Colony-stimulating Factor-1 Stimulates the Formation of Multimeric Cytosolic Complexes of Signaling Proteins and Cytoskeletal Components in Macrophages*

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Stimulation of macrophages with colony-stimulating factor-1 (CSF-1) results in the protein tyrosine phosphorylation of the CSF-1 receptor (CSF-1R) and many other, primarily cytosolic, proteins. Stimulation by CSF-1 at 4 °C was used to facilitate the purification and identification of the proteins of the cytosolic anti-phosphotyrosine (PY)-reactive fraction (aPY-RF) involved in downstream signaling pathways. Confocal microscopy revealed that the PY proteins are in close proximity to the CSF-1R at the plasma membrane. The aPY-RF contained pre-existing complexes of PY proteins and non-PY proteins which generally increased in size and PY protein content following CSF-1 stimulation. PY proteins identified by microsequencing and Western blotting include Cbl, STAT3, STAT5a, STAT5b, SHP-1, She, and two novel proteins pp57 and pp37. Other proteins included cytoskeletal/contractile proteins (paxillin, vimentin, elongation factor-1α, F-actin, tropomyosin, and myosin regulatory light chain), Ras family signaling proteins (p85 [phosphoinositide 3-kinase], Vav, Ras-GTPase-activating protein SH3 domain-binding protein, and Grb2), DnaJ-like protein, and glyceraldehyde-3-phosphate dehydrogenase. CSF-1 induced the de novo recruitment of Cbl, STAT3, STAT5a, STAT5b, p85, SHP-1, She, vimentin, and Grb2 to complexes and caused pre-existing complexes involving Vav, elongation factor-1α, and F-actin to increase in size. These studies indicate that CSF-1-induced protein tyrosine phosphorylation is associated with the reorganization of complexes of cytoskeletal, signaling, and other proteins that mediate CSF-1-regulated motility and growth.

The survival, proliferation, and differentiation of mononuclear phagocytes are primarily regulated by colony-stimulating factor-1 (CSF-1)† (reviewed in Refs. 1 and 2). CSF-1 also regulates macrophage morphology in vitro (3, 4) and in vivo (5–7). In vitro studies of regulation by CSF-1 have utilized the CSF-1-dependent mouse macrophage cell line BAC1.2F5 (8). BAC1.2F5 cells morphologically resemble primary macrophages and, like primary macrophages, require CSF-1 for both survival and proliferation (8). They also exhibit similar chemotactic and morphological responses to CSF-1 (4, 8, 9). When cultured overnight in the absence of CSF-1, BAC1.2F5 cells round up and become less mobile. In response to re-addition of CSF-1, these cells rapidly (5 min) spread and produce membrane ruffles and lamellipodia and regain their motility (4, 8, 9). The early responses to CSF-1 are associated with the reorganization of the actin cytoskeleton and the appearance of new actin cables (4, 10).

The effects of CSF-1 are mediated by a specific receptor tyrosine kinase (11–13) encoded by the c-fms protooncogene (14). Incubation of BAC1.2F5 macrophages with CSF-1 causes dimerization, activation, and tyrosine phosphorylation of the CSF-1 receptor (CSF-1R), followed by the tyrosine phosphorylation of several primarily cytoplasmic proteins (15–19). The identities of the majority of these proteins are still unknown. At 37 °C, their appearance is maximally stimulated by 60 s (18). They may represent direct substrates of the activated CSF-1R or substrates of non-receptor tyrosine kinases activated in the CSF-1 response, or alternatively, the increased tyrosine phosphorylation may be due to CSF-1-induced inhibition of protein tyrosine phosphatases. At 4 °C, these proteins are tyrosine-phosphorylated with slower kinetics than at 37 °C (16, 18), but the rates of phosphotyrosine dephosphorylation are differentially lowered. Thus, CSF-1-stimulated tyrosine phosphorylation reaches a relatively stable maximum between 90 and 180 min (16, 18), and a higher level of tyrosine phosphorylation is obtained than the level achieved by incubation for optimum periods at 37 °C. Analysis of the stimulation of cells with CSF-1 at 4 °C has enabled differences in the kinetics of the appearance of the tyrosine-phosphorylated proteins to be resolved (16). Furthermore, by stimulating BAC1.2F5 cells with CSF-1 at 4 °C for 2 h, it is possible to carry out large scale stimulation and subcellular fractionation in order to isolate sufficient amounts of anti-phosphotyrosine (PY)-reactive fraction (aPY-RF) proteins from the subcellular fractions for their identification (20). Essentially the same pattern of protein tyrosine phosphorylation is observed following CSF-1 stimulation at either 4 °C for 2 h or 37 °C for 1 min. However, at 4 °C, subsequent

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§ The abbreviations used are: CSF-1, colony-stimulating factor 1; aPY, anti-phosphotyrosine; aPY-RF, anti-phosphotyrosine reactive fraction; PY, phosphotyrosine; EF-1α, elongation factor 1-α; G3BP, Ras-GTPase-activating protein SH3 domain-binding protein; Hsp40, heat shock protein 40 kDa; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MRLC, myosin regulatory light chain; IAA, iodoacetic acid; PAGE, polyacrylamide gel electrophoresis; S6, Superose 6; RP-HPLC, reverse phase high pressure liquid chromatography; F1 3-kinase, phosphatidylinositol 3-kinase; p85, regulatory subunit of PI 3-kinase; PIPES, piperezine-N,N′-bis[2-ethanesulfonic acid]; GdnHCl, guanidine hydrochloride; FITC, fluorescein isothiocyanate; CSF-1R, CSF-1 receptor.
events, e.g. Raf activation and phosphorylation (21), are blocked. Thus, analysis of the stimulation of cells with CSF-1 at 4 °C has been very useful in analyzing the very early events in CSF-1 signal transduction.

