluble F-Actin During Myeloid Differentiation of HL-60 Cells

By Raymond G. Watts

Structurally and functionally distinct F-actin pools coexist with globular (G)-actin in a variety of eukaryotic cells, including polymorphonuclear leukocytes (PMNs). In PMNs, a Triton-soluble F-actin pool (TSF) exists as short cytoplasmic filaments capped with gelsolin, while Triton-insoluble F-actin (TIF) is a three-dimensional meshwork of F-actin associated with actin-binding protein 280 (ABP-280), α-actinin, and tropomyosin. The unique association of gelsolin with the TSF suggests a role for gelsolin in creation or regulation of TSF. To evaluate gelsolin’s role in TSF formation, the quantities of actin and gelsolin were determined by quantitative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblots in uninduced HL-60 cells (U-HL-60) and in HL-60 cells induced to myeloid differentiation with 1.25% dimethyl sulfoxide for 4 to 5 days (I-HL-60). U-HL-60 cells contain 17.7 ± 6.01 pmol actin per 10^6 cells (TIF, 5.3 ± 1.5; TSF, 2.17 ± 0.37; G, 10.3 ± 5.7; n = 5) and 0.073 pmol gelsolin per 10^6 cells (TIF, 0; TSF, 0.002 ± 0.005; G, 0.07 ± 0.01; n = 3), representing molar actin to gelsolin (A:G) ratios of 1,085:1 for TSF and 147:1 for G. After myeloid differentiation, the actin content increases 1.80-fold (31.9 ± 6.14 pmol/10^6 cells) equally in each actin pool (TIF, 3.36 ± 2.35; TSF, 3.29 ± 0.62; G, 19.28 ± 4.83). Gelsolin increases 2.4-fold overall (0.178 ± 0.02 pmol/10^6 cells) but 19-fold in TSF (0.038 ± 0.009) and only 1.9-fold in G pool (0.129 ± 0.006), resulting in A:G ratios of 87:1 in TSF and 139:1 in G. The findings of an increase in TSF gelsolin with decreased A:G ratios (1,085:1 v 87:1) with myeloid differentiation suggest shortening of TSF filaments, while the A:G ratios of unbound gelsolin are unchanged (147:1 v 139:1). Measurement of EGTA-resistant gelsolin/actin complexes in HL-60 cells shows that 95% to 100% of complexes exist in the TSF-actin pool only. These findings are consistent with a role for gelsolin in formation and organization of Triton-soluble F-actin. Furthermore, the apparent shortening of TSF-actin filaments with myeloid cellular differentiation and maturation may represent one mechanism of conversion of the nonmotile myeloblast to the motile PMN.

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Table 1. Characteristics of F-Actin Pools

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<thead>
<tr>
<th>TIF-Actin</th>
<th>TSF-Actin</th>
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<tr>
<td><strong>Basic three-dimensional</strong></td>
<td><strong>Cytoplasmic pool of shorter crosslinked and branched oligomer</strong></td>
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<tr>
<td><strong>Stable at 4°C</strong></td>
<td><strong>Depolymerizes at 4°C</strong></td>
</tr>
<tr>
<td><strong>Stable to cytochalasin D</strong></td>
<td><strong>Depolymerizes with depolymerization</strong></td>
</tr>
<tr>
<td><strong>Resistant to dilutional</strong></td>
<td><strong>Resistant to dilutional</strong></td>
</tr>
<tr>
<td><strong>Localizes to submembranous areas</strong></td>
<td><strong>Depolymerizes with depolymerization</strong></td>
</tr>
<tr>
<td><strong>Site of all FMLP-induced F-actin growth</strong></td>
<td><strong>Decreases in response to FMLP</strong></td>
</tr>
<tr>
<td><strong>Defined by association with tropomyosin, α-actinin, and ABP-280</strong></td>
<td><strong>No tropomyosin, α-actinin, or ABP-280</strong></td>
</tr>
<tr>
<td><strong>No associated gelsolin</strong></td>
<td><strong>Filaments capped with gelsolin</strong></td>
</tr>
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</table>

The formation of TIF-actin. The quantitative changes in total cellular actin and gelsolin, as well as amounts of actin and gelsolin in TIF-actin, TSF-actin, and G-actin pools of HL-60 cells before and after myeloid differentiation induced by DMSO are reported.

The results show that (1) TIF-actin and TSF-actin pools exist in undifferentiated and myeloid-differentiated HL-60 cells; (2) myeloid differentiation results in a 1.8-fold increase in total cellular actin symmetrically distributed among the three actin pools; (3) myeloid differentiation results in a 2.4-fold increase in total cellular gelsolin, which is asymmetrically distributed (19-fold increase in TIF-actin, 1.9-fold increase in G-actin pool); (4) gelsolin/actin complexes (gelsolin molecules bound to actin) are found exclusively in the TSF-actin pool of undifferentiated HL-60 cells and nearly exclusively in the TSF-actin pool of differentiated HL-60 cells; and (5) myeloid differentiation of HL-60 cells results in an apparent marked shortening of TSF-actin filaments, as reflected by a marked fall in the molar ratios of actin to gelsolin in the TSF-actin pool.

