Cellular Physiology of STAT3: Where’s the Cytoplasmic Monomer?*

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In the standard model of cytokine-induced signal transducer and activator of transcription (STAT) protein family signaling to the cell nucleus, it is assumed that STAT3 is recruited to the cytosolic side of the cell surface receptor complex from within a cytosolic monomer pool. By using Superose-6 gel-filtration chromatography, we have discovered that there is little monomeric STAT3 (91 kDa) in the cytosol of liver cells (human hepatoma Hep3B cell line and rat liver). The bulk of STAT3 (and STAT1, STAT5a, and -b) was present in the cytosol as high molecular mass complexes in two broad distributions in the size range 200–400 kDa (“statosome I”) and 1–2 MDa (“statosome II”). Upon treatment of Hep3B cells with interleukin-6 (IL-6) for 30 min (i) cytosolic tyrosine-phosphorylated STAT3 was found to be in complexes of size ranging from 200–400 kDa to 1–2 MDa; (ii) a small pool of monomeric STAT3 and tyrosine-phosphorylated STAT3 eluting at 80–100 kDa was observed, and (iii) most of the cytoplasmic DNA-binding competent STAT3 (the so-called SIF-A “homodimer”) co-eluted with catalase at 230 kDa. In order to identify the protein components of the 200–400-kDa statosome I cytosolic complexes, we used the novel technique of antibody-subtracted differential protein display using anti-STAT3 antibody. Eight polyepitides in the size range from 20 to 114 kDa co-shifted with STAT3; three of these (p60, p20a, and p20b) were co-shifted in an IL-6-dependent manner. In-gel tryptic fragmentation and mass spectrometry identified the major IL-6-dependent STAT3 co-shifted p60 protein as the chaperone GRP58/ERp57. Taken together, these data (i) emphasize the absence of a detectable STAT3 monomer pool in the cytosol of cytokine-free liver cells as posited by the standard model, and (ii) suggest an alternative model for STAT signaling in which STAT3 proteins function in the cytoplasm as heteromeric complexes with accessory scaffolding proteins, including the chaperone GRP58.

Cytokines play an important role in the communication between mammalian cells as exemplified by their role in mediating the “acute phase” response to infection and injury (1, 2). Many cytokines, such as the interferons, interleukins (IL), and hematopoietic growth factors, are currently in widespread therapeutic use against various forms of human cancer (3). Some cytokines, such as interleukin-6 (IL-6), are invariably present at the host-tumor interface (1–3). A common feature of many cytokines is that they engage the class of “non-tyrosine kinase” cell surface receptors that signal to the cell nucleus by activation of the JAK-STAT signaling pathway (Fig. 1A, the “standard” model; figure based on reviews by Heinrich and colleagues (4); Darnell (5), Leonard and O’Shea (6), and Taga and Kishimoto (7)).

The cytokine IL-6 is a major systemic mediator of the acute phase response to infection and injury which includes increased production of various acute phase plasma proteins by the liver (e.g. fibrinogen, C-reactive protein, α1-antichymotrypsin, etc) and enhanced immune function (increased B-cell differentiation and T-cell activation) (1–3). IL-6 knockout mice have a profound defect in their ability to mount an acute phase plasma protein response to sterile inflammation (reviewed in Ref. 4). The IL-6-type cytokines (which include IL-6, IL-11, leukemia inhibitory factor, oncostatin M, and cardiotrophin) signal to the cell interior through cell surface receptors which include the common gp130 signal-transducing chain (Fig. 1A) (1–7). In the case of IL-6, the cytokine binds to the α-chain (“IL-6Ra”) of the receptor (either in the cell surface-attached IL-6Ra form or the soluble IL-6Rα form), and this binary complex then binds cell surface-attached gp130 inducing dimerization of the latter (Fig. 1A) (1–7). This dimerization of gp130 triggers activation of JAK family tyrosine kinases (primarily JAK1, also JAK2 and Tyk2) associated with the cytoplasmic tail of gp130, by Tyr phosphorylation in trans of the JAK kinases thus leading to their activation (4–7). The activated JAK kinases carry out Tyr phosphorylation of up to five discrete “docking” sites in the cytoplasmic tail of gp130 (8, 9). These Tyr-phosphorylated docking sites in gp130 are thought to “recruit” monomeric STAT3 (91 kDa) and to a lesser extent STAT1 via the src homology domain 2 of the respective STAT proteins (Fig. 1A) (4). This “recruitment” event is followed by Tyr phosphorylation (PY) of the STAT proteins by the JAK kinases to give PY-STAT3, the departure of PY-STAT3 from the receptor “docking” sites in the cytoplasmic tail of gp130, and the movement from the cytoplasm to the nucleus of PY-STAT3 through an unknown mechanism (Fig. 1A) (4–7). In the nuclear compartment, PY-STAT dimers bind target DNA

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1 The abbreviations used are: IL-6, interleukin-6; IL-6R, IL-6 receptor; C, 100,000 × g cytosol (the “S100”); CP, 100,000 × g cytosol pellet (the “P100”); DTt, dithiothreitol; ELB, extract lysis buffer; EMSA, electrophoretic mobility shift assay; GRP58, glucose regulated protein 58 (synonymous with “ER-60” and “Erp57”); JAK, Janus kinases; M, mitochondrial pellet; mAb, monoclonal antibody; N, nuclear pellet; pAb, polyclonal antibody; PAGE, polyacrylamide gel electrophoresis; PIAS3, protein inhibitor of activated STAT3; PY, phosphorylation; SIE, mutant serum-inducible element from the c-fos promoter; STAT, signal transducer and activator of transcription protein family; gp, glycoprotein; FPLC, fast protein liquid chromatography; IFN, interferon.
motifs and other transcription factors and thus modulate gene expression (Fig. 1A) (4–7). Additional Ser phosphorylation of STAT proteins modulates the DNA binding competence of these signal transducers (4–7). Recently, the x-ray structures of the amino-terminal part of STAT4 (123 amino acids), of STAT3β (a partially truncated version of STAT3) bound to its cognate DNA site, and that of STAT1 bound to DNA (10–12) have been solved. STAT dimers bind DNA in the form of a pair of piers (11, 12).

