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SARS-CoV Accessory Protein 3b Induces AP-1 Transcriptional Activity through Activation of JNK and ERK Pathways

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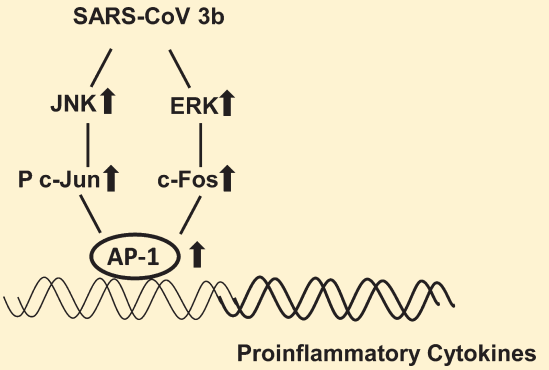
ABSTRACT: The outbreak of severe acute respiratory syndrome (SARS) in 2003 in China, characterized by atypical pneumonia, was associated with the emergence of a novel coronavirus named severe acute respiratory syndrome coronavirus (SARS-CoV). Eight accessory proteins of SARS coronavirus were the suspected players in the pathogenesis of the virus. Among them, protein 3b localizes to the nucleus and behaves as an interferon antagonist by inhibiting IRF3 activation. However, the effect of 3b on the activity of other common host transcription factors remains unexplored. In this work, we studied the effect of 3b on the transcriptional activity of AP-1. Our findings elucidate augmentation of AP-1-dependent gene expression in 3b-transfected Huh7 cells. Reporter gene and mobility shift assays depict an increase in the AP-1 transcriptional and DNA binding activity in the presence of 3b. This increase in activity correlates with the activation of ERK and JNK pathways. Furthermore, 3b expression potentiates AP-1-driven promoter activity of proinflammatory cytokine MCP-1, suggesting a plausible role for 3b as a virulence factor that might function by upregulating AP-1-dependent cytokine levels in SARS-CoV infection.



The emergence of SARS coronavirus (SARS-CoV) in 2003 once again boosted the field of coronavirus research and attracted worldwide interest. SARS-CoV has a positive strand RNA genome of 29751 bp, which encode various structural (spike, envelope, membrane, and nucleocapsid) and nonstructural proteins (Nsp1−16). Additionally, it encodes several group specific unique accessory proteins, namely, 3a, 3b, 6, 7a, 7b, 8ab, 9a, and 9b.[1,2] Among them, 3b is a predicted 154-amino acid protein transcribed from the second ORF of subgenomic RNA3.[1−3] Though the expression of 3b is dispensable for viral replication, it is suspected to play a vital role in virus pathogenesis.[4] Along with SARS6, nucleocapsid, nsp1, nsp3, and membrane protein, 3b has been reported to possess interferon antagonistic activity.[5−7] It has been demonstrated that 3b shuttles from the nucleus to mitochondria, where it inhibits induction of RIG-I and MAVS-induced type I interferons.[8] Also, 3b has been reported to inhibit interferon production and signaling by inhibiting phosphorylation-dependent activation of IRF3 transcriptional activity.[5] However, no significant effect of 3b on NF-κB activity was observed.[5] AP-1 is another virus-activated factor involved in regulating the activity of the IFN-β promoter.[9] Apart from interferons, AP-1 regulates transcription of many cytokine genes that are affected in SARS-CoV infection. Therefore, we aimed to study the effect of 3b on the transcriptional activity of AP-1.

AP-1 is composed of members of Jun and Fos families that dimerize and bind in a sequence specific manner on promoter regions. Jun and Fos family members form a variety of homo- and heterodimers that bind to a common AP-1 site.[10] AP-1 activity is regulated by mainly two mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase (ERK)[11] and c-Jun N-terminal kinase (JNK).[12−14] The activation of ERK leads to an increased level of phosphorylation of transcription factor Elk-1, which consequently induces transcription of c-Fos leading to AP-1 activity. The c-Fos thus synthesizd heterodimerizes with c-Jun in the nucleus and shifts the equilibrium from a Jun−Jun homodimer to a Jun−Fos heterodimer, which is more stable and has higher transactivation potential.[15] On the other hand, activation of JNK induces phosphorylation of c-Jun at Ser-63 and Ser-73 and potentiates its transcriptional activity. JNK also phosphorylates ATF-2 at Thr-63 and Thr-71 and stimulates its transcriptional activity in the c-Jun−ATF2 heterodimer complex.[16]

