Murine Stat2 is uncharacteristically divergent

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ABSTRACT

Characterization of the ability of human interferons (IFNs) to rapidly induce genes led to the identification of the first two members of the STAT (signal transducers and activators of transcription) family, Stat1 and Stat2. To study the unique role of this transcription factor in IFN signaling under more physiological conditions, murine Stat2 was isolated and found to be surprisingly divergent. This divergence was most striking in the C-terminal transcriptional activation domain. Studies on murine Stat2 indicate that it functions in IFN signaling. This includes IFN-α-dependent activation, nuclear translocation, DNA binding and activation of reporter genes. However, the profound divergence at the C-terminus suggests that murine Stat2 may have evolved to mediate some unique functions as well. To explore this possibility, proteins that interact with the C-termi

INTRODUCTION

Type I interferons (IFN) (α, β, ω and τ) are a group of related proteins that bind to a common receptor and exert important immunomodulatory, antiproliferative and antiviral effects. These responses are in large part achieved through the induction of genes by the JAK–STAT pathway (1–3). This entails the sequential ligand-dependent activation of two receptor-associated JAK kinases (Jak1 and Tyk2) and two STAT transcription factors (Stat1 and Stat2). Stat1 and Stat2 each become activated by the phosphorylation of a single tyrosine (4,5). These phospho-tyrosyl residues then interact with the SH2 domain of the other STAT, leading to the formation of a Stat1:Stat2 heterodimer (6,7). This heterodimer translocates to the nucleus where it associates with p48 to form a stable SGF-3 complex on the IFN-stimulated response element [ISRE; (8,9)]. p48 provides the DNA binding domain and Stat2 the transcriptional activation domain in ISGF-3 (10–14). IFN-α can also transduce signals through a second transcription factor, a Stat1 homodimer, which is formed through a similar SH2 phosphotyrosyl interaction (6,15–17). Likewise, Stat1 homodimers translocate to the nucleus and bind DNA. However, in contrast to ISGF-3, Stat1 homodimers bind directly to a distinct element known as the IFN-γ activation site [GAS; (18)]. This Stat1 homodimer also transduces important signals for IFN-γ (19–21), suggesting that Stat2 is critical for defining the unique responses to type I IFNs (e.g. IFN-α).

Stat2 shares a number of conserved domains with other members of the STAT family. This includes an N-terminal coiled-coil domain, a ‘putative’ DNA binding domain, an SH2 domain, a tyrosine activation domain, and a C-terminal transcriptional activation domain (see below). In contrast to all other STATs, Stat2 dimers [e.g. Stat1:Stat2 heterodimers and putative Stat2 homodimers; (7)] do not bind to members of the GAS family of enhancers. This raises the vexing question as to why Stat2 posses a conserved DNA binding domain.

Deletional studies, including ones on human Stat2, have mapped the STAT TAD to the C-terminus (12,14,24–26). As is the case with many transcription factors, the sequence of the TAD is not conserved between different STATs, suggesting that it may contribute to specificity. Naturally occurring STAT isoforms, in which the TAD is absent, have been reported for several STATs, but not Stat2 (14,25,27–30). This is consistent with the observation that ISGF-3 function is dependent on expression of full-length Stat2 (12). In contrast, an alternatively spliced isoform of Stat1, missing its TAD (i.e. Stat1B), is fully functional in the ISGF-3 complex (14). While most other truncated STAT isoforms are generated by an RNA processing event (31), the truncated isoform of Stat5 is generated by a unique and developmentally regulated protein processing event (28).

In an effort to develop reagents to study Stat2 in the murine system, a murine cDNA was isolated and a specific antibody generated. Although the biological response to type I IFNs is conserved between man and mouse, murine Stat2 (MuStat2) is surprisingly divergent from its human counterpart. This contrasts with the significantly higher degree of identity found between human and porcine Stat2 (i.e. 84%; GenBank accession no. 2189980). Although a number of studies indicate that the function of MuStat2 is conserved overall, it appears to bind distinct nuclear proteins. These observations may provide some insight into the remarkable divergence of murine Stat2.

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MATERIALS AND METHODS

Cell culture

293T, HeLa S3 and NIH 3T3 cells were acquired from ATCC. The 2fTGH and U6A cells were gifts from G. Stark (32). Primary mouse embryonic fibroblasts (PMFs) were isolated as previously described (33). These cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (or 10% calf serum for the HeLa and NIH 3T3 cells) and penicillin/streptomycin (Life Technologies). For metabolic labeling, HeLa cells were grown in methionine-free DMEM (Life Technologies), supplemented with 5% dialyzed calf serum (Hyclone) and 200 μCi/ml [35S]methionine (1000 Ci/mmole Easy Tag; NEN) for 16 h.

