Stat Protein Transactivation Domains Recruit p300/CBP through Widely Divergent Sequences*

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The family of signal transducer and activators of transcription (Stat) gene family has been highly conserved throughout evolution. Gene duplication and divergence has produced 7 mammalian Stat genes, each of which mediates a distinct process. While some Stat proteins are activated by multiple cytokines, Stat2 is highly specific for responses to type I interferon. We have cloned mouse Stat2 and found that while its sequence was more divergent from its human homologue than any other mouse-human Stat pairs, it was fully functional even in human cells. Overall sequence identity was only 69%, compared with 85–99% similarity for other Stat genes, and several individual domains that still served similar or identical functions in both species were even less well conserved. The coiled-coil domain responsible for interaction with IRF9 was only 65% identical and yet mouse Stat2 interacted with either human or mouse IRF9; the carboxyl terminus was only 30% identical and yet both regions functioned as equal transactivation domains. Both mouse and human transactivation domains recruited the p300/CBP coactivator and were equally sensitive to inhibition by adenovirus E1A protein. Interestingly, the Stat3 carboxyl terminus also functioned as a transactivator capable of recruiting p300/CBP, as does the Stat1 protein, although with widely differing potencies. Yet these proteins share no sequence similarity with Stat2. These data demonstrate that highly diverged primary sequences can serve similar or identical functions, and that the minimal regions of similarity between human and mouse Stat2 may define the critical determinants for function.

The IFN pathway has served as a paradigm for defining the role of Stat proteins in signaling. Type I IFN (IFNα/β) signals through Stat1 and Stat2 while type II IFN (IFNγ) signals through Stat1 alone. At least one reason why Stat1 is capable of functioning alone is its ability to form homodimers capable of binding specific DNA sequences in the enhancers of target genes, called γ-activated sites, or GAS elements. Stat2, in contrast, has not been demonstrated to exhibit DNA binding activity but rather is recruited to DNA as part of a multimeric complex containing Stat1 (3) and usually the auxiliary protein ISGF3γ (4–7), now referred to as IRF9. Stat2 homodimers are also incapable of binding DNA in the absence of IRF9 (8). Protein-DNA complexes containing Stat2 do not appear to involve direct contacts between Stat2 and DNA but rather rely on indirect interactions involving the other proteins in the complex (6, 8). Therefore, while one of the conserved domains identified in Stat proteins serves a DNA binding function (9, 10), it is unclear what role this domain plays in Stat2.

Other protein-protein interactions necessary for Stat function during IFN signaling include Stat-receptor interactions that are necessary for recruitment to Janus kinases for activation (11, 12), SH2-phosphotyrosine interactions that are involved in multimerization, interactions between Stat transactivation domains and transcriptional coactivator proteins, particularly of the p300/CBP family (13–15), and, at least for type I IFN, interactions between a Stat protein coiled-coil domain (16, 17) and IRF9 (7). Conservation of these protein interactions would suggest conservation of protein sequences, and most Stat protein homologues display considerable sequence conservation across different mammalian species. Stat2 appears to be an exception to this rule. Through cloning and characterization of the mouse Stat2 protein, we report that it is remarkably divergent in primary sequence in comparison to its human homologue. Despite this divergence, mouse Stat2 retains all the functions defined for human Stat2 and will even substitute for the human homologue when transfected into human cells, demonstrating its ability to form all protein in-

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The abbreviations used are: Stat, signal transducer and activators of transcription; IFN, interferon; SH2, Src homology domain 2; GST, glutathione S-transferase; RT-PCR, reverse transcriptase-polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis.

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teractions necessary for IFN action with both mouse and human partner proteins.

