

# Brahma-Related Gene 1-Dependent STAT3 Recruitment at IL-6-Inducible Genes<sup>1</sup>

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**IL-6 is an immunoregulatory cytokine with multiple functions in hemopoiesis, proliferation, and tumorigenesis. IL-6 triggers phosphorylation, dimerization, and nuclear translocation of STAT3, which binds to target promoters and activates transcription. Brahma-related gene 1 (*BRG1*), the enzymatic engine of the yeast-mating type-switching and sucrose-nonfermenting chromatin-remodeling complex, is essential for recruitment of STAT1 or STAT1/STAT2-containing complexes to IFN targets. We hypothesized that *BRG1* might also be required for STAT3 recruitment. In this study, we show that induction of a subset of human IL-6-responsive genes is *BRG1* dependent. *BRG1* is constitutively present at these targets and is required for STAT3 recruitment, downstream histone modifications, and IL-6-induced chromatin remodeling. IL-6-induced recruitment of STAT3 to the IFN regulatory factor 1 promoter and subsequent mRNA synthesis is *BRG1* dependent, even though IFN- $\gamma$ -mediated STAT1 recruitment to this locus is *BRG1* independent. *BRG1* also increased basal expression of IFN-induced transmembrane protein 3 and IFN- $\gamma$ -induced protein 16, and the basal chromatin accessibility at the promoter of IFN regulatory factor 1. The effect on basal expression was STAT3 independent, as revealed by small interfering RNA knockdown. Together with prior observations, these data reveal that *BRG1* has a broad role in mediating STAT accessibility at multiple cytokine-responsive promoters and exposes promoter specific differences in both the effect of *BRG1* on basal chromatin accessibility and on access of different STAT proteins to the same target. *The Journal of Immunology*, 2007, 178: 345–351.**

Chromatin remodeling is fundamental to gene regulation in eukaryotes and is mediated by two categories of enzymes that either use the energy of ATP hydrolysis to alter nucleosome structure or position, or that covalently modify histones (1). Yeast mating type switching and sucrose nonfermenting (SWI/SNF)<sup>3</sup> is one complex that uses ATP hydrolysis to regulate the position and structure of nucleosomes, which can lead to either gene activation or repression (2). There are more than 10 components of SWI/SNF (3) which can interact with a variety of activators or repressors (2).

Brahma-related gene 1 (*BRG1*) is the ATPase subunit of SWI/SNF and has been linked to differentiation, proliferation, and tumorigenesis. Homozygous inactivation of *BRG1* causes early embryonic lethality in mice and heterozygotes are prone to a variety of tumors (4–6). Previously, we showed that *BRG1* is required for induction of a subset of IFN- $\gamma$ -responsive genes (7) and subse-

quently others reported that it is also essential for induction of a subset of IFN- $\alpha$ -responsive targets (8, 9). Moreover, the BAF47 and BAF200 components of SWI/SNF are also required for IFN signaling, although the INI1 and BAF180 proteins are dispensable (10–12). These findings highlight an important role for SWI/SNF in the immune response.

In view of the findings that *BRG1* binds STAT2 and STAT3 (8, 13) and that SWI/SNF typically functions downstream of primary activators in gene induction cascades (14–21), it seemed logical that *BRG1* might act after STAT1 recruitment at IFN targets. Instead, *BRG1* is essential for STAT1 recruitment at multiple IFN- $\alpha$  and IFN- $\gamma$  targets (22). In contrast, constitutive *BRG1* recruitment is STAT1 independent (22). All of the subsequent IFN-induced events, including chromatin remodeling, histone modifications, and transcription are blocked if *BRG1* is absent (10, 22). Thus, *BRG1* has an apical role in IFN signaling.

IL-6 is an important immunoregulatory cytokine with multiple functions in hemopoietic proliferation, differentiation of B cells, and tumorigenesis. For example, it is essential for the growth and survival of B lymphocyte-derived murine plasmacytomas and human myelomas, and, most likely, IL-6 promotes such tumors by increasing proliferation and inhibiting apoptosis (23, 24). IL-6 triggers tyrosine phosphorylation and activation of JAKs. Activated JAKs phosphorylate STAT3 which forms either a homodimer or a STAT3/1 heterodimer, which translocates to the nucleus and bind to the STAT-binding site at target loci (25).

A direct link between IL-6 signaling and *BRG1* has never been established. However, *BRG1* associates with STAT3 at the p21<sup>waf1</sup> promoter (13), raising the possibility that, in addition to IFN targets, *BRG1* may have a broader role in cytokine signaling. In this study, we provide in vivo evidence that *BRG1* is required for IL-6-induced expression at a subset of target genes. As seen at IFN targets (22), *BRG1* constitutively binds to IL-6-responsive promoters and is essential for all events, including IL-6-induced STAT3 recruitment, chromatin remodeling, and covalent histone modifications. Intriguingly, *BRG1* has promoter-specific effects on basal

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<sup>3</sup> Abbreviations used in this paper: SWI/SNF, yeast mating type switching and sucrose nonfermenting; IRF1, IFN regulatory factor 1; qPCR, quantitative real-time PCR; IFI16, IFN- $\gamma$ -inducible protein 16; ChIP, chromatin immunoprecipitation; IP, immunoprecipitated; RSB, reticulocyte standard buffer; siRNA, small interfering RNA; IFITM3, IFN-induced transmembrane protein 3; MNDA, myeloid cell nuclear differentiation Ag.