The IL-6-responsive elements in the promoters of many plasma protein genes contain STAT3-binding sites, and STAT3 is the major signal transducer protein involved (reviewed in Refs. 1, 2, 4, and 7). More generally, for IL-6-type cytokines in diverse cell types, STAT3 (originally called acute phase response factor in 1993 (13)) is the major signal transducer from the cell membrane to the cell nucleus, with the liver (the hepatocyte) being a major physiological site for IL-6 action (Fig. 1). We have previously reported that the wild-type form of the p53-Val-135-inducible gene product(s) which formed a complex with STAT3 in an IL-6-dependent manner and thus “masked” its immunological accessibility (21, 22).

In this article we report experimental data indicating that almost all STAT3 in the cytosol of cytokine-free or IL-6-treated hepatocytes exists in the form of high molecular mass protein assemblies of size in the range 200–400 kDa (“statosome I”) and 1–2 MDa (“statosome II”). We also extend these observations to STAT1, STAT5a, and STAT5b for both the Hep3B and primary rat liver cytoplasmic compartments. We devised the novel technique of antibody-subtracted differential protein display using anti-STAT3 antibody to help identify protein components of the statosome I complexes. Eight polypeptides in the size range from 20 to 114 kDa co-shifted with STAT3; three of these (p60, p20a, and p20b) were co-shifted in an IL-6-dependent manner. The p60 protein was identified to be the chaperone GRP58/ER-60/ERp57. Taken together, the new data (i) emphasize the absence of a detectable STAT3 monomer pool in the cytosol of cytokine-free liver cells as posited by the standard model, and (ii) suggest an alternative model for STAT signaling in which STAT3 proteins function in the cytoplasm as complexes with novel accessory scaffolding proteins, including the chaperone GRP58.

**EXPERIMENTAL PROCEDURES**

**Hepatoma Hep3B Cell Lines**—The p53-free parental human hepatoma Hep3B cell line, a series of 11 stably transfected cell lines constitutively expressing p53-Val-135, and 7 control cell lines expressing p53 alone have been described earlier (20–22). Hep3B (Line 1) used in many of the experiments illustrated in this article is a p53-Val-135-expressing cell line (20–22). Parental Hep3B and Line 1 cells gave similar results in the experiments reported in this article.

**IL-6 Treatment**—Hep3B-derived cell lines were grown to confluence in 100-mm Petri dishes (10 ml of culture medium/dish) at 37 °C as described earlier (20–22). Cultures were then either continued at 37 °C or shifted to 32.5 °C for 18–20 h (20–22). For IL-6 treatment, cultures were washed twice with phosphate-buffered saline at the designated temperature, then replenished with 3 ml of serum-free medium, and allowed to stabilize for 4 h at the respective temperature. IL-6 (30 ng/ml) was then added for 30 min.

**Cell Fractionation**—Hep3B-derived cell lines cultured in 100-mm plastic Petri dishes as described earlier (20–22) were harvested by scraping into ice-cold phosphate-buffered saline and fractionated into cytoplasmic and nuclear compartments using hypotonic swelling in ice-cold extract lysis buffer (ELB, 10 mM Hepes, pH 7.9, 10 mM NaCl, 3 mM MgCl₂, 1 mM dithiothreitol (DTT), 0.4 mM phenylmethylsulfonyl fluoride, and 0.1 mM sodium vanadate) using a loose-fitting Dounce homogenizer essentially as described earlier (21–23). Approximately 1 ml of ELB buffer was used per cell pellet derived from one 100-mm culture. The crude cytoplasmic fraction was clarified by centrifugation at 15,000 × g for 15 min to yield the mitochondrial pellet (M) and the clarified cytoplasmic supernatant. The latter was then centrifuged for 1 h at 100,000 × g in a Beckman TLC Tabletop ultracentrifuge to yield...
the 100,000 g pellet (CP, the "P100") and the 100,000 g cytosol (C, the "S100"). The crude nuclear pellet (N) was washed twice with ESB buffer to remove the sucrose and then either extracted using an equal volume (approximately 200 ml) of 0.5 M sucrose or was in a volume equivalent to 1 ml. All cellular fractions were stored at −70 °C. In some fractionation experiments, the reducing agent DTT was omitted from the ESB buffer.

**Rat Liver Cytosol**—Rat liver cytosol was prepared at 4 °C as described previously for the purification and characterization of 20 S, 23 S, and 26 S proteasomes (25–27). Briefly, livers from male Wistar rats were minced and homogenized in 3 volumes of buffer containing 50 mM Tris-HCl, pH 7.4, 1 mM DTT, 2 mM ATP, and 0.25 M sucrose. The crude cytoplasmic extract fraction was centrifuged at 100,000 g for 1 h to remove membrane debris, organelles, and insoluble material. The soluble rat liver 100,000 g cytosol was supplemented with 20% glycerol and stored at −70 °C.

**Superoose-6 Gel Filtration Chromatography (FPLC)**—Aliquots (200 μl) of the crude cytosolic fraction were subjected to size exclusion chromatography on a Superose-6 FPLC system, with separation range from 5 MDa to 50 kDa) in the cold. For quantitation of STAT3 and PY-STAT3 in various compartments, the M, CP, and N pellets were resuspended in 0.25 ml of SDS buffer, whereas the corresponding cytosolic fractions were stored at −70 °C (350–400 μg total protein for each extract) were rapidly thawed and re-clarified by centrifugation at 15,000 g for 15 min at 4 °C just before gel filtration chromatography. The extracts were fractionated by Superose-6 sieving chromatography (Amersham Pharmacia Biotech FPLC system, with separation range from 5 MDA to 50 kDa) in the cold (4 °C). Loading and elution of the FPLC system was carried out in ESB buffer. One-milliliter fractions were collected, each divided into several 100-μl aliquots and stored at −70 °C until analyzed. For the antibody-subtracted differential protein display, 200 μl of the 100,000 g cytosol samples were incubated with 10 μg of anti-STAT3 polyclonal antibody (clarified separately at 15,000 g for 15 min at 4 °C before mixing) in 150 mM NaCl for 2 h at 4 °C prior to Superose-6 FPLC. For DNA binding assays or Coomassie Blue staining of proteins, eluate fractions were concentrated using Millipore Ultrafree Biomax-10K centrifugal filtration units (1 ml reduced to 25–30 μl).

