Hydrophobic Residues Phe<sup>751</sup> and Leu<sup>753</sup> Are Essential for STAT5 Transcriptional Activity*

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One facet of cytokine signaling is relayed to the nucleus by the activation, through tyrosine phosphorylation, of latent cytoplasmic signal transducers and activators of transcription (STAT) family members. It has been demonstrated that the C terminus of STATs contain the transactivation domain and are essential for the transactivation of target genes. To better understand the function of the STAT C terminus, we have generated a series of C-terminal mutants in STAT5α and examined their effects on transactivation, tyrosine phosphorylation, and DNA binding. Using GAL4 chimerae with the C terminus of STAT5, we have defined a 12-amino acid region essential for STAT5 transactivation. Surprisingly, deletion of these 12 amino acids in the context of the native STAT5 backbone preserved the overall transcriptional activity of the protein. Further analysis revealed that deletion of this region resulted in hyper-DNA binding activity, thus compensating for the weakened transactivation domain. Using site-directed mutagenesis, we show that within this 12-amino acid region the acidic residues were non-essential for transactivation. In contrast, the non-acidic residues were crucial for transactivation. Mutating either Phe<sup>751</sup> or Leu<sup>753</sup> to alanine abolished transactivation suggesting that these residues were essential for connecting STAT5 to the basal transcriptional machinery.

Cytokines initiate their pleiotropic effects on cells by binding to specific transmembrane receptor proteins. This association induces a conformational change in the receptor (1, 2) that signals to the nucleus via a direct pathway involving the activation of receptor-associated Janus tyrosine kinase (JAK), and the subsequent phosphorylation and nuclear translocation of members of the signal transducer and activator of transcription (STAT) family (3, 4). Once activated, JAK phosphorylates the receptor on cytoplasmic tyrosine residues providing sites for interaction with various co-activators, such as MCM5 (29) and p300/CBP (24, 30–34). Alternatively spliced variants lacking the C-terminal region as well as mutants in which the C terminus was truncated has provided evidence that this region acts as the transactivation domain (11, 26, 27). In the case of STAT5, these mutants show sustained tyrosine phosphorylation and exhibit increased levels of DNA binding activity compared with the wild-type protein but remain transcriptionally inert (26, 28). In addition, the C-terminal region of STAT5 has been described to interact with various co-activators, such as MCM5 (29) and p300/CBP (24, 30–34).

These observations suggest that the C terminus of STAT5 has pleiotropic functions and plays an important regulatory role. To better understand the function of the C terminus of STAT5, we have generated a series of C-terminal mutants in STAT5α and examined their effects on transactivation, tyrosine phosphorylation, and DNA binding. Using GAL4 chimerae with the C terminus of STAT5, we have identified a 12-amino acid region essential for STAT5 transactivation. Surprisingly, deletion of these 12 amino acids in the context of the native...
Structural Requirements of STAT5 Transactivation

Immunoprecipitations and Immunoblotting—Anti-HA antibody (1 μg) was added to cellular extracts and incubated overnight at 4 °C in the presence of protein A-Sepharose (Amersham Pharmacia Biotech). Immune complexes were washed thrice with Nonidet P-40 lysis buffer before addition of 2× Laemmli sample buffer. Bound proteins were resolved by electrophoresis on a 10% polyacrylamide gel electrophoresis (SDS-PAGE) using 6–7% gels. For direct immunoblotting, extracts (25–50 μg) were mixed with 2× sample buffer, boiled, and separated by SDS-PAGE.

Electrophoresed proteins were transferred to Immobilon-P PVDF membrane (Millipore, Bedford, MA) and blocked with 5% BSA in TBST (Tris-buffered saline plus 0.05% Tween 20). Anti-hrY(P)-STAT5 (3 μg) diluted in 1% BSA/TBST and incubated for 2 h at room temperature. Anti-HA antibody (4 μg) was added to blocking solution and incubated overnight at 4 °C. Membranes were washed four times with TBST and incubated with a 1:5000 dilution of horseradish peroxidase-conjugated anti-mouse Ig (Amersham Pharmacia Biotech) in 1% BSA/TBST for 30 min at room temperature. After four washes with TBST, immunoreactive proteins were detected using enhanced chemiluminescence (ECL) reagent (Amersham Pharmacia Biotech). RC20 blotting was performed as described previously (35). Where appropriate, membranes were stripped with a solution containing 2% SDS, 62.5 mM Tris, and 0.7% β-mercaptoethanol for 30 min at 55 °C, washed extensively with H2O2 twice with TBST, and re-blocked with 3% BSA/TBST before addition of primary antibody.