As indicated above, with the exception of the CSF-1R, a 260-kDa protein which we now know to be a multi-ubiquitinated form of the CSF-1R (22, 23) and a few additional proteins, the proteins tyrosine-phosphorylated in response to CSF-1 are predominantly cytoplasmic (18). Hence, we have focused on the identification of the PY proteins in the cytosolic fraction. In our analysis of the cytosolic aPY-RF from CSF-1-stimulated and unstimulated BAC1.2F5 macrophages, the protein tyrosine phosphatase SHP-1 was identified by microsequencing of the purified protein (20) and the proto-oncogene product Cbl, by Western blotting analysis (22). Tyrosine phosphorylation of both SHP-1 and Cbl was stimulated by CSF-1.

We and others have shown that SHP-1 is tyrosine-phosphorylated in macrophages (22) and myeloid cells (24) in response to CSF-1, and others have shown that phospholipase C-γ2 (25), p150SHIP (26), Tyk2, STAT3, STAT5a and STAT5b (27, 28) are tyrosine-phosphorylated in myeloid cells in response to CSF-1.

In this report, we describe the use of anti-PY affinity chromatography for the isolation of PY proteins and the non-PY proteins associated with them from the cytosolic fraction of CSF-1-stimulated macrophages. By using Western blotting with antibodies to known proteins and direct purification and microsequencing approaches, we have identified a number of PY and non-PY proteins in the cytosolic aPY-RF, and we demonstrate that these proteins are present in various complexes. We also demonstrate that there is significant reorganization of proteins in these complexes in response to CSF-1 stimulation.

MATERIALS AND METHODS

Cell culture, Protein Purification, and Sequencing—BAC1.2F5 macrophages (8) were cultured in 100-mm tissue culture dishes and stimulated with 13.2 nm CSF-1 (human recombinant macrophage colony-stimulating factor, a gift from Chiron Corp.) at 4 °C in the presence of 8 mM iodoacetic acid (IAA, Fluka) to increase the yield of PY proteins, as described previously (15). Subcellular fractionation of the cells after CSF-1 stimulation, purification of aPY-RF from the cytosol, tryptic digestion, and separation of tryptic peptides for microsequencing were performed exactly as described previously (20) with the exception that the C8 reverse phase-high performance liquid chromatography (RP-HPLC) was performed at 55 °C and the C18 RP-HPLC at 40 °C. Briefly, cells were disrupted by Dounce homogenization, and subcellular fractions were separated by differential centrifugation. The cells were disrupted by Dounce homogenization, and subcellular fractions were separated by differential centrifugation. The cytosolic fraction of the cells was then incubated for 1 h with donkey anti-goat antibody (3.75 μg/ml) antibodies dissolved in blocking solution were FITC-labeled) dissolved in blocking solution. After thorough washing (5 times for 5 min each time) with washing solution, the cells were washed in a medium containing 50% glycerol, 20 mM Tris-HCl, 154 mM NaCl, and 100 mg/ml 1,4-diazabicyclo-(2.2.2)octane (Sigma) and examined under a Bio-Rad MRC 600 Laser Scanning Confocal Microscope.

Immunoprecipitation—Immunoprecipitation and immunoblotting were performed as described previously (15, 20). All immunoblots were developed with horseradish peroxidase-coupled secondary antibody and ECL reagent (Amersham Pharmacia Biotech).

Two-dimensional Gradient Gel Electrophoresis—The first dimension non-denaturing electrophoresis utilized a 1-mm thick vertical slab gradient gel. Proteins were then incubated for 1 h with 0.5% Triton X-100 in buffer F for 10 min and incubated in 0.1 mM iodoacetamide in buffer F for 10 min to quench aldehydes produced during the sample preparation. The fixed cells were extracted with 0.5% Triton X-100 in buffer F for 10 min and incubated in 0.1 mM iodoacetamide in buffer F for 10 min to quench aldehydes produced during the sample preparation. The gel was then cut out with a scalpel and placed into a stacking gel buffer containing 4% stacking gel buffer, wrapped in a layer of plastic food wrap, and exposed to x-ray film for 5 h. The gel lanes comprising the first dimension were cut out with a scalpel and placed into a stacking gel buffer containing 4% stacking gel buffer, wrapped in a layer of plastic food wrap, and exposed to x-ray film for 5 h. The gel was electroblotted at 8 V/cm for 18 h at 16 °C, stained with Coomassie Blue, dried, and autoradiographed.

Non-denaturing Size Exclusion Chromatography—An S6 column equilibrated with 20 mM Hepes, pH 7.0, 200 mM NaCl, 0.1 mM sodium orthovanadate, 0.8% octyl glucoside, and 1 mM benzamidine was run at a flow rate of 0.2 ml/min at 4 °C. The αPY-RF (0.2 ml, ~1 mg/ml) was clarified by centrifugation at 13,000 × g for 15 min prior to injection. Protein concentration was monitored at 280 nm, and 0.3-ml fractions were collected. Starting from the fraction containing the protein eluted at the void volume of the column, fractions were pooled in groups.

Immunoaffinity chromatography and a 1:1 mixture of the two affinity purified antibodies specific to each peptide were purified from the sera by peptide affinity chromatography (30). Anti-human p85 (regulatory subunit of phosphoinositide 3-kinase (PI-3-kinase)) antibody was a gift from Dr. Jonathan Backer of the Albert Einstein College of Medicine. An affinity purified antibody directed against C-terminal half of the chicken gizzard myosin regulatory light chain (MRLC) was kindly provided by Dr. Kathleen Trybus of Brandeis University.