**EXPERIMENTAL PROCEDURES**

**Growth of Cells, IFN Treatment, and Antibodies**—Mouse embryonic fibroblasts from CD1 mice (18), U6A cells (19), COS cells (20), and U2OS cells (21) were cultured in Dulbecco's modified Eagle's medium supplemented with 10% bovine serum (Sigma). Stable cell lines in U6A cells were obtained by the calcium phosphate method using 30 μg of human or murine Stat2 expression constructs in the pcDNA3 vector (Invitrogen) by selection in 400 μg/ml G418 (Life Technologies) and were screened by Western blot for expression of Stat2. Human cells were treated with recombinant IFN-α2a (kind gift of Hoffmann-La Roche) and mouse cells were treated with natural type 1 IFN (Lee Biomedical). Rabbit antiserum and monoclonal antibodies were raised against a bacterial fusion protein expressing the carboxyl terminus of mouse Stat2 fused to GST using the vector pGEX-2T (22). Antibodies specific for tyrosine-phosphorylated Stat2 were developed by immunizing rabbits with a phosphopeptide representing the conserved phospho-acceptor site of Stat2. The resulting antiserum was absorbed extensively against the non-phosphorylated peptide and against phosphotyrosine to remove cross-reactive antibodies. Anti-phosphotyrosine monoclonal antibodies PY20 and 4G10 were obtained from UBI and Transduction Laboratories, respectively, and antibodies against p300 and GB1 were obtained from Santa Cruz Biotechnology.

**Cloning of Mouse Stat2 and Plasmid Constructs**—A partial clone for Stat2 was obtained by low stringency hybridization of a mouse cDNA library, as described previously (23). Sequence analysis indicated that this clone lacked two regions present in human Stat2 and later found in mouse Stat2 (see below), namely amino acids 44–98 and 157–182 (data not shown). A full-length clone was obtained by RT-PCR using RNA derived from mouse embryonic fibroblasts and 5′ and 3′ primers based on the predicted untranslated regions of the partial clone. Total RNA was isolated by Trizol reagent (Life Technologies) from mouse embryonic fibroblasts treated for 12 h with IFNα/β (500 units/ml). The following sequences were used as primers: 5′-UTR: 5′-ttgcagcgagacgactggaag-3′; 3′-UTR: 5′-tggaggtttagaagtctac-3′. The resulting PCR fragment was cloned first into pPCR (24) and then subcloned into the expression vector pcDNA3 (Invitrogen). A similar expression construct for human Stat2 was prepared in pcDNA3 (8); Gal4 fusions were created in the vector pSG424 (25) using full-length murine Stat1, murine Stat2 residues 699–923, human Stat2 residues 699–923, human Stat2 residues 716–770, and murine Stat2 residues 736–851 (human Stat2) and 670–851 (human Stat2), and recombinant protein was isolated from inclusion bodies of transformed bacteria by glutathione-agarose affinity chromatography (26). GST fusion coprecipitations were performed using nuclear extracts prepared from mouse L cells (27), as described (15). GST fusion constructs were prepared in pGEX-2T (22). Antibodies specific to different degrees (Fig. 1B). Interestingly, the region analogous to the DNA-binding domains of other Stat proteins (9, 10) was also conserved between mouse and human despite the absence of a demonstrated ability of Stat2 to bind DNA (6, 8).

However, the carboxyl terminus of human Stat2, a region necessary for the transcriptional activity of the protein (33), was only poorly conserved in the mouse. While this region of the sequence is not well conserved between distinct genes, it is nonetheless highly conserved between species for all other Stat genes characterized thus far (Table 1). All other Stat proteins show 80 to >90% cross-species identity in their carboxyl-terminal transactivation domains, while mouse and human Stat2 proteins are only 33% identical and 41% similar.

The lack of conservation between human and mouse Stat2 led us to question whether our cDNA clone was a legitimate representative of mouse Stat2 or a minor, diverged subspecies. Since other Stat genes are known to be expressed as multiply spliced variants and alternative splicing has been described for Stat2 (34), RNA extracted from mouse cells was examined by Northern blotting with the full-length mouse Stat2 cDNA as probe. A major mRNA approximately 6 kilobases in length hybridized the probe, and this mRNA was increased in abundance in response to IFNα treatment (Fig. 2A). Induction of Stat2 protein by IFNα has been previously described (35). No additional mRNA species were detected by this analysis from either the uninduced or the IFNα-induced sample. In particular, no predominant products were observed that might correspond to major splice variants, as has been described for Stat1 (36). However, additional minor products possibly representing either splice variants or splicing intermediates were detected by RT-PCR (data not shown).

To examine mouse Stat2 protein, antibodies were raised against a recombinant protein consisting of amino acids 644 to the carboxyl terminus. Western blotting detected a single protein of approximately 120 kDa in extracts from mouse fibroblasts (Fig. 2B, lane 2) which is significantly larger than human Stat2 (113 kDa). This larger size is consistent with the open reading frame predicted by sequence analysis which is also longer in mouse than in human, although both proteins migrate on SDS-PAGE with an abnormally large apparent molecular size. Transfection of human cells with an expression construct containing the mouse Stat2 cDNA produced a protein sequence has been deposited in GenBank as accession number AF088862.