**Western Blot Analyses for STAT Proteins**—Western blot procedures used were as per the protocol provided by Transduction Laboratories, Lexington, KY, and the ECL detection kit (Amersham Pharmacia Biotech, Buckinghamshire, UK). When comparing whole cell extracts, aliquots containing equal total protein amounts in the range 30–40 μg were resuspended in 0.25 ml of SDS buffer, whereas the corresponding cytosolic extracts (25–75 μg) were resuspended in 0.25 ml reaction volume; 30 min incubation at room temperature) contained 40 mM KCl, 1 mM MgCl₂, 0.1 mM EGTA, 0.5 mM DTT, 20 mM Hepes, pH 7.9, 4% (v/v) Ficoll, and 1 μg per reaction of poly(1-dC)–poly(1-dC) (Amersham Pharmacia Biotech). DNA-protein complexes were separated by electrophoresis through 5% PAGE (acrylamide/bis-acrylamide ratio, 30:0.8) in 0.2% TBE (0.02 M Tris, pH 8.3, 0.02 M boric acid, 0.25 mM EDTA, Ref. 29) at 300 V for 3 h at 4 °C. Prestained molecular weight markers were placed in adjacent lanes on each side of the lane containing the extract (203 kDa, myosin; 116 kDa, β-galactosidase, and 83 kDa, bovine serum albumin; Bio-Rad, broad range). The location of the prestained marker proteins in the respective figure indicates the relative migration of the respective proteins and not necessarily an indication of the relationship between mobility and molecular mass.) Proteins resolved through native PAGE were analyzed by electroblotting and probing using anti-STAT mAb.

**Silver Staining of Proteins in SDS-PAGE**—Aliquots of FPLC eluate fractions (100 μl boiled down to 20 μl; see above) were electrophoresed through either 7.5 or 12.5% SDS-polyacrylamide gel and stained using the Silver Stain Plus Kit from Bio-Rad.

**Protein Identification by Mass Spectroscopy**—Coomassie Blue-stained protein bands were excised from SDS-PAGE gels using a scalpel and digested in situ with trypsin (Promega, Madison, WI) as described by Rosenfeld and colleagues (30) except that the detergent Tween 20 was absent from the digestion buffers. The digests containing the gel bands were extracted in 50% acetonitrile, 0.1% trifluoroacetic acid, concentrated using a SpeedVac to less than 10 μl, and desalted using a ZipTip (Millipore, Bedford, MA). An aliquot of the digest was mixed with an equal volume of matrix material (a-cyano-4-hydroxycinnamic acid (Sigma) as a saturated solution in 50% acetonitrile, 0.05% trifluoroacetic acid), and ion masses were determined by matrix-assisted laser desorption ionization-time of flight mass spectroscopy (Kratos KOMPACT MALDI III). Data bases were searched at 0.08% mass accuracy with the MS-Fit algorithm (University of California at San Francisco web site) to identify the digested protein.

**Additional Reagents**—Human IL-6 (Escherichia coli-derived) was purchased from R & D Systems, Inc. (Minneapolis, MN), and anti-GRP58 pAb was from StressGen Biotechnologies Corp. (Vancouver, British Columbia, Canada).

RESULTS

**Cellular Compartmentation and Cytoplasm to Nuclear Trans- it of STAT3 in IL-6-stimulated Hepatocytes**—We verified the extent of movement of STAT3 from the cytoplasm to the nucleus in the Hep3B cell line in this laboratory; here we show that the reagents used for FPLC were not washed out and that the resulting binding buffer (250 mM potassium chloride, 10 mM trisodium phosphate, 0.1 mM EGTA) typically the binding buffer used in the reagent kit protocol. Briefly, the procedure was the same as for the peroxidase-based Western blot method outlined above except that an alkaline phosphatase-linked secondary antibody was used instead. The signals were amplified and detected by the Vistra ECF Substrate and scanned and quantitated by phosphorimaging.

**DNA Gel-shift Assays**—STAT-specific DNA binding activity was assayed using the mutant “SIE” oligonucleotide derived from the c-fos promoter (purchased from Santa Cruz Biotechnology, Santa Cruz, CA) which yields the typical pattern of SIF-A, -B, and -C complexes in gel-shift assays corresponding to STAT3 “homodimer,” STAT1/3 “het- erodimer,” and STAT1 “homodimer,” respectively (reviewed in Refs. 4–7). The SIE oligonucleotide was “P”-labeled at its 5’ ends using T4 polynucleotide kinase and used in electrophoretic mobility shift assays (see above). Typically the binding buffer to 1 (15-μl reaction volume; 30 min incubation at room temperature) contained 40 mM KCl, 1 mM MgCl₂, 0.1 mM EGTA, 0.5 mM DTT, 20 mM Hepes, pH 7.9, 4% (v/v) Ficoll, and 1 μg per reaction of poly(1-dC)–poly(1-dC) (Amersham Pharmacia Biotech). DNA-protein complexes were separated by electrophoresis through 5% PAGE (acrylamide/bis-acrylamide ratio, 30:0.8) in 0.2% TBE (0.02 M Tris, pH 8.3, 0.02 M boric acid, 0.25 mM EDTA, Ref. 29) at 300 V for 3 h at 4 °C and dried for autoradiography. In antibody inhibition/supershifting experiments, cell extracts were incubated with antibody preparations for 30 min at room temperature in DNA-binding buffer prior to addition of 32P-labeled oligonucleotide for another 30 min.

**Western Blotting of STAT Proteins following Native PAGE**—One-dimensional native PAGE was performed using 10% acrylamide separating gel, cast with a 1.2-mm thick Teflon comb spacer using the same buffer and electrophoresis conditions as for the DNA gel-shift assays (see above) and the Bio-Rad Protein II xi electrophoresis unit (Bio-Rad). Hep3B 100,000 g cytosolic extract (25–75 μl) prepared from cells treated with or without IL-6 for 30 min were adjusted to the composition of the DNA gel-shift binding reaction buffer with or without the addition of excess cold SIE oligonucleotide (see above) and electrophoresed through 5% native PAGE at 300 V for 3 h at 4 °C. Prestained molecular weight markers were placed in adjacent lanes on each side of the lane containing the extract (203 kDa, myosin; 116 kDa, β-galactosidase, and 83 kDa, bovine serum albumin; Bio-Rad, broad range). The location of the prestained marker proteins in the respective figure indicate solely where the respective proteins migrated and not necessarily an indication of the relationship between mobility and molecular mass.) Proteins resolved through native PAGE were analyzed by electroblotting and probing using anti-STAT mAb.