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Table I. List of primers

Gene	Region	Forward (5' to 3')	Reverse (5' to 3')
<i>IFI16</i>	Promoter	CATGTTGAGAGTCCACTATCCACG	CACTAGCTGTCCCTTTGACCATGA
	Promoter (DNase I assay)	ACCCATTAAAGTAATTTCTCATCCC	CCAGTGTTTTTAGCTTTTCGTTTCT
	Exon 9	ATGGTGTCTGAACGCAACAGA	CCTTCACCTCGGAAGACTTCATTC
<i>IRF1</i>	Promoter	GCTAAGTGTTTGGATTGCTCGGTGG	TTGCCTCGACTAAGGAGTGGCGAGC
	Promoter (DNase I assay)	CGCCCGCCGAGAGGGTGGG	CCGCGCGGGCCCACTATTGGC
	Last exon	AGGCCATTCCCTGTGCACCGTAGCA	GTCCAGCTTCTCTGCACCATATCCA
<i>ISGF3G</i>	Promoter	ATGGGATCTCTGGGACCTGTT	AACTGACACTTTGGGAGGGAAAC
	Last exon	TACGCCCTCTTTCATCTGTAAGG	GGAGGTCAGGGAAGAGGGAA
<i>MNDA</i>	Promoter	TGCTCCTGCCACTCTGTGAA	GGTAGCCGTTGAATAAGGATCTTT
	Exon 6	AACTTCGACTCTTCTGCCTTCAA	GCTGTGACTTCCACACACCAGT
<i>AIM2</i>	Promoter	AGTTCCTATGTGGCATGACTCAGA	GGGTTCCTACTAGCAGCCACAG
	Last exon	GCCAACTGGTCTAAGCAGCAT	GGAGCCTGTGAAGTGCAGCT
<i>CIITA</i>	Exon 20	ACGTCCTGACAGGCAATGCTG	GGGTCCCTAGCCCACTATTCCG
<i>IFI27</i>	Last exon	TCTCCGGATTGACCAAGTTCA	CAGGGAGCTAGTAGAACCTCGC
<i>GBP1</i>	Last exon	CCAGATGACCAGCAGTAGACAAAT	GCCAGACCAATGCCAAATAT
<i>IFITM3</i>	Promoter	ATTTGTTCGGCCCTCATCTG	GTTCCTGGTTTCTCAACAGTTTCCT
	Last exon	CCATTCGTCTCATCGTCATCC	AGAGCTCCTGGCCTCAGTGA
<i>IFIT1</i>	Last exon	AAGGCAGGCTGTCCGCTTA	TCCTGTCTCTCATCCTGAAGCT
<i>6-16 (GIP3)</i>	Last exon	CAGCAGCGTCTGTCATAGGTAAT	TCCTCATCTCTCTACTATFCGAG
<i>p21<sup>Waf1</sup></i>	Last exon	GTGACAATAACAAGGCTGGCC	CTGACAGGACACTTCTGTGTGTTGA
<i>Cyclin D1</i>	Last exon	GTTGACTTCCAGGCACGGTT	GATCCTCCAATAGCAGCAACAAT
<i>UCK2</i>	Last exon	GGAGGCATCTGTCATCTGTACA	TCAAAGGCATTTTCATCTCCATG
<i>JunB</i>	Last exon	CCAGCTCAAACAGAAGGTCATG	GACGTTCCAGAAGGCGTGTC
<i>Bcl-x<sub>L</sub></i>	Last exon	CTGGGTTCCCTTTCCCTTCCA	TCCCAAGCAGCCTGAATCC
<i>Pim 1</i>	Last exon	GTCCCCTGTCACCTCTTCCGA	GGCAAGTTCAAGTATTTCTCCAG
<i>Pim 2</i>	Last exon	GATGCAAAACACCCAGCCGAG	TTAGGGTAGCAAGGACCCAGG
<i>PITX2</i>	Promoter	CTCACACCCACACTCCACAC	CAAGAGACGGAACAAGGAC
<i>β-actin</i>	Exon 4	CCGGGACCTGACTGACTACCTCATG	CAGCTTCTCTTAAATGTACAGCAGC

chromatin remodeling and, at the IFN regulatory factor 1 (*IRF1*) locus, is required for STAT3 but not STAT1 recruitment. These results reveal a broad role for *BRG1* in mediating the induction of STAT target genes and suggest locus and transcription factor-specific differences in its mechanism of action.

## Materials and Methods

### Cell culture

Human adenocarcinoma cell line SW13 and cervical carcinoma cell lines, HeLa-Ini-11 (HeLa) and C33A cells, were grown in  $\alpha$ -MEM/10% FBS as described (7). Cells were treated with 50 ng/ml IL-6 (PHC0064; BioSource International).

### Adenoviruses

Adenoviral vectors were based on pAdlox (26). Construction details and complete sequences are available on request. Vectors were used to generate adenovirus as described (26). Each virus was plaque purified to remove contaminating normal adenovirus. Virus was amplified in the 293-derived Cre8 cell line (26).

### Western blotting

SW13 cells were lysed in radioimmunoprecipitation assay buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.25% sodium deoxycholate, 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM NaF, 1 mM PMSF, 1  $\mu$ g/ml aprotinin, 1  $\mu$ g/ml leupeptin, and 1  $\mu$ g/ml pepstatin). Fifty micrograms of cell lysate was separated by 10% SDS-PAGE gel and transferred to a nitrocellulose membrane. Proteins were incubated with Abs against STAT3 (sc-483; Santa Cruz Biotechnology), p-STAT3 (Tyr<sup>705</sup>-R; sc-7993-R; Santa Cruz Biotechnology), STAT1 (06-501; Upstate Biotechnology), *BRG1* (sc-10768; Santa Cruz Biotechnology), or  $\beta$ -actin (A4511; Sigma-Aldrich), and detected by ECL with HRP-conjugated goat anti-mouse or goat anti-rabbit Ig G. Protein intensity was quantified by MultiImager (Bio-Rad) and normalized to  $\beta$ -actin.