The results suggest that (1) distinct association of actin with actin-regulatory proteins during cellular differentiation may define the structural and functional role of actin within the cell; (2) gelsolin plays a primary role in the structure and function of the TSF-actin pool; and (3) developmentally regulated modifications in the cellular cytoskeleton of myeloid cells may underlie and result in acquisition of motile properties of the cell.

**MATERIALS AND METHODS**

**Cell culture.** HL-60 human promyelocytic leukemia cells were obtained from the American Type Culture Collection (Rockville, MD) and grown in Iscove's modified Dulbecco's medium (IMDM) supplemented with 20% bovine calf serum and 1% penicillin/streptomycin (Sigma Chemicals, St. Louis, MO) at 37°C in a 5% CO₂ humidified environment. Undifferentiated HL-60 cells (U–HL-60) were grown to a density of 2 × 10⁸ to 4 × 10⁹ cells per milliliter, collected by centrifugation, and resuspended in Hanks'/HEPES buffer (25 mmol/L HEPES, 50 mmol/L phosphate, 150 mmol/L NaCl, 40 mmol/L KCl, pH 7.15) for cellular studies. HL-60 cells were induced to myeloid differentiation (I–HL-60) by the addition of 1.25% DMSO for 4 to 5 days and then harvested as above. The differentiated phenotypic expression was assessed by light microscopic appearance after Wright-Giemsa staining and by the ability of the differentiated cells to reduce nitroblue tetrazolium (NBT). I–HL-60 cells were studied only at low passage numbers (20 to 28) to limit intrinsic differentiation and ensure uniformity of results.

**Isolation and purification of human PMNs.** Human PMNs were isolated under endotoxin-free (ETF) conditions over Percoll gradients as previously described. Cells were studied in suspension in Hanks'/HEPES buffer to a final cell concentration of 2 × 10⁶ cells per experiment.

**Determination of differentiated phenotype of HL-60 cells by light microscopy.** For morphologic confirmation of myeloid differentiation of HL-60 cells, differentiated or undifferentiated cell samples in suspension were spun onto glass slides (1,000 rpm for 5 minutes) with a Cytospin II (Shandon, Pittsburgh, PA), stained with Wright's stain, and graded morphologically as myeloblasts, promyelocytes, myelocytes, metamyelocytes, bands, or mature PMNs. Myeloblasts and promyelocytes were defined as immature cells, while myelocytes, metamyelocytes, bands, and PMNs were defined as mature cells for the purpose of determining differentiation.14,17

**Surface expression of CD11b.** The expression of CD11b (Leu 15, C3b, CR3) was used to confirm myeloid differentiation of HL-60 cells. U–HL-60 cells or I–HL-60 cells were collected from tissue culture flasks by centrifugation (1,500 rpm for 5 minutes), washed once in Hanks'/HEPES buffer, and resuspended in Hanks'/HEPES buffer to a density of 2 × 10⁶ cells per milliliter. The cells in suspension were fixed with 3.7% formaldehyde for 5 minutes. The cells were washed once, resuspended in Hanks'/HEPES buffer plus 0.5% human albumin, labeled with a 1:20 dilution of monoclonal anti-CD11b (Becton Dickinson, San Jose, CA) or control primary antibody (mouse IgG2a antibody to keyhole limpet hemocyanin; Becton Dickinson) for 30 minutes at 25°C, and then stained with fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Kirkegaard and Perry, Gaithersburg, MD) at a 1:100 dilution for 30 minutes. Stained cells were analyzed on a FACStar flow cytometer (Becton Dickinson, Mountain View, CA). The mean fluorescence of 5,000 test cells was corrected for nonspecific binding by subtracting the fluorescence of the secondary antibody only control. The fluorescence of differentiated cells is expressed relative to the fluorescence of undifferentiated cells.

**NBT slide test.** The reduction of NBT as a measure of superoxide production indicative of a myeloid differentiation phenotype was assayed by the slide NBT test as previously described. Briefly, PMNs or HL-60 cells in suspension in Hanks'/HEPES buffer with Ca⁺⁺ and Mg⁺⁺ were exposed to 0.2% NBT in saline and activated with phorbol 12-myristate 13-acetate (PMA), 0.4 μg/mL at 37°C for 25 minutes. The cells were then cytopsin onto glass slides, stained with Wright's stain, and examined for the presence of intracellular blue-black deposits indicative of NBT reduction to formazan. Results are expressed as percentage of 100 cells positive for blue-black deposits.

