Arginine/Lysine-rich Nuclear Localization Signals Mediate Interactions between Dimeric STATs and Importin α5∗

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Interferon stimulation results in tyrosine phosphorylation, dimerization, and nuclear import of STATs (signal transducers and activators of transcription). Proteins to be targeted into the nucleus usually contain nuclear localization signals (NLSs), which interact with importin α. Importin α binds to importin β, which docks the protein complex to nuclear pores, and the complex translocates into the nucleus. Here we show that baculovirus-produced -activated STAT1 homodimers and STAT1-STAT2 heterodimers directly interacted with importin α5 (NPI-1). This interaction was very stable and was dependent on lysines 410 and 413 of STAT1. Only STAT dimers that had two intact NLS elements, one in each monomer, were able to bind to importin α5. STAT-importin α5 complexes apparently consisted of two STAT and two importin α molecules. STAT NLS-dependent colocalization of importin α5 with STAT1 or STAT2 was seen in the nucleus of transfected cells. γ-Activated sequence DNA elements efficiently inhibited STAT binding to importin α5 suggesting that the DNA and importin α binding sites are close to each other in STAT dimers. Our results demonstrate that specific NLSs in STATs mediate direct interactions of STAT dimers with importin α, which activates the nuclear import process.

Signal transducers and activators of transcription (STATs) are latent transcription factors that are activated by cytokines and certain growth factors. Presently seven mammalian STAT proteins have been described. Binding of cytokines to their specific cell surface receptors leads to the activation of the Janus tyrosine kinase (JAK)-STAT pathway (1, 2). In response to type I IFN (IFN-α, -β, and -ω) stimulation, IFN-α/β receptor-associated JAK1 and Tyk2 are phosphorylated and activated (2–4). Activated JAKs in turn tyrosine-phosphorylate STAT associated JAK1 and Tyk2 are phosphorylated and activated (2–4). Activated JAKs in turn tyrosine-phosphorylate STAT1 and STAT2 at Tyr-701 and Tyr-690, respectively, which results in dimerization and nuclear translocation of STAT1-STAT2 heterodimers. In the nucleus STATs interact with IRF-9/p48 protein to form ISGF3 complexes, which bind to well conserved interferon-stimulated response elements in the promoter regions of IFN-α/β-responsive genes and activate transcription (5–8). Binding of type II IFN (IFN-γ) to its receptor leads to the activation of JAK1 and JAK2 and tyrosine phosphorylation of STAT1 (also at Tyr-701). Activated STAT1 forms homodimers, which translocate into the nucleus and bind to GAS elements and activate transcription of IFN-γ-inducible genes (1, 2). Although the structure-function relationships of STATs have been carefully analyzed, the mechanisms of nuclear import of this important group of transcription factors have remained less well characterized. Recently we and others have shown that STAT1 and STAT2 have an arginine/lysine-rich nuclear localization signal (NLS) that mediates their nuclear translocation in dimeric complexes (9, 10).

Active nuclear transport of large macromolecules occurs via the nuclear pore complex (11). Proteins to be imported into the nucleus usually contain a mono- or bipartite basic-type NLS, which binds to a specific NLS receptor, importin α (12–14). The N-terminal importin β binding (IBB) domain of importin α interacts with importin β (15), which mediates the docking of NLS-containing cargo-importin αβ complex to the cytoplasmic side of the nuclear pore, and the complex translocates into the nucleus (16, 17). Inside the nucleus RanGTPase is involved in the disassembly of the cargo-importin complex (14, 18, 19). IFN-γ-induced nuclear import of STAT1 has been suggested to be dependent on one importin α subtype, importin α5 (20), and the RanGTPase (21). However, the elements that regulate STAT-importin α5 interactions have remained elusive.

In the present work we show, by using a baculovirus-reconstituted STAT activation system, that homodimeric STAT1 or heterodimeric STAT1-STAT2 complexes directly interact with importin α5. The interaction of STAT dimers with importin α is very stable and is dependent on NLS situated in the DNA binding domain of STATs. The STAT-importin α5 complex consists of two importin α and two STAT molecules. STAT-binding GAS oligonucleotides efficiently prevent the binding of dimeric STATs with importin α. We also demonstrate by confocal microscopy that wild type STATs colocalize with importin α, whereas NLS-mutated STATs do not.