DNA

A murine thymus cDNA library, a generous gift from M. Nussenzweig, was screened with a BglII–HindIII fragment (bp 1600–2039) of the human Stat2 cDNA under low stringency conditions (5× SSC at 52°C). The largest positive clone was subcloned and sequenced with multiple overlapping sense and antisense primers (each strand was sequenced at least twice). Expression constructs of murine and human Stat2 were prepared by cloning the corresponding cDNAs into the NotI and ApuI sites of RcCMV (Invitrogen). The MuStat2–Gal4 fusion proteins were prepared by cloning amino acids 725–925 of murine Stat2 (SacI–BamHI fragment) into pSG424 (34). Glutathione S-transferase (GST) fusion proteins encoding Stat2 C-termini were prepared by cloning PCR products amplified corresponding to amino acids 691–851 and a MuStat2 cDNA template (primers 5’-CGC GGA TCC CTG AAA CAC AGG CTA ATT GGT-3’ and 5’-CGC GAA TTC CAA ACG TAT CCA CTA A-3’, corresponding to amino acids 690–925) into the BamHI and EcoRI sites of pGEX-2T (Pharmacia). All reading frames were confirmed by sequencing. The HuStat2–Gal4 fusion, which includes Stat2 amino acids 736–851, was a generous gift from D. Levy. The Gal4–luciferase and ISRE–luciferase fusion, which includes Stat2 amino acids 736–851, was a generous gift from D. Levy. The Gal4–luciferase and ISRE–luciferase fusion proteins have been described previously (34, 35).

Antibodies

A polyclonal murine Stat2 specific antibody was generated by immunizing rabbits (BabCo) with a GST fusion protein with a HindIII–BamHI fragment (amino acids 672–925) of MuStat2, as described previously (31). The polyclonal human Stat2 antibody is directed against amino acids 713–785 (7) and the Stat1 antibody, which recognizes murine and human species, is directed against amino acids 715–750 (31). An anti-phosphotyrosine specific monoclonal antibody was purchased from Upstate Biotechnology Inc. (4G10).

Protein analysis

Whole cell extracts were prepared from IFN-α/β, IL-10 (1000 U/ml, 15 min; Peptika Research Labs) or IFN-α/β (1000 U/ml, 15 min; Hoffman-LaRoche) stimulated cells by lysing them in NP-40 buffer (0.5% NP-40, 0.4 mM PMSF, 1 mM DTT and 1 mM sodium orthovanadate) as described previously (15, 27). Extracts were fractionated by 7% SDS–PAGE, either before or after immunoprecipitation (27), transferred to nitrocellulose, and immunooblotted with a 1:1000-fold dilution of primary antibody and then detected by ECL (Amersham; 27). For electrophoretic mobility shift assays (EMSA), extracts were incubated with a radiolabeled ISG15 ISRE probe (5’-gatcCTCG-GGAAAGGGAAACCGAAACTGAAGCC-3’), as reported previously (16, 39). Supershift assays were performed by pre-incubating 3–4 μl of whole cell extracts with 0.6 μl of antibody at 4°C for 30 min prior to adding shift probe, in a standard shift reaction (16, 39). For transient transfections, a total of 5 μg of DNA (per 10 cm dish) was introduced into 293T cells by the calcium phosphate/chloroquine method (40). 2fTGH, U6A and NIH 3T3 cells were transfected with a total of 20 μg of DNA (per 10 cm dish) for protein studies or 10 μg of DNA for reporter assays, by a standard calcium phosphate method (37). Reporter assays included 1 μg of TK-driven renilla luciferase reporter (Promega) for normalization in a dual luciferase assay (Tuner luminometer). Transiently transfected cells were stimulated with IFN-α for 12 h, 24 h post-transfection. GST pull-down studies entailed incubating 10 μg (as determined by Coomassie blue staining) of agarose–bound (i.e. GSH–agarose; Sigma) GST alone (pGEX-2T; Pharmacia), GST–MuStat2 (amino acids 690–925) or GST–HuStat2 (amino acids 691–851) with ~600 μg of extracts prepared from metabolically labeled HeLa cells. Briefly, 5–10 × 107 labeled cells were washed in PBS, scraped into 2 ml of RSB and homogenized by 40 strokes in a dounce homogenizer (type ‘b’ pestle) as previously described (31, 41). Nuclear pellets were resuspended in 1 vol of buffer C (20 mM HEPES, pH 7.9, 420 mM NaCl, 20% glycerol, 1.5 mM MgCl2, 0.2 mM EDTA, 0.1 mM NaVO3, 0.2 mM PMSF and 1 mM DTT) diluted to 280 mM NaCl and incubated with the GST fusion proteins at 4°C for 16 h. Beads were washed in binding buffer (five times) and eluted by boiling in SDS–PAGE loading buffer. Eluted proteins were fractionated by 10% SDS–PAGE and identified by autoradiography after the gel was fluorography enhanced (EN3 HANCE; Dupont).

