

The Sin3a repressor complex is a master regulator of STAT transcriptional activity

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Tyrosine phosphorylation is a hallmark for activation of STAT proteins, but their transcriptional activity also depends on other secondary modifications. Type I IFNs can activate both the ISGF3 (STAT1:STAT2:IRF9) complex and STAT3, but with cell-specific, selective triggering of only the ISGF3 transcriptional program. Following a genome-wide RNAi screen, we identified the SIN3 transcription regulator homolog A (Sin3a) as an important mediator of this STAT3-targeted transcriptional repression. Sin3a directly interacts with STAT3 and promotes its deacetylation. SIN3A silencing results in a prolonged nuclear retention of activated STAT3 and enhances its recruitment to the SOCS3 promoter, concomitant with histone hyperacetylation and enhanced STAT3-dependent transcription. Conversely, Sin3a is required for ISGF3-dependent gene transcription and for an efficient IFN-mediated antiviral protection against influenza A and hepatitis C viruses. The Sin3a complex therefore acts as a context-dependent ISGF3/STAT3 transcriptional switch.

STAT3 was originally identified as an IL-6-activated transcription factor in hepatocytes (1–4) and later reported to be activated by many other stimuli, including cytokines [e.g., leukemia inhibitory factor (LIF), IL-10, IFNs], growth factors (e.g., EGF), and hormones (e.g., insulin). Activated STAT3 stimulates the transcription of several genes involved in cell-cycle progression and the antiapoptotic program (5). Therefore, and for its ability to transform normal fibroblast cells and cause tumors in nude mice, STAT3 has been classified as an oncogene (6). The regulation of STAT3 transcriptional activity strongly depends on its posttranslational modification status. The functional role of phosphorylation on hallmark tyrosine and serine residues is by far best understood (7) and correlates in most cases with functional and transcriptionally active STAT3. Beside phosphorylation, STAT3 activity is tightly regulated by several other posttranslational modifications, including lysine methylation (8, 9) and acetylation (10–12). Although STAT3 methylation negatively regulates its activity, lysine acetylation is associated with a positive regulation of STAT3 activity in general, although its precise impact depends on the acetylated residues. STAT3 acetylation is efficiently reverted by histone deacetylases (HDAC)1, HDAC2, and HDAC3, which associate with STAT3 and contribute to its negative regulation (10).

Type I IFNs induce antiviral and antiproliferative responses through the activation of the ISGF3 (STAT1:STAT2:IRF9) transcriptional complex (13). IFN stimulation also leads to STAT3 phosphorylation (14), which is remarkable, given the opposed roles of ISGF3 and STAT3 in regulating cell survival and proliferation. We have previously shown that, in a cell-specific manner, IFN stimulation can induce STAT3 phosphorylation and DNA binding without triggering transcription (15). HDAC1/2 are responsible for this transcriptional repression, as interfering with their expression or activity restored the transcription of STAT3-target genes (15). On the other hand, HDAC activity is required

for transcriptional activation of ISGF3-responsive genes and IFN-induced antiviral immunity (16–18).

Here, we report that the SIN3 transcription regulator homolog A (Sin3a) complex represses STAT3 activity by modifying its acetylation status. Sin3a is instead required for IFN-stimulated gene (ISGs) transcription and an efficient antiviral response. Our results unveil a critical role for the Sin3a complex in balancing STAT functions at the transcriptional level.

Results

Genome-Wide RNAi Screen Identifies the Sin3a Complex as a Repressor of STAT3 Transcriptional Activity. Although type I IFN treatment stimulates STAT3 tyrosine phosphorylation and its binding to STAT3-responsive promoters, the subsequent transcription of canonical STAT3-responsive genes is impaired in certain cell types (15). In line herewith, IFN- α 2 stimulation of Hek293T cells failed to activate the transcription of the STAT3-responsive rat pancreatitis-associated protein 1 (rPAP1)-luciferase reporter (Fig. 1A, Upper graph). In contrast, LIF stimulation strongly activated the reporter. Because both cytokines support STAT3 phosphorylation (Fig. 1A, Lower blot), a different regulatory mechanism must account for the impaired STAT3 activity. We performed a genome-wide RNAi screen aimed at identifying putative STAT3 repressors (Fig. 1B). In this setting, the silencing of a candidate repressor would release the STAT3 transcriptional brake and induce rPAP1-luciferase activation. The normalized reporter activation corresponding to each siRNA was expressed by z-scoring (cellHTS2) (19). Strikingly, among the top candidate repressors (Dataset S1), we identified four components of the Sin3a transcriptional repressor complex: *SAP130* (z-score: 9.5), *SUDS3* (z-score: 6.3), *SFPQ* (z-score: 5.2), and *TGIF2* (z-score: 4.25) (Fig. 1C). *SIN3A* silencing itself also enhanced the reporter activation. Independent reporter-based experiments confirmed that the Sin3a complex is involved in repressing IFN- α 2-activated STAT3 (Fig. S1). Of note, silencing of the Sin3a complex components identified in the screen also led to a robust increase of the LIF-induced rPAP1-luciferase reporter activation (Fig. 1D), as well as the transcription of the