Non-denaturing Size Exclusion Chromatography—An S6 column equilibrated with 20 mM Hepes, pH 7.0, 200 mM NaCl, 0.1 mM sodium orthovanadate, 0.8% octyl glucoside, and 1 mM benzamidine was run at a flow rate of 0.2 ml/min at 4 °C. The αPY-RF (0.2 ml, ~1 mg/ml) was clarified by centrifugation at 13,000 × g for 15 min prior to injection. Protein concentration was monitored at 280 nm, and 0.3-ml fractions were collected. Starting from the fraction containing the protein eluted at the void volume of the column, fractions were pooled in groups.
of four to yield 10 large fractions of 1.2 ml each, for further analysis.

Denaturing Size Exclusion Chromatography—To 100 µl of αPY-RF (containing ~0.5 mg of protein), 110 mg of GdnHCl, 9.5 µl of 2 mM Tris-HCl, pH 8.5, and 1.4 µl of β-mercaptoethanol were added to yield 190 µl of αPY-RF in 6 mM GdnHCl, 0.1 mM Tris-HCl, pH 8.5, and 0.1 mM mercaptoethanol. The resulting solution was incubated at room temperature for 2 h and centrifuged at 13,000 × g for 15 min. The reduced, denatured, and clarified αPY-RF was then injected into an S6 column equilibrated at room temperature in 6 mM GdnHCl, 0.5% dodecyl tri-methyl ammonium bromide (Sigma), 0.1 mM β-mercaptoethanol, and 20 mM Tris-HCl, pH 6.5, at a flow rate of 0.2 ml/min. Protein concentration was monitored at 280 nm, and 0.3-ml fractions were collected.

DNase I Precipitation of G-actin—DNase I (Sigma) was coupled to Sepharose 4B (Amersham Pharmacia Biotech) at a concentration of 1 mg of protein per ml of gel by cyagen bromide activation, and the DNase I binding reaction was performed as described previously (33). Cytosolic extract (60 µg) or the αPY-RF (approximately 6 µg from stimulated and 4 µg from unstimulated cells) prepared from 6 mg of cytosolic protein was incubated with 50 µl (packed volume) of the DNase I-Sepharose beads in buffer A (2 mM Tris-HCl, pH 8.0, 10 mM NaCl, 0.2 mM CaCl₂, 0.2 mM ATP, 0.2 mM dithiothreitol, and 0.8% octyl glucoside) at 4 °C for 3 h. The beads were then washed 4 times each with 0.5 ml of buffer A containing 0.2 mM NH₄Cl (salt wash) and then two times with buffer A. The bound proteins were eluted by incubating the beads with equal volume of 2x SDS-PAGE sample buffer at 60 °C for 30 min.

Biotinylated Phalloidin Precipitation of F-actin from the Cytosolic αPY-RF—The cytosolic αPY-RF from both αPY-RF-stimulated and unstimulated cells prepared from 10 confluent 100-mm culture dishes (~80 × 10⁶ cells) was incubated with 2 nmol of either phalloidin-XK-biotin (Molecular Probes) or 2 nmol of biotin (Sigma) in buffer B (20 mM HEPES, pH 7.3, 150 mM NaCl, 0.1 mM sucrose, 0.8% octyl glucoside, 0.1 mM sodium orthovanadate, and 0.1 mM phenylmethylsulfonyl fluoride) in a final volume of 20 µl for 18 h at 4 °C. The reaction mixtures were then incubated for 2 h with 25 µl (packed volume) avidin-agarose (Equilibrated) in buffer B at 4 °C, with continuous agitation. The agarose beads were pelleted by centrifugation and washed 6 times each with 0.5 ml of buffer B at 4 °C. The initial supernantant and the first 2 washes were pooled and concentrated by microcon 30 (Amicon) to 20 µl. The thoroughly washed agarose beads were incubated with equal volume of 2% SDS and heated at 60 °C for 30 min to elute the bound proteins.

Cytchalasin D Treatment—Cytchalasin D (Sigma) was dissolved in dimethyl sulfoxide (Me₂SO, Sigma) at 5 mg/ml. Cells were incubated without CSF-1 for 18 h. Cytchalasin D solution (2.8 µl) was added to each dish (final concentration, 5 µM), and the cells were incubated for a further 3 h without CSF-1 at 37 °C. Control cells received the same volume of Me₂SO. CSF-1 was then added (final concentration, 13.2 nM), and the cells were lysed with Nonidet P-40 buffer at various times as described previously (16).

Other Techniques—Proteins were resolved by gradient SDS-PAGE (7.5–17.5% acrylamide) (20). Silver staining of protein in SDS-PAGE was carried out by the method of Morrissey (34). Protein determination was carried out as described previously (20).

RESULTS

Distribution of the CSF-1R and PY Proteins in BAC1.2F5 Cells—For all of the following experiments with the exception of experiment shown in Fig. 9, BAC1.2F5 macrophages were preincubated without CSF-1 for 18 h. Cytchalasin D solution (2.8 µl) was added to each dish for 3 h followed by replacement of CSF-1, the CSF-1R representing ~50% of the total cellular CSF-1R as determined by its lower molecular mass and by its content of N-linked oligosaccharides of the high mannose type (35). In cells stimulated with CSF-1 for 2 h, the intensity of anti-PY staining was significantly increased, and the staining was more evenly distributed in the vicinity of the plasma membrane, whereas the CSF-1R staining maintained the punctate appearance seen at 10 min of stimulation (Fig. 1). Thus the proteins exhibiting an increase in phosphotyrosine in response to CSF-1 at 4 °C, despite their predominance in the cytosolic fraction (18), are found close to the plasma membrane.