Immunofluorescence

NIH 3T3 cells were seeded into 6-well plates containing coverslips at a density of 1 × 105 well and stimulated 24 h later with IFN-α A/D (1000 U/ml for 15 min). Cells were washed in 1× BWD (125 mM NaCl, 5 mM KCl, 5 mM glucose, 10 mM NaHCO3,

RNA

Total cellular RNA was prepared by guanidinium thiocyanate lysis (36). An aliquot of 1–3 μg of poly(A)+ RNA, prepared on oligo(dT) cellulose (Boehringer Mannheim; 37) was fractionated on 0.8% agarose–formaldehyde, transferred to nitrocellulose and hybridized with a MuStat2 cDNA 5’ probe (HincII–HincII, bp 786–1090) or 3’ probe (NheI–BamHI, bp 2524–2955). RNase protection analysis was carried out as described previously (38). Riboprobes were prepared with T7 and SP6 RNA polymerase (New England Biolabs) from linearized templates, generated by cloning the Real–HindIII (bp 1689–2033) or the SacI–PvuII (bp 2066–2302) fragment of MuStat2 into pBluescript KS II (Strategene). [α-32P]UTP (5000 Ci/mmole; NEN) was incorporated to a final specific activity of 5 × 106 d.p.m./μg. These riboprobes were hybridized to total RNA for 16 h in 80% formamide at 45°C, and then digested with RNase T2 (Life Technologies) for >1 h at 37°C. The products were precipitated and resolved on a standard 6% acrylamide–8 M urea sequencing gel.

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20 mM HEPES, 1 mM KH₂PO₄, pH 7.4), fixed in 3.7% formaldehyde for 20 min at 22 °C, permeablized in 0.2% Triton (4 min) and then washed in 1× TBS (137 mM NaCl, 25 mM Tris, pH 7.4). Fixed cells were blocked in 10% milk in TBS (30 min) and stained with antibody diluted to a concentration of 1:100. Stained cells were visualized with a Cy-3-conjugated donkey anti-rabbit antibody (Jackson Immunochemicals) after excitation at 568 nm, at 400-fold magnification on a Zeiss LSM410 laser scanning confocal microscope.

RESULTS

Isolation of the murine Stat2 cDNA

The seven known human STATs consist of several conserved domains and a divergent C-terminal TAD. The conserved domains, which exhibit a high degree of identity between mouse and man (85–90%), include a multimerization (i.e. N-terminal) domain, coiled-coil domain, DNA binding domain, SH2 domain, tyrosine activation domain (*P) and the TAD are indicated (12,22,23). This sequence has been deposited in GenBank (accession no. AF187231). The first coding nucleotide represents base pair 1 in the nucleotide numbering scheme. Primers 1–5, employed for the analysis of the Stat2 gene, are indicated below the sequence with numbered arrows (1, 2020-GGG TGT TAC TAC CAG GAA-2038; 2, 2050-GAA GAA CAG AGG AAA TAT TTG AAA CA-2075; 3, 2158-GTG AA T GCA GAG CTC TTG-2175; 4, 2260-TAC TCA GTA TGG GGT CCA-2243; 5, 2269-CAA GTC CTG CTG GAG CCA-2286). Tandem repeat sequence motifs identified in murine Stat2 are denoted Mu-1–Mu-6, and those identified in human Stat2 are denoted Hu-1 and Hu-2 (see Discussion).
sequences between amino acids 564 and 767 are found on a single major transcript, and strongly argue against the possibility that the Stat2 gene gives rise to another significant murine Stat2 transcript (e.g. one with a C-terminus more homologous to HuStat2). Confirming this observation, both conserved and divergent sequences of MuStat2 map to the same genetic locus in a different strain of mouse (i.e. 129SVJ mice; data not shown).