### RNA extraction and quantitative real-time PCR (qPCR)

RNA was isolated from cells in confluent 60-mm plates using TRIzol reagent (Invitrogen Life Technologies). A 2.5- $\mu$ g aliquot of RNA was diluted in 20  $\mu$ l of diethylpyrocarbonate-treated water, heated to 90°C for 5 min, then combined with 30  $\mu$ l of first strand master mix (10  $\mu$ g of Pd(N)<sub>6</sub>,

1  $\times$  First Strand buffer (Invitrogen Life Technologies), 1 mM dNTPs, 10 mM DTT, 50 U of Superscript II reverse transcriptase (Invitrogen Life Technologies)), and incubated at 37°C for 1 h, then for 10 min at 95°C. qPCR was performed by using an Applied Biosystems PRISM 7900HT in duplicate with SYBR Green PCR master mix (Applied Biosystems) according to the manufacturer's instructions. PCR consisted of 40 cycles of 95°C for 15 s and 55°C for 30 s. A final cycle (95°C, 15 s, 60°C) generated a dissociation curve to confirm a single product. The cycle quantity required to reach a threshold in the linear range (Q<sub>t</sub>) was determined and compared with a standard curve for each primer set generated by five 3-fold dilutions of genomic DNA samples of known concentration. Expression levels were examined using primers in the last exon of each gene or exon 6, 9, or 20 in the case of myeloid cell nuclear differentiation Ag (MNDA), IFN- $\gamma$ -inducible protein 16 (IFI16), or CIITA, respectively (Table I), and normalized to those for  $\beta$ -actin.

### Chromatin immunoprecipitation (ChIP)

HeLa or SW13 cells were cross-linked with 1% formaldehyde at room temperature for 10 min, washed twice with ice-cold PBS, collected in 1 ml of PBS and centrifuged for 5 min at 5000 rpm. Cells were resuspended in 1 ml of lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris-HCl (pH 8)) plus proteinase inhibitors (aprotinin, leupeptin, and pepstatin), incubated on ice for 10 min and sonicated to an average size of 500 bp (Vibra Cell; Sonics and Materials). Chromatin was precleared with 25  $\mu$ l of Staph A (507862; Calbiochem) at 4°C for 15 min. A 100- $\mu$ l aliquot of sonicated chromatin was immunoprecipitated (IP) with 2  $\mu$ g of Abs for STAT1 (06-501; Upstate Biotechnology), STAT3 (sc-7179; Santa Cruz Biotechnology), *BRG1* (sc-10768; Santa Cruz Biotechnology), or acetylated histone H4 (06-866; Upstate Biotechnology), at 4°C overnight. IP samples were centrifuged at 13200 rpm and supernatant was incubated with 10  $\mu$ l of Staph A at room temperature for 15 min. Precipitates were washed sequentially for 3 min in 1  $\times$  dialysis buffer (2 mM EDTA, 50 mM Tris-HCl (pH 8), and 0.2% Sarkosyl) twice, and IP wash buffer (1% Nonidet P-40, 100 mM Tris-HCl (pH 9), 500 mM LiCl 1% and deoxycholic acid) four times. Samples were extracted twice with 150  $\mu$ l of elution buffer (1% SDS and 50 mM NaHCO<sub>3</sub>), heated at 65°C overnight to reverse cross-links, and DNA fragments were purified with a QIAEX II Gel Extraction kit (catalog no. 20051). A 4- $\mu$ l aliquot from a total of 50  $\mu$ l was used in the qPCR using primers listed in Table I. Q<sub>t</sub> values were compared with a standard

curve, the copy number was calculated, the amount of DNA was precipitated by an irrelevant GAL4 (06-262; Upstate Biotechnology) Ab subtracted, and the percent ChIP DNA relative to input chromatin was calculated.

#### DNase I accessibility assay

SW13 cells transduced with Ad-FG or Ad FG-*BRG1* were left untreated or exposed to IL-6. Cells were trypsinized and washed twice in PBS, and once in 1× reticulocyte standard buffer (RSB; 10 mM Tris-HCl (pH 7.5), 10 mM NaCl, 3 mM MgCl<sub>2</sub>, and 1 mM PMSF). The cell pellet was resuspended in 10 ml of 50% glycerol, 0.5% Nonidet P-40, 1 mM PMSF, and 1× RSB solution, lysed by pipetting 10 times, and incubated on ice for 5–10 min. Nuclei were spun at 4000 rpm for 10 min at 4°C, washed in 10 ml of 1× RSB plus 0.1 mM PMSF, spun at 2500 rpm for 10 min at 4°C, then resuspended in 0.3 ml of 1× RSB. DNase I stock (4 mg/ml; D5793; Sigma-Aldrich) was diluted in 1× RSB to a final concentration of 80 ng/ml. A 1/1000 volume of 1 M CaCl<sub>2</sub> was added and 100 μl of nuclei was digested with 2.5 Kunitz U/100 μl of DNase I for 3 min (IRF1) or 10 min (IFI16) at 37°C. A total of 100 μl of 2× STOP solution (0.6 M NaCl, 20 mM Tris-HCl (pH 8.0), 10 mM EDTA and 1% SDS) was added. Next, samples were incubated with 10 μl of proteinase K (25 mg/ml) at 55°C overnight. A total of 200 μl of 1× STOP solution was added, and samples were phenol/chloroform extracted and treated with RNase (Molecular Biology Grade; Sigma-Aldrich) at 37°C overnight. Samples were phenol/chloroform extracted again, ethanol precipitated, and resuspended in 100 μl of 10 mM Tris-HCl (pH 8.0) and extracted DNA was subjected to qPCR using primers listed in Table I.

#### Small interfering RNA (siRNA)

Double-stranded siRNA oligonucleotide against STAT3 (top: 5'-CAUCUGCCUAGAUCGGCUAdTdT-3') (27) and scrambled siRNA (top: 5'-ACUCUGCGCGUUGUACAACdTdT-3') were obtained from Dharmacon Research. SW13 cells in 12-well plates were transfected with 300 nM per siRNA for 3 days using DharmaFECT-1 (Dharmacon Research) according to the manufacturer's instructions. One day before harvesting, cells were transduced with Ad-FG or Ad FG-*BRG1* as described above.

