STATs Dimerize in the Absence of Phosphorylation*

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Upon activation by tyrosine kinases, members of the STAT family of transcription factors form stable dimers that are able to rapidly translocate to the nucleus and bind DNA. Although crystal structures of activated, near full-length, Stat1 and Stat3 illustrate how STATs bind to DNA, they provide little insight into the dynamic regulation of STAT activity. To explore the unique structural changes Stat1 and Stat3 undergo when they become activated, full-length inactive recombinant proteins were prepared. To our surprise, even though these proteins are unable to bind DNA, our studies demonstrate that they exist as stable homodimers. Similarly, the Stat1 and Stat3 found in the cytoplasm of unstimulated cells also exhibit a dimeric structure. These observations indicate that Stat1 and Stat3 exist as stable homodimers prior to activation.

Cytokines are important regulators of intercellular communication. They mediate pleiotropic cellular responses by binding to specific transmembrane spanning receptors (reviewed in Refs. 1 and 2). These receptors in turn activate intracellular signaling pathways, resulting in the induction of gene expression. The STAT1 (signal transducers and activators of transcription) family of transcription factors transmits signals in response to cytokines. This family consists of seven members, Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6. Structural and functional studies have led to the identification of six conserved STAT domains (3–5): 1) the amino-terminal domain (amino acids 1–125), which has been implicated in cooperativity of DNA binding to tandem gamma activation site (GAS) elements, nuclear translocation, and in receptor association (2); 2) the coiled-coil domain (amino acids 125–325), which has been shown to mediate interactions with several other proteins (2); 3) the DNA binding domain (amino acids 325–475); 4) the linker domain (amino acids 475–575); 5) the SH2 domain + tyrosine activation motif (amino acids 575–710), which is essential for STAT recruitment to the receptor, STAT activation, and dimerization (2); and 6) the carboxyl-terminal transcriptional activation domain.

The general outline for the STAT signaling paradigm elucidated a decade ago is now widely accepted (1, 2, 6). In this model, a specific interaction between a cytokine and its cognate receptor brings the cytoplasmic domains of this receptor into apposition, thereby promoting the transphosphorylation of receptor associated tyrosine kinases from the Janus kinase family. These activated Janus kinases in turn phosphorylate specific tyrosine motifs found in receptor endodomains, which then serve to mediate recruitment of the specific monomeric STATs to the receptor complex. Once at the receptor, the STATs become activated by a single tyrosine phosphorylation event. The activated STATs are then released, whereupon they dimerize through the interaction between the SH2 domain of one STAT and the tyrosine-phosphorylated tail segment of the other STAT. This renders them competent for both rapid translocation into the nucleus and DNA binding. Both the ability to translocate into the nucleus and to bind DNA are attributed to dimerization. Although countless studies support this model (7–12), a number of other studies have suggested that at least some STATs are found in poorly characterized higher molecular mass complexes prior to their activation (13–17).

The standard model of activation-dependent STAT dimerization effectively explains the acquisition of DNA binding activity, but it fails to provide any insight into how ligand-dependent nuclear translocation is regulated. To learn more about the conformational changes regulating ligand-dependent nuclear translocation, structural studies on unphosphorylated (i.e. inactive) Stat1 and Stat3 were undertaken. Unexpectedly, we found that these proteins purified as stable homodimers. Subsequent studies demonstrated that these inactive homodimers are also found in cells prior to ligand stimulation. These observations necessitate modification of the traditional STAT-signaling paradigm.