MATERIALS AND METHODS

**Cells**—Monolayers and suspension cultures of *Spodoptera frugiperda* Sf9 cells that were used for baculovirus expression were maintained in TNM-FH medium as described previously (22). Human hepatocellular carcinoma HuH7 (23) cells were maintained in minimal essential medium supplemented with 0.6 mg/ml penicillin, 60 mg/ml streptomycin, 2 mM glutamine, 20 mM HEPES buffer, pH 7.4, and 10% fetal calf serum (Integro, Zaandam, the Netherlands). In transfection experiments the cells were cultured in the growth medium supplemented with 2% fetal calf serum.

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**Antibodies**—In Western blot analysis rabbit anti-Tyk2 (H-135, 1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-STAT1 (c-24, 1:2000; Santa Cruz Biotechnology), rabbit anti-STAT2 (c-20, 1:2000; Santa Cruz Biotechnology), rabbit anti-phosphotyrosine (PY99, 1:200; Santa Cruz Biotechnology), and mouse monoclone anti-FLAG (M2, 1:500; Sigma) antibodies were used. Cell Signaling Technology antibodies were used as suggested by the manufacturer. Anti-influenza A nucleoprotein (NP) antibodies (24) were used at 1:500 dilution. GST-specific antibodies were prepared by immunizing rabbits subcutaneously with *Escherichia coli*-produced, preparative SDS-PAGE-purified GST (100 μg/rabbit/immunization) for four times at 3-week intervals. The rabbits were bled at 1 week after the last immunization. Anti-GST antibodies were used at a 1:500 dilution. In Western blotting secondary biotin-SP-conjugated goat anti-rabbit or anti-mouse antibodies (1:10,000 dilution; Jackson ImmunoResearch Laboratories) and horseradish peroxidase-conjugated streptavidin (1:2000 dilution; Jackson ImmunoResearch Laboratories) were used.

For confocal laser microscopy mouse anti-phosphotyrosine)-STAT1 (clone 4G10, 1:1000) and 2H7 (1:100; kindly provided by D. Pale), and anti-FLAG M5 (1:500; Sigma) antibodies were used. FITC- and TRITC-labeled goat-mouse and rabbit-immunoglobulins were used as secondary antibodies (1:100 dilution; Cappel, Organon Teknika Co., West Chester, PA and Jackson ImmunoResearch Laboratories, respectively).

**Puriﬁcation**—The wild type and mutant STAT gene constructs in Flag-tagged (25) pCDNA 3.1(+) expression vector (Invitrogen) were as described previously (9). Human importin α gene (Ref. 26, GenBankTM accession number NM_002264) was PCR-modified with oligonucleotides AAAAAAAGTACCCAGTACCCCAAGGAAAAGACTTTTTGATCCCTTCAAGCTGGAAACCTCCATAAGGG (3’ oligonucleotide) to create BamHI cloning sites (in both on both sides of the gene coding region). BamHI digestion the insert was cloned into Flag-tagged pCDNA 3.1(+) vector (25). All DNA manipulations were performed according to standard protocols, and the newly created gene constructs were partially sequenced. Point mutations to genes were done directly in Flag-tagged expression vectors using the QuikChangeTM site-directed mutagenesis kit (Stratagene, La Jolla, CA).

**Baculovirus Expression**—Human Tyk2 cDNA—baculovirus expression plasmid pVL1923 was kindly provided by Dr. Sandra Pellegren (Institute Pasteur, Paris, France). Recombinant Tyk2 baculovirus was obtained by cotransfection of Sf9 cells (22) with the expression plasmid and BaculoGold DNA reagent (BD PharMingen). wt STAT1, wt STAT2, and mutants STAT1 K410A,K413A and STAT1 Y701A baculovirus constructs were as described previously (9). Influenza A (PR8) virus NP was produced similarly. Virus-infected Sf9 cells were coinfected and recombinant viruses were obtained by plaque purification as described previously (22). For protein production Sf9 cells were mixed with E. coli cell extracts containing Flag-tagged -produced GST-importin α. Sepharose-immobilized GST-importin α. Sepharose-immobilized GST-importin α-bound proteins were boiled in Laemmli sample buffer, separated by SDS-PAGE, and analyzed by Western blotting as described above.