PhosphoSTAT5 Mobility Shift Assay—Samples (5 μg) of extracts were used for STAT5 EMSA. EMSA was performed with a STAT5 oligonucleotide probe from the β-casein promoter element (top strand: 5'-GTGATTTCTAGGAATTTCAAGA-3') as described previously (35). Association and dissociation of the STAT5-DNA complex was determined using modifications to an established protocol (39). Briefly, to measure association, 5 μg of extracts were incubated at 0 °C with labeled probe before mixing with a 100-fold excess of specific competitor DNA, followed by pre-incubation with 5 μg of extracts with labeled probe for 30 min at 0 °C, then a 100-fold excess of unlabeled probe was added before incubating at 30 °C. Dissociation was dependent on the addition of unlabeled probe after polyn(dI·poly(dC): a nonspecific competitor DNA, failed to compete with the STAT5-DNA complex (data not shown). For both association and dissociation experiments, samples were immediately loaded at the indicated times onto continuously running 6% non-denaturing polyacrylamide, 0.5× TBE gels. For more details, see legend to Fig. 6. All quantitation was done by phosphorimage analysis using a GS-525 molecular imaging system (Bio-Rad).

RESULTS

Internal Deletions within the STAT5 C Terminus Abolish the Transcriptional Activity of GAL4 Chimerae—Truncation analysis of the C terminus of STAT5 had indicated that the region between amino acids 750 and 772 played an important role in transactivation (26). To further understand the contribution of this region to transactivation, we generated a series of C-terminal mutants of STAT5 (Fig. 1A). The C termini of these STAT5 backbone preserved the overall transcriptional activity of the protein. Further analysis revealed that deletion of this region resulted in hyper-DNA binding activity, thus compensating for the weakened transactivation domain. Using site-directed mutagenesis, we show that, within this 12-amino acid region, acidic residues were non-essential for transactivation. In contrast, the non-acidic residues, especially the hydrophobic residues Phe751 and Leu753, were crucial for transactivation.

EXPERIMENTAL PROCEDURES

Chemicals, cDNAs, and Antibodies—Recombinant murine IL-3 was purchased from R&D (Minneapolis, MN). Recombinant murine prolactin was obtained from the National Hormone and Pituitary Program (NIDDK, National Institutes of Health, Bethesda, MD). Staurosporine was obtained from Sigma, dissolved in dimethyl sulfoxide (Me2SO), and purchased from R&D (Minneapolis, MN). Recombinant murine prolactin was generously provided by Bernd Groner (Institute for Experimental Cancer Research, Malmo, Sweden). Mouse anti-phosphotyrosine-specific STAT5 monoclonal antibody (ST5P-4A9) was purchased from Zymed Laboratories Inc. (South San Francisco, CA).

Cells—COS-7 and NIH3T3 cells were grown continuously in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum (HyClone, Logan, UT), penicillin G (50 units/ml), streptomycin (100 μg/ml), 4-methylsulfonyl fluoride, 1 mM Na3VO4, and 10% FCS. Cells were transfected using a calcium phosphate/DEAE-dextran method (37). Cos-7 cells were plated at 100,000 cells per well of a 384-well plate (Nunc, Naperville, IL) the evening before transfection. NIH3T3 cells were plated at 100,000 cells per well the day prior to transfection. Approximately 50% confluent plates were transiently transfected for 5 h at 37 °C with 2 μg of (STAT5RE)-61pE and 3 μg of STAT5 cDNAs per dish using LipofectAMINE/Opti-MEM. 100 ng of a Renilla luciferase construct was included to normalize for transfection efficiency. After 24 h, cultures were split and cultured in either the presence or absence of 10 μg of recombinant murine prolactin for 16–20 h. Cells were harvested and assayed for luciferase activity using a dual luciferase assay kit (Promega, Madison, WI). Activity was normalized with Renilla luciferase activities and expressed relative to that obtained with prolactin-stimulated wild-type STAT5 (100%). For GAL4 fusion experiments, NIH3T3/ProR cells were prepared as described earlier before lipofection with 6 μg of the GAL4-fusion cDNA, 4 μg of the GAL4 reporter gene pFR-LUC (Stratagene), and 100 ng of Renilla luciferase construct. After 16–20 h of cell harvest, luciferase activities determined. BaF3 cells (5 × 106) were prepared as described previously (36) before being electroporated at 350 V and 960 microfarads with 5 μg of the GAL4-fusion cDNA, 7.5 μg of pFR-LUC, and 100 ng of Renilla luciferase construct. After 16–20 h of cell harvest and luciferase activities determined. Activity was normalized with Renilla luciferase activities and expressed as a percentage of GAL4 WT STAT5 activity.