MATERIALS AND METHODS

**Cell Culture and Cell Extracts**

Cell culture reagents were purchased from Invitrogen. 3T3, 293T, SF9, and HeLa cells were obtained from ATCC and grown in Dulbecco’s modified Eagle’s medium (3T3, HeLa, and 293T cells) or Grace’s medium (SF9 cells), supplemented with either 10% fetal calf serum (3T3, 293T, SF9 cells) or calf serum (HeLa cells). To prepare extracts, cells were washed in cold (4 °C) phosphate-buffered saline and resuspended in ice-cold lysis buffer (20 mM Tris, pH 8.0, 10 mM NaCl, 6 mM MgCl2, 4 mM DTT, 0.8 mM phenylmethylsulfonyl fluoride), adjusted to 150 mM NaCl and 20% glycerol, lysed by Dounce homogenization (25 strokes), and then cleared by centrifugation. For cross-linking studies the 20 mM Tris, pH 8.0, was substituted with 20 mM Hepes, pH 7.4.

**Preparation of Recombinant Stat1 and Stat3**

Human Stat1- and murine Stat3-encoding baculoviruses (8, 18) were plaque-purified and then expressed in SF9 cells (7.5 × 107 cells/ml) at a multiplicity of infection of 1–2. After 43 h, cells were harvested and lysed by Dounce homogenization (15 strokes) in ice-cold non-detergent “lysis buffer” (see above). All subsequent steps were performed at 4 °C unless noted otherwise. Stat1, precipitated from lysates with solid ammonium sulfate (25–35%; Sigma), was dissolved in 20 mM Tris, pH

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* The abbreviations used are: STAT, signal transducers and activators of transcription; DTT, dithiothreitol; DSS, disuccinimidyl suberate; IFN-α, interferon-α; BSA, bovine serum albumin.

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8.0, 75 mM NaCl, 4 mM DTT, 0.8 mM phenylmethanesulfonyl fluoride, 0.2 mM EDTA, and 10% glycerol (Buffer A). After dialysis against 1 liter of Buffer A, the material was cleared (100,000 rpm, 30 min, 4 °C) and loaded onto an Uno-Q1 anion-exchange column (Bio-Rad) equilibrated in Buffer A. Stat1 was eluted with a linear salt gradient (75–500 mM NaCl) and identified by immunoblotting (9, 19). Minor contaminating levels of phosphorylated Stat1, either detected with a phospho-specific Stat1 antibody (New England Biolabs) or an electrophoretic mobility shift assay (20, 21), were found to elute at higher salt concentrations and thus easily separated from unphosphorylated Stat1. The purity of the Stat1 containing fractions was assessed by Coomassie Blue staining (see Fig. 1) and mass spectrometry. Yields from 1 liter of SF9 cell culture were typically ~1.5 mg for Stat1. Stat3 was purified through a similar procedure, except that it was precipitated in 0–25% ammonium sulfate fraction, and Buffer A was adjusted to 50 mM NaCl. One liter of SF9 cell culture typically yielded ~5 mg of Stat3.

Prior to analytical ultracentrifugation, STAT samples (~200 μl at 1–3 mg/ml) were fractionated by size-exclusion on a Superose 12 HR 10/30 column (Amersham Biosciences; 22 °C), equilibrated with 20 mM Tris, pH 8.0, 150 mM NaCl, and 4 mM DTT. Fractions containing Stat1 or Stat3 were pooled, concentrated to 1 mg/ml (Centricon 30,000 dalton molecular mass cutoff), and dialyzed overnight against equilibration buffer. The column was calibrated using thyroglobulin (bovine, 670 kDa), γ-globulin (bovine, 155 kDa), ovalbumin (chicken, 44 kDa), myoglobin (horse, 17 kDa), and vitamin B12 (1.35 kDa; Bio-Rad). The purity of purified proteins was determined by UV spectrophotometry at 280 nm (Amersham Biosciences). Molar extinction coefficients of 109,300 for Stat1 and 115,300 for Stat3 were calculated based on the number of tryptophans and tyrosines present in each sequence.

Biochemical Studies

Native PAGE⁄Ferguson Plots—Molecular mass determination by non-denaturing gel electrophoresis was carried by a standard protocol that entails fractionation on 5, 6, 7, 8, and 9% native gels (7). The migration of purified preparations of Stat1, Stat3, HeLa, and/or 293T cell extracts was evaluated by Coomassie Blue staining or immunoblotting.