**Gei Filteration and Protein Oligomerization Analysis**—Baculovirus-infected Sf9 cells or E. coli lysates were gel ﬁltrated in the above lysis buffer followed by 24-ml Superose 12 fast protein liquid chromatography (FPLC) (Amersham Biosciences) gel filtration column. To study STAT-importin α complex formation STAT protein-containing baculovirus cell extracts were mixed with E. coli cell extracts containing GST-importin α. Proteins in gel filtration fractions were separated by 8% SDS-PAGE and transferred to nitrocellulose filters followed by staining with anti-GST, anti-STAT1, anti-phospho-STAT1, and anti-phosphotyrosine antibodies as described above. To estimate the relative amounts of STAT and importin α proteins in STAT1/STAT2-importin α complexes, the proteins in gel ﬁltration fractions were separated by SDS-PAGE followed by Coomassie Blue staining. Quantitation of Coomassie Blue-stained protein bands was carried out with the Kodak phosphorimager documentation and analysis system 120. The MW-GF-200 kit for molecular weights (Sigma) was used as gel ﬁltration markers.

**Transfections and Confocal Laser Microscopy**—HuH7 cells were grown on glass coverslips or transfected with importin α and wt or mutant Flag-STAT1- or Flag-STAT2 pCDNA 3.1(+) gene constructs (9) using FuGENETM 6 transfection reagent (Roche Molecular Biochemicals). At 48 h after the transfection the cells were treated with human leukocyte IFN-α (1000 IU/ml; Ref. 28) for 30 min. Cells were fixed and stained as described previously (29) using monoclonal anti-phosphotyrosine)-STAT1, anti-STAT2 (monoclonal anti-FLAG), and rabbit anti-importin α antibodies and FITC- and TRITC-conjugated secondary antibodies. The cells positive for STAT or importin α protein were visualized on a Leica TCS NT confocal microscope.

**RESULTS**

**Expression and Tyrosine Phosphorylation of STAT Proteins in Baculovirus System**—To study possible interactions of wild type or NLS-mutated STATs with importins we used a baculovirus expression system to reconstitute the STAT activation system. We created a Tyk2 baculovirus construct that was found to express Tyk2 protein in relatively high levels. Coinfection of Sf9 cells with recombinant Tyk2 baculovirus and STAT1, STAT2, or NLS mutant STAT1 protein-expressing baculoviruses resulted in efficient expression and tyrosine phosphorylation of wt STAT1, wt STAT2, and STAT1 Y701A proteins, whereas the STAT1 Y701A mutant protein completely lacked tyrosine phosphorylation (Fig. 1). Tyrosine-phosphorylated wt STAT1 or STAT1 K410A,K413A formed dimers (result not shown and Ref. 9), which enabled us to analyze potential interactions of dimeric STAT complexes with importins.

**Binding of STAT1 Homodimers or STAT1/STAT2 Heterodimers to Importin α Is Regulated by a Lysine-rich NLS of STAT1—IFN-induced nuclear import of STAT1 has been suggested to be mediated by importin α (20). We recently proposed that STAT proteins have a well conserved arginine/lysine-rich NLS in their DNA binding domain that regulates IFN-induced nuclear import of STAT1 has been suggested to be mediated by STAT1 (20). We recently proposed that STAT proteins have a well conserved arginine/lysine-rich NLS in their DNA binding domain that regulates IFN-induced nuclear import of STAT1 has been suggested to be mediated by importin α (20). We recently proposed that STAT proteins have a well conserved arginine/lysine-rich NLS in their DNA binding domain that regulates IFN-induced nuclear import of STAT1. In Western blot analysis rabbit anti-Tyk2 (H-135, 1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-STAT1 (c-24, 1:2000; Santa Cruz Biotechnology), rabbit anti-phosphotyrosine (PY99, 1:200; Santa Cruz Biotechnology), and mouse monoclone anti-FLAG (M2, 1:500; Sigma) antibodies were used. Cell Signaling Technology antibodies were used as suggested by the manufacturer. Anti-influenza A nucleoprotein (NP) antibodies (24) were used at 1:500 dilution. GST-specific antibodies were prepared by immunizing rabbits subcutaneously with *Escherichia coli*-produced, preparative SDS-PAGE-purified GST (100 μg/rabbit/immunization) for four times at 3-week intervals. The rabbits were bled at 1 week after the last immunization. Anti-GST antibodies were used at a 1:500 dilution.
modimeric STAT1 K410A,K413A, or heterodimeric STAT1 K410A,K413A-STAT2 complexes completely failed to bind to importin. Tyrosine-phosphorylated STAT2 was also devoid of importin binding activity suggesting that STAT2 can only dimerize with STAT1. No binding of STATs to importin was seen (see Fig. 7 and results not shown).