**RESULTS**

**Mouse and Human Stat2 Are Homologous Except for a Divergent Carboxyl Terminus**—A mouse Stat2 cDNA clone was initially isolated from a library screen for Stat clones based on hybridization with the conserved SH2 domain (Ref. 23 and data not shown). Conceptual translation of the sequence of this clone demonstrated homology with human Stat2 at the amino terminus and throughout much of the sequence except for two gaps and a divergent carboxyl terminus (data not shown). Further characterization of the mouse Stat2 gene was obtained using specific primers, 3′ and 5′ rapid amplification of cDNA end techniques, and PCR amplification from RNA isolated from mouse embryonic fibroblasts (“Experimental Procedures”). The longest clones obtained by these procedures were approximately 3 kilobases in length, and sequence analysis showed the presence of 5′- and 3′-untranslated regions and an open reading frame encoding 923 amino acids (Fig. 1A). The DNA sequence has been deposited in GenBank as accession number AF088862.

Comparison of the mouse Stat2 sequence with human Stat2 demonstrated that the two proteins are co-linear, with conservation of all the major features previously identified in Stat proteins (16, 17). In particular, the amino-terminal domain shown to be important for interaction between Stat dimers (29, 30), the coiled-coil domain involved in interaction with ISGF3γ (p48 (IRF9) (7), the SH2 domain involved in dimer formation (31), and the peptide containing the phosphotyrosine-acceptor site (32) could all be recognized, although they were conserved to different degrees (Fig. 1B). Interestingly, the region analogous to the DNA-binding domains of other Stat proteins (9, 10) was also conserved between mouse and human despite the absence of a demonstrated ability of Stat2 to bind DNA (6, 8).
detected by the antiserum of equal size to endogenous mouse Stat2 (Fig. 2B, lane 1). However, protein expressed from our initial partial Stat2 cDNA clone (lane 3) was significantly smaller than either the endogenous protein or protein expressed from the full-length clone (lane 1). Protein expressed from the full-length cDNA clone comigrated with endogenous mouse Stat2, suggesting that this clone indeed contained the entire Stat2 coding region. Consistent with the sequence divergence between mouse and human Stat2, the antiserum raised against mouse Stat2 and several monoclonal antibodies raised against the same immunogen failed to recognize human Stat2 (data not shown).

A characteristic of Stat proteins is their ability to be activated by tyrosine phosphorylation (1, 37). Recombinant mouse Stat2 was coexpressed in human embryonic kidney 293T cells along with mouse Stat1 and the tpr-met-activated tyrosine kinase (38) which efficiently phosphorylates Stat proteins. Coexpression of mouse Stat2 and tpr-met resulted in phosphorylation of Stat2, as revealed by immunoblotting with antibody specific for tyrosine-phosphorylated Stat2 (Fig. 2C, lane 1). In the absence of coexpressed kinase (lane 2), no phosphorylated Stat2 was detected. Protein expressed from the shorter, mStat2-DN clone was similarly phosphorylated (lane 3). Endogenous mouse Stat2, recognized by antisera prepared against the recombinant protein, was tyrosine phosphorylated in response to IFNα/β treatment, as revealed by the phospho-specific Stat2 antibody (lane 6). Activation of Stat proteins results in their ability to dimerize, and phosphorylated Stat1 and Stat2 co-precipitated when coexpressed along with kinase (data not shown), indicating SH2 domain-phosphotyrosine interactions. This interaction led to formation of the ISGF3 multimeric complex when mouse Stat2 was coexpressed with mouse Stat1 and ISGF3γ5p48 (IRF9) in the presence of kinase (Fig. 2D, lane 1). This complex was efficiently super-shifted by monoclonal antibody against mouse Stat2 (lane 2) and comigrated with mouse ISGF3 isolated from IFN-treated fibroblasts.