#### Statistical analysis

A paired Student's *t* test was used for statistical analysis.

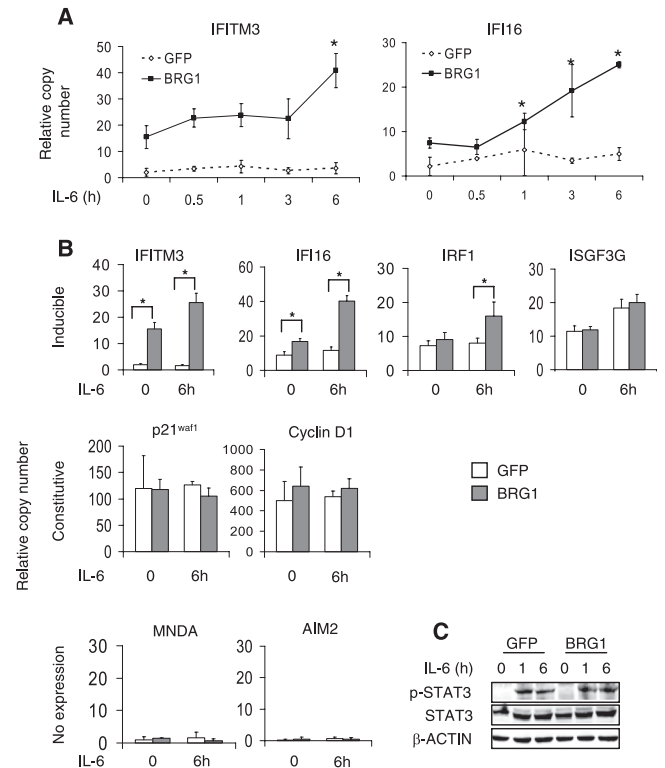
## Results

### A subset of IL-6 targets is BRG1 dependent

To study the role of *BRG1* in IL-6-mediated gene induction, we used SW13 and C33A cells, which do not express either *BRG1* or the closely related BRM protein (3, 28, 29). These cells were transduced with an adenovirus vector (Ad FG-*BRG1*) expressing *BRG1* fused to an N-terminal Flag-GFP tag, or a control virus (Ad FG), expressing Flag-tagged GFP. The infection was optimized in SW13 cells under conditions where >90% cells were transduced after 24 h and the level of *BRG1* expression was similar to the amount of *BRG1* seen in HeLa cells (22). Transduced cells were either left untreated or exposed to IL-6. qPCR was used to measure the expression levels of several potential IL-6-inducible genes using primers at the 3' end of each transcript (Table I). These genes were selected because they are known IL-6 and/or STAT3 targets or, based on the considerable overlap between cytokine-responsive genes, were known IFN targets (30–36).

First, we studied the known IL-6-responsive gene *IFI16* and the potential novel target IFN-induced transmembrane protein 3 (*IFITM3*) (Fig. 1A). *BRG1* increased the basal levels of both genes, especially of *IFITM3* ( $p < 0.01$ ; Fig. 1A). Induction of these genes was only seen in the presence of *BRG1*, and was more rapid for *IFI16* relative to *IFITM3*, which were increased at 1 or 6 h, respectively ( $p < 0.01$ ; Fig. 1A). Therefore, 6 h of IL-6 treatment was used to analyze additional targets.

Of a total of 17 genes tested in SW13 cells, 4 (*IFI16*, *IRF1*, *IFITM3*, and *ISGF3G*) were IL-6 responsive, all of which exhibited *BRG1* dependency except *ISGF3G* (Table II and Fig. 1B). Unlike *IFITM3* and *IFI16*, *BRG1* did not increase basal expression of *IRF1* (Fig. 1B). Like *IFITM3*, *ISGF3G* is a new IL-6-responsive



**FIGURE 1.** *BRG1* is required for IL-6-induced gene expression. **A**, Time-course analysis of *IFITM3* and *IFI16* induction. SW13 cells were transduced with Ad-FG or Ad FG-*BRG1* and exposed to IL-6 for the indicated times. qPCR was used to assess the level of mRNA. \*, Significant increase in expression compared with nontreated, *BRG1*-expressing cells ( $p < 0.05$ ). **B**, *BRG1*-dependent IL-6-inducible gene expression: SW13 cells were infected as in **A** and left untreated or exposed to IL-6 for 6 h. qPCR was used to assess the level of mRNA. \*, Significant *BRG1*-dependent increases in basal or IL-6-induced expression ( $p < 0.05$ ). Samples in **A** and **B** were normalized to  $\beta$ -actin and presented in arbitrary units. Results are the average of three independent experiments  $\pm$  SD. Selected targets are shown, see Table II for a complete list. **C**, *BRG1* does not affect STAT3 levels or activation. Ad-FG or Ad-FG-*BRG1*-transduced SW13 cells were left untreated or exposed to IL-6 for the indicated times. Proteins levels were examined by Western blot using the indicated Abs. Results are representative of three independent experiments.

gene. *BRG1* did not affect the levels or activation (i.e., phosphorylation) of STAT3 (Fig. 1C), indicating that it facilitates IL-6 activity by another mechanism (see below). The *BRG1* dependence of IL-6-mediated *IRF1* induction is noteworthy given that IFN- $\gamma$  induction of this gene is *BRG1* independent (7, 22). Seven genes (*p21<sup>waf1</sup>*, *cyclin D1*, *UCK2*, *JUNB*, *Bcl-x<sub>L</sub>*, *Pim 1*, and *Pim 2*) were constitutively expressed in SW13 cells but not further induced by IL-6, and the remaining 6 genes, such as *MNDA* and *AIM2*, were expressed at negligible levels (Table II and Fig. 1B). None of 10 genes tested in C33A cells were induced by IL-6 (Table II). Of these, 7 (*IRF1*, *p21<sup>waf1</sup>*, *cyclin D1*, *JunB*, *UCK2*, *Pim 1*, and *Pim 2*) were constitutively expressed and 3 (*CIITA*, *IFI27*, and *IFIT1*) were silent (Table II).