RESULTS
mutants, starting from residue 694, was fused to the GAL4 DNA-binding domain (DBD) (Fig. 1B) and the relative ability of these constructs to transactivate a GAL4-responsive luciferase reporter gene was examined by ectopically expressing these chimerae in BaF3 cells. As shown in Fig. 2A, fusing the wild-type (WT) STAT5 C terminus to the GAL4-DBD resulted in luciferase expression 10–20-fold higher than the GAL4-DBD alone. A C-terminal truncation of the last 57 amino acids (D737) removed all of STAT5’s ability to transactivate confirming that the STAT5 transactivation domain is localized within its C terminus. Internal deletions of 29 (TDΔ29) and 12 (TDΔ12) amino acids also dramatically decreased the ability of these mutant chimerae to transactivate. However, unlike the truncated mutant, these internally deleted mutants, especially TDΔ12, were not completely inert. A potential SH3 binding site within the 29-amino acid region was destroyed by mutating four proline residues to alanine (P4/A4). This mutation had no effect on the ability of the GAL4-chimera to transactivate compared with the WT fusion protein, suggesting the structural contributions of these proline residues were non-essential for transactivation. Identical results were obtained when we repeated these experiments in NIH3T3 cells (Fig. 2B). Since the P4/A4 mutant had 100% WT activity in BaF3 cells, we did not test this mutant in NIH3T3 cells.

**Internal Deletions within the C Terminus Preserve Transcriptional Activity of STAT5 Mutants**—To extend these observations, we placed these mutations in the context of the STAT5 backbone and we examined the relative ability of these constructs to transactivate a STAT5-responsive promoter in NIH3T3 cells stably expressing the prolactin receptor (NIH3T3/ProR cells). STAT5 constructs were co-transfected into these cells with a STAT5 luciferase reporter gene, and luciferase expression was measured following activation of the ectopically expressed STAT5 proteins in response to prolactin treatment. As shown in Fig. 3, expression of WT STAT5 resulted in a 4-fold increase in luciferase expression following prolactin treatment. This increase was specific as the Y694S mutant, which cannot be phosphorylated (and therefore cannot dimerize and bind DNA),
failed to transactivate the reporter gene. Furthermore, the transfection of pcDNA3 failed to induce luciferase expression following prolactin treatment (data not shown), indicating that prolactin-induced luciferase expression was dependent on ectopically expressed STAT5. The P4/A4 mutant also transactivated the reporter gene, although in this case to 70% of WT levels in response to prolactin treatment. Prolactin activation of the Δ737 mutant failed to significantly increase luciferase expression above that of the untreated sample, confirming our earlier experiments that the C terminus contains the transactivation domain. Unexpectedly, we observed that both the TD29 and TDΔ12 mutants transactivated the STAT5 reporter gene to 75% and 100% of WT levels following prolactin treatment, respectively. This was in contrast to our data using GAL4 fusion proteins, which clearly demonstrated that these deletions affect the activity of the transactivation domain per se (Fig. 2) and suggested that deletion of this region of the C terminus, in the context of the native protein, had additional effects on STAT5 function.

