Differential Roles of C-terminal Activation Motifs in the Establishment of Stat6 Transcriptional Specificity*

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Members of the Stat transcription factor family are specifically activated by cytokines, and each Stat mediates its biological effects through the trans-activation of a unique profile of target genes. This specificity is achieved even when Stat proteins mediating opposite transcriptional effects bind to the same palindromic Stat sites in genes. We show here that the non-conserved sequences of Stat transcription activation domains (TADs) contribute to specificity in promoter activation. Chimeric proteins in which the Stat6 TAD was replaced by that from Stat1α or Stat5 exhibited normal interleukin-4-inducible DNA binding activity, but at best modest trans-activation of reporters containing Stat6 binding sites, and a failure to activate the endogenous CD23 promoter in primary B cells. The p160 coactivator nuclear coactivator-1 (Src-1) was specifically recruited by and coactivated Stat6 but not the chimeric Stat6 molecules. Strikingly, transcriptional responses exhibited distinct requirements for the nuclear coactivator-1 interaction motif of the Stat6 C terminus. Together, these findings indicate that the Stat6 TAD contributes to promoter specificity by the differential recruitment of and requirement for a p160-class coactivator.

The cytokine-activated Stat family of transcription factors mediate biologically distinct and specific functions, yet each member of the family has a conserved overall structure and in most cases binds to the same elements (reviewed in Refs. 1 and 2). A fundamental question is how the transcriptional specificity of each factor is achieved. Each of the seven mammalian Stat family members undergoes cytokine-induced phosphorylation of a conserved tyrosine residue by Janus kinases non-covalently associated with cytokine and growth factor receptors (reviewed in Refs. 1 and 2). Phosphorylated Stats dimerize, translocate to the nucleus, and bind to palindromic DNA elements of the sequence TTC(N2–4)GAA. One source of biological specificity arises through preferential association of certain cytokine receptors with a particular member of the Stat family (1). Thus, the IFN-γ receptor induces only Stat1α, whereas the IL-4 receptor is linked specifically to Stat6 (3, 4). Some functional specificity is achieved through differences in binding to particular cis-acting elements (5, 6). However, because of the highly conserved nature of the binding domain and target DNA sequence, there is considerable overlap among the promoter elements to which the different Stats bind (5). For example Stat1α and Stat6 both bind to the Stat binding site (SBS) of an IL-4-inducible CD23 promoter, but only Stat6 activated by IL-4 induces CD23 (7). Conversely, IFN-γ-induced Stat1α activates the IRF-1 promoter through a Stat binding site to which Stat6 binds equally well, yet IL-4-induced Stat6 fails to activate this promoter and instead inhibits the action of IFN-γ by a competition-independent mechanism (8, 9). The finding that DNA binding specificity cannot account for these differences between Stat1α and Stat6 function raises the question through what mechanism(s) a particular Stat factor mediates promoter specificity when binding to a consensus DNA element. We hypothesized that their unique transcription activation domains (TADs) may contribute to transcriptional specificity by recruiting distinct forms of coactivator complex.

Coactivators containing histone acetyltransferase activity recruited by activation domains play a role in mediating promoter specificity (reviewed in Refs. 10 and 11). Histone acetylation provides for enhanced transcription rates by altering nucleosome structure within chromatinized DNA (12). Recent evidence suggests that specific transcription factors recruit distinct profiles of coregulators (reviewed in Ref. 10). These molecules are in part recruited by the TADs of transcription factors. Thus, the specific composition of coactivators assembled by the TAD may dictate the relative ability of a transcriptional complex to overcome the inherent repressive effects of nucleosomal chromatin and lead to activation of a particular promoter. Both the p300/CREB and p160/NCoA families of transcription coregulators have been implicated in Stat-mediated transcription (12–14). However, their role in transcriptional specificity is not well understood and in particular has not been tested in relation to the expression of endogenous Stat-dependent genes.