In summary, our data reveal an important role for *BRG1* in mediating induction at a subset of IL-6 targets, highlight a difference in the requirement for *BRG1* in responding to IFN- $\gamma$  or IL-6 at the *IRF1* locus, and reveal that many genes induced by IL-6 in other cell types are unaffected in SW13 and/or C33A cells.

Table II. Responsiveness and BRG1 dependency of IL-6 targets in different cell types

Cells	SW13	C33A	Other <sup>b</sup>
<i>IFI16</i>	+ <sup>b</sup>	NT	+ <sup>c</sup>
<i>IFITM3</i>	+ <sup>b</sup>	NT	NT
<i>IRF1</i>	+ <sup>b</sup>	- <sup>e</sup>	+ <sup>e</sup>
<i>ISGF3G</i>	+	NT	NT
<i>GBP1</i>	-	NT	NT
<i>CIITA</i>	-	-	NT
<i>IFI27</i>	NT	-	NT
<i>IFIT1</i>	-	-	NT
6-16 ( <i>GIP3</i> )	-	NT	NT
<i>UCK2</i>	- <sup>d</sup>	- <sup>d</sup>	+ <sup>c</sup>
<i>Cyclin D1</i>	- <sup>d</sup>	- <sup>d</sup>	+ <sup>e</sup>
<i>p21<sup>Waf1</sup></i>	- <sup>d</sup>	- <sup>d</sup>	+ <sup>f</sup>
<i>JunB</i>	- <sup>d</sup>	- <sup>d</sup>	+ <sup>g</sup>
<i>Bcl-x<sub>L</sub></i>	- <sup>d</sup>	NT	+ <sup>h</sup>
<i>Pim 1</i>	- <sup>d</sup>	- <sup>d</sup>	+ <sup>i</sup>
<i>Pim 2</i>	- <sup>d</sup>	- <sup>d</sup>	+ <sup>i</sup>
<i>MNDA</i>	-	NT	NT
<i>AIM2</i>	-	NT	NT

<sup>a</sup> BRG1 dependency is not known in these cell types. +, BRG1-independent induction; -, silent and not induced. NT, Not tested.

<sup>b</sup> BRG1-dependent induction.

<sup>c</sup> Splenocytes and NIH 3T3 (30).

<sup>d</sup> Constitutively expressed but not induced by cytokines.

<sup>e</sup> Macrophages (32).

<sup>f</sup> HepG2 and NGP (33).

<sup>g</sup> HepG2 (36).

<sup>h</sup> 293T (34).

<sup>i</sup> G277, G133, G68, and G25 cells (STAT3-mediated induction) (35).

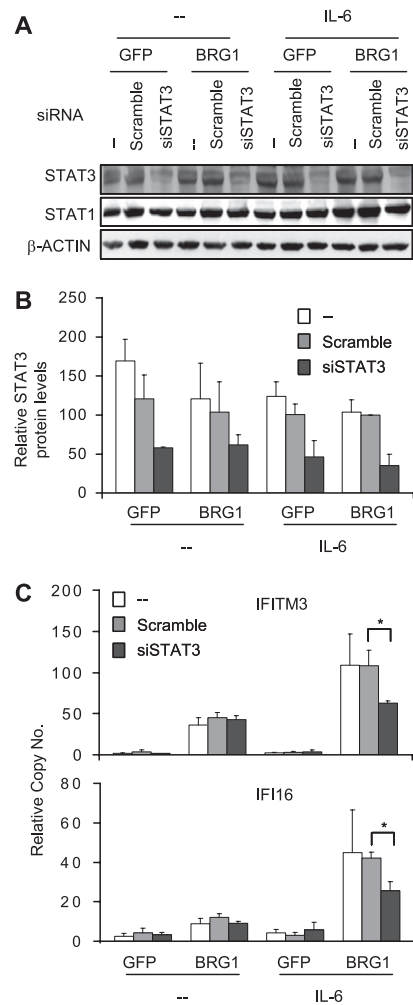
### STAT3 is required for IL-6-induced, but not BRG1-enhanced basal gene expression

To confirm that STAT3 mediates IL-6 activity, and to address whether it is required for the BRG1-dependent increase in basal expression, RNA interference was used to reduce STAT3 protein levels. SW13 cells were left untransfected with STAT3 or scrambled siRNAs then transduced with BRG1 or GFP virus and left untreated or exposed to IL-6. STAT3 protein levels were significantly reduced by STAT3 but not scrambled siRNA (Fig. 2, A and B). STAT3 siRNA had no effect on the levels of STAT1 or  $\beta$ -actin (Fig. 2A). As expected, STAT3 knockdown significantly reduced IL-6-induced *IFITM3* and *IFI16* mRNA (Fig. 2C). In contrast, STAT3 knockdown had no effect on BRG1-enhanced basal expression of either *IFITM3* or *IFI16* (Fig. 2C).

### BRG1-dependent STAT3 recruitment at IL-6 target promoters

Next, we asked whether STAT3 recruitment is BRG1 dependent at IL-6-inducible genes. IFN- $\gamma$  stimulates STAT1 binding to the promoters of several of the genes studied here (22), so we tested the same regions for IL-6-induced STAT3 binding. First, we used ChIP to assess the kinetics of STAT3 recruitment at two IL-6 target promoters in the presence or absence of BRG1. SW13 cells were transduced with Ad FG-BRG1 or Ad FG and left untreated or exposed to IL-6 for 0.5, 1, 3, 6, and 24 h. Sheared cross-linked chromatin was IP with STAT3 Abs and qPCR used to assess enrichment of specific target sequences.