**Separation of actin pools.** F-actin content in individual actin pools was determined as cytoskeletal-associated actin (CAA) by a modification of the technique of Phillips et al19 and White et al,9 as previously reported in detail. Briefly, the TIF-actin pool is defined as that F-actin retained in the low-speed pellet (16,000g for 2 minutes) after cell lysis with Triton X-100 for 15 minutes at 25°C [1% in imidazole 10 mmol/L, KCl 40 mmol/L, and EGTA 10 mmol/L, pH 7.15, with 7 mmol/L diisopropylfluorophosphate (DFP) to limit proteolysis].20 After low-speed centrifugation, the supernatant is separated into TIF-actin and G-actin by ultracentrifugation in a TL 100 ultracentrifuge (Beckman, Palo Alto, CA) at 366,000g for...
5 minutes, as previously described. After high-speed centrifugation, the supernatant contains G-actin and is concentrated by precipitation with 20% trichloroacetic acid. Samples were solubilized in a Tris buffer [0.625 mol/L in 2% sodium dodecyl sulfate (SDS), 10% glycerol, and 5% 2-mercaptoethanol] and evaluated by gradient (5% to 15%) SDS-polyacrylamide gel electrophoresis (SDS-PAGE), using the Mini Protean II system (Biorad, Richmond, CA).

**Purification of actin.** Actin was isolated from rabbit skeletal muscle according to the technique of Spudich and Watt and was further purified over a Sepharose 2B chromatography column, as previously described.

Quantification of F-actin by gel scanning. Protein bands were quantified by densitometric gel scans of Coomassie blue-stained gels via laser densitometry (LKB Ultrascan XL, Bromma, Sweden) using rabbit skeletal muscle actin as standards. Control experiments (data not shown) defined the linear range of detectability for actin standards as Coomassie blue staining falling between 0.25 and 5.0 μg. Gelsolin was purified from human plasma by affinity chromatography using monoclonal anti-human gelsolin antibody25 linked to cyanogen bromide-activated Sepharose 4B beads. The plasma gelsolin bound to the antibody-coated beads was eluted by alkaline pH, concentrated, and solubilized in SDS sample buffer (see above). Purity was confirmed by Coomassie-stained SDS-PAGE gels and immunoblot analysis. This purification technique yields milligram amounts of gelsolin.

**Immunoblot analysis.** The quantity of gelsolin in individual actin pools was determined by quantitative immunoblot technique in a Mini-Protean transblot system (Biorad) using a previously described monoclonal antigelsolin antibody as primary antibody and peroxidase-tagged anti-mouse IgG (Kirkegaard and Perry, Gaithersburg, MD). Purity of human plasma gelsolin was verified on the same nitrocellulose sheets. Control experiments not shown defined the linear range of detectability for immunoblot analysis of gelsolin as falling between 5 and 100 ng, allowing construction of standard curves for each individual immunoblot.

**Calculation of moles of actin and gelsolin.** Actin was quantified in microgram amounts by quantitative densitometric gel scans of Coomassie blue-stained SDS-PAGE gels, and gelsolin was quantified in nanogram amounts by quantitative densitometric gel scans of peroxidase-labeled immunoblots as described above. The results were converted to moles of protein using the molecular weights of 43,000 D for actin and 90,000 D for gelsolin. Results shown are normalized to 1 X 10^6 cells.

**Measurement of gelsolin/actin complexes.** Determination of EGTA-resistant gelsolin/actin complexes in HL-60 cell supernatants was performed as previously described. Briefly, U-HL-60 cells and 1–HL-60 cells were harvested by centrifugation, washed twice in Hanks’/HEPES buffer, and resuspended in Hanks’/HEPES buffer with Ca^2+ and Mg^2+ to a final concentration of 100 X 10^6 cells per milliliter. Cell suspensions were lysed for 15 minutes with Triton lysis buffer as described above. Triton-soluble supernatants containing both TSF-actin and G-actin were prepared by centrifugation of lysed cell suspensions at 16,000g for 5 minutes. The G-actin pool was separated from the TSF-actin pool by centrifugation of lysed cell suspensions at 366,000g for 5 minutes. Thus, Triton-soluble supernatants and G-actin pools from both undifferentiated and differentiated HL-60 cells were available for comparison. Respective pools were transferred to 1.5-mL Eppendorf tubes (Brinkmann Instruments, Westbury, NY) and immunabsorbed onto cyanogen bromide-activated Sepharose 4B beads (Pharmacia LKB, Uppsala, Sweden) conjugated with a monoclonal antihuman IgG, as previously described. After 2 hours mixing at 4°C, the beads were centrifuged at 16,000g for 5 minutes and washed successfully with 1 mL of Triton lysis buffer, 1 mL of Tris/saline/EGTA buffer (10 mmol/L Tris, 100 mmol/L NaCl, 1 mmol/L EGTA, pH 7.4) X 2, and, in some cases, 1 mL of 0.3 mol/L MgCl_2. The washed beads were resuspended in SDS solubilizing buffer as described above, boiled for 5 minutes, and analyzed on 5% to 15% gradient SDS-PAGE gels. Representative gels demonstrate four primary bands: actin, approximately 25 kD, the heavy chain of the antigelsoin antibody (approximately 50 kD), actin (43 kD), and the light chain of the antigelsoin antibody (approximately 25 kD). The intensities of gelsolin and actin bands were measured by scanning laser densitometry as described above, and relative molar ratios of gelsolin to actin were calculated as described.