Here we tested the hypothesis that Stat6 C terminus contributes to specificity in promoter activation by recruitment of a set of coactivators distinct from that of heterologous Stat TADs. To do so, we studied the chimeric transcription factors in which the C-terminal portion of the Stat6 molecule was replaced by the Stat1α TAD (15) or the Stat5 TAD (16). Structural evidence suggests that the C termini of Stat transcription...
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MATERIALS AND METHODS

Plasmids and Generation of Constructs—The Stat6C-Stat1TAD and Stat6C-Stat5TAD expression plasmids were engineered by subcloning PCR products corresponding to amino acids 701–750 of Stat1 and 722–794 of Stat5 into Stat6C-pCdnA3 (19). Stat1C was generated by digesting the Stat1–pRc/CMV plasmid (20) with XbaI and Apal, end-filling the overhangs, and religating. PCR products corresponding to amino acids 661–847 of Stat6 were subcloned into Stat1ΔC-Pr/CMV to generate Stat1ΔC-Stat6CT. The Stat6(1–18) mutant was generated by engineering a stop codon at position 792 using the QuickChange site-directed mutagenesis kit (Stratagene). Briefly, complementary oligonucleotides corresponding to portions of Stat6 with a stop codon at position 792 were designed and used for PCR with plasmids containing wild-type Stat6 and Fsu DNA polymerase. The PCR products were treated with DpnI to eliminate the wild-type DNA template and then transformed into Escherichia coli to amplify the mutants. All constructs were reconfirmed by restriction digest analyses and sequencing..

Transient Transfections and Promoter Assays—HepG2 and human embryonic kidney 293 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 50 units/ml penicillin, 50 μg/ml streptomycin, 3 mM glutamine, and minimum Eagle’s medium nonessential amino acids (Invitrogen) (Dulbecco’s modified Eagle’s medium (DMEM) at 37 °C in a humidified CO2 incubator. The indicated expression and reporter plasmids, along with a constitutive β-galactosidase reporter, were transfected into cells using SuperFect reagent (Qiagen, Chatsworth, CA) as described (8). Cells were divided equally at 24 h post-transfection and were incubated overnight with either human IL-4 (10 ng/ml) or IFN-γ (1 μg/ml), and promoter activity was determined by performing luciferase assays as described (8). The transfection efficiency in samples was normalized to the level of β-galactosidase activity generated by this constitutive reporter.

Electrophoretic Mobility Shift Assays, Immunoprecipitation, and Immunoblotting—After transient transfection with expression vectors and treatment with IL-4 as indicated, HepG2 cells were disrupted using lysis buffer (0.5% Nonidet P-40, 0.01 M Tris-Cl, pH 7.4, 150 mM NaCl, 50 mM NaF, 1 mM dithiothreitol, 0.1 mM sodium vanadate, 0.4 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, and 1 mg/ml leupeptin) for 40 min at 4 °C. DNA binding reactions were performed with 10 μg of soluble cellular proteins as described previously (8). The Stat6 binding site derived from the mouse IgH chain germ line ε promoter (21) was used as a probe in the mobility shift reactions. For immunoblot analysis, clarified lysates were resolved by SDS-PAGE and blotted onto nitrocellulose membranes, which were then probed with the indicated antibodies and visualized using appropriate secondary antibody conjugated to horseradish peroxidase followed by enhanced chemiluminescence reagents (Amersham Biosciences). Similarly, 293T cells were co-transfected with the indicated Stat6 expression constructs together with an expression vector with or without the cDNA encoding HA-tagged CBP (22) or HA-NCoA-1 (23). After treatment of the transfected cells with IL-4, whole-cell extracts were prepared as described above. Cellular proteins (1 μg) were incubated overnight with anti-CBP (Clone C-20, Santa Cruz Biotechnology, Santa Cruz, CA) or anti-Stat6 (Clone M-200, Santa Cruz Biotechnology) and protein A-conjugated agarose beads at 4 °C. After washing the agarose beads with phosphate-buffered saline, the bound proteins were eluted in loading dye, resolved on SDS-PAGE, and blotted onto nitrocellulose membranes. Transferred proteins were then probed with either anti-Stat6 (Clone ST6–3E4, Zymed Laboratories, San Francisco, CA) or anti-HA (Roche Applied Science) antibodies and visualized as described above.

RESULTS

Stat6 or Stat5 TADs Can Restore Trans-activation Function to a Mutant of Stat6 Lacking Activation Domains—Earlier work has shown that a Stat-binding element activated by IFN-γ and Stat1 was repressed by IL-4 and Stat6 even though the Stat proteins bound equally well to the cis-acting element (8, 9). Therefore, to investigate whether Stat6 TADs contribute to the transcriptional specificity of IL-4-regulated gene expression, we generated chimeric Stat6 proteins. The C-terminal portion of Stat6 was replaced by the activation domain from IFN-γ and Stat1 repressed by IL-4 and Stat6 even though the Stat proteins bound equally well to the cis-acting element (8, 9).