In this study, we investigate the effect of transiently expressed 3b on AP-1 transcriptional and DNA binding activity in human hepatoma Huh7 and Vero cells, which are permissive with respect to SARS-CoV infection. We demonstrated activation of ERK and JNK signaling cascades in 3b-expressing cells, leading to an increase in AP-1 activity and upregulation of AP-1-mediated activity of the MCP-1 promoter. Taken together, our results suggest an important role for SARS-CoV 3b protein in the stimulation of AP-1-dependent genes, especially pro-inflammatory cytokines responsible for the cytokine storm generated during SARS virus pathogenesis.

MATERIALS AND METHODS

Reagents and Plasmids. Luciferase assay kits (E1500 and E2810) were purchased from Promega. ERK inhibitor U0126,

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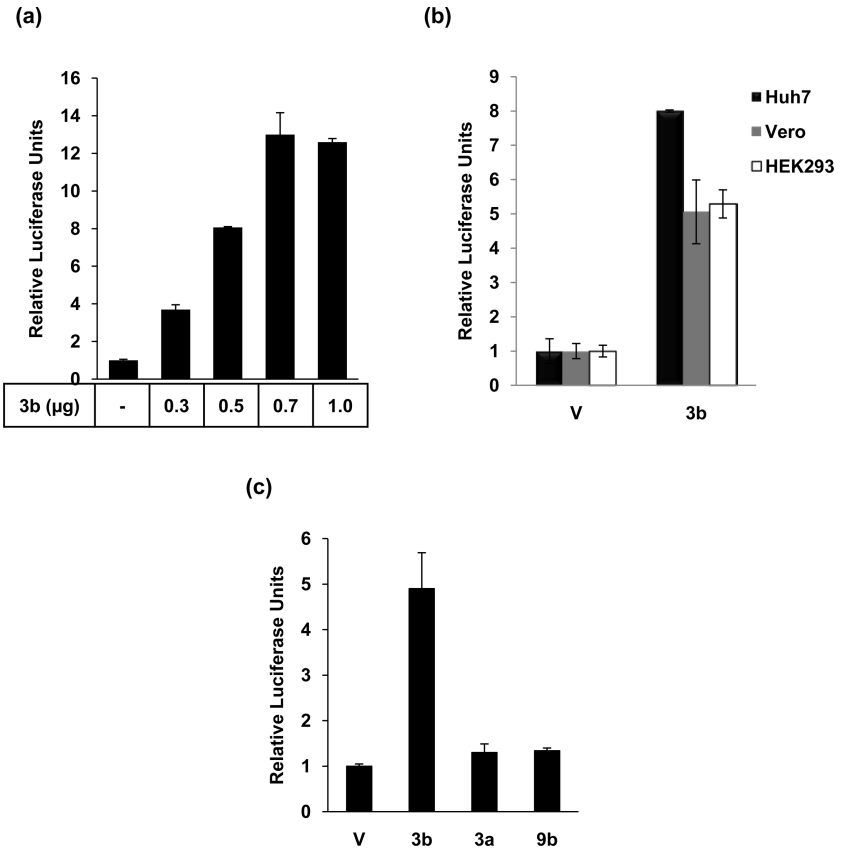

Figure 1. Augmentation of AP-1 transcriptional activity by 3b. (a) Huh7 cells were transfected with 2× TRE-Luc, pRLTK, and the indicated amounts of 3b-pcDNA expression plasmids. Relative luciferase activity was calculated after 48 h. (b) Huh7, Vero, and HEK293 cells were transfected with 2× TRE-Luc and 3b-pcDNA (800 ng). Relative luciferase activity was calculated 48 h post-transfection. (c) Huh7 cells were transfected with 2× TRE-Luc and 3b/3a/9b/pcDNA mammalian expression plasmids, and luciferase activity was calculated after transfection for 48 h. Results represent the mean ± SD of at least three experiments performed in triplicate. $p < 0.005$.