PY Proteins in the Cytosol Are in Multimeric Complexes That Change following Stimulation with CSF-1—The existence of multimeric complexes in the cytosolic αPY-RF of the BAC1.2F5 macrophages was demonstrated by two-dimensional gradient gel electrophoresis. In the first dimension, proteins in 3P-labeled cytosolic αPY-RF were separated according to size by non-denaturing gradient gel electrophoresis. The gel lanes from the first dimension were cut out, denatured, and reduced in SDS sample buffer containing mercaptoethanol and placed horizontally on an SDS gradient gel to separate the proteins present in each complex. The resulting autoradiogram (Fig. 2) showed that CSF-1 not only stimulated an increase in phosphorylation of existing proteins but also the assembly of larger complexes involving phosphorylated proteins of 37, 57, 75, 120, and 160 kDa.

Purification and Identification of Proteins in the Cytosolic αPY-RF—To understand better the relationship between CSF-1-stimulated protein tyrosine phosphorylation and formation of these complexes, proteins were identified by a combination of Western blotting with antibodies to known proteins and direct microsequencing of purified proteins in the αPY-RF. The first step in the purification of these proteins was non-denaturing anti-PY affinity chromatography. To be sure of the identity of proteins exhibiting CSF-1-stimulated tyrosine phosphorylation, purification was carried out in parallel from cytosolic fractions of both CSF-1-stimulated and unstimulated cells. To increase the yield of PY and associated proteins, cells used for purification of αPY-RF were incubated with CSF-1 at 4 °C for 2 h in the presence of 8 mM IAA. As shown in Fig. 3A, incubation with IAA increased the overall intensity of cytosolic protein tyrosine phosphorylation in both stimulated and unstimulated cells, without changing the number of tyrosine-phosphorylated bands. In fact, tyrosine phosphorylation of some proteins, for example at 57 and 37 kDa, was augmented so profoundly by IAA that the effect of CSF-1 stimulation was...
no longer apparent. Comparison of the silver-stained protein profiles of the αPY-RF (Fig. 3B) with the anti-PY Western blotting profiles (Fig. 3A) of the same fractions reveals that the αPY-RF prepared from the cytosol of BAC1.2F5 cells contained many proteins that were not tyrosine-phosphorylated. IAA treatment not only increased the amount of PY protein in this fraction but also increased the amount of associated non-PY protein to a similar degree, suggesting that these non-PY proteins are specifically associated with the PY proteins. Furthermore, despite the fact that some of the non-PY proteins were abundant cytoplasmic proteins (see below), the αPY-RF obtained by repeated anti-PY affinity chromatography (Fig. 4) comprised only 0.1% of the total cytosolic protein (Fig. 4 and
Table I, indicating that the non-PY proteins that co-purify with the PY proteins are specifically associated with the latter and their presence is not due to contamination.

Fractions from the S6 denaturating chromatography of the cytosolic aPY-RF, containing proteins between 18–25, 30–50, and 50–70 kDa (Fig. 5) were pooled. Each pool was purified by C<sub>8</sub> RP-HPLC. Each purified protein was digested with trypsin, and the tryptic peptides were separated by C<sub>8</sub> RP-HPLC and sequenced. We obtained definitive tryptic peptide sequences from 11 proteins. Except for tyrosine-phosphorylated proteins of ~37 kDa (pp37) and ~57 kDa (pp57), 9 of these are known proteins. Table II shows the sequences of tryptic peptides from the known proteins and the corresponding matching regions of the published amino acid sequences. Western blotting analysis of the aPY-RF with the relevant antibodies confirmed the existence of these proteins (Fig. 6B). The presence of Cbl, Vav, STAT3, STAT5a, STAT5b, p85, paxillin, and Grb2 was also demonstrated by Western blotting (Fig. 6B).

Analysis of the Multimeric Complexes in the Cytosolic aPY-RF—When the S6 size exclusion chromatography elution profiles of the non-denatured proteins in the cytosolic aPY-RF (Fig. 6A, upper) are compared with those of denatured proteins (Fig. 5), it was apparent that many of the proteins were present in complexes of different sizes. One of these was actin. Western blotting analysis of fractions from the non-denaturing S6 size exclusion chromatography (Fig. 6B) showed that actin (44 kDa) is present in complexes varying in apparent M<sub>r</sub> from >6 million (Fig. 6B, fraction 1, at void volume of the column) down to ~100,000 (Fig. 6B, fraction 8). In fractions 1–4, which contained protein complexes of 669 kDa or larger, more actin was detected in the CSF-1-stimulated preparation. However, more actin was detected in the unstimulated preparation for fractions containing protein complexes of 250 kDa or less. In the original cytosolic aPY-RF, there was little change (Fig. 6B) or a decrease (Fig. 7, lanes 1 and 2) in actin content with stimulation. Vav, EF-1α, GAPDH, and pp37 showed a very similar distribution to actin in that their presence in the high molecular size fractions (>334 kDa) was increased by CSF-1, whereas their levels in the lower molecular size fractions (<334 kDa) were decreased by CSF-1 stimulation. Although it was not obvious that Vav was tyrosine-phosphorylated (Fig. 6B), immunoprecipitation of Vav and Western blotting with aPY antibody indicated that it was tyrosine-phosphorylated in the response of

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**TABLE I**

| Yield of proteins in the aPY-RF from each of three consecutive anti-PY affinity purifications of the same preparation of cytosol from BAC1.2F5 cells, either CSF-1 stimulated (+) or unstimulated (−), in the presence of IAA |
|-----------------|-----------------|-----------------|-----------------|
|                 | Cytosol         | aPY-1           | aPY-2           | aPY-3           |
| CSF-1           |                 |                 |                 |                 |
| −               | 427             | 0.215           | 0.098           | 0.015           |
| +               | 439             | 0.269           | 0.113           | 0.014           |