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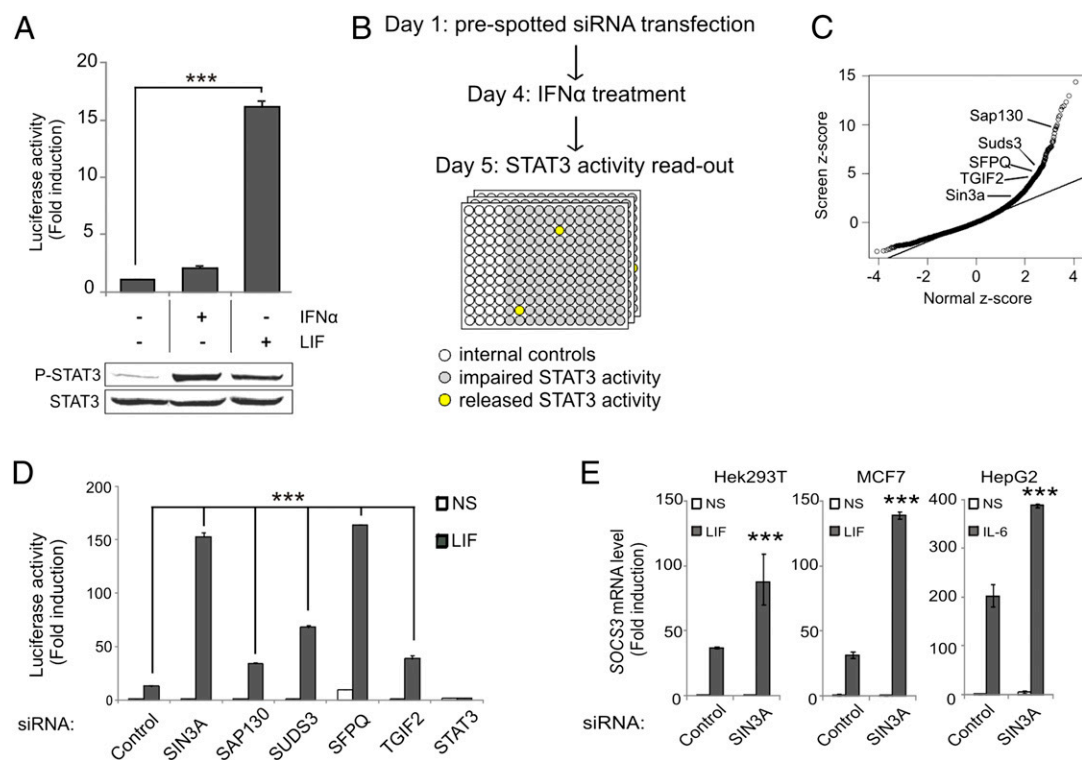


Fig. 1. A genome-wide RNAi screen identifies the Sin3a complex as negative regulator of STAT3 transcriptional activity. (A) Hek293T cells were transiently transfected with the rPAP1-luciferase reporter. After 24 h, cells were left nonstimulated (NS) or stimulated with LIF or IFN- α 2 for 24 h for the luciferase assay or 30 min for phospho-STAT3 detection. Luciferase readout is expressed as a ratio between stimulated and unstimulated values. $***P < 0.001$; *t* test. Total cell extracts were blotted with anti-phospho STAT3 (Tyr705) and anti-STAT3 antibody. (B) Genome-wide RNAi screen strategy. Hek293 cells stably expressing the rPAP1-luciferase reporter were used. siRNAs targeting potential STAT3 repressors will release STAT3 transcriptional brake and activate the rPAP1-luciferase reporter (wells in yellow), but STAT3 activity will be impaired in wells containing unrelated siRNAs (in gray). Wells colored in white were used for the internal controls. The detailed screening procedure is described in the *SI Materials and Methods*. (C) Most siRNA phenotypes behave as normally distributed data (line). The tail deviating from normal distribution represents siRNA with significant phenotypes (repressor candidate genes). The Sin3a complex components identified in the screen are highlighted. (D) Hek293T cells were transiently transfected with the indicated siRNA and with the rPAP1-luciferase reporter after 24 h. *Renilla* luciferase siRNA was used as control. After 48 h, cells were left nonstimulated or stimulated with LIF. Luciferase readout is expressed as a ratio over the control-silenced NS condition. $***P < 0.001$; one-way ANOVA with Bonferroni test. (E) Hek293T, MCF7, and HepG2 cells were transfected with control (*Renilla* luciferase) or a *SIN3A* siRNA. After 72 h, cells were cultured 4 h without FCS, then left nonstimulated or stimulated with LIF or IL-6 for 1 h. Graphs represent mRNA levels relative to NS sample. $***P < 0.001$; one-way ANOVA with Bonferroni test. Results are representative of three independent experiments. Error bars indicate SD from triplicates

endogenous STAT3-responsive *SOCS3* gene in both LIF-induced Hek293T and MCF7 cells, and IL-6-stimulated HepG2 cells (Fig. 1E). These results unveil a unique role for the Sin3a complex as a negative regulator of STAT3 transcriptional activity, regardless of the activating stimulus.