**RESULTS**

**Table 2. Characteristics of HL-60 Cells**

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<th>% NBT Reduction (n = 4)</th>
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<tr>
<td>U–HL-60 cells</td>
<td>Mature: 8 ± 3</td>
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<tr>
<td>I–HL-60 cells</td>
<td>Mature: 87 ± 6</td>
<td>84 ± 5</td>
</tr>
<tr>
<td></td>
<td>Immature: 13 ± 6</td>
<td>0 ± 0.4</td>
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Mature: myelocytes, metamyelocytes, bands, and PMNs; immature: myeloblasts and promyelocytes. P < .0001.

**Documentation of myeloid differentiation of HL-60 cells.** As the experiments reported here examined quantitative changes in the amounts of and segregation of the important cytoskeletal proteins actin and gelsolin with myeloid differentiation of HL-60 cells, stringent differentiation criteria were chosen to define the myeloid phenotype. After exposure of HL-60 cells to DMSO for 4 to 5 days, induction of myeloid differentiation was defined by (1) morphologic maturation to more mature myeloid forms,(14,17) (2) the ability to reduce NBT,(14,17) and (3) the expression of CD11b.30 Documented here the phenotypic change of HL-60 cells was measured by the Student’s t test or the standard error of the difference between means.28,29

**RESULTS**

**Statistical evaluation.** Statistical evaluation of changes in actin and gelsolin content in undifferentiated and differentiated HL-60 cells was used to determine the Student’s t test or the standard error of the difference between means.38,29

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ROLE OF GELSONIN IN TSF-ACTIN FORMATION

Quantification of actin in distinct actin pools in U–HL-60 and I–HL-60 cells and in human PMNs. Previous studies of actin content in differentiating HL-60 cells have examined changes in the amount of total cellular actin with either monocytic or myeloid differentiation. In human PMNs and other non-muscle cells, distinct structural and functional pools of F-actin in equilibrium with G-actin are described and operationally defined. Whether similar pools of F-actin exist in undifferentiated or differentiated HL-60 cells and how these pools are formed is unclear. To determine the actin pool composition of U–HL-60 and I–HL-60 cells, control U–HL-60 cells in suspension and I–HL-60 cells were isolated, actin pools were separated as defined operationally, and quantitative SDS-PAGE of TIF-actin, TSF-actin, and G-actin pools was performed. Results in U–HL-60 and I–HL-60 cells were compared with similar studies previously reported for mature human PMNs.

As shown in the representative SDS-PAGE gel shown in Fig 1, U–HL-60 (Fig 1A) and I–HL-60 (Fig 1B) cells contain defined pools of actin previously noted in human PMNs. When quantified by gel electrophoresis and expressed as picomoles of actin per million cells (Fig 2A), the total cellular actin content of U–HL-60 cells is noted to be $17.8 \pm 6.01 \text{ pmol/10}^6 \text{ cells}$ or, when expressed as micrograms actin per 100 micrograms total cellular protein, $4.78 \pm 0.81 (n = 3)$. These findings of actin content in U–HL-60 cells at low passage number are comparable with those of previous studies. The apportionment of actin between actin pools in U–HL-60 cells is shown in Fig 2A. The TIF-actin pool contains $5.3 \pm 1.5$, the TSF-actin pool $2.2 \pm 0.37$, and the G-actin pool $10.3 \pm 5.7 \text{ pmol of actin per 10}^6 \text{ cells} (n = 5)$. Note a predominance of G-actin and a small pool of TSF-actin.

After myeloid differentiation with DMSO (Fig 2B), there is an approximately 1.80-fold increase in total cellular actin content to a total of $31.9 \pm 6.14 \text{ pmol/10}^6 \text{ cells}$ ($P < .05$), in agreement with previous studies of relative changes in actin amount. As shown in Fig 2B, myeloid differentiation of HL-60 cells results in a statistically significant ($P < .05$ compared with undifferentiated cells), symmetric increase in actin content in all three defined actin pools: TIF-actin $9.4 \pm 2.35$, TSF-actin $3.3 \pm 0.62$, and G-actin $19.3 \pm 4.83 \text{ pmol of actin per 10}^6 \text{ cells} (n = 3)$.

In comparison, as previously reported, human PMNs isolated from adult donors contain a total of $23.9 \pm 1.8 \text{ pmol of actin per 10}^6 \text{ cells}$, divided as TIF-actin, $8.4 \pm 1.7$; TSF-actin, $3.5 \pm 1.0$; and G-actin, $12.0 \pm 1.1 \text{ pmol actin per 10}^6 \text{ cells} (n = 4$; shown graphically in Fig 2C). When compared with human PMNs, differentiated HL-60 cells contain significantly ($P < .05$) more total cellular actin and G-actin. In contrast, there is no significant difference in the quantity of TIF-actin or TSF-actin between differentiated HL-60 cells and human PMNs.