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from cytoplasm to nucleus was evaluated by Western blotting. Briefly, Hep3B cells (Line 1) treated with IL-6 (30 ng/ml) for 30 min (the same time point as the maximal appearance of DNA binding activity in the nuclear compartment in these cells; see Refs. 21 and 22) were fractionated by Dounce homogenization and differential centrifugation to yield the following fractions: 15,000 × g mitochondrial pellet (MP), a 100,000 × g cytoplasmic pellet (CP), 100,000 × g cytosolic fraction (C), and the nuclear pellet purified through a 1.5 M sucrose cushion (N). Aliquots from each of these fractions were solubilized in buffer containing 0.1% SDS and 0.5% Triton (24) and evaluated directly with no intervening immunoprecipitation step by Western blotting for STAT3 and PY-STAT3 following SDS-PAGE separation using Superose-6 seiving chromatography, and the presence STAT3 protein in the eluted fractions was assayed by SDS-PAGE under reducing conditions and Western blotting without any intervening immunoprecipitation step (Fig. 3, A and B). Similarly, 200-μl aliquots of extracts of Brij-58 (0.5%) washed nuclei prepared from Hep3B cells without or with IL-6 treatment for 30 min were also fractionated through Superose-6 FPLC, and STAT3 protein was assayed in the eluates by Western blotting (Fig. 3, C and D).

The data in Fig. 3A show that cytosolic STAT3 in cytokine-free Hep3B cells was distributed in two broad size classes, 200–400 kDa and 1–2 MDa. Very little, if any, monomeric STAT3 was observed in the 80–100-kDa region. Fig. 3D shows the marked increase of 200–400-kDa and some 1–2-MDa STAT3 in nuclear extracts from IL-6-treated cells.

It was unexpected to find that the latent STAT3 complexes in the cytoplasm of cytokine-free Hep3B cells (Fig. 3A) were at least as large as the conventionally accepted STAT3 “dimer” in the nuclear extract (Fig. 3D), supposedly of size at least 180 kDa. Also, as shown in Fig. 5, “monomeric” STAT3 displayed the expected elution at approximately 90–100 kDa in Superose-6 FPLC. Additionally, Fig. 3E is a reprobing of the Hep3B cytosol blot in Fig. 3A using an mAb to a protein designated as HC8 which is a subunit of the 20 S proteasome, a complex of approximately 700 kDa (27, 31). This internal control, eluting at the expected location in fractions 10 and 11, further validates the size fractionation obtained by Superose-6 FPLC in this experiment.

The inclusion or exclusion of the reducing agent DTT from the ELB hypotonic lysis buffer and the subsequent FPLC buffer yielded data similar to those shown in Fig. 3A. Additional controls have included the addition of an ATP-generating system and buffer containing 2 mM ATP in the hypotonic swelling buffer, without significant resulting changes in the gel-filtration profile of the STAT3. The almost complete absence of monomeric STAT3 in the cytosol was a surprise (see the standard model in Fig. 1A). While recognizing that there was a continuum of STAT3 complexes in the size range from 200-kDa to 2MDa, for
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In these experiments, little monomeric STAT3 was detected in the cytosol. In order to increase the sensitivity of the procedure to detect even small amounts of monomeric STAT3 in the cytosol, we immunoprecipitated 0.5-ml aliquots of each eluate fraction using anti-STAT3 pAb in detergent-containing buffer and then used an anti-STAT3 mAb for probing the Western blots. Fig. 5A confirms the detection of a broad size distribution of the bulk of the STAT3 in Hep3B cytosol (1 MDa to 200 kDa). By using immunoprecipitation to concentrate eluate fractions, a small pool of monomeric STAT3 was detected in the cytosol of Hep3B cells eluting at 80–100 kDa but only upon IL-6 treatment.

Inspection of the anti-PY-STAT3 pAb-probed blot in Fig. 4B (and its longer exposures) revealed the presence of some PY-STAT3 in complexes >400 kDa (in fractions 10–14). By using immunoprecipitation with anti-STAT3 pAb to concentrate eluate fractions, PY-STAT3 can be readily observed in STAT3 complexes >400 kDa (Fig. 5B). Comparing Figs. 4B with 5B, the PIAS3-containing fraction (fraction 17 in Fig. 4B) remained resolvable from the bulk of PY-STAT3. Finally, the free STAT3 monomer pool in IL-6-treated cells included detectable PY-STAT3 (arrowhead in Fig. 5B). These data are similar to the transient detection in the cytoplasm of interferon-γ-treated HeLa cells of monomeric PY-STAT1 eluting at 80–100 kDa off Superose-12 FPLC (32).

Where Does the Cyttoplasmic Homodimeric DNA-binding Competent STAT3 Complex (“SIF-A”) Elute Off Superose-6 FPLC?—In order to detect DNA-binding competent STAT3 in the cytosolic compartment, we carried out electrophoretic mobility shift assays (EMSA) using the mutant serum-inducible element (SIE) oligonucleotide probe derived from the c-fos promoter (4–7). By using nuclear extracts from cytokine-treated cells, the SIE probe typically gives a set of three complexes called SIF-A, -B, and -C that are known to correspond to STAT3 homodimer, STAT1/3 heterodimer, and STAT1 homodimer (reviewed in Refs. 4–7). Fig. 6A shows the detection of SIF-A, -B, and -C complexes in EMSA carried out using Hep3B 100,000 × g cytosolic extracts prepared from cells treated for 30 min with IL-6 but not in the absence of IL-6. Antibody inhibition assay using anti-STAT3 pAb confirmed the inclusion of STAT3 in the IL-6-treated SIF-A and SIF-B complexes derived from the cytoplasm (Fig. 6A; also see Fig. 7C). We have also confirmed that, as expected, the formation of the SIF-C complex using the IL-6-treated cytosol is completely inhibited by anti-STAT1 mAb (data not shown).

Superose-6 eluate fractions (1 ml) derived from chromatography of cytosol (200 μl) of IL-6-treated Hep3B cells were either
Western blotted directly to locate the distribution of STAT3 using 100-μl aliquots (Fig. 7A), and the remainder (900 μl) individually concentrated using the Biomax-10K centrifugal system to a volume of 25–30 μl, and DNA binding activity assayed using the SIE oligonucleotide probe (Fig. 7B). Fig. 7B shows that most of the SIF-A and -B complexes co-eluted in a peak with catalase at approximately 232 kDa, approximately coincident with the major STAT3-containing statosome I peak (200–400 kDa). The antibody inhibition data in Fig. 7C confirm the inclusion of STAT3 in the SIF-A complexes co-eluting with catalase. The SIF-C, STAT1-containing complex, co-elutes with ferritin at 440 kDa (Fig. 7B). Reprobing the blot in Fig. 7A with anti-STAT3 pAb shows a distribution of PY-STAT3 in this experiment coincident with the distribution of SIF-A DNA complexes (data not shown). The data in Fig. 7, A and B, show clearly and directly that the bulk of cytoplasmic STAT3 is in complexes at least as large as, and larger than, the DNA-binding competent STAT3 and again emphasize the absence of a major STAT3 monomer pool in the cytosol.