Sin3a Directly Interacts with STAT3 and Promotes Its Deacetylation.

Sin3a associates with several transcription factors promoting their deacetylation and modulating their activity (20). Hence, we asked whether the modulation of STAT3 activity by Sin3a involves a stimulus-dependent physical interaction. Coimmunoprecipitation analysis in Hek293T cells demonstrated that endogenous STAT3 interacts with endogenous Sin3a in a stimulus-dependent manner (Fig. 2A). To identify the functional domain of STAT3 involved in Sin3a binding, Etag-STAT3 deletion mutants were generated and overexpressed in Hek293T along with GFP-Sin3a. Only the constructs containing the STAT3 DNA-binding domain (DBD) coprecipitated with GFP-Sin3a, revealing the importance of the STAT3-DBD for this interaction (Fig. 2B). To verify if Sin3a directly interacts with STAT3, a GST pull-down assay was performed. In vitro produced Flag-Sin3a directly interacted with the GST-tagged C-terminal part of STAT3 (amino acids 131–770) containing the DBD (Fig. 2C), in concordance with the previous experiment. Next, we asked whether Sin3a modified the

posttranslational profile of STAT3. Because posttranslational modifications alter the protein isoelectric point (pI), we compared the pI distribution of STAT3 isoforms in cells transfected with control or *SIN3A*-specific siRNA, using the NanoPro technology (21, 22). *SIN3A* silencing resulted in a clear pI-shift of STAT3 isoforms toward more acidic values, suggesting increased STAT3 acetylation (Fig. S24). Confirming this hypothesis, we observed a marked STAT3 hyperacetylation in LIF-stimulated *SIN3A*-silenced cells, but the phosphorylation status was not significantly altered (Fig. 2D). It has been previously reported that HDACs bind to and negatively regulate acetylated STAT3 (10, 23), so we asked whether the Sin3a-STAT3 interaction could be regulated by STAT3 acetylation. We found that Sin3a interacted more strongly with the acetyl-mimicking STAT3K49/87Q mutant compared with STAT3 WT or the other STAT3 variants (Fig. 2E), indicating that binding of Sin3a preferentially occurs with N-terminally acetylated STAT3. Further analysis on single N-terminal lysine STAT3 mutants identified K87 acetylation as the main regulator of the STAT3-Sin3a interaction (Fig. S2B).

Sin3a Complex Regulates a Subset of STAT3-Responsive Genes. Sin3a is a conserved multifunctional repressor protein complex that regulates gene transcription (20). To gain more insight into the STAT3-responsive genes that are regulated by the Sin3a