To study the stability of STAT-importin complex we washed Sepharose-bound importin-STAT complexes with buffers containing high concentrations of NaCl or urea. Some reduction in the amounts of importin-bound STAT1 or STAT1/STAT2 dimers was seen after washing with 1 or 2 M NaCl, whereas 2 M urea was not able to disrupt the STAT-importin complex (Fig. 3). In higher urea concentrations Sepharose-bound STATs were released, but apparently this was due to the release of GST-importin fusion protein from the resin (Fig. 3). Influenza A virus NP that is known to bind to importin (26) was used as a positive control in binding stability experiments.

Colocalization of STATs with Importin α—Biochemical evidence suggested that STAT1 binding to importin α5 takes place via lysine residues at positions 410 and 413 of the STAT1 protein. To study whether the arginine/lysine-rich NLS of STAT1 or STAT2 regulates STAT interactions with importin α5 also in cultured cells we carried out colocalization experiments with transfected STAT and importin α5 gene constructs.

We used confocal laser microscopy to analyze the colocalization of wt and NLS-mutated STATs in transfected human HuH7 hepatoma cells. In transiently transfected and IFN-α-treated (1000 IU/ml, 45 min) cells tyrosine-phosphorylated STAT1 clearly colocalized with STAT2 in the cell nucleus (Fig. 4). When nuclear import-defective STAT1 K410A,K413A or STAT2 R409A,K415A were cotransfected with heterologous wt STAT gene constructs no IFN-α-induced nuclear accumulation of STATs was seen. However, mutant and wt STAT forms colocalized in the cell cytoplasm (Fig. 4) suggesting that STAT1 and STAT2 directly interact with each other. The data indicates that NLS-defective STAT1 functions as a dominant negative for nuclear import of wt STAT2 and vice versa.

Next we transfected HuH7 cells with importin α5 and wt or NLS-mutated STAT gene constructs. Transfected cells were

Fig. 1. Production of dAK STAT proteins by baculovirus expression system. Sf9 insect cells were infected with Tyk2, STAT1, STAT2, or STAT mutant protein-expressing recombinant baculoviruses for 42 h in combinations as shown in the figure. The proteins in Sf9 cell extracts (10 μg) were separated by 8% SDS-PAGE, transferred to nitrocellulose filters, and stained with anti-STAT1, anti-Tyk2, anti-phosphotyrosine-STAT1, anti-STAT2, or anti-phosphotyrosine antibodies. P, phospho; —, constructs at left coexpressed with Tyk2.

Fig. 2. Binding of Tyk2-activated STATs to importin α5. Baculovirus-infected Sf9 cell extracts containing Tyk2 and STAT proteins in various combinations (as shown) were allowed to bind to Sepharose-immobilized GST-importin α5 for 2 h at +4 °C. Unbound protein was washed away, Sepharose-bound proteins were dissolved in Laemmli sample buffer, and proteins were separated by 8% SDS-PAGE. Proteins were transferred to nitrocellulose filters and stained for STAT1 (A) or STAT1 and STAT2 (B) proteins as indicated in the figure. A similar gel was also stained with Coomassie Brilliant Blue (C) to visualize the amount of Sepharose-immobilized GST-importin α5 as well as STAT1 and STAT2. P, phospho; imp, importin; —, constructs at left coexpressed with Tyk2.

Fig. 3. Stability of STAT-importin α5 complexes. Baculovirus-infected Sf9 cell extracts containing activated STAT1 or STAT1/STAT2 dimers or influenza A virus NP protein were allowed to bind to Sepharose-immobilized GST-importin α5. The beads were washed with the IP buffer, 1 M NaCl, 2 M NaCl, 2 M urea, or 4 M urea followed by SDS-PAGE and Western blotting with antibodies as indicated in the figure. The Coomassie Brilliant Blue-stained gel is shown to visualize the amount of Sepharose-bound GST-importin α5. P, phospho; imp, importin.
treated with IFN-α for 45 min, and the cells were fixed and stained with STAT- and importin α5-specific antibodies. wt STAT1 or STAT2 was found to colocalize with importin α5 especially in the cell nucleus. Such a colocalization was not observed between importin α5 and NLS-mutated STAT1 K410A,K413A proteins (Fig. 5) suggesting that STAT1-importin α5 interaction is mediated by lysines 410 and 413 of STAT1 also in living cells. Similar results were observed with NLS-mutated STAT2 R409A,K415A protein. While in IFN-α-stimulated cells wt STAT2 was transported into the nucleus with intrinsic STAT1, the NLS-mutated STAT2 was not (Fig. 5).