**Fig. 5.** Gradient SDS-PAGE of fractions from denaturing S6 chromatography of the cytosolic aPY-RF. aPY-RFs prepared from equal amounts of cytosol from BAC1.2F5 cells incubated with (+) and without (−) CSF-1 were denatured, reduced, and separated on an S6 column equilibrated in a buffer containing 6 M guanidine HCl and 0.1 M β-mercaptoethanol as described under “Materials and Methods.” Proteins eluted from the column were monitored at 280 nm (+CSF-1, −; −CSF-1, − −). Proteins in each fraction were analyzed by gradient SDS-PAGE and silver staining. The apparent discrepancy between the A<sub>280</sub> (O.D.) profile and silver staining profile of the very high molecular weight proteins in the gel is due to their failure to enter the separation gel. Arrows indicate proteins identified by peptide microsequencing. Orig, unfractionated aPY-RF.
BAC1.2F5 cells to CSF-1.\(^2\) Grb2, p85, SHP-1, Cbl, STAT3, STAT5a, STAT5b, Shc, vimentin, and pp57 showed a CSF-1-stimulated increase in all fractions in which they were present (Fig. 6B). The three isoforms of Shc showed significant differences in their distribution as follows: p68 Shc was eluted in fractions of 150–400 kDa, p58 Shc in all fractions from 44 kDa to 6 million Da, and p54 Shc in fractions of 150 kDa to 6 million Da. p54 Shc and p68 Shc were dramatically increased in the CSF-1-stimulated fractions. p58 Shc exhibited a similar dramatic CSF-1-stimulated response in the very high molecular weight fractions (Fig. 6B, 1 and 2). Whereas p58 Shc was lower in increased in the low molecular weight fractions, CSF-1 stimulated a smaller fold increase in p58 Shc because a significant amount of p58 Shc was present in the unstimulated fractions. Vimentin, a 56-kDa intermediate filament protein, was present only in fractions of proteins >669 kDa (Fig. 6B). SHP-1 was present in CSF-1-stimulated fractions of both high and low molecular weight (Fig. 6B). There was no effect of CSF-1 on the amount of complexed tropomyosin, paxillin, or MRLC. Tropomyosin was eluted at 350 kDa, paxillin at 200 kDa, and the MRLC at 80–44 kDa (Fig. 6D). The F-actin severing and capping protein, gelsolin, could not be detected in the cytosolic aPY-RF. The analysis of fractions from two additional non-denaturing S6 columns yielded similar results.

Analysis of Actin and Identification of Actin-associated Proteins in the Cytosolic aPY-RF—Actin is one of the major silver-stained proteins present in all non-denaturing S6 chromatography fractions (Fig. 6A, upper). As we considered it likely that actin forms multiple complexes with several proteins in the cytosolic aPY-RF, it was studied further. Actin can exist as G-actin or short F-actin fragments in the cytosol. DNase I has been shown to bind G-actin but not F-actin (33). To characterize the form of actin present in the cytosolic aPY-RF, DNase I affinity precipitation was performed (Fig. 7). DNase I could not bind the actin present in the aPY-RF from either stimulated or unstimulated cells (Fig. 7, lanes 1–6), despite the fact that it bound about 70% of the actin in the cytosol (Fig. 7, lanes 10–13). Actin of the cytosolic aPY-RF was quantitatively recovered in the DNase I flow-through (Fig. 7, lanes 7 and 8). These results suggest that the actin present in the aPY-RF is F-actin and that G-actin, an abundant cytosolic protein, is absent.

Antibodies that efficiently immunoprecipitate actin have not been reported. However, phalloidin has been shown to bind F-actin specifically and will not sterically hinder interaction with other macromolecules (36). By incubating biotin-tagged phalloidin with the cytosolic aPY-RF and precipitating the resulting phallloidin F-actin complex with avidin-coupled agarose (Fig. 8), a number of tyrosine-phosphorylated proteins (37, 57, 60, 64, 110, and 120 kDa) were found to be co-precipitated with F-actin (Fig. 8A, lanes 7 and 8). In contrast, when the same molar concentration of biotin was used instead of phallloidin-biotin, insignificant amounts of F-actin (Fig. 8B, lanes 11 and 12) and tyrosine-phosphorylated proteins (Fig. 8A, lanes 11 and 12) were precipitated. Although the amount of phallloidin-biotin was in at least a 10-fold molar excess over the F-actin concentration of the sample and the avidin-agarose could precipitate at least 3 times the biotin conjugate used, the quantity of actin precipitated only accounts for between 5 and 10% of the total actin present in the original sample. This low efficiency of precipitation may be explained by the slow on-rate of phallloidin F-actin binding (37) and the possibility that the efficiency of binding to some large complexes may be decreased by the presence of the associated proteins. Western blots showed that the proteins associated with F-actin that were precipitated by phallloidin included STAT3, STAT5b, p58 Shc, p54 Shc, SHP-1, pp37, pp57, Grb2, paxillin, actin, Vav, and vimentin (Fig. 8B).