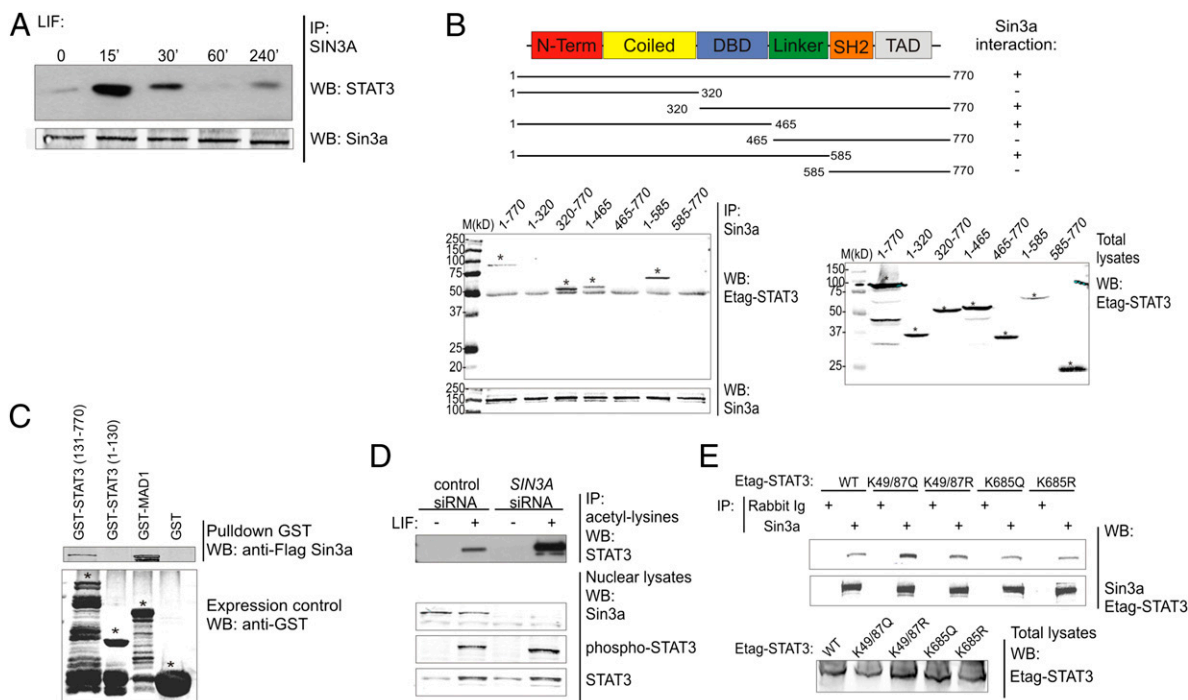


Fig. 2. Sin3a interacts with STAT3 and modifies its acetylation pattern. (A) Hek293T cells were cultured 4 h without FCS and then stimulated with LIF for the indicated time points. Nuclear lysates were prepared, endogenous Sin3a was immunoprecipitated, and coprecipitated endogenous STAT3 was revealed with an anti-STAT3 antibody. Precipitated Sin3a was revealed using an anti-Sin3a antibody. (B) Schematic representation of Etag-STAT3 truncated mutants. DBD, DNA binding domain; SH2, Src Homology 2; TAD, transactivation domain. Hek293T cells were transfected with plasmids coding for GFP-Sin3a and Etag-STAT3 truncated mutants. Sin3a was immunoprecipitated and coprecipitated Etag-STAT3 mutants were revealed with anti-Etag antibody. Total lysates were blotted as loading control. Stars indicate the specific bands. (C) *Escherichia coli* BL21DE3 cells were transformed with plasmids coding for GST-STAT3 N-terminal (1-130), C-terminal (131-770), GST-MAD1 (positive control), or GST alone (negative control). Cell lysates were incubated with the in vitro transcribed and translated Flag-Sin3a protein. Complexes were precipitated using glutathione-agarose beads and revealed with anti-flag antibody. GST-transformed bacterial lysates were blotted with anti-GST antibody as control of protein production and solubility. Stars indicate the specific bands. (D) Hek293T cells were transfected with control (*Renilla* luciferase) or *SIN3A* siRNA. After 72 h, cells were cultured 4 h without FCS and then stimulated with LIF for 30 min. Nuclear extracts were prepared and acetylated lysines were immunoprecipitated. Coprecipitated STAT3 was revealed with anti-STAT3 antibody. Endogenous Sin3a and STAT3 levels in the nuclear lysates were detected using specific antibodies. (E) Hek293T cells were transfected with plasmids coding for GFP-Sin3a and Etag-STAT3 WT, acetyl-mimicking (K49/87Q and K685Q), or acetyl-deficient (K49/87R and K685R) mutants. Sin3a was immunoprecipitated and coprecipitated Etag-STAT3 mutants were revealed with an anti-Etag antibody. Total lysates were blotted as loading control. Results are representative of three independent experiments.

complex, we performed an Affymetrix microarray-based screen on LIF-treated MCF7 cells that had been transfected with a control siRNA or with *SIN3A* siRNA. Knockdown of *SIN3A* and LIF-induced STAT3 phosphorylation were confirmed during sample preparation (Fig. S3). As represented in Fig. 3A, 14 LIF-responsive genes were up-regulated in the near absence of Sin3a. Results were confirmed by independent quantitative RT-PCR experiments, highlighting that the Sin3a complex negatively regulates the transcription of a subset of STAT3-responsive genes. The expression profile of three representative Sin3a-regulated LIF-induced genes (*SOCS3*, *FGG*, and *AGT*) is shown in Fig. 3B. Of note, both early and late genes were subjected to regulation by Sin3a. The expression profile of the remaining LIF-induced Sin3a-regulated genes is summarized in Table 1.

Sin3a Complex Regulates STAT3 Nucleocytoplasmic Distribution and Its Recruitment at the *SOCS3* Promoter. STAT3 activity depends on its nuclear translocation and DNA binding. Interestingly, we found that the LIF-induced nuclear residence time of STAT3 was prolonged in cells transfected with *SIN3A*-targeting siRNA (Fig. 4A). Thus, we examined whether Sin3a could influence STAT3 binding to responsive promoters. The *SOCS3* promoter was used as a representative model for a STAT3-responsive region because its transcription was found to be significantly up-regulated in *SIN3A* knocked-down cells (Fig. 3B). ChIP assays were performed on Hek293T cells stably transfected with control

or *SIN3A* shRNAmir (Fig. S4). We found that LIF treatment induced the recruitment of Sin3a at the *SOCS3* promoter (Fig. 4B), although a basal amount of Sin3a was already present in absence of stimulation. LIF-induced STAT3 recruitment was significantly increased upon *SIN3A* knockdown (Fig. 4C). The basal levels of phospho-RNA pol II present at the promoter in the uninduced state increased upon LIF stimulation (Fig. 4D) and were further enhanced upon *SIN3A* silencing. Finally, we observed an enrichment of histone 3 K27 (H3K27) acetylation in *SIN3A*-silenced cells (Fig. 4E), indicating a more accessible chromatin status. In conclusion, we found that the Sin3a complex regulates *SOCS3* transcription, modulating the recruitment of STAT3 and phospho-pol II at the responsive regions of its promoter and influencing the acetylation of associated histones. Silencing of Sin3a complex components leads to an enhanced promoter recruitment of STAT3 and phospho-RNA pol II, histone hyperacetylation, and concomitant enhanced transcription.