JNK inhibitor SP600125, and PI3K inhibitor LY294002 were purchased from EMD Biosciences. Antibodies against phospho MKK4 (Ser-257/Tyr-261), phospho JNK (Thr-183/Tyr-185), phospho ERK (Thr-202/Tyr-204), phospho Elk-1 (Ser-383), and JNK were purchased from Cell Signaling. Antibodies against phospho Jun (Ser-63/73), c-Jun, MKK4, c-Fos, ERK-1, phospho Akt (Ser-473), Akt, and c-myc were purchased from Santa Cruz. The SARS-CoV 3b gene (25689−26153 bp) was amplified via polymerase chain reaction (PCR) from the SARS-CoV genome (NC_004718) and cloned at *Nhe*I and *Afl*II sites in mammalian expression vector pcDNA3.1(−)myc/his. The reporter construct containing the luciferase gene under the control of two copies of TPA responsive element (2× TRE-Luc) was generously provided by M. Karin, [17] and the construct expressing the dominant negative mutant of Fos (FosDN) was generously provided by C. Vinson and has been described previously.[18] The MCP-1-Luc construct was a gift from A. Garzino-Demo.[19]

Cell Culture and Transfection. Huh7 human hepatoma cells, HEK293 human embryonic kidney 293 cells, and Vero cells were maintained in Dulbecco's modified Eagle's medium (DMEM). All cell lines were maintained in DMEM supplemented with 10% FCS (fetal calf serum, v/v) and antibiotics (penicillin and streptomycin). Transient transfections in cells were conducted using either FuGENE 6 (Roche) or Lipofectamine 2000 (Invitrogen), following the manufacturer's protocol.

Luciferase Assay. The luciferase assay was performed following the manufacturer's protocol (Promega). For the assay, cells were transfected with 100 ng of reporter plasmid (2× TRE-Luc or MCP-1-Luc), 10 ng of pRL-TK, and the indicated amounts of expression plasmid pcDNA-3b; 200 ng of FosDN was added where indicated. All transfections were normalized for the amount of total DNA by adding vector pcDNA3. Luciferase activity was measured 48 h post-transfection and normalized against Renilla luciferase activity. Drug (U0126, 10 μM, and SP600125, 20 μM) treatment was performed 16 h prior to the cells being harvested. Relative luciferase activity was expressed as the mean ± standard deviation (SD) of three independent observations. Statistical significance was calculated using a Student's *t* test. *p* values are given in the figure legends. Error bars represent the SD.

Western Blotting. The method for Western blotting has been described previously.[20] Actin was used as a loading control.

Nuclear Fractionation and Electrophoretic Mobility Shift Assay (EMSA). Transfected cells were harvested in 800 μL of buffer A [10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EGTA, 0.1 mM EDTA, and 1 mM DTT] with 1× protease inhibitor cocktail. Lysates were incubated on ice for 15 min, followed by addition of 50 μL of 10% NP40, and immediately vortexed for 10 s. Samples were centrifuged at maximal speed for 30 s to eliminate the cytosolic fraction. The pellet was resuspended in buffer B (buffer A with 400 mM NaCl and 20% glycerol) with 1× protease

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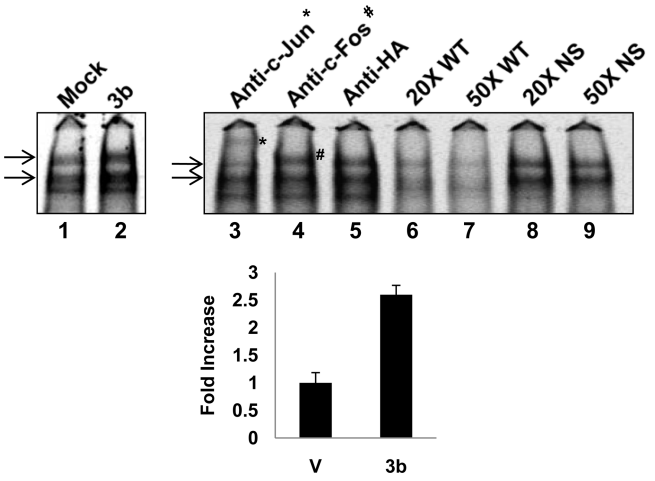