Characterization of the murine Stat2 protein

Having provided strong evidence that the murine Stat2 gene gives rise to a single surprisingly divergent transcript, we proceeded to characterize the protein it encodes. An antibody, directed against both conserved and divergent amino acids (i.e. 672–925), was generated and evaluated on extracts prepared either from human or murine cell lines (see Fig. 3). A human cell line (i.e. 2TGH) transfected with the murine Stat2 cDNA was included in the analysis (lane 9). The murine-specific antibody detected a single ~130 kDa protein in each of the wild-type strains represented by the murine cell lines examined. This larger molecular weight contrasts with the 113 kDa size of the human Stat2 protein, but is consistent with the larger open reading frame. Notably, this protein was absent in ES cells in which both alleles of the Stat2 gene had been targeted for deletion by homologous recombination (lane 4; C.Park and C.Schindler, manuscript in preparation). Moreover, the ~130 kDa endogenous murine Stat2 band is identical in size to the one detected in human cells transfected with the murine Stat2 cDNA (lane 9). Some additional minor bands, evident in PMEFs, are likely to represent proteolytic breakdown products, as their presence varies considerably between preparations. When this filter was reprobed with the HuStat2 antibody (Fig. 3A, lower panel), a distinct 113 kDa band was detected in each of the human cell lines. A minor band of ~100 kDa was detected in each of the murine extracts. However, this band was also found in Stat2 null cells and is therefore likely to represent a non-specific band.

To determine whether the endogenous MuStat2 protein can become activated (i.e. tyrosine phosphorylated), extracts were prepared from NIH 3T3 cells before and after stimulation with IFN-α. When these extracts were immunoprecipitated with the murine-specific antibody, MuStat2 was found to be tyrosine phosphorylated in stimulated human cells (lanes 6 and 7). Likewise, control preparations of HuStat2 were tyrosine phosphorylated in IFN-α stimulated cells (Fig. 3B, lanes 4 and 5). Again, both antibodies were species specific. These studies demonstrate that endogenous MuStat2 is specifically tyrosine phosphorylated in response to stimulation with IFN-α, and provide additional evidence that it is bona fide murine Stat2.

Next, the ability of these proteins to participate in the formation of ISGF-3 was examined. Extracts were prepared from 2TGH (human) cells expressing MuStat2 before and after stimulation with IFN-α. Control extracts were prepared from IFN-α-stimulated 2TGH or NIH 3T3 (murine) cells. Consistent with the larger size of murine Stat2, murine ISGF-3 (MuISGF-3) exhibits a notably slower mobility than human ISGF-3 (HuISFG-3; Fig. 4A). Moreover, expression of MuStat2 in 2TGH cells (Fig. 4A) leads to the induction of a new ‘larger’ DNA binding complex, which co-migrates with MuISGF-3. A faster migrating HuISGF-3 complex is also evident in these extracts. Antibody addition (i.e. ‘supershift’) studies confirm that the faster...
migrating complex is composed of human Stat1 and human Stat2, whereas the slower migrating complex is composed of human Stat1 and murine Stat2. Under these conditions, the HuStat2 antibody does exhibit some modest reactivity to MuStat2. Similar results were obtained when native MuISGF-3 and HuISGF-3 were evaluated by antibody addition (see Fig. 4B).

Another property of STATs is their ligand-dependent nuclear translocation. To determine whether endogenous MuStat2 translocates to the nucleus in response to stimulation with IFN-α, immunofluorescence studies were carried out. NIH 3T3 cells were stained with the murine Stat2 and control Stat1 antibodies before and after stimulation with IFN-α (Fig. 5).

As previously reported for HuStat2 (15), MuStat2 is predominantly cytoplasmic in unstimulated cells (Fig. 5C). Following IFN-α treatment, there is a dramatic redistribution of MuStat2 to the nucleus (Fig. 5D). Control experiments with an antibody that recognizes murine Stat1 provided similar results (Fig. 5A and B). As previously reported, some Stat1 was found in the nucleus of unstimulated cells (Fig. 5A; 15,19). This may reflect the role of Stat1 in transducing other signals (42). The studies in this section demonstrate that the protein encoded by the MuStat2 cDNA is activated by IFN-α.