Comigration of Importin α5-STAT Complexes in Gel Filtration—As shown above we have demonstrated that dimeric STATs bind to importin α5 in an NLS-dependent manner (Fig. 2). To estimate the molecular size and protein composition of importin α5-STAT complexes we carried out comigration experiments using gel filtration. We mixed Tyk2-activated STAT cell extracts with E. coli-produced importin α5 and analyzed the migration pattern of these complexes by FPLC using a Superose 12 gel filtration column. First GST-importin α5 (by itself) was subjected to gel filtration analysis, and it was found in fractions corresponding to 70–90-kDa proteins (Fig. 6A)

indicating that importin α existed as a monomer. Unphosphorylated STAT1 and phosphorylated STAT1 dimers eluted in the range of 90 and 160–200 kDa, respectively (Fig. 6A).

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In the importin/H9251/H11001 complex, (stained with Coomassie Brilliant Blue, and quantitated. Blue dextran B STAT1/STAT2 complexes (tyrosine or anti-GST antibodies. by 8% SDS-PAGE followed by Western blot analysis with anti-phosphotyrosine column. Protein samples in gel filtration fractions were separated the complex contained twice as much GST-importin/H9251/H11001 Sf9 cells were infected with STAT1, Tyk2/H9251/H11001 STAT2 protein-expressing recombinant baculoviruses. After 42 h the cells were collected, cell extracts were prepared, and protein samples were subjected to a Superose 12 gel filtration column by themselves or after binding the proteins to Sepharose-immobilized E. coli-produced GST-importin/α5. The gel filtration profile of GST-importin/α5 was also analyzed. Samples from gel filtration fractions were subjected to 8% SDS-PAGE followed by Western blot analysis with anti-GST, anti-STAT1, anti-phospho(tyrosine)-STAT1, or anti-phosphotyrosine antibodies as shown in the figure. A, gel filtration patterns of Sf9 cell-produced monomeric STAT1 (unphosphorylated, expressed alone) and phosphorylated STAT1 dimers (coexpressed with Tyk2 and E. coli-produced GST-importin/α5 protein. B, gel filtration pattern of GST-importin/α5-STAT1/STAT2 complexes. STAT1/STAT2 complexes were allowed to bind to immobilized importin/α5. Glutathione-Sepharose-bound GST-importin/α5-STAT1/STAT2 complexes were released by glutathione treatment followed by analysis on a Superose 12 gel filtration column. Protein samples in gel filtration fractions were separated by 8% SDS-PAGE followed by Western blot analysis with anti-phosphotyrosine or anti-GST antibodies. C, peak fractions of importin/α5-STAT1/STAT2 complexes (B, arrow) were separated by 8% SDS-PAGE, stained with Coomassie Brilliant Blue, and quantitated. Blue dextran (void), β-amylase (200 kDa), and bovine serum albumin (66 kDa) functioned as molecular mass markers. p, phospho; imp., importin.

In the present work we have demonstrated that activated STAT dimers are able to directly bind to two importin/α5 (NPI-1) molecules with relatively high affinity. STAT1-importin/α5 interaction was regulated by dimerization of STATs and by a lysine-rich element in the DNA binding domain of STAT1 since the mutation of lysines 410 and 413 to alanines completely abolished STAT1 binding to importin/α5. By confocal laser microscopy we also found that in IFN-α-stimulated cells STAT1 and STAT2 colocalized with importin/α5 in the cell nucleus. Consistent with biochemical analysis NLS-mutated STAT1 or STAT2 failed to show colocalization with importin/α5. In addition, we observed that STAT1-specific GAS oligonucleotide was able to inhibit STAT1-importin/α5 interaction suggesting that STAT1 binding sites to importin/α5 or target DNA elements are very close to each other.