These studies show that U–HL-60 cells contain distinct pools of F-actin similar to those demonstrated in mature PMNs and that myeloid differentiation of HL-60 cells approximates the differentiation of human PMNs in vivo in regard to cytoskeletal structure and actin quantity and pool distribution. These results suggest that HL-60 cells may serve as a useful model for examination of the roles of changes in actin-binding protein amount in the structure and formation of distinct actin pools.
Quantification of gelsolin in distinct actin pools in U-HL-60 and I-HL-60 cells and in human PMNs. As shown in Fig 3A, U-HL-60 cells contain very low amounts of gelsolin (0.073 ± 0.02 pmol/10⁶ cells; n = 3), with essentially all gelsolin (97% ± 7%; n = 3) localized to the G-actin pool (TIF-actin, 0; TSF-actin, 0.002 ± 0.005; G-actin, 0.070 ± 0.001 pmol/10⁶ cells). The gelsolin localized in the G-actin pool, by definition, presumably represents free gelsolin not bound to F-actin, while the small amount of gelsolin in the TSF-actin represents gelsolin bound to F-actin.

After myeloid differentiation with DMSO, the amount of total cellular gelsolin increases 2.4-fold, as previously described,²⁸ to 0.178 ± 0.02 pmol/10⁶ cells (n = 3; Fig 3B). However, in marked contrast to actin, myeloid differentiation of HL-60 cells results in an asymmetric increase in gelsolin between the actin pools. As in undifferentiated cells and mature PMNs, no gelsolin is found in the TIF-actin pool of differentiated HL-60 cells. The amount of free gelsolin in the G-actin pool increases 1.9-fold to 0.139 ± 0.006 pmol/10⁶ cells, while the amount of gelsolin bound to F-actin in the TSF-actin pool dramatically increases 19-fold to 0.009 pmol/10⁶ cells. The growth of gelsolin in the TSF-actin and total cell fractions is statistically significant (P < .05) when compared with U-HL-60 cells. This asymmetric growth of gelsolin in the TSF-actin pool results in an increase in total gelsolin bound to F-actin in the TSF-actin pool from 3% ± 7% in undifferentiated cells to 21% ± 5% after myeloid differentiation. Conversely, the percentage of gelsolin found free in the G-actin pool decreases from 97% ± 7% to 79% ± 5% with myeloid differentiation.

In comparison, mature human PMNs contain a total of 0.241 ± 0.042 pmol gelsolin per 10⁶ cells, divided in the actin pools as follows: TIF-actin, 0; TSF-actin, 0.073 ± 0.002; and G-actin, 0.190 ± 0.021 pmol/10⁶ cells (n = 4; Fig 3C). When expressed as percent gelsolin bound to F-actin in the TSF-actin pool versus percent free gelsolin in the G-actin pool, human PMNs are noted to have 30% ± 4% bound and 70% ± 4% free. These results agree with measurement of gelsolin/actin complexes in ETO human PMNs by Deaton et al.,²⁷ who found 32% ± 4% of gelsolin bound to F-actin in basal PMNs.

Measurement of gelsolin bound to actin in U-HL-60 and I-HL-60 cells. The results above suggest a role for gelsolin
in the determination of TSF-actin filament structure. Interpretation of the results, however, assumes that gelsolin/actin complexes exist only in the TSF-actin pool and that the gelsolin found in the G-actin pool represents free gelsolin (gelsolin not bound to actin). To test these assumptions, EGTA-resistant gelsolin/actin complexes were measured in the Triton-soluble supernatant (which contains both the TSF-actin and G-actin pools) and in the G-actin pool alone in U-HL-60 and I-HL-60 cells. This method measures actin bound to gelsolin by immunoabsorption of gelsolin or gelsolin-actin complexes onto antigelsolin-conjugated beads. As shown in Fig 4 (lanes 1 and 3), gelsolin/actin complexes are found in the Triton-soluble supernatant of both U-HL-60 and I-HL-60 cells. The Triton-soluble supernatant contains both TSF-actin and G-actin. If the TSF-actin pool is removed by high speed centrifugation before immunoa-
bsorption (lanes 2 and 4), gelsolin is found in the G-actin pool as expected, but essentially no actin coisolates with gelsolin in U-HL-60 cells, thereby indicating that the gelsolin in the G-actin pool is free of actin. After myeloid differentiation of HL-60 cells, a minimal number of gelsolin/actin complexes are found in the G-actin pool, accounting for 3.3% ± 1.8% (n = 3) of total cellular gelsolin/actin complexes. Whether this finding represents the presence of actin monomer to gelsolin binding in the G-actin pool or is an experimental artifact is uncertain. Clearly, however, in U-HL-60 cells 100% of gelsolin/actin complexes are found in the TSF-actin pool, and in I-HL-60 cells greater than 95% of HL-60 gelsolin/actin complexes are found in the TSF-actin pool.