Sin3a Is Necessary for the Transcription of Several ISGs and for Maximal Type I IFN-Induced Resistance to Influenza A and Hepatitis C Viruses. Although HDAC1 and HDAC2 negatively modulate STAT3 signaling (10, 12, 24), their activity is required for IFN-triggered ISGs transcription and antiviral response (16–18). Because the activity of the Sin3a complex depends on its association with HDAC1/2, we asked whether *SIN3A* silencing would affect the transcription of ISGs involved in the antiviral defense.

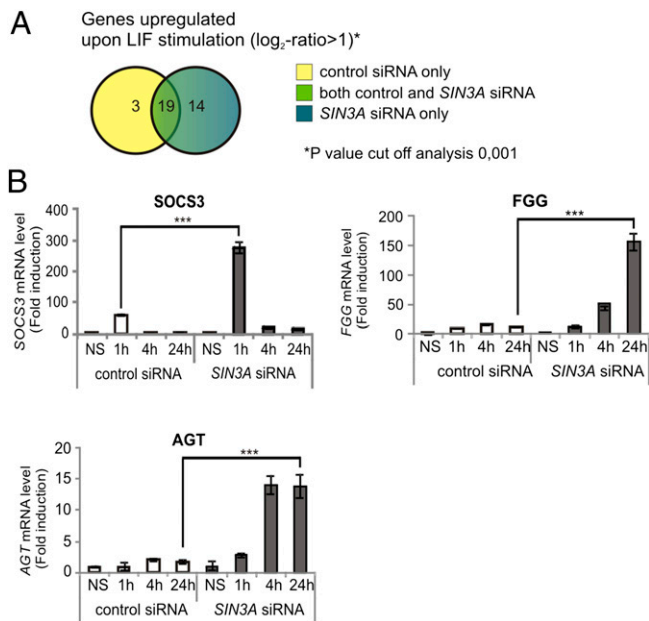


Fig. 3. The Sin3a complex negatively regulates the transcription of a subset of STAT3-dependent genes. (A) Venn diagram showing the number of LIF-induced genes in cell transfected with control siRNA (*Renilla* luciferase, yellow), with *SIN3A* siRNA (blue), or in both the conditions (green). As cutoff, a $\log_2\text{-ratio} > 1$ (more than two times up-regulated) was applied to analyze the gene expression profile. (B) MCF7 cells were transfected with control (*Renilla* luciferase) or *SIN3A* siRNA. After 72 h, cells were left nonstimulated or stimulated with LIF. Graphs represent mRNA levels relative to NS sample. Results are representative of three independent experiments. Error bars indicate SD from triplicates. *** $P < 0.001$; one-way ANOVA with Bonferroni test.

Interestingly, the basal transcription of several ISGs was found to be down-regulated by *SIN3A* silencing in the microarray experiment (Fig. 5A). We therefore asked whether *SIN3A* silencing would also affect the IFN-induced ISG transcription. As summarized in Table 2, expression of all tested ISGs was strongly impaired in *SIN3A*-silenced cells. Similar results were obtained using a different and nonoverlapping *SIN3A*-specific siRNA (Fig. S5A). Of note, *SIN3A* silencing strongly impaired transcription of all three IFN-inducible transmembrane (IFITM) proteins (Fig. 5B). IFITM proteins play a key role in the antiviral

defense against influenza A virus and hepatitis C virus (HCV) infection (25, 26). To verify if Sin3a is involved in the IFN-triggered antiviral resistance, control or *SIN3A*-silenced Hek293T cells were exposed to IFN- α 2 before influenza A virus (A/PR8/34, H1N1 subtype) infection. Fig. 5C shows that IFN- α 2 treatment potentially reduced the infection rate in cells transfected with a control siRNA (-37.7%), but only weakly in *SIN3A*-silenced cells (-10.8%). *SIN3A* silencing also increased infectability in untreated and IFN- α 2-treated MCF7 cells (Fig. S5B). The key role played by Sin3a in the IFN-induced antiviral response was also observed in HCV-infected Huh7.5 hepatoma cells (Fig. 5D). As for the influenza A virus infection, the antiviral protection triggered by IFN- α was reduced in *SIN3A*-silenced cells, resulting in an enhanced infection rate compared with control cells. In summary, our data show that the Sin3a complex is required for basal and IFN- α -dependent transcription of several ISGs and, as a consequence, for efficient IFN- α -induced protection against viral infection.

Discussion

STAT signaling is tightly regulated and imbalances between STAT1 and STAT3 activity can lead to pathologies including immune disorders, cardiomyopathies, and cancer (27). STAT1 and STAT3 reciprocally regulate each other's expression and activities, thus contributing to maintain the specificity of cytokine signaling (28–30). HDAC1 and HDAC2 exert opposite effects on STAT1- and STAT3-dependent transcription: they negatively regulate STAT3 signaling (23), and they are required for ISGs activation (16–18). Interfering with the activity of those HDACs shifts the ISGF3-dependent IFN response toward a STAT3-dependent response (15).