To verify the expression of each recombinant molecule, cells with low levels of endogenous Stat6 (19) were transfected with expression plasmids containing the hybrid Stat cDNAs. Comparable levels of expression and IL-4-inducible binding activity were observed for each construct when cell extracts were analyzed by Western blotting and mobility shift assays (Fig. 1, B and C). To compare the trans-activation capabilities of the Stat6 variants, reporter assays were performed in this cell line using plasmids containing either homo-oligomeric Stat binding sites from a human CD23 promoter (28) or a germ line immunoglobulin heavy chain ε locus promoter (13, 21). Previous work with other transcription factors has shown that a small change in the spacing between palindromic repeats can cause a profound shift in the function of a binding site (29). Therefore, we used both a CD23 Stat binding site containing an N3 spacer and also the G of a binding site (29). Therefore, we used both a CD23 Stat binding site containing an N3 spacer and also the G of a binding site (29).
activation function to Stat6ΔC, which, although statistically significant, was substantially weaker than that of the Stat6 C terminus. A similar pattern of results was obtained using the reporter containing the dimeric (not shown) or trimeric Stat6 binding sites from the germ line promoter of the IgH locus (Fig. 1D, right panel), as well as for a tetramer of sites with different spacing (not shown). Furthermore, placement of a cis-acting C/EBP-like element next to the Stat binding site and co-transfection of C/EBPβ along with the Stat cDNAs only increased the disparity between Stat6 and the chimeras between its DNA binding domain and heterologous activation domains (data not shown). Taken together, these data indicate that the TAD of either Stat1α or Stat5 could restore at best very weak trans-activation to a crippled mutant of Stat6.
that each of these C termini was dramatically less competent to provide activation domain functions than that of Stat6.

Stat6 and the Stat6ΔC Chimeras Recruit Comparable Levels of CBP/p300 but Not NCoA-1—The experiments outlined above indicate that the chimeric Stat molecules bearing a Stat1Δ or Stat5 TAD lack sufficient trans-activation potential to mediate robust induction of multiple Stat6-responsive promoters. We hypothesized that this inadequacy of the Stat6 chimeric molecules reflects inefficient recruitment of coactivators (13, 30, 31). Association with CBP/p300, a known coactivator of Stat6, was tested by immunoprecipitation experiments in which 293 cells were co-transfected with plasmids encoding the wild-type and mutant Stat6 molecules along with an expression vector with or without HA-tagged CBP cDNA. Anti-HA immunoprecipitates of extracts from cells transfected with only the Stat6 variants serve as the negative control for each sample. B, cotransfections with the Stat6 variants were performed as in A, but a plasmid encoding HA-tagged NCoA-1 (23) was used instead of CBP. Extracts from these transfectants were immunoprecipitated with anti-Stat6 and then probed with anti-HA and anti-Stat6 as indicated.