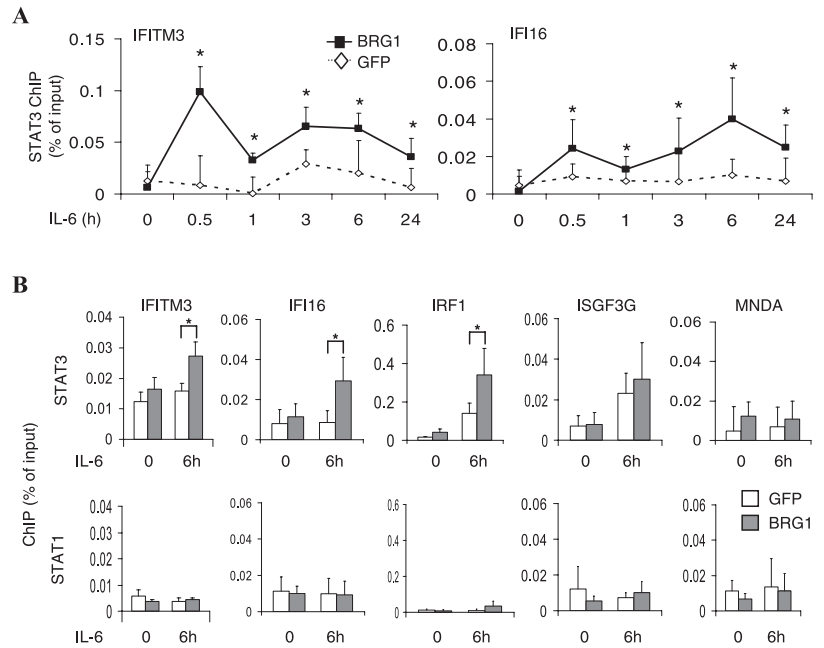
In BRG1 but not GFP-expressing cells, STAT3 was detected at the *IFITM3* and *IFI16* promoters within 30 min of IL-6 treatment (Fig. 3A). Binding diminished after 1 h, but peaked a second time at 6 h which fell again by 24 h (Fig. 3A). This cyclical recruitment of STAT3 is reminiscent of the activated estrogen receptor which also binds in cycles coincident with transcriptional initiation (37). We also found that BRG1 was required for STAT3 recruitment at the IRF1 promoter ( $p < 0.05$ ; Fig. 3B). In contrast, STAT3 re-



**FIGURE 2.** STAT3 is required for IL-6-induced expression but not BRG1-enhanced basal expression. A, STAT3 siRNA reduces STAT3 protein levels. SW13 cells were left untransfected, or transfected with STAT3 or scrambled siRNA. Two days later, cells were transduced with Ad-FG or Ad FG-BRG1 then 1 day after that cells were either left untreated or exposed to IL-6 for 6 h. Western blots were performed using Abs against STAT3, STAT1, or  $\beta$ -actin. B, Quantification of STAT3 protein levels. Western blots were quantified using the MultiImager (Bio-Rad). The level of STAT3 in IL-6-treated SW13 cells transduced with Ad-FG-BRG1 and transfected with scrambled siRNA was set as 100%. Results were normalized to  $\beta$ -actin and are the average of three independent experiments  $\pm$  SD. C, STAT3 is required for IL-6-enhanced, but not BRG1-increased basal gene expression. RT-qPCR was conducted for the RNA from the cells treated as in A. Results were normalized to  $\beta$ -actin and presented in arbitrary units. The average of three independent experiments  $\pm$  SD is shown.

ruitment to the *ISGF3G* promoter was BRG1 independent (Fig. 3B), matching the expression analysis (Fig. 1B). The latter result proves that the inability of STAT3 to bind other targets is not due to aberrant STAT3 activity in BRG1-deficient cells. STAT3 binding at the silent MNDA promoter was negligible (Fig. 3B). Activated STAT3 can form heterodimers with STAT1 (25), but ChIP results did not show significant STAT1 recruitment at any of the tested promoters (*IFITM3*, *IFI16*, *IRF1*, *ISGF3G*, or *MNDA*) (Fig. 3B). In summary, these data indicate that BRG1-dependent induction of a subset of IL-6 targets correlates perfectly with BRG1-dependent recruitment of STAT3 to their promoters.

**FIGURE 3.** *BRG1*-dependent recruitment of STAT3 at IL-6 targets. **A**, Kinetics of *BRG1*-dependent STAT3 recruitment at *IFITM3* and *IFI16* promoters. SW13 cells were transduced with Ad-FG or Ad FG-*BRG1* and left untreated or exposed to IL-6 for the indicated times. ChIP assays were performed with STAT3 Abs and isolated DNA analyzed by qPCR. Results are the average of six independent experiments  $\pm$  SD. **B**, *BRG1*-dependent recruitment of STAT3 at IL-6 targets. SW13 cells were treated with IL-6 for 0 or 6 h, and STAT3 (*top panel*) or STAT1 (*bottom panel*) ChIP-qPCR performed for the indicated promoters. The STAT3 ChIP data for *IFITM3* and *IFI16* from (A) at 0 and 6 h are repeated here to allow straightforward comparison with the other targets and with the STAT1 control ChIPs. Results are the average of six (*IFI16*, *IRF1*, and *MNDA*) or three (*IFITM3* and *ISGF3G*) separate experiments  $\pm$  SD. \*, Significant *BRG1*-dependent IL-6-induced STAT3 recruitment ( $p < 0.05$ ).