Type I IFN-induced STAT3 phosphorylation was widely reported (14) yet, in some cell types, IFN-activated STAT3 fails to trigger the transcription of canonical STAT3-responsive genes (15). The transcriptional brake on STAT3 observed upon IFN stimulation allowed us to perform a genome-wide RNAi screen aimed at the identification of novel STAT3 repressors. The Sin3a complex was identified as a repressor of STAT3 activity independently from the STAT3-activating stimulus. Our study provides a rationale for the previous identification of STAT-specific *cis*-regulatory elements in a subset of Sin3a-regulated genes (31). Although interplay between STAT3 and Sin3a has been suggested for the *c-MYC* (32) and for glial fibrillary acidic protein (*GFAP*) (33) promoters, direct proof, and detailed insight into the precise modalities of this interaction have so far

Table 1. Gene-expression analysis identifies several Sin3a-repressed STAT3-dependent genes

| Gene symbol | Gene ID | RL siRNA | | | | | | SIN3A siRNA | | | | | |
|----------------|---------|----------------|-----|-----|----------------|-----|-----|-----------------|------|------|------------------|------|------|
| | | 1 h LIF | | | 24 h LIF | | | 1 h LIF | | | 24 h LIF | | |
| | | Fold-induction | SD+ | SD– | Fold-induction | SD+ | SD– | Fold-induction | SD+ | SD– | Fold-induction | SD+ | SD– |
| <i>SOCS3</i> | 9021 | 38.19 | 0.5 | 0.5 | 7.14 | 1.4 | 1.2 | 70.77** | 11.7 | 10.0 | 6.99 | 0.8 | 0.7 |
| <i>FGG</i> | 2266 | 8.03 | 0.4 | 0.4 | 11.35 | 0.4 | 0.4 | 12.95 | 1.8 | 1.6 | 155.42*** | 14.7 | 13.4 |
| <i>AGT</i> | 183 | 1.01 | 0.7 | 0.4 | 1.74 | 0.4 | 0.3 | 2.90 | 0.3 | 0.3 | 13.69*** | 1.9 | 1.7 |
| <i>GADD45G</i> | 10912 | 2.56 | 0.2 | 0.2 | 1.12 | 0.8 | 0.5 | 12.25*** | 1.7 | 1.5 | 7.19*** | 0.6 | 0.5 |
| <i>CEBPD</i> | 1052 | 3.59 | 0.2 | 0.2 | 2.12 | 0.1 | 0.1 | 5.92** | 0.4 | 0.4 | 3.33 | 0.8 | 0.6 |
| <i>KLF10</i> | 7071 | 6.21 | 1.3 | 1.1 | 1.61 | 0.4 | 0.3 | 6.34 | 1.3 | 1.1 | 4.04 | 0.3 | 0.3 |
| <i>CD14</i> | 929 | 1.95 | 0.2 | 0.1 | 0.64 | 0 | 0 | 8.28*** | 0.2 | 0.6 | 3.26*** | 0.7 | 0.6 |
| <i>SOX9</i> | 6662 | 2.36 | 0.2 | 0.1 | 1.13 | 0.1 | 0.1 | 5.50*** | 0.5 | 0.5 | 4.79*** | 0.4 | 0.3 |
| <i>RND1</i> | 27289 | 2.75 | 0.7 | 0.6 | 0.82 | 0.1 | 0.1 | 5.13** | 0.5 | 0.5 | 0.96 | 0.1 | 0.1 |
| <i>STAT3</i> | 6774 | 0.76 | 0 | 0 | 2.38 | 0 | 0 | 1.50*** | 0 | 0 | 5.12*** | 0.3 | 0.3 |
| <i>ZFP36</i> | 7538 | 8.06 | 0.3 | 0.3 | 1.56 | 0.1 | 0.1 | 8.37 | 0.7 | 0.6 | 3.283* | 0.9 | 0.7 |

MCF7 cells were transfected with control (*Renilla* luciferase) or *SIN3A* siRNA. After 72 h, cells were left nonstimulated or stimulated with LIF. Fold-inductions represent mRNA levels relative to the NS sample. Results are representative of three independent experiments. SD from triplicates. *** $P < 0.001$, ** $P < 0.005$, * $P < 0.01$; one-way ANOVA with Bonferroni test and are highlighted in bold.