### Table 1

| Sequence   | Full-length | Carboxyl terminus |
|------------|-------------|-------------------|
|            | % Similar   | % Identical       | % Similar   | % Identical |
| Stat1      | 93.7        | 92.4              | 93.3        | 93.3        |
| Stat2      | 78.1        | 68.6              | 38.6        | 29.7        |
| Stat3      | 99.1        | 98.8              | 98.4        | 96.8        |
| Stat4      | 96.0        | 94.7              | 94.2        | 94.2        |
| Stat5a     | 97.1        | 96.3              | 89.6        | 89.6        |
| Stat6      | 87.3        | 85.1              | 83.4        | 78.8        |

### Fig. 1. Sequence comparison of mouse and human Stat2. Panel A, sequence alignment of the mouse and human Stat2 produced by the GCG computer program Pileup. Identical amino acids are underlined and in uppercase. Panel B, domain comparison of mouse and human Stat2. Functional domains defined for the Stat protein family are compared with percent amino acid similarity indicated.
against mouse Stat2.

SDS-PAGE analyzed by immunoblotting using a monoclonal antibody lane 2 293T cells or mouse embryo fibroblasts (partial clone, mStat2- the same mobility as endogenous mouse Stat2. 293T cells were tran-

ment. 40 treated (a single mRNA for muStat2 is induced following IFN treat-

lanes 1 and 4 (Fig. 3 A, lanes 2 and 3) and with antibody against Stat2 (lanes 3 and 4)). Therefore, by the criteria that recombinant protein expressed from this clone comigrates with endogenous Stat2, is tyrosine phosphorylated, associates with Stat1 and IRF9, and is capable of generating an antibody that recognizes endogenous mouse Stat2, we conclude that it is an authentic cDNA clone. Mouse Stat2 Can Substitute for Human Stat2 Despite Its Sequence Divergence—Because human and mouse Stat2 are more divergent than other human-mouse Stat homologues, we tested whether mouse Stat2 could substitute for its human counterpart. U6A cells fail to respond to IFNα treatment because they lack the Stat2 protein, but they can be comple-

mented by transfection of human Stat2 (19). We transfected U6A cells or the parental 2TGH cells with the IFN-responsive reporter construct ISG54-luc (8). The wild type parental cells responded to IFNα treatment by an increase in luciferase ac-

kines (lanes 1 and 3) and analyzed by Western blotting using an antibody specific for tyrosine-phosphorylated Stat2. Stat2 is phosphorylated in response to IFN treatment. Stat2 was detected in extracts of untreated (lane 5) or IFN-treated fibroblasts (lane 6) by probing with antibodies against phospho-Stat2 (upper panel) and with antibody against Stat2 (lower panel). Panel D, mouse Stat2 forms the ISGF3 complex with Stat1 and IRF9. Extracts from 293T cells transfected with expression constructs for mouse Stat1, Stat2, IRF9, and tpr-met (lanes 1 and 2) or extracts from fibroblasts treated with IFN for 1 h (lanes 3 and 4) were analyzed by EMSA using an ISRE probe from the ISG15 gene. Antibody against Stat2 was added to lanes 2 and 4 to produce a supershift. No ISGF3 was observed in extracts from cells not treated with IFN (not shown). Nonspecific bands that are not affected by the antibody are present in the extracts from 293T cells (lanes 1 and 2).

(Fig. 3, Mouse Stat2 is a transcriptional activator. Panel A, mouse Stat2 restores IFN response to U6A cells. U6A cells were stably transfected with vector (U6, lane 2) or with mouse Stat2 (mStat2, lane 3), as indicated. 2TGH cells (lane 1) or U6A stable transfectants (lanes 2 and 3) were transiently transfected with ISG54-luc. 16 h after transfection, cells were left untreated or treated with human IFN-α (1000 units/ml) for 6 h, and cell lysates were assayed for luciferase activity. Data represent fold induction in response to IFN relative to untreated cells and are the average of duplicate experiments and the range of each determination. Values were normalized for co-transfected β-galactosidase. Panel B, mouse Stat2 is a potent transactivator. COS cells were transfected with UAS-luc reporter along with Gal4 (lane 1) or Gal4 fusion proteins for human (lane 2) and mouse Stat2 (lane 3) or mouse Stat3 (lane 4), as indicated. 36 h after transfection, cell extracts were assayed for luciferase activity and represented as fold activation over reporter alone. Values presented are the mean of triplicate samples normalized for co-transfected β-galactosidase. Equivalent expression of each Gal4 construct was verified by electrophoretic mobility shift assay (data not shown).