Internal Deletion of the STAT5 C Terminus Enhances DNA Binding Activity—In an effort to explain the discrepancy in the previous experiments, we expressed the C-terminal mutants in COS-7 cells and examined whether the mutations altered the function of STAT5 by affecting DNA binding or tyrosine phosphorylation. Co-expression of the Δ737 mutant and JAK2 in COS-7 cells resulted in a marked increase in DNA binding activity compared with cells expressing WT STAT5 (Fig. 4A). Transfection of either WT STAT5 or JAK2 cDNAs alone did not result in detectable STAT5 DNA binding activity, indicating that the co-expression of both STAT5 and JAK2 is necessary for measurable STAT5 DNA binding activity in COS-7 cells (data not shown). Since STAT5 is normally phosphorylated on Tyr694 upon activation (40), we confirmed that phosphorylation of Tyr694 was essential for STAT5 DNA binding activity, as mutation of this residue to serine (Y694S) abolished all phosphorylation and association profile closely parallels that of WT STAT5 (Fig. 5A). Interestingly, an associated phosphoprotein (pp59) was consistently observed to co-precipitate with HA-tagged STAT5 proteins expressed in BaF3 cells. Its phosphorylation and association profile closely parallels that of either mutant or WT STAT5 phosphorylation. Although we have not yet identified pp59, our data suggest that it is unlikely to be a degradation product of STAT5 (data not shown). To
Further confirm that the 29-amino acid deletion affects the rate of dephosphorylation rather than that of phosphorylation. WT or TD29 STAT5 cDNAs were co-transfected into COS-7 cells with JAK2. After 24 h, staurosporine was added to inhibit JAK2 activity required for phosphorylation of the ectopically expressed STAT5 proteins. Although WT STAT5 DNA binding activity could no longer be detected after 1 h of staurosporine treatment, the DNA binding activity of TD29 was unaffected up to 4 h after treatment, indicating a slower rate of tyrosine dephosphorylation (Fig. 5B). Together, these experiments indicate that TD29 is dephosphorylated on Tyr694 more slowly than WT STAT5. Furthermore, the resulting increase in steady state STAT5 dimers due to prolonged phosphorylation partially explains the enhanced DNA binding activity of the mutant protein.

Careful examination of mutants which exhibit a markedly enhanced DNA binding characteristic (i.e. Δ737, TD29, TDΔ12) revealed that they do so in a manner that is proportionately greater than their respective enhanced levels of Tyr694 phosphorylation would predict (Fig. 4). This suggested that, in addition to altering the kinetics of dephosphorylation, these mutations were in some way affecting DNA binding, perhaps affecting their DNA binding affinity. To explore this possibility, we determined the respective “on” and “off” rates of DNA binding for TDΔ12 and WT STAT5 proteins. Surprisingly, neither the on-rate nor the off-rate of DNA binding was significantly affected by the 12-amino acid deletion (Fig. 6). Thus, it appears that the mutations do not affect DNA binding per se. We believe that the mutations favor the rate of dimerization once the mutant proteins are phosphorylated. This would effectively raise the concentration of DNA-binding complex relative to what is normally observed with WT STAT5.

Non-acidic Residues, but Not Acidic Residues, within the 12-Amino Acid Region Are Crucial for Transactivation by STAT5—To better understand how this 12-amino acid region affects STAT5 function, we made additional mutations within this region. The 12-amino acid domain, which is essential for transactivation, contains one serine (Ser756). Since serine phosphorylation has been shown to be important for maximal transactivation by STATs (29, 42), we mutated this residue to glycine (S756G; Fig. 7A). Additionally, since acidic residues have been reported to be important for transactivation (43–46), we made two additional mutants in which we mutated either all of the acidic (TDacid/Ala) or all of the non-acidic (TDnon-acid/Ala) residues within this region to alanine residues (Fig. 7A).

Using the GAL4 chimera transactivation assay, we observed that the S756G mutation resulted in levels of transactivation 45% and 70% of WT when tested in BaF3 and NIH3T3 cells, respectively (Fig. 7, B and C). The acidic mutant (TDacid/Ala), although inactive in BaF3 cells, retained nearly 50% of WT activity when expressed in NIH3T3 cells. We favor the explanation that this difference reflects differential expression of the acidic mutant in the two cell lines. Indeed, it is the only mutant for which we see reduced levels of protein expression when expressed in COS-7 cells (see below), suggesting it is less stable than the WT or other mutant proteins.

When placed in context of the STAT5 backbone and expressed in fibroblasts, the S756G mutation resulted in 75% of WT levels of transactivation following prolactin treatment (Fig. 7D), similar to that observed in fibroblasts using the GAL4 chimera. Interestingly, despite weakening the transactivation domain in the GAL4 chimera, the TDacid/Ala mutation resulted in greater than WT levels of transactivation when placed in the STAT5 backbone, suggesting that this mutation might also affect DNA binding activity. Somewhat to our surprise, we found that the TDnon-acid/Ala mutation abolished transactivation either in the context of the GAL4 chimera or in the context of the STAT5 molecule (Fig. 7, B–D).
levels of protein expression and Tyr694 phosphorylation resulted in enhanced DNA binding activity, similar to that of the WT protein. Hence, the function of the C terminus of STAT5 is pleiotropic with somewhat “plastic” properties such that considerable modulation in this region can be tolerated before an overall loss of function is observed.