The MuStat2 C-terminus contains a TAD

The C-terminus of HuStat2 has been shown to encode a TAD that is required for ISGF-3 function (12). Since ISGF-3 appears to be critical in mediating the biological response to type I IFNs in both the murine and human systems, the divergent C-terminus of MuStat2 should be expected to encode a TAD as well. To test this, the C-terminal 201 amino acids of MuStat2 were fused to the DNA binding domain of transcriptionally inactive GAL4 (34). The ability of this chimeric transcription factor to drive expression of a GAL4-driven reporter was then assayed in NIH 3T3 cells (24,25). As shown in Figure 6A, the MuStat2–GAL4 fusion promoted a robust ~50-fold induction of the luciferase reporter. This compared favorably with the transcriptional activation potential of the control VP16–GAL4 fusion (24). Likewise, a HuStat2–GAL4 fusion construct that included the C-terminal 116 amino acids, known to encode a TAD [D.Levy, personal communication; (12)], also potently induced the luciferase reporter (~30-fold).

To evaluate the transcriptional activation potential of MuStat2 under more physiological conditions, Stat2-defective human U6A cells (32) were reconstituted with either MuStat2 or HuStat2 cDNAs. The ability of these cells to respond to IFN-α was then evaluated by induction of a well-characterized ISRE-driven luciferase reporter (35). As has been previously demonstrated (32), HuStat2 fully restored the ability of U6A cells to promote the IFN-α-dependent induction of the ISRE-driven reporter gene (~7-fold above unstimulated cells). Consistent with the preceding GAL4 fusion assay (Fig. 6A) and EMSA (Fig. 4), MuStat2 was also able to fully reconstitute the ability of U6A cells to respond to IFN-α (also ~7-fold above unstimulated cells; Fig. 6B). In contrast, U6A cells transfected with a MuStat2 construct missing the most C-terminal 44 amino acids were unable to respond to IFN-α (data not shown). These studies indicate that despite its sequence divergence, MuStat2
those that bound both the C-termini equivalently; those that
interact with these C-termini are the same. However, proteins
with molecular weights of ~250 and ~52 kDa preferentially
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These observations suggest that although these divergent Stat2
C-termini bind to many similar proteins, they also exhibit
distinct binding preferences. It is intriguing to speculate that a
need for Stat2 to bind other species-specific proteins may have
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**DISCUSSION**

Studies over the past 30 years have highlighted the important
role IFNs play in providing protection against viral infections
in both the murine and human system (1,43). This response is
dependent on the induction of genes. Characterization of the
ability of human type I IFNs to induce target genes led to the
identification of the first two members of the STAT family of
transcription factors (31,44,45). Subsequent studies have iden-
tified seven mammalian members of the STAT family, which
transduce vital signals for all cytokines (2,3,46). Although
STAT family members vary in sequence, murine and human
homologs exhibit a high degree of sequence homology. In fact,
the similarity between the sequences and chromosomal locations
of STATs in these two species has led to the hypothesis that
multiple duplications of a single primordial STAT gene gave
rise to the entire family of seven genes (47).

Until recently, IFN-induced STAT signals have been charac-
terized predominantly in the human system. However, in mice,
which offer a number of advantages in the study of signaling
cascades, IFNs stimulate an analogous biological response.
Recently for example, deletion of the IFN-α receptor and Stat1
gene in mice have provided important insights into IFN
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**The C-termini of murine and human Stat2 bind distinct
proteins**