Figure 2. Increased level of AP-1 DNA binding on the AP-1 consensus element in 3b-expressing cells. Nuclear extracts were prepared from Huh7 cells transfected with 3b and vector constructs and used for an EMSA. Lanes 1 and 2 shows in vitro DNA binding of the AP-1 complex on ^{33}P-labeled consensus element in 3b and control vector nuclear extracts, respectively. Arrowheads indicate the positions of Jun−Jun homodimers and Jun−Fos heterodimers. The 3b nuclear extract was subjected to a supershift assay using anti-c-Jun (lane 3), anti-c-Fos (lane 4), and anti-HA (lane 5) antibodies. The specificities of the complexes were ascertained by a competition assay using 20−50-fold molar excesses of cold wild-type (WT) and nonspecific (NS) oligos. The asterisk and number sign represent supershifted bands with anti-c-Jun and -c-Fos antibodies, respectively. The *x*-fold increase is the mean ± SD of three independent experiments, assessed by densitometry.

inhibitor cocktail and incubated at 4 °C for 45 min on a rocker. The nuclear extract was obtained by centrifuging the suspension at 5000 rpm for 5 min. Protein was quantitated, and aliquots were stored at −70 °C.

For the EMSA, 3−5 μg of nuclear extract was preincubated with 200 ng of dIdC and 10 μg of BSA in binding buffer [20 mM HEPES (pH 7.9), 60 mM KCl, 1 mM DTT, 3.5 mM $MgCl_2$, 0.2 mM EDTA, and 20% glycerol] for 10 min at 4 °C. To the reaction mix was added 80−100 fmol of [γ-^{33}P]AP-1 consensus probe, and binding was allowed for 20 min at room temperature. Protein−DNA complexes were resolved on an 8% nondenaturing polyacrylamide gel and electrophoresed at 100 V in 0.5× TAE at 4 °C. The sequences of the AP-1 consensus probes were 5′ CGCTTGATGAGTCAGCCGGAA 3′ (forward) and 5′ TTCCGGCTGACTCATCAAGCG 3′ (reverse).[21,22] For competition experiments, unlabeled oligonucleotides were added in 20- and 50-fold molar excess in the reaction mixture before the addition of labeled probe. Sequences of non-AP-1 probe used in competition experiments were 5′ TTGCGTTTGTCCACCACAACAGGCTGCTTAC 3′ (forward) and 5′ GTAAGCAGCCTGTTGTGGTGGACAAACGCAA 3′ (reverse). For supershift assays, reaction mixtures were incubated with 1 μg of antibody for 10 min prior to the addition of labeled probe.

RESULTS

3b Induces AP-1 Transactivation Potential. To analyze the effect of 3b expression on the activity of the AP-1 complex, we used an AP-1 responsive luciferase reporter construct (2× TRE-Luc) and SARS-CoV permissive Huh7 cells.[23] Huh7 cells transiently transfected with a constant amount of 2× TRE-Luc showed a significant increase in luciferase activity (3−12-fold) with an increasing dose of 3b (0.3−1.0 μg), reflecting a 3b-mediated increase in the level of AP-1 transactivation (Figure 1a). This effect could be replicated in Vero and HEK293 cell lines, although to a lesser extent (Figure 1b).

No significant increase in the level of AP-1 transactivation was observed when Huh7 cells were transfected with other SARS-CoV accessory proteins, 3a and 9b (Figure 1c), suggesting that the increase was specific to 3b.