It is well established that STATs have to undergo cytokine receptor-mediated tyrosine phosphorylation by JAKs and dimerization via phosphotyrosine residues and Src homology 2 domains of each of the monomers before nuclear translocation can take place (2, 31). The key regulatory event controlling the nuclear import of STATs appears to be dimerization (32, 33). Sekimoto and coworkers (20) demonstrated that importin/α5 interacted with activated STATs, but in their work specific STAT NLSs were not identified. Now we know more of the details of the regulation of nuclear import and export of STATs. Mutational analyses revealed that lysines 410 and 413 of STAT1 and corresponding basic residues of STAT2 regulate IFN-induced nuclear import of STAT1 homodimers and STAT1/STAT2 heterodimers (9, 10). Several groups have also shown IFN-independent nuclear localization of STAT1, which of STAT1 is situated immediately adjacent to the STAT1 DNA binding site (9, 30). It is thus possible that STAT1-specific GAS DNA elements would compete with the binding of STAT complexes to importin/α5. Preincubation of activated STAT1 with different concentrations of GAS oligonucleotide clearly inhibited the binding of STAT1 dimers to Sepharose-immobilized importin/α5. Consensus NF-κB oligonucleotide that was used as a control DNA did not inhibit STAT1 binding to importin/α5 (Fig. 7).

**DISCUSSION**

In the present work we have demonstrated that activated STAT dimers are able to directly bind to two importin/α5 (NPI-1) molecules with relatively high affinity. STAT1-importin/α5 interaction was regulated by dimerization of STATs and by a lysine-rich element in the DNA binding domain of STAT1 since the mutation of lysines 410 and 413 to alanines completely abolished STAT1 binding to importin/α5. By confocal laser microscopy we also found that in IFN-α-stimulated cells STAT1 and STAT2 colocalized with importin/α5 in the cell nucleus. Consistent with biochemical analysis NLS-mutated STAT1 or STAT2 failed to show colocalization with importin/α5. In addition, we observed that STAT1-specific GAS oligonucleotide was able to inhibit STAT1-importin/α5 interaction suggesting that STAT1 binding sites to importin/α5 or target DNA elements are very close to each other.

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appears to occur constitutively and by a different mechanism than that of IFN-induced import of STAT1 (9, 10, 34). Nuclear export of STAT1 is regulated by CRM1/exportin 1 protein, which binds to a DNA-free form of STAT1 via a leucine-rich consensus-like nuclear export signal situated at positions 400–409 of STAT1 (35). It is of great interest that the STAT1 nuclear export signal is in the immediate vicinity of its NLS residing at positions 410–413 (9, 35). Mutations in the STAT1 nuclear export signal may also interfere with STAT1-importin α interaction (36).

In the present work previous cell biological observations (9, 10) were extended to a biochemical level. For these studies we chose to use the baculovirus expression system since it has been shown to efficiently produce biologically active components of the JAK-STAT pathway (9, 37). Here we show that importin α can directly bind to STAT1 homodimers or STAT1/STAT2 heterodimers evidently with a relatively high affinity since even high molar concentrations of NaCl or urea are not able to disrupt the interaction of STAT dimers with importin α. We also show that no binding of monomeric STAT1 to importin α is taking place. In addition, neither monomeric nor dimeric STAT binding site. The major binding site (arm 2–4) functions as the binding site for STAT1, but rather it is the C-terminal end of importin α that regulates STAT1-importin α interaction. To reveal the question of STAT binding site(s) in importin α a more fine-tuned mutational analysis should be carried out. Alternatively, a three-dimensional structural analysis of the STAT-importin α complex should be obtained.

In our previous study we observed that the nuclear import-defective STAT1 K410A,K413A mutant was also defective in its DNA binding activity to GAS or interferon-stimulated response elements (9). This prompted us to study whether GAS oligonucleotides would be able to interfere with STAT-importin α interaction. We observed that high concentrations of GAS DNA was able to almost completely inhibit STAT1 dimer binding to importin α. This suggests that STAT1 binding to importin α or target DNA occurs at sites that are very close to each other.

In the present work we have taken a clear step forward in understanding the mechanisms of nuclear import of STATs. We have evidence that dimeric STAT complexes interact with two importin α molecules via a lysine-rich conformation NLS within the STAT1 DNA binding domain. However, it still remains an open question why only STAT dimers are able to bind to importin α and which of the NLS binding sites of importin α are involved in this interaction.

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