The most divergent region of mouse Stat2 relative to human is the carboxyl terminus which encodes the transcriptional domain of the human protein. The transcriptional potential of the mouse Stat2 carboxyl terminus was tested and compared with that of its human counterpart. The carboxyl termini of the two proteins were fused with the DNA-binding domain of the yeast Gal4 protein (25), and these constructs were tested for transcriptional activity by co-transfection with a Gal4 UAS-luciferase reporter in COS cells. Both human and mouse Stat2 fusion proteins induced high levels of luciferase activity (Fig. 3B, lanes 2 and 3) relative to the Gal4 DNA-binding domain protein expressed alone (lane 1). A similar fusion protein containing the carboxyl terminus of the mouse Stat3 protein induced a moderate response from the reporter construct (lane 4). Therefore, despite their high degree of sequence divergence, both human and mouse Stat2 transcriptional domains encode comparable activities.

Both Human and Mouse Stat2 Interact with p300/CBP Co-activators—It has been reported that human Stat2 recruits p300/CBP as a coactivator during transcriptional activation (15). The sequence divergence between human and mouse Stat2 raised the possibility that mouse Stat2 might activate transcription by a distinct mechanism. Recombinant human and mouse Stat2 carboxyl termini were purified from bacteria as glutathione S-transferase fusion proteins. When mixed with nuclear extracts from mouse L cells, both human and mouse Stat2 protein fragments were capable of selectively precipitating p300/CBP as determined by immunoblotting (Fig. 4A, lanes 2 and 3). GST displayed only background binding of p300/CBP (lane 4).

Interaction of mouse Stat2 with p300 was also tested in vivo. A Gal4-Stat2 construct was co-transfected with an epitope-

tagged human p300 expression construct. For comparison, Gal4-Stat1 and Gal4-Stat3 constructs were also co-transfected with p300. Immunoprecipitation of p300 from extracts of transfected cells also recovered each Gal4 construct, detected by

(data not shown).
immunoblotting with an antibody against the Gal4 DNA-binding domain (Fig. 4B). Because p300 interacts with both the amino and carboxyl termini of Stat1 (13), the entire protein was fused to Gal4. The carboxyl termini of both mouse Stat2 and Stat3 efficiently interacted with p300, despite these protein fragments displaying no significant sequence similarity, as did the divergent human Stat2 carboxyl terminus (not shown). Therefore, p300 is capable of interacting with considerably dissimilar sequences: Stat1, the divergent carboxyl termini of mouse and human Stat2, and the unrelated carboxyl terminus of mouse Stat3.

Adenovirus E1A Inhibits Stat-dependent Transcription—Adenovirus E1A inhibits IFN-dependent transcription (40), possibly due to its ability to bind and sequester p300/CBP (13–15). Because both human and mouse Stat2 and mouse Stat3 interact with p300/CBP, we tested the functional significance of this interaction by co-transfection with E1A (Fig. 5). Increasing amounts of the co-transfected E1A 12S gene inhibited reporter gene expression driven by each of the Gal4-fusion constructs by 8–16-fold. To determine whether the action of E1A correlated with its ability to sequester p300/CBP, various mutant versions of E1A were tested. Deletion of amino acids 2–36 from E1A blocks its interaction with p300 (41), while deletion of amino acids 38–67 impairs its interaction with Rb (42) and to some extent p300 (43). These two mutants were tested for their ability to disrupt transcriptional induction by mouse Stat2 and Stat3 fusion proteins. Both Stat2 and Stat3 transactivation domains responded similarly to E1A and the E1A mutants. Loss of p300 binding by E1A prevented it from interfering with either Stat2 or Stat3 transcriptional induction (Fig. 5, B and C, lanes 3), suggesting a role for p300/CBP coactivators in this process. Interestingly, the E1A mutant 38–67 was also partially impaired in blocking Stat-mediated transcription (lanes 4), mimicking the requirement of this region to block IFN-stimulated gene expression (43), possibly reflecting its reduced interaction with p300/CBP.