Proteins within this family interact directly with p300/ CBP coactivators and thereby can be recruited independent of contacts with DNA-binding transcription factors (32–34). However, other studies provide evidence of direct interactions between members of the p160/NCoA family and transcription factors and further suggest preferential associations of a given factor with particular NCoA family members (35, 36). These findings suggest two different mechanisms by which NCoAs could participate in cytokine-induced transcriptional activation. NCoA molecules could be recruited by Stat-associated CBP, in which case there would be little specificity because all Stats interact with CBP, or by direct interaction with particular Stat proteins, in which case the NCoA could help mediate cytokine specificity. To test whether NCoA-1 (Src-1) associates with the Stat6 but not Stat1Δ or Stat5 TADs, we performed immunoprecipitation experiments with Stat6ΔC chimeras and compared these with the association with wild-type Stat6. Cells were transfected with the Stat6 chimera, with or without co-transfection of an HA-tagged construct encoding a full-length NCoA-1 molecule (23). Stat6 immunoprecipitates probed with antibodies against HA or Stat6 showed that full-length NCoA-1 associated with Stat6ΔC and compared these with the association with wild-type Stat6. Cells were transfected with the Stat6 chimera, with or without co-transfection of an HA-tagged construct encoding a full-length NCoA-1 molecule (23). Stat6 immunoprecipitates probed with antibodies against HA or Stat6 showed that full-length NCoA-1 associated with Stat6ΔC and compared these with the association with wild-type Stat6. Cells were transfected with the Stat6 chimera, with or without co-transfection of an HA-tagged construct encoding a full-length NCoA-1 molecule (23). Stat6 immunoprecipitates probed with antibodies against HA or Stat6 showed that full-length NCoA-1 associated with Stat6ΔC and compared these with the association with wild-type Stat6. Cells were transfected with the Stat6 chimera, with or without co-transfection of an HA-tagged construct encoding a full-length NCoA-1 molecule (23). Stat6 immunoprecipitates probed with antibodies against HA or Stat6 showed that full-length NCoA-1 associated with Stat6ΔC and compared these with the association with wild-type Stat6. Cells were transfected with the Stat6 chimera, with or without co-transfection of an HA-tagged construct encoding a full-length NCoA-1 molecule (23). Stat6 immunoprecipitates probed with antibodies against HA or Stat6 showed that full-length NCoA-1 associated with Stat6ΔC and compared these with the association with wild-type Stat6. Cells were transfected with the Stat6 chimera, with or without co-transfection of an HA-tagged construct encoding a full-length NCoA-1 molecule (23). Stat6 immunoprecipitates probed with antibodies against HA or Stat6 showed that full-length NCoA-1 associated with Stat6ΔC and compared these with the association with wild-type Stat6.
stable complex involving Stat6 if the sequences specific to the Stat6 C terminus are available.

The Stat6 C Terminus Is Required for NCoA-1-mediated Coactivation—To extend these biochemical data we tested whether NCoA-1 could coactivate IL-4-induced transcription mediated by the Stat6 variants. Consistent with the biochemical data, NCoA-1 coactivated gene expression mediated by wild-type Stat6 but not by Stat6ΔC or the Stat6 chimeras containing the Stat1α or Stat5 TAD (Fig. 3A). These findings indicate that NCoA-1 can function as a coactivator of Stat6 in a manner requiring the C terminus to provide interactions for which the known capacity of CBP to bind NCoA-1 is insufficient. To determine the extent to which the C-terminal region of Stat6 is sufficient for coactivation by NCoA-1, NCoA-1 enhancement of transcription was tested using a promoter activated by IFN-γ but repressed by IL-4 (8, 41). Using a 1.3-kb promoter from the IRF-1 gene, IFN-γ-induced transcription mediated by Stat1α was not increased by NCoA-1, whereas a reproducible increase was mediated by a chimeric Stat1α in which TAD was replaced by the Stat6 C terminus (Fig. 3B). Consistent with these functional analyses, NCoA-1 was able to associate with the chimeric molecule containing the Stat6 C terminus but not Stat1α (Fig. 3C). As Stat1α is known to bind at the CD23 promoter (7), the ability of NCoA-1 to coactivate the Stat1α chimera was tested on a reporter containing the CD23 SBS. No IFN-γ-dependent trans-activation or NCoA-1-mediated coactivation was observed for either Stat1α or Stat1αC-6CT, suggesting that in addition to the Stat6 TAD, N-terminal portions of Stat6 are also required to activate the CD23 promoter (Fig. 3B). Further, Stat6 contains an LXXL motif at amino acid 802–806, and such sequences often mediate heterotypic interactions between transcription factors and coactivators (31, 36). We tested the ability of NCoA-1 to coactivate IL-4 induction of a transiently transfected reporter via Stat6 molecules from which this region had been deleted. Like Stat6ΔC, this deletion mutant supported no NCoA-1-enhanced induction (Fig. 3D), whereas wild-type Stat6 was coactivated as in previous experiments. Unlike the CBP and p300 association observed for Stat6ΔC, neither of the two deletion mutants of Stat6 associated with NCoA-1 in co-immunoprecipitation assays (Fig. 3E). Together, these results indicate that specific sequences within the Stat6 C terminus can mediate transcriptional activity of Stat6 through interactions with NCoA-1, and the TADs from heterologous Stat transcription factors cannot substitute for this contribution. However, the same chimera was not coactivated by NCoA-1 when tested with an IFN-γ-unresponsive promoter using the CD23-derived Stat binding element (Fig. 3B). These findings further suggest that promoter specificity of Stat6 is mediated in part by NCoA-1.