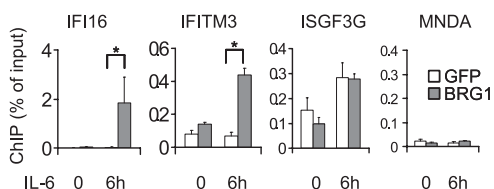


*IL-6-induced histone acetylation is BRG1 dependent*

Histone lysine acetylation is linked to gene activation and previous studies indicated that STAT3 and *BRG1* bind to at least one target (p21<sup>waf2</sup>) in HepG2 cells, resulting in histone acetylation (13). We and others demonstrated that *BRG1* is required for histone acetylation at IFN target promoters (10, 22). Therefore, we asked whether histone acetylation at IL-6 target promoters is also *BRG1* dependent. An Ab that binds to tetra-acetylated histone H4 was used in ChIP assays with chromatin from SW13 cells infected with Ad FG-*BRG1* or Ad FG, in the presence or absence of IL-6. Acetylation was induced at the *IFI16* and *IFITM3* promoters in the presence of *BRG1* and, importantly, was blocked in its absence (Fig. 4). Thus, as seen at IFN targets, IL-6-inducible histone acetylation is downstream of and dependent on *BRG1*. As expected, IL-6-induced histone acetylation at the *BRG1*-independent *ISGF3G* promoter was unaffected by the absence of *BRG1* and negligible levels of acetylation were seen at the silent and unresponsive *MNDA* promoter (Fig. 4).

*BRG1 binds constitutively to IL-6 target promoters*

*BRG1* is associated with several IFN-responsive promoters even in the absence of cytokine (10, 22), so we tested whether this is also the case at IL-6 targets. These assays were performed with chromatin from HeLa cells, which express *BRG1*. Cells were left un-

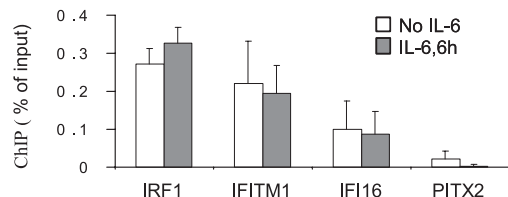


**FIGURE 4.** *BRG1*-dependent histone acetylation at IL-6 target promoters. SW13 cells were transduced with Ad-FG or Ad FG-*BRG1* and left untreated or exposed to IL-6 for 6 h. ChIP assays were performed with acetylated histone H4 Ab and isolated DNA analyzed by qPCR using the indicated promoter primers. Results are the average of three independent experiments  $\pm$  SD. \*, Significant *BRG1*-dependent IL-6 induced histone acetylation ( $p < 0.05$ ).

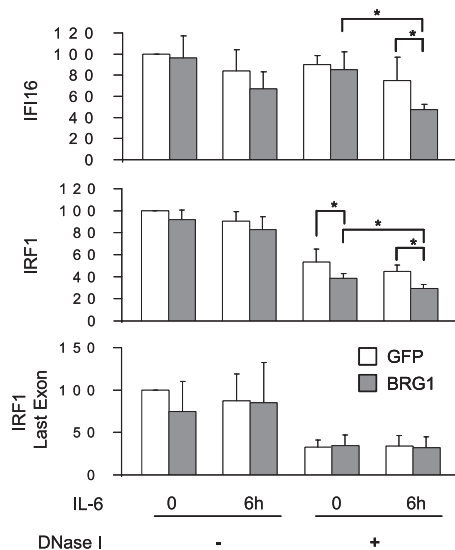
treated or exposed to IL-6 for 6 h and ChIP assays performed with anti-*BRG1* Ab. These assays showed that *BRG1* was constitutively bound to the *IRF1* and *IFI16* promoters at similar levels before or after IL-6 stimulation (Fig. 5). *BRG1* was also present at the *IFITM1* promoter (Fig. 5), as reported previously (10). Negligible binding was observed at the irrelevant *PITX2* promoter (Fig. 5). Thus, *BRG1* binds constitutively to IL-6-responsive promoters.

*BRG1-dependent chromatin remodeling*

*BRG1* does not affect the basal level of DNase I and/or restriction enzyme accessibility at the IFN target genes *CIITA*, *GBP1*, or *IFI27* but is essential for IFN-induced remodeling at these loci (7, 22). However, *BRG1* does increase basal accessibility at the IFN-responsive *IFITM1* (also called 9-27 or *IFI17*) locus (10). We used DNase I digestion coupled with qPCR to quantify accessibility at *IFI16* and *IRF1* promoters in SW13 cells in the presence or absence of *BRG1* and IL-6. SW13 cells were transduced with Ad FG-*BRG1* or Ad FG, left untreated or exposed to IL-6, nuclei incubated with DNase I, and levels of intact DNA at the *IFI16* and *IRF1* promoters were determined by qPCR. In *BRG1*-deficient cells, *IFI16* promoter accessibility was identical plus or minus IL-6 (Fig. 6). *BRG1* did not affect basal accessibility to DNase I at this promoter, but increased accessibility



**FIGURE 5.** Constitutive recruitment of *BRG1* at IL-6 targets. Chromatin was prepared from HeLa cells left untreated or exposed to IL-6 for 6 h. ChIP assays were performed with *BRG1* Ab and isolated DNA analyzed by qPCR using the indicated promoter primers. Results are the average of three independent experiments  $\pm$  SD. *BRG1* binding at all three IL-6-inducible promoters was significantly above the *PITX2*-negative control ( $p < 0.05$ ).



**FIGURE 6.** *BRG1*-dependent chromatin remodeling at IL-6 target promoters. SW13 cells transduced with Ad-FG or Ad FG-*BRG1* were left untreated or exposed to IL-6 then nuclei were prepared and exposed to 2.5 Kunitz U/100  $\mu$ l of DNase I for 3 min (*IRF1*) or 10 min (*IFI16*). DNA was purified and analyzed by qPCR using the indicated promoters or a control region (*IRF1* last exon). Results are the percentage of the level of intact DNA in GFP-transduced cells that were not exposed to IL-6 and are the average of three independent experiments  $\pm$  SD. \*, A significant increase in accessibility ( $p < 0.05$ ).

in the presence of IL-6 ( $p < 0.05$ ; Fig. 6). At the *IRF1* promoter, *BRG1* increased basal accessibility ( $p < 0.05$ ) and further enhanced IL-6-induced accessibility ( $p < 0.05$ ; Fig. 6). In contrast, DNase I accessibility at the last exon of *IRF1* was identical in untreated or IL-6-treated cells transduced with Ad FG-*BRG1* or Ad FG (Fig. 6). These data suggest that, as seen at IFN target genes (10, 22), *BRG1* increases basal promoter accessibility at some and IL-6-induced promoter accessibility at all tested *BRG1*-dependent targets.