Quantification of actin:gelsolin molar ratios shows that the percent of gelsolin molecules bound to actin is unchanged by myeloid differentiation (ratio of actin/gelsolin complexes in undifferentiated: differentiated cells, 1.00 ± 0.09; n = 3). However, gelsolin content in the Triton-soluble supernatant (which contains TSF-actin and G-actin) and G-actin pools significantly increases (2.56 ± 1.44-fold, n = 3; and 1.74 ± 0.30-fold, n = 3; respectively) with cellular differentiation, as confirmed by the previously reported immunoblot results (Fig 3). Likewise, the actin content in the TSF-actin pool measured as complexed to gelsolin in these experiments increases 1.51-fold ± 0.52-fold (n = 3) with differentiation (quantitatively identical to the change noted in Fig 2). The findings of increased gelsolin and actin content in TSF-actin pool coupled with the finding of an unchanged percent of gelsolin bound to actin with cellular differentiation would result in a net increase in the absolute number of gelsolin/actin complexes in the TSF-actin pool.

Calculation of molar ratios of actin to gelsolin in HL-60 actin pools. As the TSF-actin pool is defined as gelsolin-
Measurement of gelsolin bound to actin in U–HL-60 and I–HL-60 cells. Shown is a Coomassie blue-stained 5% to 15% SDS-PAGE of gelsolin/actin complexes isolated from U–HL-60 (lanes 1 and 2) and I–HL-60 (lanes 3 and 4) cells. Triton-soluble supernatants (containing both TSF-actin and G-actin pools) (lanes 1 and 3) and G-actin pools (produced by high-speed sedimentation (366,000g for 5 minutes) to remove TSF-actin; lanes 2 and 4) were immunoabsorbed to antigelsolin-conjugated beads, as described in Materials and Methods. Four bands are evident: G, gelsolin; HC, heavy chain of Ig; A, actin; and LC, light chain of Ig. Note that in both U–HL-60 and I–HL-60 cells, actin coimmunoabsorbs with gelsolin in the Triton-soluble supernatants (lanes 1 and 3). Removal of the TSF-actin pool by centrifugation results in removal of 95% to 100% of the actin bound to gelsolin (lanes 2 and 4). Thus, nearly all cellular gelsolin/actin complexes are found in the TSF-actin pool of HL-60 cells. Lane 5 is a 1.0-μg rabbit skeletal muscle actin standard. (Representative of three experiments.)

![Image of SDS-PAGE gel](image-url)

Table 3. Moles of Actin Per Moles of Gelsolin

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<th>U–HL-60 Cells</th>
<th>I–HL-60 Cells</th>
<th>PMNs</th>
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<tbody>
<tr>
<td>TSF-actin</td>
<td>1,085</td>
<td>87</td>
<td>50</td>
</tr>
<tr>
<td>G-actin</td>
<td>147</td>
<td>139</td>
<td>63</td>
</tr>
<tr>
<td>Whole cell</td>
<td>243</td>
<td>180</td>
<td>100</td>
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</table>

capped filaments free of the three-dimensional crosslinked meshwork of the TIF-actin pool, calculation of the molar ratios of actin to gelsolin in the TSF-actin pool should provide a reflection of TSF-actin filament length because (1) all TSF-actin exists as filaments, (2) all TSF-actin filaments are capped by a gelsolin molecule, (3) free gelsolin does not cosediment with the TSF-actin pool,10 and (4) greater than 95% of gelsolin/actin complexes are found in the TSF-actin pool. Likewise, comparison of molar ratios of actin to gelsolin in the G-actin pool should provide a reflection of the number of gelsolin molecules coexisting, but not bound to G-actin monomers. The quantitative molar data for actin and gelsolin shown in Figs 2 and 3 provide the data necessary to compare these ratios.

As shown in Table 3, U–HL-60 cells are characterized by a molar ratio of actin to gelsolin of 243:1, indicating 243 actin molecules per gelsolin molecule when the whole cell is measured. The molar ratio of actin to gelsolin in the TSF-actin pool is 1,085:1, while the molar ratio in the G-actin pool is 147:1. These results suggest that the TSF-actin pool of U–HL-60 cells consists of a low number of very long, gelsolin-capped filaments that are an average of 1,085 monomers in length. However, myeloid differentiation of HL-60 cells results in a decrease in the whole-cell actin-to-gelsolin ratio to 180:1, indicative of the relatively greater increase in gelsolin content compared with actin after differentiation. The difference in gelsolin-to-actin ratio is localized exclusively to the TSF-actin pool with differentiation, where the actin-to-gelsolin ratio falls to 87:1, indicating a marked shortening of the filaments of the TSF-actin pool by a factor of 12. Conversely, the molar ratio of actin to gelsolin in the G-actin pool is not significantly changed with differentiation, remaining at 139:1. For comparison, the molar ratios of actin to gelsolin in actin pools of mature human PMNs is also shown in Table 3. The maturation of HL-60 cells towards the myeloid lineage is further noted by the relative similarity between differentiated HL-60 cells and PMNs in these measurements. The human PMN demonstrates an overall lower molar ratio of actin to gelsolin compared with I–HL-60 cells, reflecting the lower total actin content and higher total gelsolin content in PMNs compared with differentiated HL-60 cells (Figs 2 and 3). Despite these differences, the molar ratio of actin to gelsolin in the TSF-actin pool of PMNs is remarkably similar to that in HL-60 cells and suggests similar structure of the actin pools in these two cells. The results confirm that myeloid differentiation of HL-60 cells results in a marked decrease in the molar ratios of actin to gelsolin in the TSF-actin pool, which reflects an apparent marked shortening of TSF-actin filaments from an average length of 1,085 actin monomers in U–HL-60 cells to 87 actin monomers after myeloid differentiation.
ROLE OF GELSOLIN IN TSF-ACTIN FORMATION