The enhanced DNA binding activity seen with several STAT5 mutants in this study is largely due to an increase in phosphorylation of Tyr694. This increase in DNA binding activity was critically dependent on Tyr694, since mutation of this residue to serine rendered the double mutant proteins incapable of being phosphorylated and binding DNA. We have shown previously that dephosphorylation of Tyr694 is the most likely mechanism for the inactivation of STAT5 (35). In this regard, we found that the TDΔ29 mutant had a slower rate of tyrosine dephosphorylation, which is consistent with that seen with variants of STAT5 lacking the C terminus (28). Thus, a delay in dephosphorylation causes a slower inactivation of TDΔ29 and consequently a steady-state accumulation of active dimers leading to an increase in DNA binding activity. Furthermore, the DNA binding activity and Tyr694 phosphorylation of both our truncated (Δ737) and deletion (TDΔ12) mutants are similarly affected. Hence, the extreme C terminus of STAT5 is not involved in the normal regulation of DNA binding or Tyr694 phosphorylation; rather, the essential 12 amino acids are.

**DISCUSSION**

Using deletion and truncation mutants, we have provided further evidence that the STAT5 transactivation domain is localized within its C terminus. Specifically, GAL4-chimeras have defined a crucial 12-amino acid region, which, when deleted, results in only residual levels of transactivation. Surprisingly, when we deleted these 12 amino acids from the entire STAT5 molecule, we found that transactivation by this mutant was preserved. Biochemical analysis of this mutant in COS-7 cells revealed that deletion of these 12 amino acids resulted in a marked increase in DNA binding activity. Therefore, this 12-amino acid deletion has two effects. First, it weakens, but does not abolish all transactivation activity, and second, it markedly enhances DNA binding activity. The combination of these two effects compensates for each other, resulting in a STAT5 mutant with net activity similar to the WT protein. Hence, the function of the C terminus of STAT5 is pleiotropic with somewhat “plastic” properties such that considerable modulation in this region can be tolerated before an overall loss of function is observed.

Loss of Acidic Residues within the 12-Amino Acid Region Enhances DNA Binding Activity—Ectopic expression of the S756G mutant in COS-7 cells affected neither DNA binding activity nor Tyr phosphorylation when compared with WT STAT5 (data not shown). In contrast, expression of the non-acidic mutant resulted in an increased level of DNA binding activity compared with WT STAT5. However, this increase was less dramatic than that observed with the TDΔ12 mutant (Fig. 8). Furthermore, it correlated very well with an increase in phosphorylation of Tyr694. Expression of the acidic mutant also resulted in enhanced DNA binding activity, similar to that observed with the non-acidic mutant. However, unlike the non-acidic mutant, the acidic mutant had significantly decreased levels of protein expression and Tyr phosphorylation compared with WT STAT5 (Fig. 8). Despite its reduced expression and phosphorylation, the acidic mutant was still capable of binding more DNA than the WT protein, indicating that loss of the acidic residues within the 12-amino acid domain significantly augments DNA binding activity (possibly through an increased rate of dimer formation).

**Hydrophobic Residues Phe751 and Leu753 Are Essential for STAT5 Transactivation**—The loss of transcriptional activation associated with the TDnon-acid/Ala mutation led us to more closely investigate the role of hydrophobic residues within the 12-amino acid region. When we compared the minimal transactivation domain of the herpes simplex virus transcription factor, VP16, with our 12-amino acid region of STAT5, we found that there was a homologous sequence EFDLD common to both proteins (Fig. 9A). Phenylalanine and leucine residues in this region were shown to be critical for transactivation by VP16 (47, 48). Interestingly, the same residues in STAT5 had been mutated as part of the non-acidic mutation that abolished transactivation. We therefore tested whether both Phe751 and Leu753 of STAT5 might also be critical for transactivation. We made three additional mutants in which Phe751 or Leu753 or both were mutated to alanine residues (Fig. 9B). Since the non-acidic mutant was transcriptionally inert using either a GAL4 chimera or the STAT5 backbone, we only tested the activity of these mutants using GAL4 chimeras in BaF3 cells. As seen in Fig. 9C, mutating either Phe751 or Leu753 to alanine reduced transactivation to 25% and 30% of WT STAT5, respectively. The double mutant (F751A/L753A) did not result in any further decrease in transcriptional activity compared with the F751A mutation, suggesting that Phe751 may be more essential than Leu753. These results indicate that the hydrophobic residues within the 12-amino acid region of STAT5, especially Phe751 and Leu753, are crucial for transactivation.
did so in a manner that was disproportionately greater than their respective levels of Tyrε94 phosphorylation would predict (Fig. 4). For example, the TDA12 mutant showed a 2–3-fold increase in Tyrε94 phosphorylation, yet bound more than 5 times as much DNA as WT STAT5. Therefore, we examined the possibility that these mutations might also be affecting the affinity of DNA binding. However, we found that neither the rates of association or of dissociation of the TDA12 mutant are affected when they are compared with those of WT STAT5 (Fig. 6).