Cross-linking—Cross-linking studies were performed with DSS (disuccinimidyl suberate; Pierce). Stat1 containing extracts were dialyzed into 20 mM Hepes, pH 7.4, 150 mM NaCl, 4 mM DTT, and 10% glycerol and then treated with 1 or 5 mM DSS for 2 h (4 °C). The reaction was quenched through the addition of 80 mM Tris, pH 7.4 (20 min at room temperature).

Sucrose Gradient Analysis—These sedimentation studies were performed on a Beckman TL-100 ultracentrifuge. 100 μl of HeLa whole cell extracts, prepared before or after treatment with IFN-α (2000 units/ml, 30 min), were loaded onto 2.2 ml of 10–40% sucrose gradients (22). These gradients were spun at 50,000 rpm for 16 h in a TLS55 rotor. Fractions (40 μl) of fractions were collected and run on 10% SDS-PAGE for analysis. Proteins were either visualized with Coomassie Blue staining or by immunoblotting with Stat3 (C20; Santa Cruz Biotechnology) or phosphospecific Stat3 (Cell Signaling Technology) antibodies.

Sedimentation Equilibrium Studies—These studies were carried out in a Beckman/Coulter XLI analytical ultracentrifuge. For studies on solutions with higher protein concentrations, Stat1 and Stat3 were prepared in 20 mM Tris, pH 8.0, 150 mM NaCl, and 4 mM DTT. Stat1 at the specific concentrations of 0.75 mg/ml (8.2 mM for Stat1 and 8.7 mM for Stat3), 0.5 mg/ml (5.5 mM for Stat1 and 5.8 mM for Stat3), and 0.2 mg/ml (2.2 mM for Stat1 and 2.3 mM for Stat3) were loaded into a six-channel cell with sapphire windows. Water blanks, taken before and after the experiment, were subtracted from the interference data sets. The partial specific volume, \( \bar{v} \), and the solution density, \( \rho \), were calculated using SEDINTERP, version 1.05 (43). For Stat1, \( \bar{v} \) was 0.7301 ml/g, and \( \rho \) was 1.00794 g/ml. For Stat3, \( \bar{v} \) was 0.7277 ml/g, and \( \rho \) was 1.00794 g/ml.

Sedimentation Velocity Studies—These studies were also carried out in a Beckman/Coulter XLI analytical ultracentrifuge with Rayleigh interference optics. Stat1 and Stat3 were prepared in 20 mM Tris, pH 8.0. 150 mM NaCl, and 4 mM DTT at a concentration of 1 mg/ml for Stat1 and 0.35 mg/ml for Stat3. 400 μl of sample was loaded into a double-sector cell and centrifuged at 40,000 rpm (20 °C). Interference scans were taken at intervals of 30 s for Stat1 and 20 s for Stat3. Best-fit profiles according to the continuous distribution c(5) Lamm equation model from SEDFIT (44) were overlaid on the experimental data using every 5th scan for Stat1 and every 10th scan for Stat3 (24).

RESULTS

Biochemical Analysis of Purified Stat1 and Stat3—Stat1 and Stat3 were expressed in SF9 cells and purified to apparent homogeneity (Fig. 1, see insets). The absence of contaminating tyrosine-phosphorylated protein was confirmed by electrophoretic mobility shift assay, immunoblotting with Stat1 and Stat3-phospho-specific antibodies, and mass spectrometry (data not shown). As a final step in purification, Stat1 and Stat3 were fractionated by size exclusion chromatography. Unexpectedly, each protein eluted as a single peak with an apparent molecular mass greater than the 158-kDa marker (i.e. γ-globulin; Fig. 1). Stat1 eluted with an apparent molecular mass of 176 kDa and Stat3 with an apparent molecular mass of 167 kDa. Of note, no or little monomer was detected at concentrations of 1–3 mg/ml (i.e. 11–33 μM). These observations suggested that purified preparations of unphosphorylated Stat1 and Stat3 associate to homodimers. As expected, these preparations of Stat1 and Stat3 exhibited no DNA binding activity (9, 25).