Effect of Cytochalasin D on CSF-1-stimulated Protein Tyrosine Phosphorylation—To assess the importance of the actin cytoskeleton for the normal CSF-1 response, we examined the effect of cytochalasin D-mediated depolymerization of cytoskeleton on CSF-1-stimulated protein tyrosine phosphorylation. Preincubation of BAC1.2F5 cells with 5 \(\mu\)m cytochalasin D significantly decreased CSF-1-stimulated protein tyrosine phosphorylation of most proteins in the Nonidet P-40 soluble cell lysate, although the kinetics of stimulation was not affected (Fig. 9). The effect of cytochalasin D was more marked...
for some proteins than for others. Tyrosine phosphorylation of proteins at 190, 165 (CSF-1R), 75, 70, and 37 kDa was substantially reduced, whereas the tyrosine phosphorylation of 85- and 47-kDa proteins was completely inhibited (Fig. 9). Tyrosine phosphorylation of 66 and 36.5 kDa proteins was not affected. The effect of cytochalasin D on cell shape and protein tyrosine phosphorylation was reversible. Cells treated with 5 μM cytochalasin D for 3 h rounded up within 1 h. When the medium was replaced with normal growth medium, the cells rapidly spread again and continued to grow normally. This result implies that efficient and specific CSF-1-stimulated protein tyrosine phosphorylation depends on the integrity of the actin cytoskeleton.

DISCUSSION

This study has focused on the characterization of the cytosolic αPY-RF which contains the majority of non-CSF-1R PY proteins in CSF-1-stimulated macrophages. Several PY proteins and associated non-PY proteins have been identified and their organization and reorganization in response to CSF-1 is described.

Identification of Proteins in the Cytosolic αPY-RF—Among the PY proteins identified in the cytosolic αPY-RF were several known signaling proteins, including Cbl (22, 38), STAT3, STAT5a, STAT5b (27, 28), SHP-1 (20, 39), Shc (40), and Vav (41, 42). Despite an earlier report demonstrating activation of STAT1 by CSF-1 (27), we were unable to demonstrate the presence of STAT1 in the cytosolic αPY-RF by anti-STAT1 Western blotting. Previous studies have shown that all seven of the PY proteins detected are rapidly tyrosine-phosphorylated in the response of macrophages to CSF-1 (20, 22). Apart from these known signaling proteins, we also obtained internal peptide sequences of two novel PY proteins, pp37 and pp57. cDNA clones encoding pp37 have recently been isolated and predict a novel member of a protein tyrosine phosphatase substrate family that plays an important role in cytoskeletal regulation. The fact that seven of the nine PY proteins identified are known to have important roles in growth factor signal transduction suggests that pp37 and pp57 will have important signaling roles and validates this approach as one likely to lead to the identification of additional novel signaling proteins.

The αPY-RF of the cytosol also contained many non-PY pro-

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Fig. 6. Gradient SDS-PAGE of fractions from non-denaturing S6 chromatography of the cytosolic αPY-RF. αPY-RFs prepared from equal amounts of cytosol from CSF-1 stimulated (+) or unstimulated (−) BAC1.2F5 cells were chromatographed in an S6 column under non-denaturing conditions as described, and the resulting fractions were analyzed by gradient SDS-PAGE. Gels were either silver-stained (upper panel A) or transferred to membrane for Western blotting with antibodies against phosphotyrosine (lower panel A) and the indicated proteins (B). No band of less than 31 kDa was observed in αPY Western blots of the αPYRF [see Fig. 3A]. Orig, unfractionated αPY-RF.
proteins that co-purified with the PY proteins. Three lines of evidence indicate that these non-PY proteins are not contaminants of the cytosolic α-PY-RF, despite the abundance of some of them in the cytosol. First, both the total amount of PY protein and non-PY protein in the cytosolic α-PY-RF were increased similarly by treatment of the cells with IAA (Fig. 3). Second, the amount of protein in the α-PY-RF during repetitive anti-PY affinity chromatography of the same preparation of cytosol was successively and rapidly reduced with each step (Fig. 4). Thus the total α-PY-RF protein, including both PY and non-PY proteins of the cytosol, is finite, representing only ~0.1% of the total cytosolic protein (Table I). Finally, free G-actin, a very abundant cytosolic protein (Fig. 8), is absent from this fraction, all the actin being recovered as F-actin. Thus within the α-PY-RF the non-PY proteins are specifically associated, directly or indirectly with the PY proteins.

Of the 11 non-PY proteins identified, 6 (actin (43), vimentin (44), paxillin (45), tropomyosin (46), MRLC (47), and EF-1α (30)) are cytoskeletal/contractile components, three (Grb2 (48), G3BP (49), and p85 (50)) are involved in Ras or Ras family effector stimulation, and two (GAPDH (51) and the DnaJ-like protein/Hsp40 (32)) are cytoskeletal/contractile components, three (Grb2 (48), G3BP (49), and p85 (50)) are involved in Ras or Ras family effector stimulation, and two (GAPDH (51) and the DnaJ-like protein/Hsp40 (32)) are involved in Ras or Ras family effector stimulation. Two-dimensional gradient gel electrophoresis of 32P-labeled cytosolic α-PY-RF showed that CSF-1 not only stimulated a dramatic increase in phosphorylation of proteins in this fraction but also an increase in the apparent M, of many phosphorylated proteins. Consistent with these observations, in higher molecular weight fractions of the non-denaturing S6 size exclusion chromatography of the cytosolic α-PY-RF, many of the PY and non-PY proteins were present at a higher level in the CSF-1-stimulated fractions than in the corresponding fractions from unstimulated cells. However, both results also show that most of the PY and non-PY proteins in the cytosolic α-PY-RF from unstimulated cells have higher apparent molecular weights on non-denaturing separation than on denaturing separation. These findings indicate that protein complexes composed of different PY and non-PY proteins pre-exist in the unstimulated cells and that CSF-1 stimulation causes significant increases in their size. Furthermore, the fact that the total protein of the α-PY-RF only increases by ~20% with CSF-1 stimulation, whereas the PY content increases by more than 5-fold, is also consistent with the pre-existence of complexes in which protein components are tyrosine-phosphorylated and to which PY proteins are differentially recruited, upon CSF-1 stimulation. Although we have demonstrated the existence of protein complexes in the α-PY-RF, we may not have detected low affinity complexes which dissociate in the detergent-containing buffer system we have used.