How does the C terminus modulate DNA binding activity? We favor the possibility that the C terminus affects the rate of dimer formation. Since only phosphorylated STAT dimers can bind DNA it is possible that certain C-terminal mutations (e.g. TDA12), although not affecting the DNA binding affinity per se, result in a shift in the equilibrium toward the phosphorylated dimer, effectively increasing the concentration of STAT5 dimers capable of binding DNA. One potential mechanism might be through the interaction of protein inhibitor of activated STAT (PIAS) proteins (49, 50). PIAS proteins associate with the phosphorylated tyrosine of STATs via their SH2 domains and reduce STAT transactivation through inhibition of STAT dimerization and DNA binding. Although a PIAS protein has not been identified for STAT5, it is possible that the mutations that result in enhanced DNA binding activity do so by decreasing the association between PIAS and STAT5. The cloning of a STAT5-specific PIAS protein would allow us to directly test this hypothesis.

The S756G mutation within the critical 12-amino acid region resulted in an overall reduction in STAT5 transactivation. Attempts to show phosphorylation at this site in vivo have failed (data not shown), consistent with a previous study on serine phosphorylation of STAT5 proteins (51). Indeed, phosphorylated serine residues other than Serε756 have been reported for both STAT5a and STAT5b (51). Contrary to what has been reported for STAT-1 and -3 (29, 42), mutation of these serine residues had no effect on the transcriptional activity of STAT5a and only a modest effect on STAT5b (51). Since both DNA binding and phosphorylation of Tyrε94 were unaffected by the S756G mutation, the effect of this mutation on transactivation is most likely due to structural changes in the 12-amino acid region.

Although the acidic nature of transactivation domains is important for the activity of many transcription factors (43–46), we were surprised that the acidic residues within the 12-amino acid region of STAT5 were not essential for transactivation, as mutation of these residues to alanines only reduced transactivation of the GAL4 chimera to about 50% of WT activity in NIH3T3 fibroblasts. Although the mutant GAL4 chimera failed to elicit activity in BaF3 cells, we believe that this is likely a consequence of reduced protein expression since the acidic mutant was the only mutant for which we have seen
differential expression. When the identical mutation was introduced in the context of the STAT5 backbone, we found that it led to increased activity in fibroblasts compared with the WT protein. Despite having a reduced Tyr^{694} phosphorylation, this mutant exhibited an increased capacity to bind DNA compared with WT STAT5, possibly through augmented dimer formation. Again, a partially active transactivation domain combined with WT STAT5, as described in Fig. 2. Each bar represents the mean ± S.E. of four independent experiments.

One common feature shared by several acidic transactivation domains, including that of VP16, is that under physiological conditions these regions adopt an unordered structure (55–61). However, when exposed to more hydrophobic or mildly acidic conditions, they have the propensity to transform into regions rich in secondary structure (55, 57–61). Only the crystal structure of STATs lacking their C termini has been reported (18, 19), suggesting that the transactivation domain of STATs also adopts an unordered structure. Despite this prediction, it is clear from our experiments that a single hydrophobic point mutation in or near the transactivation domain of STAT5 is highly destructive. It is likely that these mutations disrupt secondary structure(s) and affect the ability of this region to contact or stabilize contact with components of either co-activator complexes or of the basal transcriptional machinery. In this regard, these point mutants will help to elucidate the mechanism by which STATs transactivate target genes. Furthermore, these transcriptionally weak mutants will be useful in identifying novel interactors with the C terminus of STAT5 by both biochemical and genetic approaches.

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REFERENCES
1. Remy, I., Wilson, I. A., and Michnick, S. W. (1999) Science 283, 990–993
2. Livnah, O., Stura, E. A., Middleton, S. A., Johnson, D. L., Jolliffe, L. K., and...
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