**Statosome Complexes Containing STAT1 and STAT5b in Hep3B Cell Cytosol**—To test whether STAT1 and STAT5b (Hep3B cells lack detectable STAT5a; see Refs. 21 and 22) also occur in cytosolic complexes, as was observed for STAT3 (Figs. 3–7), Western blots of Superose-6 elution fractions derived from the 100,000 × g cytosol from Hep3B cells (no IL-6 treatment) were re-probed using anti-STAT1 and then anti-STAT5b mAb. Fig. 8, A–C, shows that like STAT3 STAT1 also occurs in complexes in the cytosol from Hep3B cells untreated or treated with IL-6 (30 ng/ml) for 30 min were mixed with 10 μg of anti-STAT3 pAb (C-20 from Santa Cruz Biotechnology) and incubated for 60 min at 4 °C. A, aliquots (7 μl) of the antibody-treated extracts or antibody-free extracts were assayed for DNA binding activity using the SIE probe. B, aliquots (30 μl) adjusted to the composition of DNA-binding reaction buffer were electrophoresed in adjacent lanes of the same native gel as in A, and Western-blotted using a murine anti-STAT3 mAb. C, reprobing the Western blot in B using a murine anti-STAT1 mAb. The balance of the antibody-treated and antibody-free aliquots, approximately 210 μl each, were used for fractionation through Superose-6 FPLC in the experiment in Fig. 8.

**Superose-6 FPLC of Cytosolic STAT Proteins in Rat Liver Hepatocytes**—To verify the generality of the detection of cytosolic statosome I and II complexes in cytosol of Hep3B cells, we investigated the presence of STAT3 complexes in 100,000 × g cytosolic extracts prepared from rat liver. The preparation of these extracts was carried out using procedures previously used in this laboratory for preparing cytosolic rat liver proteasome preparations (25–27). Fig. 9 shows the properties of STAT3 protein present in 100,000 × g rat liver-derived cytosol (no ATP addition) as assayed by Superose-6 FPLC. It is evident that (i) little monomeric STAT3 was observed (elution fractions from 21 to 40 of Fig. 9A were also negative for STAT3 or fragments thereof; not shown), and (ii) STAT3 was present in...
The remainder (approximately 210 μl) of each of the four Hep3B cell line-derived cytosolic extracts illustrated in Fig. 6 (without and with IL-6 treatment of the cells; each without and with the addition of rabbit anti-STAT3 pAb) were fractionated through Superose-6 FPLC. As a control, 10 μg of the rabbit anti-STAT3 pAb was also subjected to Superose-6 FPLC. Eluate fractions (100 μl each) were assayed by SDS-PAGE and Western blotting for STAT3, STAT1, and STAT5b using murine anti-STAT mAb as the probe and for rabbit IgG using a goat-anti-rabbit pAb as the probe. This figure illustrates data obtained from cytosol extracts of IL-6-free Hep3B cells with and without prior incubation with anti-STAT3 pAb. Similar results were obtained for antibody-induced supershifting of STAT3 in the IL-6-treated extracts (data not shown). Fr. No., fraction number.

**FIG. 8. Antibody supershifting of STAT3 as evaluated by Superose-6 FPLC.** The remainder (approximately 210 μl) of each of the four Hep3B cell line-derived cytosolic extracts illustrated in Fig. 6 (without and with IL-6 treatment of the cells; each without and with the addition of rabbit anti-STAT3 pAb) were fractionated through Superose-6 FPLC. As a control, 10 μg of the rabbit anti-STAT3 pAb was also subjected to Superose-6 FPLC. Eluate fractions (100 μl each) were assayed by SDS-PAGE and Western blotting for STAT3, STAT1, and STAT5b using murine anti-STAT mAb as the probe and for rabbit IgG using a goat-anti-rabbit pAb as the probe. This figure illustrates data obtained from cytosol extracts of IL-6-free Hep3B cells with and without prior incubation with anti-STAT3 pAb. Similar results were obtained for antibody-induced supershifting of STAT3 in the IL-6-treated extracts (data not shown). Fr. No., fraction number.

**FIG. 9. Superose-6 FPLC analyses of STAT proteins in rat liver cytosol.** An aliquot (200 μl) of rat liver cytosol (the 100,000 g supernatant) was fractionated through Superose-6 FPLC, and the elution of various STAT proteins was evaluated by SDS-PAGE and Western blotting of 100-μl aliquots of each eluate fraction as described under "Experimental Procedures" and in the legend to Figs. 3 and 8. A–D, show the elution of STAT3, STAT1, STAT5a, and STAT5b. E, shows the elution of murine anti-STAT3 mAb (IgG) off the same column in a separate run. Fr. No., fraction number.

![Image](https://example.com/image.png)

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Two broad distributions of size 200–400 kDa and 1–2 MDa. These data, replicated both without and with the addition of 1 mM ATP to the extracts (as used in the preparation of 20 S and 2 S proteasome preparations, respectively (25, 26)), indicate the generality of the observation that cytosolic STAT3 occurs in statosome complexes in rat liver hepatocytes.

![Image](https://example.com/image.png)

**Fig. 9B shows that little monomeric STAT1 was detected in the rat liver cytosol.** STAT1 was also included in complexes in the 200–400-kDa and 1–2-MDa size range. Additionally, the STAT1-containing 200–400-kDa complexes were shifted to the higher side of the STAT3 profile in the 200–400-kDa region suggesting that STAT1 and STAT3 were in different complexes.

As in the Hep3B data in Fig. 8 above). Reprobing the blot in Fig. 9A with anti-STAT5a and anti-STAT5b mAb revealed that these STAT proteins were also present largely in complexes in the size range 200–400 kDa, with detectable STAT5a and STAT5b in the 1–2-MDa region (Fig. 9, C and D). As an additional control, anti-STAT3 mAb (IgG) eluted through Superose-6 FPLC at the expected position corresponding to approximately 160-kDa. Fig. 9 shows that most STAT1, -3, -5a, and -5b in the rat liver cytosol was in complexes larger than IgG.