DISCUSSION

The studies reported here (1) confirm the presence of distinct F-actin pools in undifferentiated and myeloid-differentiated HL-60 cells, (2) provide the first quantitative study of changes in the amounts and segregation of actin and gelsolin between individual actin pools during myeloid differentiation of HL-60 cells, (3) provide clear evidence for a primary role for gelsolin in the formation and organization of Triton-soluble F-actin, and (4) suggest one morphologic microfilamentous cytoskeletal change induced by maturation and differentiation of HL-60 cells to a myeloid phenotype, which may in part explain how myeloid differentiation of HL-60 cells results in conversion of the cells from a nonmotile to a motile phenotype.

Distinct F-actin pools with unique structural and functional properties clearly exist in mature human PMNs. Similar pools of F-actin are known to exist in squid axon, human platelets, and fibroblasts. As shown in Fig 1, immature U–HL-60 cells likewise contain similar distinct F-actin pools, suggesting that the cellular or molecular features that provide distinctive characteristics to TIF-actin and TSF-actin are present in early leukocyte development. However, though present in U–HL-60 cells, the actin pools differ, quantitatively and perhaps structurally, from those characterized in mature human PMNs. As shown in Figs 2 and 3, U–HL-60 cells contain a lower total amount of cellular actin (17.8 v 23.9 pmol/10⁶ cells) and a lower amount of cellular actin as F-actin (7.47 v 11.9 pmol/10⁶ cells; either TIF-actin or TSF-actin) compared with human PMNs. In contrast, U–HL-60 cells contain equivalent amounts of G-actin as mature PMNs (10.3 v 5.7 v 12.0 ± 1.1 pmol/10⁶ cells). This finding suggests that the additional actin generated by cellular differentiation to mature PMNs, though produced as actin monomer, is converted to actin filament likely by simultaneous maturation of actin-regulatory proteins. As shown in Fig 2, myeloid differentiation of HL-60 cells results in statistically significant increases in total cellular actin, TIF-actin, TSF-actin, and G-actin when compared with U–HL-60 cells. When compared with mature PMNs, I–HL-60 cells contain equivalent amounts of TIF-actin and TSF-actin, but significantly more G-actin and, therefore, total actin. HL-60 cells induced to differentiate to a myeloid phenotype by DMSO do not become a monotonous population of mature PMNs. Instead, less mature forms of myeloid differentiation, including metamyelocytes, myelocytes, and band forms, predominate. This imperfect correlation of differentiated HL-60 cells to PMNs perhaps explains why the quantity and structure of actin pools in DMSO-differentiated HL-60 cells does not exactly match that of mature PMNs. With this one exception, however, differentiated HL-60 cells closely and quantitatively mimic human PMNs in their actin pool structure and organization.

Similarly, gelsolin, an important and well-characterized actin-regulatory protein, manifests significant changes with myeloid differentiation of HL-60 cells. Gelsolin is a native 84-kD F-actin-binding protein (migrates at 90 kD on SDS-PAGE) named for its ability to inhibit gelation in vitro. Although gelsolin was initially purified from rabbit pulmonary tissues, including all leukocytes. In vitro, gelsolin in a Ca²⁺ regulated manner affects F-actin in three ways: it (1) shortens preformed actin filaments (severing), (2) binds to the fast-growing (+) end of the filament (capping), and (3) serves as a nucleus for actin polymerization. The in vitro effects of gelsolin on actin are recapitulated in PMNs. Gelsolin severing, capping, and actin nucleation occur in temporal association with actin polymerization and depolymerization.

These cellular effects of gelsolin on actin are critical to phagocytic motility, as demonstrated by the findings of enhanced motility of fibroblasts with gelsolin overexpressed by gene transfection and impaired motility of phagocytes from gelsolin null mutant transgenic animals. The importance of gelsolin to actin pool structure, as outlined in Table 1 and discussed in the introduction, bears reemphasis. The TSF-actin pool is defined as that F-actin that exists in the cellular cytoplasm as stable filaments free of the complex branched and crosslinked actin filament network of the TIF-actin pool. All TSF-actin filaments are apparently capped on their barbed end by gelsolin, and essentially all cellular gelsolin/actin complexes are found in the TSF-actin pool. The TSF-actin pool is understood to function as short oligomers of F-actin available for rapid reorganization of the microfilamentous cytoskeleton in response to cellular activation.