## Discussion

### *A broad role for BRG1 in regulating access of STAT proteins to promoters*

Previously, we demonstrated that recruitment of the STAT1 homodimer to multiple IFN- $\gamma$ -inducible promoters requires *BRG1* (22). We also showed that recruitment of the STAT1/STAT2/IRF9 (ISGF3) trimer at various IFN $\alpha$ -inducible promoters is *BRG1* dependent (22). In this study, we extend these observations, revealing that recruitment of a third STAT family member, STAT3, to several IL-6-responsive targets is also *BRG1* dependent. Taken together, these studies reveal a broad role for *BRG1* in mediating the access of STAT proteins to promoters. This finding underscores the importance of *BRG1* for immune function.

In addition to STAT binding, all downstream events at IFN targets are blocked in the absence of *BRG1*, including chromatin remodeling, histone modifications, and recruitment of other transcription factors (7, 10, 22). Likewise, we found that both histone acetylation and chromatin remodeling require *BRG1* at IL-6 target genes. Thus, *BRG1* has an apical role at multiple STAT target genes and is necessary to set up the entire cascade of events that lead to transcription. This scenario is distinct from many other genes, where *BRG1* acts downstream of activators that initiate promoter assembly (14–21). This primary role for *BRG1* at cytokine-responsive targets is possible through constitutive recruitment to

IFN- and IL-6-inducible promoters. Constitutive binding of *BRG1* to target genes is STAT independent (this study and Ref. 22). Numerous other mechanisms may recruit *BRG1* to cytokine target genes, including association with transcription factors, various chromatin regulatory complexes, and modified histones; for a detailed discussion, see Refs. 2 and 22).

### *Distinct roles for BRG1 in controlling basal and/or inducible promoter accessibility*

Our initial study linking *BRG1* to IFN-responsive genes focused on *CIITA*. At this locus, we found that *BRG1* did not affect basal DNase I or restriction enzyme accessibility at the promoter, but was absolutely essential for IFN- $\gamma$ -induced chromatin remodeling (7). Recently, we repeated and extended this finding by showing that *BRG1* does not alter basal chromatin accessibility at the *CIITA*, *GBP1*, or *IFI27* promoters, but is critical for IFN- $\gamma$ -induced remodeling at these loci (22). In contrast, others reported that *BRG1* increases both basal and IFN- $\alpha$ -induced accessibility at the *IFITM1* promoter (10). In this study, we showed that *BRG1* increases basal DNase I accessibility at the *IRF1* but not the *IFI16* promoter and enhances IL-6-induced chromatin remodeling at both promoters. Thus, there appear to be two distinct classes of *BRG1*-dependent promoters: chromatin structure at uninduced type A promoters (e.g., *CIITA*, *GBP1*, *IFI27*, *IFI16*) is apparently unaffected by *BRG1*, whereas basal accessibility is increased at type B promoters (e.g., *IFITM1*, *IRF1*). *BRG1* enhances cytokine-induced chromatin accessibility at both type A and B promoters.

How does *BRG1* facilitate access of STAT complexes to promoters where it does not seem to affect basal chromatin structure? Because *BRG1* interacts with STAT proteins (8, 13) it might act as a platform to facilitate STAT1 recruitment. However, this cannot be its only role as *BRG1* ATPase activity is essential for induction of genes like *CIITA* (7). Another possibility is that *BRG1* acts primarily on distant elements that influence promoter accessibility following cytokine treatment. However, the role of distant elements at cytokine-regulated genes is unclear, but it will be important to investigate this possibility.

### *BRG1-dependent and -independent STAT3 or STAT1 recruitment at IRF1*

Previously, we showed that IFN- $\gamma$  induces *IRF1* independent of *BRG1* (7). Indeed, STAT1 recruitment was unimpaired at this locus in *BRG1*-deficient cells (22). Surprisingly, however, we found that IL-6-induced STAT3 recruitment at the *IRF1* promoter was greatly enhanced by *BRG1*. This unexpected result suggests that STAT1 dimers and STAT3 dimers do not engage this promoter in an identical fashion. Both STAT1 and STAT3 can bind TTN<sub>5</sub>AA motifs, but STAT3 also binds TTN<sub>4</sub>AA motifs, and the GAS (TTCCCCGAA) in the *IRF1* promoter is a TTN<sub>5</sub>AA motif that binds well to all STAT family members (38). This observation suggests that the specific requirement for *BRG1* for STAT3 access to the *IRF1* promoter is not linked to the sequence of the DNA-binding motif. The crystal structures of DNA-bound STAT1 or STAT3 are virtually superimposable (39, 40), but were created using truncated molecules that lack the extreme N- and C-terminal portions. These regions bind common but also distinct proteins. For example, the N-terminal region of STAT3 but not STAT1 binds and cooperates in gene induction with c-Jun (41). Thus, it is feasible that differences in the protein complexes associated with STAT3 or STAT1 could block and/or facilitate entry in the absence of *BRG1*, respectively.

In summary, there is a broad role for *BRG1* in regulating the access of STAT proteins to DNA. Although many promoters are

*BRG1* dependent, others are *BRG1* independent, and among the former there are differences in the effects of *BRG1* on basal chromatin accessibility and the requirement for *BRG1* to promote access of difference STAT family members. Given the crucial role STAT and BRG1 proteins play in immunity and cancer, it will be important to uncover the mechanisms that underlie these differences.

## Disclosures

The authors have no financial conflict of interest.

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