3b Increases AP-1 DNA Binding Activity. To study whether the 3b-induced AP-1 activity parallels its increased level of DNA binding, we performed an EMSA. A 2.5-fold higher level of AP-1 complex binding was observed using nuclear extracts from 3b-transfected cells in comparison to control transfected cells (lanes 1 and 2, Figure 2). The specificity of the complex was proven by competition experiments using wild-type (WT, consensus) and nonspecific (NS, containing no AP-1 binding site) cold oligos in which the presence of 20- and 50-fold molar excesses of WT oligos competitively displaced the complex (lanes 6 and 7), whereas no effect on band intensity was observed with nonspecific oligos (lanes 8 and 9). Two bands for the AP-1 complex observed in the EMSA, as reported previously,[24] correspond to the Jun−Jun homodimer (upper band) and the Jun−Fos heterodimer (lower band). The composition of the complex was assayed by supershift using c-Jun, c-Fos, and nonspecific antibodies (lanes 3−5, Figure 2), confirming the Fos−Jun heterodimer and the Jun−Jun homodimer as the components of the complex.

3b Induces Phosphorylation of c-Jun (Ser-63) through Activation of the JNK Pathway. Activation of AP-1 depends on either enhanced synthesis or an increased level of phosphorylation of Fos and Jun family members that potentiates its transcriptional activity.[16] 3b-expressing cells showed a significant increase in phospho c-Jun levels as compared to that in GFP-expressing control cells, while total c-Jun levels remained unaffected (Figure 3a).

Because c-Jun phosphorylation at Ser-63 is governed by JNK, activation of JNK pathway components in 3b-expressing cells was assessed by analyzing levels of active phosphorylated forms of JNK and its upstream kinase MEK4. An enhanced phosphorylation of JNK and MEK4 was observed in cells expressing 3b as compared to control cells expressing GFP (Figure 3a). Equal amounts of protein loading were confirmed by probing with JNK and MEK4 antibodies. Treatment of cells with JNK specific inhibitor SP600125 (20 μM) for 1 h led to inhibition of phospho JNK and phospho c-Jun levels, whereas no change was noticed in DMSO-treated 3b-expressing cells (Figure 3b), suggesting that 3b induces phosphorylation of c-Jun at Ser-63 through activation of the JNK pathway. A similar activation of JNK pathway components was also observed in 3b-transfected HEK293 cells (data not shown), indicating that the phenomenon is not cell line specific.

3b Augments c-Fos Levels by Activating the ERK Pathway. Next our aim was to determine whether 3b transfection in Huh7 cells affects c-Fos levels. Higher c-Fos levels were observed in 3b-expressing cells than in GFP-expressing control cells (Figure 4a).

An increased level of c-Fos synthesis is attributed to an increased level of phosphorylation of transcription factor complex Elk-1/TCF on c-Fos promoter, which in turn is the result of ERK pathway activation.[16] Hence, ERK and Elk-1 activation was checked in 3b-expressing cells. An increase of ∼2.0-fold in phospho ERK (Thr-202/Tyr-204) levels in 3b-expressing Huh7 cells as compared to GFP-transfected control cells (Figure 4a) was observed. This increase in phospho ERK levels was also

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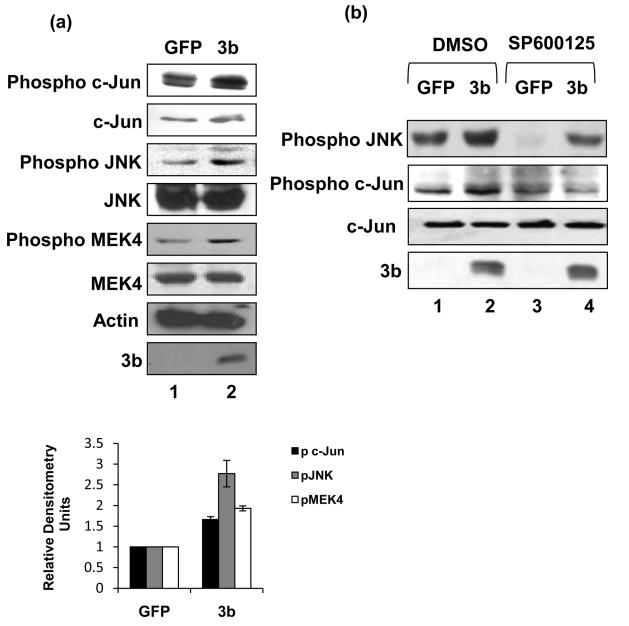