Endogenous Gene Expression Programs Require the Stat6 C Terminus but Exhibit Differential Requirements for Its NCoA-1-interacting Segment—The dose-response relationship between trans-activation potential in reporter assays and induction of endogenous Stat6 target genes and whether there are any threshold effects are unknown. To test whether the 5–8-fold increase in reporter transcription mediated by the chimeric Stat6 molecules was sufficient at an endogenous IL-4/Stat6-responsive promoter, we measured their ability to mediate IL-4-induced hyperexpression of the CD23 gene in primary B cells. To measure CD23, pools of lipopolysaccharide lymphoblasts were transduced with an IRES-GFP retrovirus containing the recombinant Stat6 molecules and analyzed by fluorescence-activated cell sorter. After gating on transduced B cells (positive for both GFP and B220), the percentage of GFP-positive B cells hyperexpressing CD23 upon IL-4 treatment was calculated from fluorescence histograms of cell size versus CD23-phycoerythin (Fig. 4A; numbers indicated within panels). The level of CD23 expression was further quantitated by determining the mean fluorescence intensity for each transduction (Fig. 4A; numbers to the right of each panel). Stat6−/− cells that were transduced with retroviruses encoding GFP alone and those co-expressing Stat6ΔC with GFP showed no IL-4-mediated up-regulation of the CD23 gene (Fig. 4A). In contrast, cells co-expressing Stat6 and GFP showed increased surface staining for the CD23 molecule, which is known to reflect increased gene transcription (42). Addition of the Stat1α or Stat5 TAD was unable to restore any CD23 trans-activation function to Stat6ΔC. Similar levels of each recombinant Stat protein were expressed following lymphoblast transduction with IRES-GFP retrovectors encoding the recombinant Stat6 molecules (as in Fig. 1) (Fig. 4B). These data indicate that IL-4-induced hyperexpression of the endogenous CD23 gene specifically requires C-terminal Stat6 sequences that cannot be replaced by the Stat1 or Stat5 TAD. To determine the importance of the NCoA-1 interaction region for Stat6-dependent biological responses in these primary cells, we compared IL-4 induction following retroviral transduction of Stat6, Stat6ΔC, and Stat6Δ792 into Stat6-deficient lymphocytes. In Stat6−/− B cells, the ability of Stat6Δ792 to mediate IL-4 induction of CD23 was reduced to one-quarter of that mediated by wild-type Stat6 (Fig. 4A) despite equivalent levels of protein expression directed by the wild-type and Stat6Δ792 retrovectors constructs. These findings indicate that even though NCoAs may interact with Stat6-bound CBP or with other transcription factors used at this promoter, the portion of Stat6 with which NCoA-1 can interact is crucial for transcriptional induction of this response by IL-4.

We have observed previously IL-4-induced reporter gene transcription mediated by a Stat6 molecule lacking this region (8). Therefore, we hypothesized that there is a differential requirement for the NCoA-1 interaction site of Stat6 (i.e. residues distal to amino acid 792). As with IL-4 induction of CD23, the induction of IL-4 production capacity in T cells (Th2 differentiation) requires Stat6 and cannot be replaced by activation of any other Stat transcription factor. Consistent with the trans-activation experiments and CD23 hyperexpression results, neither Stat6ΔC nor the ectopic trans-activation domain fusions promoted IL-4 production by transduced Stat6-deficient T cells (Fig. 4C). In contrast to CD23, however, the Stat6Δ792 mutant promoted Th2 differentiation in response to IL-4 at a level similar to wild-type Stat6. Thus, there is a differential requirement for the ability of the Stat6 activation domains to interact directly with NCoA-1 in mediating this Stat6-dependent response to IL-4 as compared with CD23 induction.