TADs are believed to promote transcription by interacting with
co-activators and the basal transcriptional machinery. To
determine whether the divergent C-termini of murine and
human Stat2 bind to a similar or differing set of nuclear
proteins, GST pull-down studies were carried out. GST fusions
with the C-termini of HuStat2 (i.e. amino acids 691–851) and
MuStat2 (i.e. amino acids 690–925) were incubated with nuclear extracts prepared from metabolically labeled
(i.e. [35S]methionine) NIH 3T3 and HeLa cells. For both cell
types, several categories of interacting proteins were identified:
those that bound both the C-termini equivalently; those that
bound specifically with the murine target; and those that bound
specifically with the human target. Some differences in the
proteins that bind each of the GST fusion proteins were observed when HeLa and NIH 3T3 cell extracts were
compared. Moreover, IFN-α stimulation did not affect the
pattern of binding (data not shown). A representative experiment
with extracts prepared from unstimulated HeLa cells, which
always provided a stronger signal (i.e. because they grow at a
significantly higher density), is presented in Figure 7. Consistent
with the functional equivalence between the C-termini of murine and human Stat2 (see above), the majority of proteins that
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with molecular weights of ~250 and ~52 kDa preferentially
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**Figure 4.** Murine Stat2 forms a distinct DNA binding complex. (A) Recombinant
murine Stat2 is a component of ISGF-3. Whole cell extracts were prepared
from 2TGH cells transfected with a murine Stat2 cDNA (2TGH/MuSt2
cDNA) before (−) and after (+) stimulation with HuIFN-α (t = 15 min) as indicated.
DNA binding activity was evaluated with an ISG15 ISRE probe either before
(Prel.) or after the addition of antibodies specific for murine Stat2 (α-MuSt2),
Stat1 (α-St1), or human (α-HuSt2) as indicated. The mobility of murine (Mu)
and human (Hu) ISGF-3 is indicated in the left margin. (B) Endogenous
murine Stat2 is a component of ISGF-3. Whole cell extracts were prepared
from HeLa (Hu) cells or murine splenocytes (Mu) before (Unstim.) and after
stimulation with IFN-α (t = 15 min) as indicated. DNA binding activity was
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associates with Stat1, translocates to the nucleus, and participates in the formation of ISGF-3. As anticipated, when Tyr689, which corresponds to the tyrosine known to mediate the activation of HuStat2 [i.e. Y690; (5)] is mutated to Phe, MuStat2 fails to become activated (J.Valens and C.Schindler, unpublished observation). Additionally, studies in 2fTGH cells confirm that murine Stat2 is responsible for the significantly slower mobility of murine ISGF-3 in DNA binding studies.

Biological responses to type I IFNs, including the activation of target genes, are well conserved between the murine and human species. Studies in human cells have demonstrated that the C-terminus of Stat2 provides the TAD for ISGF-3-regulated genes. Likewise, GAL4 fusion experiments and studies in U6A cells demonstrate that the divergent murine Stat2 C-terminus encodes a potent TAD (Fig. 6). Consistent with studies on human Stat2 (12), experiments with C-terminally truncated MuStat2 (i.e. deletion of the terminal 44 amino acids), or with a chimeric Stat2 where the murine and human C-termini have been exchanged at amino acid 672, indicate that the murine C-terminal residues are critical for transcriptional activation (C.Park and C.Schindler, unpublished observation). Closer inspection of the murine Stat2 sequence reveals that much of the divergence can be attributed to six tandem copies of a novel 16 amino acid Leu/Pro-rich repeat (APQVLLEP·APQVQLEP) between amino acids 756 and 851 (see Fig. 1, repeats Mu-1–Mu-6). This repeat appears to represent a less well conserved eight amino acid tandem duplication. This is particularly evident in the most divergent repeat 5 (denoted Mu-5a and Mu-5b). HuStat2 does not encode such a repeat motif, but there are two shorter (i.e. 10–11 amino acid) and less well conserved, Leu/Pro-rich motifs between amino acids 714 and 737 (see Fig. 1, repeats Hu-1 and Hu-2). The role of these repeat motifs in transcriptional activation is not clear, since deletional studies have mapped the TAD distal to these repeats. Perhaps these repeats mediate the interaction with species-specific proteins important for some aspects of Stat2 biology (see below).

STAT sequence comparisons have determined that Stat2 contains the largest C-terminus, far more than appears to be required for an active TAD. In fact, only the most C-terminal residues are likely to represent the TAD that associates with the basal transcriptional apparatus as well as an association with transcriptional activators [e.g. YY-1, p300/CBP and MCM5; (50–53)]. The remaining residues (i.e. those more amino proximal) may mediate other, potentially more species-

**Figure 5.** Murine Stat2 translocates into the nucleus in response to IFN-α. NIH 3T3 cells, before (A and C) or after (B and D) stimulation with IFN-α A/D (t = 15 min), were stained with either a Stat1 (α-Stat1, A and B)- or murine Stat2 (α-Stat2, C and D)-specific antibodies. Cells were visualized using a Cy-3-conjugated secondary donkey anti-rabbit antibody and visualized after excitation at 568 nm by a Zeiss LSM410 laser scanning confocal microscope.
specific protein–protein interactions. Consistent with the possibility that murine and human Stat2 evolved to mediate interaction(s) with additional proteins that are unique to each species, murine and human Stat2 C-terminal GST fusion proteins bind to overlapping, but distinct sets of nuclear proteins. These observations support a model in which the specific proteins provided the evolutionary pressure for these sequences to rapidly diverge. However, despite these differences, murine and human Stat2 mediate a conserved biological response to type I IFNs.

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