To further explore the oligomeric state of purified unphosphorylated Stat1 and Stat3, the proteins were studied by native polyacrylamide gel electrophoresis (PAGE). Previous studies have determined that there is a direct relationship between the molecular mass of a protein (or complex) and its relative migration through a series of gels that vary in acrylamide concentration (7). Thus, the relative migration of purified Stat1 and Stat3 was compared with that of several molecular mass standards in 5, 6, 7, 8, and 9% native polyacrylamide gels (see Fig. 2). Analysis of the relative migration of each protein through these gels yielded a linear series of retardation coefficients, which were directly proportional to molecular mass. These Ferguson plots indicated that purified unphosphorylated Stat1 migrated with an apparent molecular mass of 170 kDa and that Stat3 migrated with an apparent molecular mass of 160 kDa. Although it was not apparent why the Stat1 results differed from a previously published study (7), they were quite consistent with the other biochemical approaches outlined in the current study.

Next, Stat1 and Stat3 were subjected to equilibrium sedimentation studies. Purified preparations of both unphosphorylated STATs were loaded at three concentrations and then spun to equilibrium at three different speeds (see Fig. 3). A global non-linear least squares fit of the nine data sets for Stat1 yielded a molecular mass of 182.5 kDa, which is within 5% of the expected size of a Stat1 dimer (i.e. 174.7 kDa, as calculated from the amino acid sequence; Table I). Analysis of the nine corresponding data sets for Stat3 yielded a molecular mass of 182.8 kDa, which differed less than 4% from the calculated molecular mass for the Stat3 homodimer (176.1 kDa; see Table 1). Neither the data set for Stat1 nor the data set for Stat3 could be fit to a monomer equilibrium sedimentation model (with molecular mass fixed at 87.3 kDa for Stat1 and 88.1 kDa for Stat3). Both data sets were also poor fits to a monomer plus dimer equilibrium model. However, both data sets exhibited a good fit to a dimer-tetramer equilibrium model, where the
dimer molecular mass was either fixed or fit as a separate variable (see Fig. 3). Of note, in both cases the data indicated that tetramers constituted only a minor fraction of the total protein pool and only at the highest protein concentrations. These observations indicate that both unphosphorylated Stat1 and Stat3 form stable homodimers. Moreover, since the lower limit for dissociation constants that can be measured by ultracentrifugation is in the low nanomolar range, and the monomer form was not detected, it is reasonable to assume the $K_d$ of the dimer was low (i.e., less than 10 nM; see Table I).

The oligomeric states of Stat1 and Stat3 were also evaluated by sedimentation velocity studies. Sedimentation profiles obtained from purified preparations of Stat1 and Stat3 were evaluated by a continuous distribution $c(S)$ Lamm equation model (see Fig. 4). When the sedimentation coefficient distribution was determined for Stat1, a single major peak at 6.27 S ($s_{20, w} = 6.39$ S) was revealed. This corresponded to a molecular mass of 192.5 kDa and was consistent with homodimer formation. Importantly, a smaller species was not detected, highlighting an absence of Stat1 monomers. Velocity sedimentation data were also exploited to crudely model the shape of the Stat1 and Stat3 dimers. Assuming a hydration coefficient of 0.403 g-water/g-protein and a prolate ellipsoid shape, a maximal axial ratio of 8.6 was determined for Stat1 and 8.0 for Stat3 from the $S$ values. These values are consistent with a relatively elongated shape of 36.4 × 4.2 nm for Stat1, and 34.5 × 4.3 nm for Stat3. In summary, these observations provide compelling evidence that purified preparations of Stat1 and Stat3 exist as stable ho-
modimers and that they are likely to exhibit an elongated ellipsoid shape.