DISCUSSION

Members of the Stat family of transcription factors achieve diametrically opposed functions despite the conserved features of their architecture, a common mechanism of ligand-mediated activation and similar DNA binding specificity (5). Stat1α and Stat6 both bind to the IRF-1 IFN-γ-activated sequence and CD23 SBS, but the IRF-1 promoter is activated only by Stat1α and not Stat6, whereas the converse is true for CD23. At such promoters, Stat factors must mediate specificity for promoter activation by mechanisms apart from induction at the cytokine receptor or DNA binding specificity. We have shown here that the non-conserved sequences of Stat TADs can dictate promoter-specific functions. The transcriptional activity of chimeras between a Stat6 DNA-binding moiety and the Stat1 or Stat5 TADs was compared with the ability of wild-type Stat6 to mediate induction of various IL-4-responsive promoters. The chimeric molecules were marginally functional at promoters
containing homo-oligomeric Stat6 binding sites, but only the Stat6 TADs were able to mediate the induction of an endogenous IL-4-responsive gene in primary cells. Furthermore, the p160 class coregulator NCoA-1 interacted preferentially with the Stat6 TADs and coactivated IL-4-induced transcription. However, there was a differential effect of an NCoA-1-interact-
ing segment of Stat6 in analyses of gene expression programs. These findings indicate that NCoA-1 is part of a mechanism by which sequences unique to the Stat6 TADs mediate specific activation of IL-4-responsive genes.

The specific transcriptional induction of a gene target requires the assembly of a complex of activators that is strong enough to overcome the intrinsic repressive effects of nucleosomes and of negative coregulators. Our observations indicate that for some Stat6-dependent target genes, a certain threshold is required of the Stat-containing complex for it to activate a native promoter. In neither case (CD23 or IL-4 production) was a Stat1 or Stat5 activation domain able to surpass this threshold for an endogenous expression program, and the requirement for NCoA-1-interacting sequences distal to amino acid 792 of Stat6 differed depending on the readout. In assays using composite Stat5-dependent promoters, it was suggested that the Stat6 activation domain is in some way more potent than that of Stat5 (43), but no mechanism underlying this difference.

**Fig. 4.** Differential requirement for NCoA-1-interacting sequences of Stat6 in IL-4-induced biological responses of primary lymphoid cells. A, lipopolysaccharide-lymphoblasts derived from Stat6−/− splenocytes were infected with replication-defective bicistronic retroviruses encoding the indicated Stat6 variants and GFP. Two days later, IL-4-mediated CD23 hyperexpression on GFP positive B cells was monitored by fluorescence-activated cell sorter analysis after staining with fluorochrome-conjugated antibodies directed against B220 and CD23. Fluorescence histograms of GFP versus CD23-phycocerythrin for IL-4-treated cells gated for GFP+. B220 positive B cell populations are shown. The percentage of GFP positive B cells hyperexpressing CD23 is indicated within each panel, and the numbers next to each panel represent the mean fluorescence intensity (CD23 expression levels). B, retrovector expression of Stat6 constructs. Bicistronic retrovector plasmids encoding the indicated cDNAs were transfected into packaging cells followed by infection of lymphoblasts with the retrovirus-containing supernatants. Stat6 was detected by immunoblotting with anti-Stat6 (N-terminal epitopes), using lysates of the lymphoblasts (lower panel) as well as of the packaging cells (upper panel) to reveal expression levels free from a nonspecific band (NS) comigrating with wild-type Stat6 when using lymphoblast lysates. C, activated T lymphocytes from Stat6−/− animals were infected with the same retroviruses as in A. After infection, the cells were cultured for 5 days under Th2 differentiation conditions and then re-stimulated 48 h later as indicated (anti-CD3/anti-CD28). IL-4 production by these cells was measured by enzyme-linked immunosorbent assay using the culture supernatants. Statistical comparison between wild-type and Stat6Δ792 samples showed no significant difference to be present (p > 0.18), whereas the differences between wild-type and Stat6ΔC were highly significant (p < 0.01).
was determined. Our data with the Stat6 chimeras are consistent with the view that the Stat6 TAD is more potent in activating transcription than the TAD of Stat1α and Stat5 (Fig. 1D). These observations suggest that one mechanism that contributes to achieving promoter specificity is a function of the strength of the activation domain the Stat factor brings to a promoter. Thus, the stronger Stat6 TAD may recruit sufficient coactivators. As discussed below, our data suggest that the preferential recruitment of NCaA-1 by the Stat6 TAD, and not Stat1α or Stat5 TAD, contributes to this enhanced potency of the Stat6 TAD.