The individual proteins identified differed in their involvement in the CSF-1-stimulated reorganization of complexes (Fig. 6). Cbl, STAT3, STAT5a, STAT5b, pp57, vimentin, and MRLC were recovered in CSF-1-stimulated non-denaturing S6 chromatography fractions. In contrast, the levels of paxillin, tropomyosin, and MRLC in these fractions remained unchanged by CSF-1 stimulation. However, Vav, EF-1α, actin, pp37, and GAPDH were all increased in higher molecular weight fractions but decreased in lower molecular weight fractions in response to CSF-1. These phenomena suggest that CSF-1 1) induces de novo association of Cbl, STAT3, STAT5a, STAT5b, pp57, Vav, EF-1α, and actin to form larger complexes by association with proteins that are modified following CSF-1 stimulation. It is not surprising, therefore, that most of the proteins exhibiting CSF-1-induced de novo association have been reported to associate with other proteins following growth factor stimulation (42, 54–56).

The widely variable distribution of both the PY and the non-PY proteins within the non-denaturing S6 size exclusion chromatography fractions suggests that the composition of these complexes is diverse. Tropomyosin, paxillin, vimentin, Cbl, STAT3, STAT5a, STAT5b, and MRLC were recovered in very narrow size ranges, whereas Vav, p85, Src, pp57, EF-1α, actin, pp37, GAPDH, and Grb2 are present in complexes of a wide range of sizes. It is noteworthy that the three isoforms of Src distribute very differently from each other, suggesting that these isoforms form complexes with different proteins and may function differently. This is consistent with the recent demonstration that the higher molecular weight Src isoform, in contrast to the two lower molecular weight Src isoforms, does not transform fibroblasts, does not increase EGF activation of mitogen-activated protein kinases, and inhibits fos promoter activation (40). Furthermore, despite the fact that SHP-1 is present in both the very high molecular weight and the 150-kDa fractions, its tyrosine phosphatase activity can only be demonstrated in the 150-kDa fraction, indicating that the activity of the enzyme is altered when it is associated in the higher molecular weight complexes and inferring that the two states of the enzyme may have different functions.

**Complexes Involving Actin**—As actin is a major silver-stained protein present in all fractions in the non-denaturing S6 size exclusion chromatography, this protein may be the common component of several complexes of the cytosolic α-PY-RF. We were unable to demonstrate the presence of any DNase I-binding G-actin in this fraction. Although the total amount of F-actin in the cytosolic α-PY-RF was usually slightly decreased

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4. D. B. Einstein, unpublished data.
by CSF-1 stimulation, CSF-1 stimulation led to an increase in its concentration in the higher molecular weight fractions from the non-denaturing S6 column and a decrease in its concentration in the lower molecular weight fractions. Should these changes involve the actin component of the complexes, it is possible that the CSF-1-induced increase in F-actin in the higher molecular weight fractions is governed by the sequestration of cytoskeletal actin filaments (57) and that the decrease in F-actin in the lower molecular weight fractions is due to the translocation of some of the lower molecular weight actin complexes to the membrane or cytoskeleton. However, de novo assembly and disassembly of the F-actin complexes cannot be excluded as an explanation of these observations.

We were able to isolate F-actin from the αPY-RF by phalloidin affinity chromatography and demonstrate F-actin association with pp37, pp57, Shc, vimentin, Grb2, SHP-1, STAT3, and to a lesser extent Vav, paxillin, STAT5b, and Cbl. The association of p64 Shc, p68 Shc, Cbl, STAT3, STAT5b, SHP-1, Grb2, and paxillin with actin was induced by CSF-1. Despite the associations demonstrated by phalloidin affinity chromatography, antibodies to Grb2 and Shc that were able to precipitate 70% Grb2 and 80% Shc co-precipitated only a small proportion of the PY proteins in the cytosolic αPY-RF. Furthermore, antibodies to Cbl and p85, which were highly efficient immunoprecipitating antibodies when used with whole cell lysates, precipitated only a small proportion of their cognate antigens from the cytosolic αPY-RF. The un-precipitated proteins recovered contain all the remaining PY proteins and actin (data not shown). These results could reflect the blocking of epitopes via the association of p85, Cbl, Grb2, and Shc with other proteins. However, given the fact that this phenomenon was observed for five different polyclonal antibodies, two of which were directed to large segments or fusion proteins, it is also possible that the actin-associated proteins are encased in a cytoskeletal framework that is relatively inaccessible to antibodies.