DISCUSSION

Sequence Divergence between Mouse and Human Stat2—The Stat gene family has been conserved throughout evolution, having its origin at the beginning of metazoans or before (44). There is a Stat homologue in Drosophila sp. (45, 46) and Anopheles sp. (47) as well as a possible ancestral gene in the slime mold Dictyostelium sp. (48). Conservation of sequence and function has resulted in the ability to recognize Stat proteins on the basis of primary amino acid sequence and for highly divergent members to be able to recognize the same DNA target sequence. In mammals, the Stat family consists of 7 genes clustered in 3 chromosomal positions (49), clearly the result of gene duplication, translocation, and divergence. The conservation and/or recent divergence of the gene family is such that a high degree of sequence identity has been noted between mouse and human homologues. We report the characterization of the last member of the known Stat genes from the mouse and have found that it is also the least conserved in relation to its human counterpart.

There are several aspects of Stat2 that are distinct from other Stat proteins. First, it is the only Stat protein that has not been found to bind DNA as a homodimer. While homodimers of Stat2 can form following activation in response to IFNα treatment (8), they have not been demonstrated to directly bind DNA. Rather, Stat2 binds DNA in conjunction with its partner protein, IRF9, either as a homodimer or as a heterodimer with Stat1 (50). Even as a multimer in conjunction with IRF9, Stat2 does not appear to directly contact DNA but instead relies on DNA contacts provided exclusively by IRF9 and Stat1 (6, 8). This lack of requirement for direct interaction with DNA may allow divergence of the protein region analogous to the DNA-binding domain of the more conventional Stat proteins. Surprisingly, this region of Stat2 (amino acids 318–465, see Fig. 1B) is actually one of the more conserved portions between the human and mouse sequences. This conservation may reflect a function for this domain other than contacting DNA, for example, as part of the dimer interface (16). Alternatively, this conservation of sequence may indicate that Stat2 retains the ability to bind DNA at an as yet unidentified target site.

A second attribute of Stat2 is its interaction with a member of the IRF family, an activity that has been mapped to the coiled-coil domain of human Stat2 (7). This function has been conserved by both mouse and human Stat2 homologues; in fact, both mouse and human Stat2 proteins are capable of interacting with either mouse or human IRF9 proteins (data not shown). Surprisingly, however, the sequence of the region involved in this function has not been conserved between human and mouse more than other regions of the protein. Indeed, the coiled-coil domain is one of the least conserved regions, second only to the transactivation domain (Fig. 1B). Even the minimal region of human Stat2 shown to interact with IRF9 by yeast two-hybrid, amino acids 156–189 (7), is only 68% similar (59% identical) to the analogous portion of mouse Stat2. Two lysine residues identified as important for ISGF3 formation in human Stat2 are also not conserved in the mouse; Lys161 is an arginine in mouse Stat2 and Lys178 is a threonine. Despite this lack of sequence conservation, the coiled-coil region of mouse Stat2 is
likely to be the IRF9-binding site because the protein expressed from the ΔN-Stat2 cDNA clone, which lacks amino acids 157–182 within the coiled-coil domain, failed to interact with IRF9 or form an ISGF3 complex (data not shown). The amino-terminal third of Stat2 has also been reported to be involved in necessary interactions with the IFNα receptor (12). Presumably, functional interactions are conserved despite the sequence divergence because mouse Stat2 was activated by IFNα in human cells (Fig. 3A).

A third feature of Stat2 is its extended carboxyl-terminal domain which is longer than all other Stat proteins with the exception of Stat6. Mouse Stat2 has the longest transactivation domain yet, making it also the largest Stat protein identified with a predicted molecular weight of 105,416. Similar to other Stat proteins, mouse Stat2 migrates in SDS-PAGE anomalously with an apparent molecular weight of greater than 120,000 (Fig. 2B). The carboxyl-terminal region is the most divergent between mouse and human Stat2 and is more divergent than similar regions of other Stat proteins (Table I). Most of the difference in size between mouse and human Stat2 can be accounted for by a repeated amino acid motif within the carboxyl-terminal domain. The sequence APQVLLEP is repeated 12 times with minor variation between amino acids 755 and 850 in mouse Stat2 but is not present in the human sequence. However, this repeat does not explain the sequence divergence in this region because even when eliminated from the analysis, similarity is not increased.