**Evaluation of the Oligomeric State of Native Stat1 and Stat3**—To determine whether unphosphorylated Stat1 and Stat3 dimerize under more physiologic conditions, extracts of unstimulated HeLa and 293T cells were evaluated. The first set of studies compared the migration of native Stat1 and Stat3 from HeLa and 293T cells to that of the corresponding purified STAT protein.

![Sedimentation equilibrium analysis of unphosphorylated Stat1 and Stat3](image)

**Fig. 3.** Sedimentation equilibrium analysis of purified recombinant Stat1 and Stat3. A, equilibrium analysis of unphosphorylated Stat1 at 0.5 mg/ml spun at 11,000 rpm. The top panel indicates the residuals for this single data set. The bottom panel shows both the actual experimental data (open circles) and the fit of this data set to a dimer-tetramer equilibrium model, where dimer is represented by black circles and tetramer by light gray circles. B, residuals for all nine Stat1 data sets. Residuals in units of mg/ml for least squares fits to a Stat1 dimer-tetramer reversible equilibrium of nine data sets with 0.75, 0.50, and 0.20 mg/ml loading concentrations with the dimer molecular mass fixed to the value calculated from the amino acid sequence and with rotor speeds of 8,000 (○), 11,000 (□), and 15,000 (■) rpm. C, equilibrium analysis of unphosphorylated Stat3 at 0.75 mg/ml spun at 11,000 rpm. The top panel indicates the residuals for this single data set. The bottom panel shows both the actual experimental data (open circles) and the fit of this data set to a dimer-tetramer equilibrium model, where dimer is represented by black circles and tetramer by light gray circles. D, residuals for all nine Stat3 data sets. Residuals in units of mg/ml for least squares fits to a Stat1 dimer-tetramer reversible equilibrium of nine data sets with 0.75, 0.50, and 0.20 mg/ml loading concentrations with the dimer molecular mass fixed to the value calculated from the amino acid sequence and with rotor speeds of 8,000 (○), 11,000 (□), and 15,000 (■) rpm.

| Table 1 | Sedimentation equilibrium centrifugation fitting statistics |
|---------|-----------------------------------------------------------|
| Data set Data set | Model | Molecular mass | ln Kd | Kd | SRV' |
| Stat1 | 0.2, 0.5, 0.75 mg/ml | Single | 182.5 (180.0, 185.0) | 174.7 | -4.046 | 197 | 17.9 |
| Stat1 | Interference-blank | Fixed (d/t) | 172.2 (169.0, 175.6) | 87.3 | 66.9 | 24.8 |
| Stat1 | 0.2, 0.5, 0.75 mg/ml | Fixed (m/d) | 87.3 | 66.9 | 24.8 |
| Stat3 | Single | 182.8 (180.2, 185.5) | 176.1 | -3.738 | 144 | 13.3 |
| Stat3 | Interference-blank | Float (d/t) | 168.4 (164.9, 172.2) | 88.1 | 121.7 | 17.1 |
| Stat3 | Fixed (m/d) | 88.1 | 121.7 | 17.1 |

- Three rotor speeds (8,000, 11,000, and 15,000 rpm) are included for each loading concentration.
- Denotes fitting to a single species, a dimer-tetramer equilibrium (d/t) with the dimer molecular mass fixed to the correct sequence mass, a dimer-tetramer equilibrium (d/t) where the dimer molecular weight was allowed to float as a separate variable, or a monomer-dimer (m/d) equilibrium with the monomer molecular mass fixed to the correct sequence mass.
- Dimer molecular mass from the fit (95% confidence interval) or the fixed value used in the two species fit.
- Square root of the variance in fringes.
through a series of native gels (i.e. a Ferguson analysis). Relative Stat1 and Stat3 migration was evaluated by a sensitive immunoblotting technique. As shown in Fig. 5, in each case all of the native Stat1 and Stat3 co-migrated with the corresponding purified STAT, previously shown to migrate as homodimers (see Fig. 2). Moreover, the bands detected by the Stat1- and Stat3-specific antibodies migrated to differing locations in each gel (see also Fig. 2), arguing strongly against the existence of significant quantities of Stat1-Stat3 heterodimers in the unstimulated extracts. The failure to detect additional complexes in these cellular extracts suggested that the vast majority of the Stat1 and Stat3 proteins exist as homodimers in unstimulated cells.