Different architectures of promoters containing Stat sites, native (endogenous CD23 promoter) versus homo-oligomeric, are associated with quantitatively different requirements for activation domains. In a recently proposed model of transcription activation at the IFN-β gene (44), cooperative binding of NF-κB, IRFs, and ATF-2/c-Jun at the IFN-β promoter is thought to create a novel activating surface, followed by the binding of GCN5 and subsequent SWI/SNF (switch/sucrose non-fermenter)-mediated chromatin remodeling as the essential checkpoint for the recruitment of CBP/polymerase II. Subsequent to chromatin remodeling by SWI/SNF, TFIIID was recruited to the IFN-β "enhancosome" to activate transcription. Analogous to the cooperative activation mediated by NF-κB, IRFs, and ATF-2/c-Jun at the IFN-β promoter, Stat6 and C/EBP collaborate to trans-activate IL-4 responsive genes (19, 45). Consistent with this analogy, we have observed that in this collaborative interaction the Stat6 TAD cannot be replaced by either the Stat1α or Stat5 TAD. Thus, the Stat6 TADs may confer promoter specificity in part because of a requirement for collaboration with C/EBP to allow formation of a unique activating surface. This surface would be responsible for recruitment of coactivators such as NCaA-1, which would be required for the subsequent steps in the formation of the pre-initiation complex and transcription activation. From this viewpoint, the inactivity of the chimera Stat6 molecules detectably to activate a native promoter is because of a lack of suitable structural interaction between a coactivator and the heterologous TADs on Stat6C. However, it is likely that still further structural factors will prove important in achieving promoter specificity. For instance, a chimera of the Stat6 C terminus fused to the Stat1 dimerization/DNA binding domains was able to mediate NCaA-1 effects on IFN-γ activation and NCaA-1 coactivation of the IRF-1 promoter but not that for CD23 (Fig. 3B). This finding may reflect a need for structural coordination between specific sequences of Stat N and C termini at some promoters.

Our data indicate that the preferential ability of Stat6 to interact with the NCaA-1 coactivator is an important determinant of specific IL-4-induced promoter activity under normal physiologic conditions, in that loss of the interaction attenuated trans-activation of an endogenous gene in normal B lymphoid cells. NCaA-1 (Src-1) is one member of a family of coactivators in which each can bind directly to CBP and be recruited to promoters through CBP binding rather than direct interaction with the transcription factor (32–34). Because each Stat protein can recruit CBP, at least two mechanisms of NCaA recruitment by Stat transcription factors are possible. In the first, direct interaction of the NCaA with a Stat protein is dispensable, because CBP/p300 proteins can mediate sufficient recruitment. Further, there is evidence of functional redundancy among NCaAs, so that any of these p160 proteins can coactivate interchangeably (46). If so, NCaA-2 or NCaA-3 would be able to substitute for NCaA-1. An alternative is that portions of Stat6 are required for promoter activity because they participate directly in enhancing the interaction with a p160-class coactivator. Key findings of our study are difficult to reconcile with a general applicability of the first model. First, Stat6ΔC was unable to activate IL-4 responses in primary cells yet was apparently as efficient in its interaction with CBP and p300 as the wild-type protein (Fig. 2A). Second, chimeras between Stat6C and the C-terminal activation domains of Stat1 or Stat5 each interacted with CBP and weakly trans-activated an array of Stat6-dependent reporter genes (Fig. 1D). Nonetheless, they could not be coactivated by NCaA-1 and were unable to induce endogenous responses dependent on Stat6. Our finding that the Stat6 chimeras were unable to mediate induction of endogenous gene expression suggests two types of functional specificity distinguishing the different p160/NCaA proteins at Stat-responsive genes. Specifically, NCaA-2 was unable to coactivate either wild-type Stat6 or the chimeras with Stat1 or Stat5 activation domains (data not shown). These findings provide evidence that NCaA-1 can serve as a non-redundant coactivator of IL-4-induced Stat6 activation at an endogenous gene locus. Importantly, however, we determined that two biological responses differ in their requirement for a portion of the Stat6 activation domains, which is essential for direct contacts with NCaA-1. Thus, the ability of Stat6 to interact directly with this coactivator is dispensable for a key biological function of IL-4 (47, 48). Because Stat6ΔC was incapable of promoting IL-4 production by T cells, whereas Stat6Δ792 led to essentially a wild-type response, we inferred that a critical interaction site for coactivation of this response maps between amino acid 661 and 791, a finding akin to data that spatially separated domains in a transcription factor such as ZEB can serve distinct biological functions through their interactions with coregulators (49).

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