**Cellular Proteins “Co-shifted” with STAT3, IL-6-independent, and IL-6-dependent “Antibody-subtracted Differential Protein Display”—** We developed a new strategy to identify cellular proteins present as components of stateosome complexes by adapting the customary technique of antibody supershifting of DNA complexes in EMSA to protein supershifting (Fig. 6, B and C, and Fig. 8). In the experiment illustrated in Fig. 6B adjacent lanes from the same native PAGE gel used to carry out the EMSA (Fig. 6A) were soaked with SDS-containing buffer, electroblotted to polyvinylidene difluoride membrane, and Western-blotted for total STAT3 using an anti-STAT3 mAb. The bulk of cytosolic STAT3 was of apparent mobility >200 kDa by native PAGE. Preincubation of the cytosolic extracts with anti-STAT3 pAb “supershifted” the STAT3 protein (Fig. 6B) but not STAT1 (Fig. 6C).

The STAT3-protein supershifting by anti-STAT3 pAb was then adapted to Superose-6 FPLC gel filtration. The data in Fig. 8D show supershifting by anti-STAT3 pAb of STAT3-containing complexes out of the stateosome I (200–400 kDa) region into a larger sized complex. STAT5b is at least partially co-shifted with anti-STAT3 pAb (Fig. 8F), whereas the bulk of STAT1 is not co-shifted (Fig. 8E). In control experiments, unrelated pAb did not supershift STAT3 (not shown).

The anti-STAT3 pAb protein co-shifting as adapted to FPLC (Fig. 8) suggested a technique for the identification of cellular proteins present in the 200–400-kDa stateosome I STAT3-containing complexes. This technique can be viewed as an antibody-subtracted differential protein display procedure (Fig. 10). Proteins present in the Superose-6 gel filtration fractions in the 200–400-kDa region (stateosome I) were displayed by SDS-PAGE (12.5 and 7.5%) and silver staining. Replicate FPLC elute fractions derived from the 100,000 g cytosol extracts of IL-6-treated or untreated Hep3B cells, each with or without anti-STAT3 pAb supershifting (the same fractions illustrated in Fig. 8), were analyzed comparatively in order to identify the subset of proteins co-shifted with anti-STAT3 pAb in an IL-6-independent or IL-6-dependent manner. Extensive antibody-subtracted differential protein display of this kind has led to the data summarized in Fig. 10 and Table I.

**Fig. 10A shows that a set of at least five proteins in the size range 114 to 91 kDa were co-shifted with STAT3 in an IL-6-independent manner.** As expected from Fig. 8, and verified by Western blotting of Coomassie-stained gels (not shown), among these were STAT3 (included in band 5) and STAT5b (included in bands 3, 4) (Table I). Fig. 10, B and C, shows an additional three proteins that were co-shifted with STAT3 but in an IL-6-dependent manner (p60, and the p20a and p20b doublet). The specificity of the antibody co-shifting experiment in helping to detect proteins associated in a complex with STAT3 is suggested by the fact that the majority of the proteins that co-eluted from the Superose-6 column with STAT3 in the 200–400 kDa region did not “co-shift” (Fig. 10 and data not shown). Furthermore, the addition of unrelated pAb to the samples did not produce any supershifting of the proteins in the stateosome I region (not shown). Also, when these elution fractions were re-probed in Western blot format for rabbit pAb, the IgG (150–180-kDa) was observed in elution fractions 17–20, which are
readily resolvable from the statosome I and II regions (Fig. 8G).

**Identification of IL-6-dependent Statosome III co-shifted p60 Protein as the Chaperone GRP58/ER-60/ERp57**—Concentrates of FPLC eluate fractions corresponding to fractions 13, 14, and 15 as in Figs. 8 and 10 were electrophoresed through 12.5% SDS-PAGE and stained with Coomassie Blue, and the major STAT3-co-shifted p60 protein in lane 13 was excised from the gel and subjected to in-gel tryptic fragmentation. Analysis of the digest by mass spectroscopy identified eight strong signals in the mass range 700 to 2000 m/z. Data base search using MS-Fit identified the top four proteins (6/8 masses included) as different entries for the same human gene product as follows: the protein P58 (GenBank™ accession number 2507461)/ER-60 (GenBank™ accession numbers 1085373) and a protein disulfide isomerase (GenBank™ accession number 1085373). As a control, a similar analysis of a protein of the same apparent molecular weight observed in an adjacent lane (marked by an asterisk in Fig. 10B), which was only weakly affected by anti-STAT3 pAb, was identified as the human gene product aldehyde/dehydrogenase I (GenBank™ accession numbers 2183299 and 118495). Low levels of GRP58/ER-60/ERp57 tryptic fragments were also detected in this digest. The identification of aldehyde dehydrogenase I, a tetramer of size 240-kDa, as a protein that co-elutes with the STAT3 200–400-kDa peak further validates the size estimate of the STAT3-containing statosome I complexes.

Fig. 11 illustrates the inhibitory effect of an anti-GRP58 pAb on the formation of the cytosolic SIF-A DNA-binding complex, compared with anti-STAT3 pAb and control rabbit serum. The anti-GRP58 pAb used was as effective in inhibiting formation of the SIF-A complex as was anti-STAT3 pAb. Similar data were obtained using two additional anti-GRP58 antisera (not shown).

**Discussion**

A search for preformed cytoplasmic complexes containing STAT3 in Hep3B cells and in the rat liver cytosol using Superose-6 FPLC led to the unexpected discovery that cytosolic STAT3 in cytokine-free cells existed in complexes of size in the range from 200 to 400 kDa (statosome I) and 1–2 MDa (statosome II) with little or no detectable free STAT3 monomers. Similar high molecular mass cytosolic complexes were observed for STAT1, STAT5a, and STAT5b, again with little detectable monomers. These observations contrast with the assumption contained in the "standard" model for JAK-STAT signaling (Fig. 1A) which customarily depicts the cytosolic STAT protein pool to be monomeric (4–7). Furthermore, although the standard model (Fig. 1A) implies an increase in size of STAT3 upon cytokine stimulation consequent to Tyr phosphorylation of STAT3 (the monomer to dimer transition), the bulk of cytosolic PY-STAT3 had similar Superose-6 gel filtration properties (200–400 kDa; "statosome I-PY") as did the bulk of non-PY-containing STAT3 in the 200–400 kDa region. An antibody supershifting technique (antibody-subtracted differential protein display) provided data suggesting that the STAT3-containing statosome I complex contained approximately eight different polypeptides, three of which associated in an IL-6-dependent manner. A major IL-6-dependent STAT-3 co-shifted protein was identified as the chaperone GRP58/ER-60/ERp57.