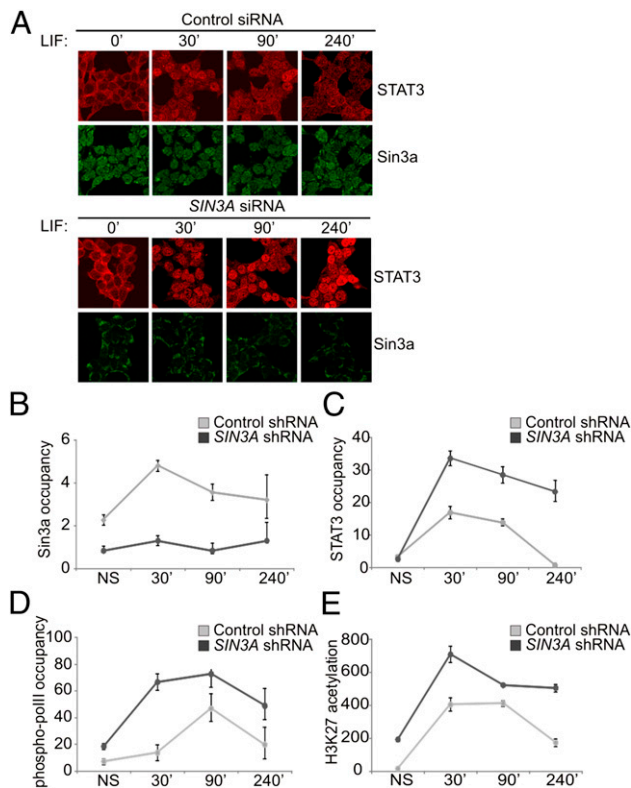


Fig. 4. The Sin3a complex negatively regulates STAT3 nuclear distribution and binding to the *SOCS3* gene promoter. (A) Hek293T cells were transfected with control (*Renilla* luciferase) or *SIN3A* siRNA. After 72 h, cells were cultured 4 h without FCS and then stimulated with LIF for the indicated times. Cells were fixed and stained and the localization of STAT3 and Sin3a was assessed by confocal analysis. Immunofluorescence of representative cell fields is shown. (Magnification: 63 \times .) (B–E) Hek293T cells were stably transfected with a scrambled or a *SIN3A*-targeting shRNAmir. Transfection and silencing efficiency are reported in Fig. S3. Transfected cells were cultured 4 h without FCS and then stimulated with LIF for the indicated time points. ChIP assays were performed to examine the occupancy of Sin3a (B), STAT3 (C), phospho-pol II (D), and the acetylation status of histone 3 (H3) K27 acetylation (E) on the *SOCS3* promoters. Immunoprecipitated DNA was quantified by quantitative PCR. Graphs represent occupancy levels relative to NS sample (fold-induction). Results are representative of three independent experiments. Error bars indicate SD from duplicates.

been lacking. We show that Sin3a directly interacts with STAT3, promoting its deacetylation, and nuclear exclusion. The stimulus-dependency of the Sin3a-STAT3 interaction and the preferential binding of Sin3a to the acetyl-mimicking STAT3K49/87Q mutant suggest that Sin3a docks to and inhibits N-terminal acetylated STAT3. Consistently, the transcription of the *AGT* gene, one of the strongest up-regulated genes identified in our microarray analysis in *SIN3A*-silenced MCF7 cells, was reported to be enhanced by STAT3 acetylation on K49 and K87 and repressed by HDAC1 overexpression (11).

ChIP assays revealed that Sin3a and STAT3 are recruited to the *SOCS3* promoter within the same time-frame after LIF stimulation, indicating that these proteins may be recruited to the consensus sequences as a complex. In addition, *SIN3A* silencing correlates with enhanced recruitment of STAT3 and phospho-RNA pol II to the *SOCS3* promoter, which is likely a direct consequence of the STAT3 hyperacetylation status (24). Although the H3K27 hyperacetylation observed in *SIN3A*-silenced cells may be a direct consequence of the reduced HDAC activity, it was previously described that N-terminal STAT3 acetylation leads to increased recruitment of p300 at the responsive sequences (24), which in

turn trigger histone hyperacetylation. Interestingly, among the LIF-induced Sin3a-repressed genes we identified, three (*FGG*, *AGT*, and *CD14*) are known acute-phase response mediators, revealing a possible role for the Sin3a complex in the regulation of this process. However, *SIN3A* silencing had barely no effect on the induction of these APR genes in IL-6 or LIF-stimulated Huh7 hepatoma cells (Table S1), indicating that the regulation exerted by Sin3a on STAT3 transcriptional activity may depend on the cellular context. Cellular specificity in the Sin3a-dependent modulation of transcription was recently highlighted for the transcription factors Myc and E2F, the target genes of which are derepressed in *SIN3A*-deficient mouse embryonic fibroblast cells (31), but are repressed in *SIN3A*-deficient immature myeloid cells (34), compared with WT cells. Such specificity may depend on the complex network of associated transcriptional corepressors and coactivators, essential to guarantee a diverse and cell-type specific response to a unique stimulus.

Although most of the research on the Sin3a complex has focused on its role as a corepressor, increasing evidence suggests that Sin3a may also activate gene transcription (20). Here, we show that Sin3a is required for the transcription of several ISGF3-responsive genes and, in particular, for the basal and IFN-induced transcription of the IFITM proteins, which are crucial mediators of the IFN-induced antiviral response to several pathogens, including influenza A virus (25) and HCV (26). Consistent with these reports, we observed a reduced IFN-mediated protection against these viruses in *SIN3A*-silenced cells.