Cross-linking agents provide another approach to evaluate complex formation. As anticipated, a single 91-kDa Stat1 immunoreactive band was detected when either purified Stat1 or unstimulated HeLa cell extracts were fractionated on SDS-PAGE (see Fig. 6). When the purified Stat1 was incubated with DSS, a second slower migrating complex, which is likely to represent of cross-linked dimer, was detected (note that cross-linked complexes can migrate with anomalously large apparent molecular masses (26, 27)). In addition, this cross-linked Stat1 complex represented only about 5% of the total input material, whether DSS was used at 0, 1, or 5 mM concentration. Cross-linking studies with extracts from unstimulated HeLa cells produced an analogous set of results (Fig. 6). Again, native Stat1 migrated with an apparent molecular mass of 91 kDa.
Upon DSS treatment, the same slower migrating complex representing only a modest fraction of the total immunoreactive Stat1 was formed. Although data from cross-linking studies provide little insight into the structure of the cross-linked complex, they do demonstrate that native Stat1 behaves similarly to that of the purified material, which is known to be dimeric. Identical results were obtained when cell extracts were prepared with an Nonidet P-40 based lysis buffer (data not shown). These cross-linking experiments support the observations made in the native gel electrophoresis studies.

To further corroborate these findings, a continuous sucrose gradient analysis was carried out on the Stat3 found in HeLa cell extracts. A preparation of purified inactive Stat3 was sedimented along with other molecular mass standards, serving as an important control. The purified Stat3 was found to sediment between the BSA monomer (~66 kDa) and the aldolase tetramer (~158 kDa) after 16 h of centrifugation (see Fig. 7A). A second slightly faster comigrating band represented a Stat3 species that had lost its carboxyl terminus through proteolysis (data not shown). Immunoblotting fractions from a concurrent gradient loaded with unstimulated HeLa cell extracts determined that native Stat3 sedimented to the same region in the gradient as purified recombinant Stat3 (Fig. 7, compare A and B), confirming the results from the native gel electrophoresis study (see Fig. 5). The relative migration of activated Stat3 was also concurrently evaluated in stimulated extracts with a phospho-specific Stat3 antibody. This analysis indicated that active Stat3 dimers sedimented to a similar region in the gradient as purified recombinant Stat3 (Fig. 7, compare A and B), confirming the results from the native gel electrophoresis study (see Fig. 5).

relative density of this dimer does not appear to change significantly when it becomes an active dimer.

**DISCUSSION**

The STAT family of transcription factors transduces signals for many members of the cytokine family of ligands. Studies over the last decade have led to a well accepted signaling paradigm in which activated (i.e. phosphorylated) STATs dimerize, translocate to the nucleus, and bind DNA. Data supporting this model include a number of compelling genetic and biochemical studies (reviewed in Refs. 1, 2, and 6). Evidence supporting the idea that tyrosine phosphorylation promotes dimerization includes numerous coimmunoprecipitation, plasmolin resonance, glycerol gradients, native gel electrophoresis, point mutagenesis, and crystallization studies (3, 4, 7, 9, 10, 28, 29). Moreover, both the tyrosine phosphorylation dependent nature of STAT nuclear translocation and DNA binding activity provide additional support for this model. They, however, fail to provide much insight into how dimerization capacitates the ability of activated STATs to translocate into the nucleus. Over the last several years, there have also been a number of studies that contradict the standard model of STAT signal transduction. They have suggested that prior to activation, STATs reside in poorly characterized large molecular mass complex(es) ranging in size from ~200 kDa to 2 Mda (13–17). Consistent with this, our efforts to understand the conformational changes associated with STAT activation have led us to conclude that Stat1 and Stat3 associate as stable dimers prior to activation. As outlined below, these observations are likely to extend to other STAT family members and necessitate a modification of the classical STAT-signaling paradigm.