Although the complete identification of all the individual statosome subunits and their relative stoichiometry remains to be determined, the new data indicate that the standard model

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**Table I**

| Polypeptide | Comment |
|-------------|---------|
| A. IL-6-independent | 1 p114 |
| | 2 p101 |
| | 3 p97 includes STAT5b |
| | 4 p95 |
| | 5 p91 includes STAT3 |
| B. IL-6-dependent | 6 p60 GRP58/ER-60/ERp57 |
| | 7 p20a |
| | 8 p20b |

**Fig. 10.** Antibody-subtracted differential protein display. Aliquots (100 μl) each from eluate fractions 13–15 from each of A and D in Fig. 8 (IL-6-free) and fractions 13–15 from the corresponding Superose-6 FPLC elutions of extracts from IL-6-treated cells (as in Fig. 3 and 8) were subjected to SDS-PAGE through 7.5 and 12.5% polyacrylamide gels followed by silver staining of the displayed proteins. A, section of a 7.5% SDS-PAGE showing differential co-shifting of proteins by anti-STAT3 pAb in extracts from IL-6-free Hep3B cells. Data illustrated are derived from fractions 13–15 in Fig. 8, A and D, and the corresponding eluate fractions derived from cytosolic extracts of IL-6-treated Hep3B cells. B, the p60 protein was identified by mass spectroscopy of tryptic peptides as GRP58/ER-60/ERp57, whereas the protein marked with an asterisk was identified as the subunit of the 240-kDa aldehyde dehydrogenase I tetramer.

**Fig. 11.** Inhibition of the formation of the IL-6-induced cytoplasmic SIF-A STAT3 complex by anti-GRP58 pAb. A, cytosol from IL-6-treated Hep3B cells (5 μl) was mixed with 0.5, 1, or 2 μl of anti-GRP58 antiserum (StressGen) or control rabbit serum (NRS) for 30 min at room temperature, and the residual DNA binding activity was assayed using the SIE oligonucleotide probe. B, the effect of 2 μl each of an anti-STAT3 pAb (H190; Santa Cruz Biotechnology), the anti-GRP58 pAb, and control rabbit serum on the generation SIE-DNA complexes by cytosol of IL-6-treated Hep3B cells (5 μl per binding reaction) were compared.
depicted in Fig. 1A and generally viewed as the accepted paradigm in the JAK-STAT signaling field (4–7) is missing scaffolding/chaperone components that are likely involved in regulating STAT protein recruitment to the receptor complex and the subsequent transit of PY-STAT to the nucleus (Fig. 1B). STATs are activated in the cytoplasm, but they exert their gene regulation function in the nucleus (Fig. 1) (4–7). We and others (4–7, 22, 23) have verified cytoplasmic to nuclear translocation of STAT3 by immunofluorescence microscopy by following the transfer of metabolically labeled STAT3 from the cytoplasm to the nucleus, by following the transfer of Western-blotted total STAT3 from the cytoplasm to the nucleus, and by following the transit of Western-blotted PY-STAT3 (using an anti-PY-STAT3 Ab). Experiments carried out by us and other investigators (4–7, 22, 23) show that at best 10–30% of total cytoplasmic STAT1 or STAT3 translocates from the cytoplasm to the nucleus upon cytokine stimulation. Thus, in most cell types, including the hepatocyte, it is only a minority fraction of cytoplasmic STAT3 that undergoes nuclear translocation. Additionally, not all cytoplasmic PY-STAT3 translocates to the nucleus; a substantial fraction (30–50%) of PY-STAT3 remains in the cytoplasm of IL-6-stimulated Hep3B cells (Fig. 2 and Ref. 22).

The mechanism by which a subfraction of the cytoplasmic of PY-STAT pool enter the nucleus is unknown. STAT proteins lack a so-called nuclear localization signal sequence, at least in the conventional sense (4–7). Recently, it was shown that Tyr phosphorylation of STAT1 and GTPase active of Ran was crucial for the nuclear import of STAT1 (35). The importin-a family member NPI-1 was identified as the nuclear localization signal for STAT1, suggesting that at least STAT1 enters the nucleus via the conventional import pathway (36). No information is available for the nuclear import pathway for STAT3.

The activation of STATs by cytokines, including the residence of DNA-binding competent STATs in the nuclear compartment, is transient, with increase in nuclear DNA-binding competent STAT3 occurring by 15–30 min and its decay by 60–120 min (4–7, 22, 23, 37, 38). Studies of interactions between STAT proteins and other cellular proteins have included investigating functional and protein-protein interactions in the nuclear compartment. Functional interactions have been reported with C/EBP/pNF-IL-6 (39, 40), NF-kB (41), AP-1 (39, 42–44), and the glucocorticoid receptor (45, 46). Direct protein-protein interaction between STAT3 and other factors have been reported with c-Jun and the glucocorticoid receptor (4, 5, 46). STAT1 also associates with members of the IRF-1 family and Sp1 (47–49). Multimerization (tetramerization) was recently demonstrated for STAT1 in the context of tandem STAT-binding sites in target DNA, and the amino terminus of STAT1 was found to be essential for this process (50, 51). Studies of how STAT proteins activate transcription have led to the identification of interactions between STAT1 and STAT3 with basal transcription factors such as CBP and p300 (4, 52–54).

A mechanism to down-regulate the JAK-STAT pathway in the cytosolic compartment has been recently discovered with the cloning and identification of a family of SOCS (suppressor of cytokine signaling) proteins (now more than 20 members) also referred to as JAB (JAK-binding protein) or SSI (STAT-induced STAT inhibitors) (55–58). SOCS family members inhibit tyrosine phosphorylation of gp130, STAT1, and STAT3 by binding to and inhibiting JAK family kinases (55–58). However, at the present time, there is no evidence for a direct protein-protein interaction between SOCS family members and STAT3 (55–58).

Direct interaction between IFN-γR1 and STAT3 monomer as an adapter linking the cytokine-stimulated IFN-γ receptor to PI3-kinase has been recently detected in cross-immunoprecipitation experiments (59). A role has also been suggested for monomeric STAT1 in "constitutively" mediating the expression of gene product(s) required in tumor necrosis factor-induced apoptosis (60). How such a monomeric STAT1 would function in cells to regulate gene expression is unclear. Neither of these investigators (59, 60) provided evidence for the existence of monomeric STAT1 as such.