**Transactivation by Stat Proteins: Conserved Function but Divergent Sequence**—Despite the divergence between mouse and human transactivation domains, the 2 domains provide similar functions. Mouse Stat2 was capable of complementing the absence of Stat6 in the mutant human cell line U6A (Fig. 3A), activating transcription to levels similar to wild-type cells. Both mouse and human carboxyl-terminal domains induced similar levels of transcription as Gal4 fusion proteins (Fig. 3B). And both mouse and human Stat2 transactivation domains interacted with p300/CBP from either mouse (Fig. 4A) or human cells (Fig. 4B). Interestingly, the Stat3 transactivation domain was also capable of recruiting p300/CBP despite sharing essentially no sequence similarity with either the human or mouse Stat2 transactivation domains. Indeed, recruitment of p300/CBP may be a universal property of Stat proteins; interaction has been previously documented for Stat1, human Stat2, Stat5, and Stat6 (13–15, 51, 52) and in this report for mouse Stat2 and Stat3.

Recruitment of p300/CBP may play a functional role in Stat transactivation as indicated by inhibition by adenovirus E1A proteins. Both Stat2 and Stat3 transactivation abilities were blocked by coexpression of the E1A 125 gene product, but not by mutant versions impaired in p300/CBP binding (Fig. 5). Deletion of amino acids 2–36 which completely abrogates the interaction between p300 and E1A while retaining its interaction with Rb prevented inhibition of Stat2 and Stat3 transactivation. Deletions in the amino-terminal portion of conserved region 1, which block Rb binding and partially impair p300 binding, also inhibited Stat2 and Stat3 transactivation, but less well than wild type E1A. These results could indicate that sequestration of p300 by E1A blocked the activity of the Stat transactivation domains. However, it has recently been shown that E1A can interact directly with Stat1, specifically with the carboxyl-terminal transactivation domain of that protein (53). Therefore, the inhibition by E1A may result from a direct negative effect on the Stat proteins rather than by preventing p300 binding. In either case, however, whether transcriptional inhibition is due to prevention of p300 binding or to direct interaction with E1A, there is virtually no homology among all the Stat transactivation domains. While Stat1, Stat3, and Stat4 share a short related motif (LPMS) within the transactivation domain, Stat2 and Stat6 lack this sequence. In addition, this motif has recently been shown to be a target for the MCM5 protein (54) rather than for p300. There is no evidence that this sequence mediates E1A binding.

The analysis of mouse Stat2 and Stat3 demonstrates the degree to which amino acid sequences can diverge and still serve the same function. The most highly conserved region of human and mouse Stat2 is the SH2 domain, while the coiled-coil domain and especially the transactivation domain are highly diverged. Nonetheless, all these domains mediate equivalent functions and have retained the ability to interact with their partner proteins from both species, even though these have diverged in sequence as well. Most surprising perhaps is the almost complete divergence of the primary sequence of the

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**Fig. 5.** **Adenovirus E1A 125 protein represses Stat transactivation.** Panel A, increasing amounts of E1A 12S protein represses Stat transactivation. U2OS cells were co-transfected with UAS-luc reporter along with Gal4-Stat constructs in the absence or presence of E1A 12S. Lane 1, Gal 4 vector alone; lanes 2–5, Gal4-mouse Stat2 (10 ng) alone or with increasing concentrations of E1A (10, 30 and 100 ng); lanes 6–9, Gal4-human Stat2 (10 ng) alone or with increasing E1A; and lanes 9–12, Gal4-mouse Stat3 (200 ng) alone or with increasing E1A. Fold repression is reported as luciferase values normalized to no E1A after correcting for transfection efficiency. All transfections were performed in triplicate and standard errors are shown. **Panels B and C,** mouse Stat2 (panel B) and Stat3 (panel C) transactivation is repressed by wild type E1A 12S, partially by E1A ΔΔ8–67 (Rb mutant), but not by E1A ΔΔ2–36 (p300 mutant). Transfections were performed as described for panel A, except a single concentration of E1A was used (250 ng for panel B, 100 ng for panel C). Lanes 1, vector; lanes 2, E1A; lanes 3, E1A ΔΔ2–36; lanes 4, E1A ΔΔ8–67. Assays were performed in triplicate and standard errors are shown.
transactivation domains while retaining virtually identical transactivation potentials and apparent mechanisms. Similarly, the Stat3 carboxyl terminus is capable of acting as a transactivation domain and recruiting p300/CBP while sharing no sequence similarity with Stat2. It is hoped that further comparison of these domains and their interactions with recruited partners will increase our understanding of the structural basis for these activities.

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