Our initial evidence that Stat1 and Stat3 associate as stable homodimers came from studies on purified preparations of recombinant protein. This conclusion is based on a number of distinct, but complementary techniques, including size exclu-
sion chromatography, native gel electrophoresis (i.e. Ferguson Plots (7)), cross-linking, and both velocity and equilibrium sedimentation studies. These latter two sets of studies not only provide compelling evidence that inactive Stat1 and Stat3 exist as homodimers, but they also provide some insight into the nature of these complexes. The velocity sedimentation studies, for example, suggest that the Stat1 and Stat3 homodimers exhibit a relatively elongated ellipsoid shape that is 36.4 × 42 nm for Stat1, and 34.5 × 43 nm for Stat3. The equilibrium sedimentation studies indicate that unphosphorylated Stat1 and Stat3 homodimers are quite stable, both with a K_{d} of less than 10 nM. Moreover, equilibrium sedimentation studies of an amino and carboxyl terminally truncated Stat3 mutant revealed a significant increase in the presence of a monomeric species (at concentrations ranging from 0.2 to 1 mg/ml; data not shown), thus indicating a marked decrease in dimer stability. Of note, the Stat3 mutant employed in these studies was the same one successfully employed in crystallization studies (4, 30). These preliminary studies raise the possibility that the missing amino terminus (and/or carboxyl terminus) normally serves to stabilize the homodimerization of inactive STATs.

Recognizing the possibility that purified proteins may exhibit nonphysiological behavior, native gel electrophoresis, continuous sucrose gradients, and cross-linking studies were carried out to compare the oligomeric state of purified Stat1 and Stat3 with that of the native proteins found in unstimulated cells. These studies determined that the vast majority of these native STATs exhibited an oligomeric pattern that was identical to that of the purified proteins, already established to be homodimers. Moreover, preparations of inactive native Stat3 sedimented to the same position in the sucrose gradient as those of the purified proteins, already established to be homodimers. The failure of these amino and carboxyl terminally truncated Stat3 mutant, which indicated that the truncated species forms less stable homodimers (data not shown), published studies on chimeric STAT proteins have concluded that the amino terminus is likely to stabilize homodimerization (36, 37). In these latter studies, the amino-terminal domains of either Stat1 or Stat4 were substituted with the corresponding structurally conserved region of another STAT. The failure of these proteins to interact with the corresponding wild type proteins suggested that the amino terminus might direct the formation of homotypic dimers and prevent the formation of heterotypic dimers (36, 37). Recent structural studies on purified preparations of the amino terminus of Stat4 strongly support the notion that this domain promotes the formation of stable homotypic dimers (5). Although these studies were interpreted to support the notion that these amino-terminal interactions promote cooperative binding of STATs to tandem GAS elements, they certainly did not exclude the possibility that these domains may also promote homotypic dimerization prior to activation. This homotypic dimerization may serve an important regulatory function by delivering appropriate STAT dimers to their cognate receptor, whereupon they are then activated and form the desired active homodimers. This raises an important question over how inactive dimers may regulate the IFN-α-dependent activation of Stat1 and Stat2 and the subsequent formation of Stat1:Stat2 heterodimers (9, 38–40). Intriguingly, not only does STAT activation at the IFN-α receptor remain controversial (2), but recent studies have highlighted tissue-specific differences in the capacity to activate Stat1 and Stat2 (39). Perhaps differences in the nature of the inactive STAT dimers found in these tissues account for differences in receptor recruitment and activation.

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