Many of the cytosolic αPY-RF proteins that we have shown to be associated with actin have previously been shown to interact with or regulate F-actin. p46 Shc and p52 Shc (p54 and p58 in our gel system) have been shown to bind directly to F-actin through their N-terminal domains, and these interactions were our gel system) have been shown to bind directly to F-actin through their N-terminal domains, and these interactions were identified, p85, Grb2, and G3BP, are involved in the Ras pathway. Indeed, the binding of Vav to F-actin via this domain whose calponin homology domain mediates association with actin (30). Furthermore, EF-1α has been shown to co-localize rapidly with F-actin at the leading edge of de novo lamellipods in MTLn3 cells after EGF stimulation, suggesting a growth factor-stimulated association of EF-1α with actin (30). Furthermore, EF-1α has been shown to regulate both the on and off rates for actin polymerization in vitro (58). Vav has been shown to be an F-actin binding protein whose calponin homology domain mediates association with F-actin. Indeed, the binding of Vav to F-actin via this domain has been postulated to correlate with its regulation of Ras family members like Rac and Rho, which in turn are involved in regulating the polymerization of actin to produce stress fibers, lamellipodia and filopodia (41). Other proteins we have identified, p85, Grb2, and G3BP, are involved in the Ras pathway and are likely to have a role in the regulation of the actin

| Cytochalasin D: | - | - | - | + | + | + |
| CSF-1 Time (min): | 0 | 1 | 4 | 15 | 0 | 1 | 4 | 15 |

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**FIG. 8.** Phalloidin affinity precipitation of F-actin and associated proteins from the cytosolic αPY-RF. The cytosolic αPY-RFs from CSF-1-stimulated (+) and unstimulated (−) BAC1.2F5 cells (from 1 or 10 100-mm dishes, as indicated) were incubated overnight at 4 °C with biotin-coupled phalloidin. Phalloidin with bound F-actin and the F-actin-associated proteins was collected with avidin-coupled agarose beads. The phalloidin-bound (Phalloidin bound) and -unbound (Phalloidin P.F.T.) proteins were analyzed by Western blotting with antibodies to the indicated proteins. Controls for stability in Western blot with anti-PY antibodies; B, Western blot with antibodies to the indicated proteins. Controls for stability include the cytosolic αPY-RF stored at −20 °C (untreated, lanes 1 and 2) and the cytosolic αPY-RF incubated overnight at 4 °C (O/N 4 °C, lanes 3 and 4). Incubation of the cytosolic αPY-RF with biotin alone prior to collection with the avidin-coupled agarose beads (biotin-bound and -unbound, lanes 11–14) represents a control for specificity. Similar results were obtained in a separate experiment.

**FIG. 9.** Effect of cytochalasin D treatment on CSF-1-stimulated protein tyrosine phosphorylation. Cells were preincubated with (+) and without (−) cytochalasin D for 3 h prior to stimulation with CSF-1 at 37 °C as described under “Materials and Methods.” The Nonidet P-40 soluble proteins were separated by gradient SDS-PAGE and analyzed by Western blotting with anti-PY antibody.

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cytoskeleton (48–50, 59). Further analysis of the actin complexes may provide information about molecules involved in growth factor-regulated actin nucleation and polymerization.

The importance of the cytoskeleton for efficient CSF-1 signaling is evident from the significant inhibition of CSF-1-induced tyrosine phosphorylation observed in cytchalasin D-treated cells. Like CSF-1, EGF stimulates rapid protein tyrosine phosphorylation of an array of cellular proteins as well as membrane ruffling. Simultaneously, EGF also causes co-localization of F-actin, the EGF receptor, phospholipase Cγ1, and some PY proteins in A431 cells, and it was suggested that the actin microfilament system acted as a matrix for growth factor-stimulated signal transduction (reviewed in Ref. 60). However, since CSF-1 stimulates association of both cytoskeletal and signaling proteins with the cytoplasmic oPY-RF complexes, the cytoskeleton seems to possess a more dynamic role than simply serving as a matrix for signaling.

Functional Significance of PY Complexes in Cellular Signaling—As shown by confocal microscopy in the present study (Fig. 1), the tyrosine-phosphorylated proteins accumulate in close proximity to the plasma membrane suggesting that tyrosine phosphorylation and complex formation/restructuring following CSF-1 stimulation occur at the plasma membrane. The complexes formed are diverse and should have specific functions. It is likely that in the resting cells, multisomers of cytosolic complexes formed are diverse and should have specific functions. This covalent modification changes their conformation and/or binding properties, causing them to associate with other signaling proteins and complexes. These complexes, in turn, mediate cytoskeletal changes, the activity of specific enzyme systems, and gene expression. The present findings demonstrate that CSF-1R signal transduction involves the integrated participation of cytoskeletal and other proteins in organized, pre-existing complexes. The analysis of these complexes and their novel components, e.g. pp57 and pp37, should significantly increase our understanding of this process.

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REFERENCES
1. Stanley, E. R. (1994) in The Cytokine Handbook (Thomson, A. W., ed) pp. 367–418, Academic Press, San Diego.
2. Pollard, T. W., and Stanley, E. R. (1996) Adv. Biochem. Pharma. 43, 153–193.
3. Tushinski, R. J., Oliver, I. T., Guilbert, L. J., Tynan, P. W., Warner, J. R., and Stanley, E. R. (1982) Cell 28, 71–81.
4. Boscocek, C. A., Jones, G. E., Stanley, E. R., and Pollard, J. W. (1989) J. Cell. Sci. 93, 447–456.
5. Pollard, J. W., Hunt, J. W., Wiktor-Jedrzejczak, W., and Stanley, E. R. (1991) Dev. Biol. 148, 273–287.
6. Naito, M., Hayashi, S., Yoshihisa, H., Nishikawa, S., Shultz, L. D., and Takahashi, K. (1991) J. Ass. Pathol. 139, 657–667.
7. Cecchini, M. G., Dominguez, M. G., Mocci, S., Wetterwald, A., Felix, R., Fleisch, H., Chiholm, D., Hofstetter, W., Pollard, J. W., and Stanley, E. R. (1994) Development 120, 1357–1372.
8. Morgan, C. J., Pollard, J. W., and Stanley, E. R. (1987) J. Cell Physiol. 130, 420–427.
9. Webb, S. E., Pollard, J. W., and Jones, G. E. (1996) J. Cell Sci. Cell. Biol. 109, 793–803.
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