By using yeast two-hybrid screens to detect interactions between STAT1 and various target proteins, Shuai and colleagues (33, 34) isolated a protein called PIAS1 which interacted with STAT1. By using PIAS1 as a probe, these investigators isolated a related 68-kDa protein called PIAS3, which interacted exclusively with PY-STAT3 (in Brij-58 detergent containing cytoplasmic extracts) and inhibited the latter’s ability to bind DNA (33). The physiological significance of PIAS3 in regulating STAT3-dependent signaling is unknown. Four human and two murine "PIAS" family proteins have been cloned thus far. In detergent (Brij-58) containing cross-immunoprecipitation assays, PIAS3 family members interact with only their cognate PY-STAT protein (33, 34). Our data (Fig. 4) show that PIAS3 in the cytosol can be resolved away from the bulk of cytosolic PY-STAT3.

Recently, Stanley and colleagues (61) reported the detection of multimeric cytosolic complexes of signaling proteins and cytoskeletal components in macrophages stimulated with colony-stimulating factor-1. These investigators treated a macrophage cell line (BAC1.2F5) with colony-stimulating factor-1 for 2 h in the cold (at 4 °C) and isolated PY-containing cytosolic proteins that bound (directly or indirectly) to an anti-PY-mAb immunoaffinity chromatography column. Gel-filtration sieving of such PY-containing cytosolic proteins through a Superoxene-6 column showed these to be in two broad distributions of size 1–5 MDa and between 300 and 400 kDa. STAT3, STAT5a, and STAT5b were detected by Western blotting in these 1–5 MDa and 300–400-kDa PY-containing CSF-1-stimulated cytosolic complexes. Evidence was obtained for the association of STAT3 to and to a lesser extent STAT5b to F-actin. This represents the first report of the detection of STAT3 and STAT5a and -b in high molecular mass complexes (300–400 kDa and 1–5 MDa) derived from the cytosolic compartment of cytokine-stimulated cells. The paradigm for JAK-STAT signaling occurring in the context of scaffolding/chaperone proteins (Fig. 1B) gains support from the observations of Stanley and co-workers (61). "Preassociation" of STAT1 with other STAT proteins in the cytosolic compartment of HeLa and 3T3-F442A cells prior to cytokine stimulation has been detected using cross-immunoprecipitation assays in the absence of added detergents (62).

We were unable to discern any significant size-shift upon Tyr phosphorylation of STAT3 in the cytosol of IL-6-treated Hep3B cells as assayed by Superoxene-6 FPLC. A small pool of monomeric STAT3, including PY-STAT3, was observed in the cytosol of IL-6-treated Hep3B cells. The main peak of DNA-binding competent STAT3 which yielded the SIF-A homodimeric complex co-eluted with the major PY-STAT3 pool and with catalase at approximately 230 kDa.

Recently, Lackmann et al. (32) have reported that STAT1 in detergent-free cytosolic extracts from HeLa cells was observed to be in high molecular weight latent complexes of size 200–300 kDa as evaluated by Superoxene-12 gel filtration. Bulk STAT1 in the cytoplasm was of apparent size similar to that of bulk PY-STAT3 in nuclear extracts, the presumed dimer, and thus of size at least 180 kDa. Overall, the data presented by Lackmann et al. (32) are similar to our observations for the STAT3-containing statosome I. Additionally, these investigators detected the transient presence of small amounts of monomeric
PY-STAT1 in the cytosol 5 min after interferon-γ addition to HeLa cells. As with the present studies, from a technical standpoint, these authors emphasized that the elution of monomeric STAT1 at 81–108 kDa through Superose-12 chromatography was consistent with the expected mass of monomeric STAT1.

Our gel-filtration data (Fig. 5) and those of Lackmann et al. (Fig. 3, B and D, in Ref. 32) verifying, respectively, that monomeric STAT3 and monomeric STAT1 (including PY-STAT1 monomers) in detergent-free cytoplasmic extracts have the expected 90–100-kDa elution size through Superose-6 or Superose-12 chromatography are at variance with conclusions contained in reports from the Darnell laboratory (51, 63–65). In contrast to the data of Lackmann et al. (32), Shuai et al. (63) reached the conclusion that bulk STAT1 in the cytoplasm of cells was a monomer. This conclusion was based upon STAT1 protein sizing by glycerol gradient sedimentation of detergent-treated cytoplasmic extracts (Fig. 2 in Ref. 63), and upon size estimates of DNA affinity purified STAT1 derived using native PAGE (Fig. 1 in Ref. 63). This conclusion was, in part, based upon a calibration curve in the sedimentation analysis that was inconsistent with that reported earlier for a similar analysis by the same group (compare Fig. 2C in Ref. 63 with Fig. 2A in Ref. 64). The native PAGE Western blot data in Fig. 1 of Ref. 63 relate to STAT1 extensively fractionated through DNA affinity chromatography and not to fresh detergent-free cytosolic extracts. Faced with gel filtration data for STAT1 in detergent-treated cytoplasm that indicated a size larger than the molecular weight assignment for a monomer, Qureshi et al. (65) expressed a "belief" that the gel-filtration calibrations may be inapplicable to STAT proteins based upon a comment concerning the sum of the molecular sizes of the proteins co-shifted with was identified to be the chaperone GRP58/ER-60/ERp57. From the kDa size estimate for statosome I complexes, we anticipate the discovery that the bulk of cytosolic STAT proteins exist in protein assemblies in the size range 200–400 kDa to 1–2 MDa suggests that the initiation and transmission of STAT signaling through the cytosolic compartment includes novel regulatory, scaffolding, and chaperone proteins (Fig. 1B).

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GRP58 is a major target for tyrosine phosphorylation by the Lyn kinase (76), a kinase that can be activated by IL-6 through gp130 signaling (77, 78). By using an oligonucleotide probe corresponding to an interferon-responsive element from the 2′-5′ oligo(A) synthetase gene promoter in cell-free DNA-binding assays, Deisseroth and colleagues (79) have previously drawn attention to the ability of cytoplasm-derived GRP58 to modulate in vitro the inducible DNA binding activity present in nuclear extracts prepared from interferon-treated myeloid cell lines. The present identification of GRP58 as an IL-6-dependent STAT3-co-shifted protein suggests the involvement of this chaperone in regulating the transit of PY-STAT3 from the cell membrane to the nucleus through the expanse of the cytosolic compartment. The discovery that the bulk of cytosolic STAT proteins exist in protein assemblies in the size range 200–400 kDa to 1–2 MDa suggests that the initiation and transmission of STAT signaling through the cytosolic compartment includes novel regulatory, scaffolding, and chaperone proteins (Fig. 1B).
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