With myeloid differentiation of HL-60 cells, as shown in Fig 3, total cellular gelsolin content increase approximately 2.4-fold. However, unlike actin which adds symmetrically to HL-60 cell actin pools with differentiation, gelsolin is added preferentially to the TSF-actin pool. TSF-actin pool-associated gelsolin increases 19-fold, while free gelsolin in the G-actin pool increases only 1.9-fold. Thus, gelsolin induction with differentiation appears to represent a regulated production of gelsolin to perform a specific organizational and structural role, as instead of being nonspecifically added to the cellular actin network, gelsolin is specifically targeted to a distinct F-actin pool, the TSF-actin pool. The consequences of this asymmetric addition of gelsolin to the TSF-actin pool is a marked shortening of TSF-actin filaments from a calculated average filament length of 1,085 monomers to a calculated filament length of 87 monomers. This shortening of TSF-actin filaments would markedly alter the cellular actin dynamics.

Undifferentiated HL-60 cells are nonmotile cells characterized by large size, decreased deformability, increased cell stiffness, poor motility, and impaired chemotaxis. Myeloid differentiation of HL-60 cells with DMSO results in a well-documented attainment of a motile phenotype characterized by decrease in cell size, increased deformability, decreased cell stiffness, enhanced motility through filters, chemotaxis, and decreased cellular viscosity. The cellular and molecular cytoskeletal changes underlying this attainment of motility of differentiated HL-60 cells is poorly characterized. The quantities of several actin-regulatory proteins are known to increase with HL-60 cell differentiation. These proteins include gelsolin, talin, α-actinin, vinculin,
lipocortin I and II, and ABP-280. However, the mechanism(s) by which these changes in cytoskeletal proteins induce a motile phenotype remain obscure. The findings reported here of changes in actin and gelsolin in actin pools with differentiation offer one potential explanation of how differentiation results in motility.

In a recent bioreologic study of HL-60 cell differentiation, Hallows and Frank noted that DMSO-induced myeloid differentiation of HL-60 cells resulted in an average 80% decrease in cell viscosity, a decrease in cell size, and an increase in whole-cell deformability of HL-60 cells in association with shortened cell transit time through a 5.5-µm pore filter. These consequences of myeloid differentiation on motility were mimicked by treatment of the undifferentiated cells with dihydrocytochalasin B, an F-actin—depolymerizing agent. Dihydrocytochalasin B effected these motile changes without affecting cell volume but by decreasing the viscosity of the cells. As the investigators found a change in cell viscosity with differentiation but no change in the concentration of F-actin within the cells, they speculated that the change in deformability must be due to a change in the actin network conformation via the action of an actin-binding protein. Our findings of apparent shortening of TSF-actin filaments with myeloid differentiation due to an asymmetric addition of gelsolin to the TSF-actin pool would explain these findings. The shortened TSF-actin filaments would result in decreased cell viscosity and increased deformability and would allow for more rapid and sensitive cytoskeletal rearrangement for motility. Cytochalasin’s ability to mimic these findings is likely via actin filament depolymerization or shortening. It is interesting to note that in Yin and StosSEL’s original description of gelsolin, gelation in vitro (comparable to viscosity in vivo) was maximally inhibited at actin-to-gelsolin molar ratios of 166:1. Myeloid differentiation of HL-60 cells results in a decrease in actin-gelsolin molar ratios in the TSF-actin pool from 1,085:1 to 87:1, similar to molar ratios found to limit viscosity (gelation) in vitro.

It is probable that other differentiation-induced changes in the actin cytoskeleton are involved in attainment of motility of HL-60 cells. Other possible effectors of motility include the monomer-binding proteins profilin and thymosin B4 and the F-actin—regulatory proteins tropomyosin, α-actinin, and ABP-280, as well as second messengers including the phosphoinositides. Also, the apparent changes in TSF-actin filament length with cellular differentiation are not yet directly measured. Despite these limitations, our findings offer exciting new insights and clues to the mechanisms by which cellular maturation and differentiation result in changes in cellular function and organization.

In summary, these studies offer important and unique new information concerning the HL-60 cell cytoskeleton, including the first defined quantitative measurement of pool-specific actin and gelsolin content in undifferentiated and differentiated cells, a characterization of the role of gelsolin in formation and organization of the TSF-actin pool, and one explanation for how myeloid cell differentiation and maturation results in phenotypic change. These findings offer an important background for future gene insertion experiments in HL-60 cells by providing a clear delineation of how the microfilamentous cytoskeleton is quantitatively organized and changes with differentiation. On a more global level, these studies offer important clues to explain aberrant migration, size, and morphology of leukemia cells and an opportunity to understand how cellular differentiation may circumvent the malignant phenotype.

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REFERENCES

ROLE OF GELSOLIN IN TSF-ACTIN FORMATION

27. Deaton JD, Guerrero T, Howard TH: Role of gelsolin interaction with actin in regulation and creation of actin nuclei in chemotactic peptide activated polymorphonuclear neutrophils. Mol Biol Cell 3:1427, 1992
Role of gelsolin in the formation and organization of triton-soluble F-actin during myeloid differentiation of HL-60 cells

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