Figure 3. Activation of the JNK pathway by 3b. Huh7 cells were transfected with 3b and GFP expression plasmids. 3b expression was detected using an anti-c-myc antibody. (a) Western blot analysis of phospho JNK, phospho c-Jun, and phospho MEK4. Blots were stripped and reprobed with Jun, JNK, and MEK4 antibodies to assess equal loading. The *x*-fold increase is the mean ± SD of three independent experiments, assessed by densitometry. (b) Transfected cells were treated with SP600125 (20 μM) and DMSO. Western blot analysis of phospho JNK, phospho c-Jun, and c-Jun.

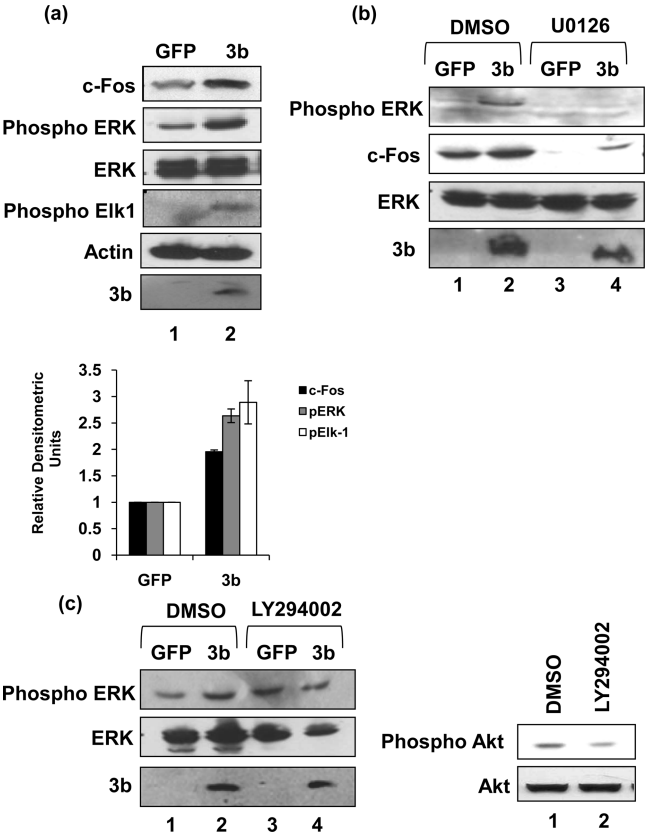

Figure 4. Activation of the ERK pathway by 3b. Huh7 cells were transfected with 3b and GFP expression plasmids. 3b expression was detected using the anti-c-myc antibody. (a) Western blot analysis of c-Fos, phospho ERK, and phospho Elk-1. ERK and actin levels were probed for equal loading. The *x*-fold increase is the mean ± SD of three independent experiments, assessed by densitometry. (b) Transfected cells were treated with U0126 (10 μM) and DMSO. Western blot analysis of phospho ERK and c-Fos. ERK levels were probed for equal loading. (c) Transfected cells were treated with LY294002 (50 μM) and DMSO. Western blot analysis of phospho ERK and ERK. The side panel shows the effectiveness of LY294002 in Huh7 cells. Western blot of phospho Akt and Akt.

observed in 3b-transfected Cos7 and HEK293 cells (data not shown). Phosphorylation levels of Elk-1 (Ser-383), the downstream substrate of ERK, were also found to be increased in 3b-expressing Huh7 cells (Figure 4a).

Treatment of transfected cells with U0126 (10 μM), a selective inhibitor of MEK1, blocked 3b-induced ERK activation and c-Fos levels (Figure 4b), whereas no change was observed upon treatment with PI3K/Akt inhibitor, LY294002 (Figure 4c). These results showed that 3b-mediated ERK activation is dependent on the Raf/MEK pathway but not the PI3K/Akt pathway.