In conclusion, the research described here clearly implicates the Sin3a complex as a cell-specific repressor of STAT3 activity and a crucial player in the innate immune defense against viral infections. Exerting opposite regulation on STAT3 and ISGF3-

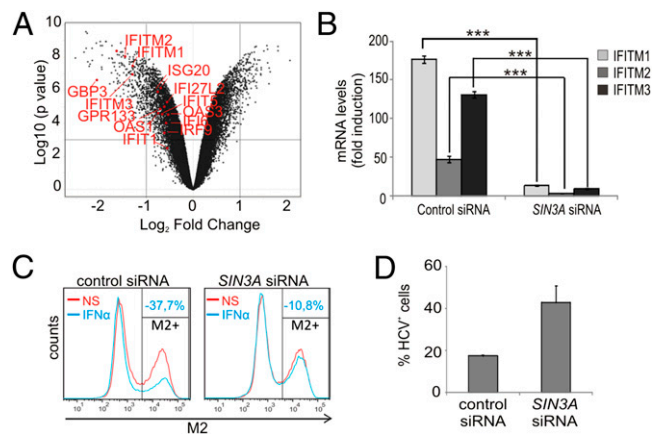


Fig. 5. The Sin3a complex is required for IFN-stimulated ISGs transcription and efficient antiviral response against influenza A and hepatitis C viruses. (A) Volcano plot representing the differential gene expression profiles (expressed as log₂ fold-change) of *SIN3A*-silenced versus control-silenced MCF7 cells. Down-regulated ISGF3-responsive genes are highlighted. (B) Hek293T cells were transfected with a control siRNA (*Renilla* luciferase) or *SIN3A* siRNA. After 72 h, cells were stimulated with IFN- α 2 for 24 h and the mRNA levels of the IFITMs were evaluated. Error bars indicate SD from triplicates. *** P < 0.001; one-way ANOVA with Bonferroni test. (C) Hek293T cells were transfected with a control siRNA (*Renilla* luciferase) or *SIN3A* siRNA. After 72 h, cells were left nonstimulated or stimulated with IFN- α 2 for 24 h before 14-h exposure to influenza A/PR8/34 virus. Infection efficiency was assessed by immunostaining of the viral protein M2 followed by flow cytometric analysis. (D) Huh7.5 cells were transfected with a control siRNA (*Renilla* luciferase) or *SIN3A* siRNA. After 72 h, cells were stimulated with IFN- α 2 (1 ng/mL) for 24 h before 48-h exposure to HCV. The efficiency of the infection was evaluated by immunostaining of the viral protein NS5A. Results are representative of at least two independent experiments.

Table 2. Down-regulated ISGF3-responsive genes in SIN3A-silenced Hek293T cells

| Gene symbol | Gene ID | Control siRNA | | | Sin3a siRNA | | |
|----------------|---------|-----------------|-------|-------|------------------|-------|-------|
| | | Fold-induction | SD+ | SD– | Fold-induction | SD+ | SD– |
| <i>ISG54</i> | 3433 | 2,574.36 | 40.21 | 39.59 | 303.38*** | 51.54 | 44.06 |
| <i>IFIT1</i> | 3434 | 265.03 | 12.61 | 12.04 | 62.03*** | 1.27 | 1.24 |
| <i>2'5'OAS</i> | 4938 | 188.71 | 8.72 | 8.34 | 34.42*** | 1.94 | 1.84 |
| <i>IFITM1</i> | 8519 | 176.68 | 4.73 | 4.60 | 13.22*** | 0.99 | 0.92 |
| <i>6–16</i> | 2537 | 163.14 | 7.76 | 7.41 | 5.94*** | 0.39 | 0.37 |
| <i>IFITM3</i> | 10410 | 130.69 | 4.17 | 4.04 | 9.32*** | 0.46 | 0.44 |
| <i>IFITM2</i> | 10581 | 46.69 | 4.48 | 4.09 | 3.19*** | 0.21 | 0.20 |
| <i>IFI27</i> | 3429 | 46.53 | 0.73 | 0.72 | 2.41*** | 0.44 | 0.37 |
| <i>ISG15</i> | 9636 | 32.00 | 4.38 | 3.85 | 5.43*** | 0.25 | 0.24 |
| <i>ISG20</i> | 3669 | 4.07 | 0.36 | 0.33 | 1.97* | 1.44 | 0.83 |

Hek293T cells were transfected with a control (*Renilla* luciferase) siRNA or *SIN3A* siRNA. Seventy-two hours after transfection, cells were stimulated with IFN- α 2 for 24 h and ISGs transcription was evaluated. Fold-inductions represent mRNA levels relative to the NS sample and are highlighted in bold. *** $P < 0.001$, * $P < 0.01$; one-way ANOVA with Bonferroni test.

dependent transcription, Sin3a represents a unique control level determining cytokine-specific responses in different tissues or cells.

Materials and Methods

Detailed protocols are provided in *SI Materials and Methods*. Hek293T, MCF7, and HepG2 cells were purchased from ATCC, HCV-permissive HuH7.5-RFP-NLS-IPS cells were kindly provided by Charles M. Rice (The Rockefeller University, New York, NY). The list of primer pairs used in this work is in [Table S2](#). The Dharmacon siGenome SMARTpool library was used for the RNAi screen. Microarray datasets generated in this study are available in National Center for Biotechnology Information's Gene Expression Omnibus (GEO) repository (<http://www.ncbi.nlm.nih.gov/geo/>), accession no. GSE35696.

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