Taken together, our results lead to the conclusion that 3b expression induces phosphorylation of c-Jun and c-Fos levels by activating JNK and ERK pathways, respectively.

3b-Induced AP-1 Activity Is Mediated by Activation of JNK and ERK Pathways. Activation of JNK and ERK pathways in 3b-expressing Huh7 cells suggests the involvement of these cascades in mediating 3b-induced AP-1 activity. To confirm our hypothesis, we performed luciferase assays in Huh7 cells transfected with 2× TRE-Luc and 3b or control vector expression plasmids in the presence or absence of the dominant negative Fos mutant (FosDN) construct. FosDN has an acidic amphipathic protein sequence that heterodimerizes with the basic sequence of c-Jun and prevents it from DNA binding, thus abolishing AP-1 activity. Coexpression of FosDN nearly abolished the luciferase activity induced by 3b (Figure 5a), suggesting that the Fos−Jun heterodimer is an essential factor in mediating 3b-induced AP-1 activity. Next, the involvement of ERK and JNK pathways in 3b-mediated AP-1 induction was confirmed by treating cells with U0126 and SP600125. Huh7 cells transfected with 2× TRE-Luc and 3b or control vector were treated with U0126 and SP600125 for 16−18 h. The control set was treated with DMSO for the same period of time. Results depicted 70% inhibition of 3b-induced AP-1 activity in SP600125 and 50% in U0126-treated cells as compared to DMSO-treated 3b-expressing cells (Figure 5b). This suggests that 3b-induced ERK and JNK pathway activation leads to an increase in AP-1 activity in cells.

3b Potentiates AP-1-Dependent MCP-1 Promoter Activity. The role of AP-1 in the regulation of cytokines and chemokines in the case of viral infections is well-documented.[25−28] Monocyte chemoattractant protein-1 (MCP-1) is one such chemokine reported to be upregulated in SARS infection, and its promoter is regulated by AP-1, NF-κB, and SP1 transcription factors.[19] To elucidate the effect of 3b on the AP-1-driven activity of the MCP-1 promoter, the MCP-1-Luc reporter construct was used. MCP-1-Luc contains the luciferase gene cloned under a 213 bp fragment of the MCP-1 promoter (residues −213 to 1).[19] For the assay, Huh7 cells were transfected with MCP-1-Luc and 3b or vector expression plasmids. An increase in MCP-1 promoter activity was observed with increasing amounts of 3b plasmid (Figure 6a). This increase in 3b-induced promoter activity was

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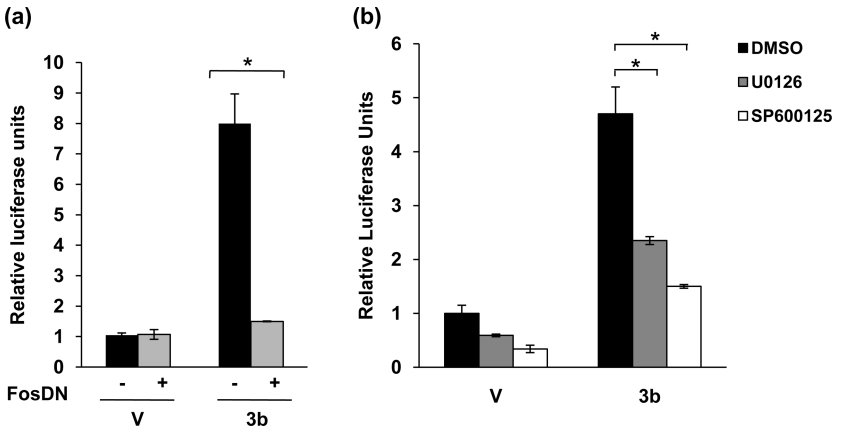

Figure 5. Upregulation of AP-1 activity by 3b is dependent on ERK and JNK pathways. Huh7 cells were transfected with 2× TRE-Luc, 3b expression construct, and FosDN (a) or treated with DMSO, U0126, and SP600125 (b). Relative luciferase activity was calculated and is shown as the mean ± SD of three independent experiments performed in triplicate. $p < 0.005$.

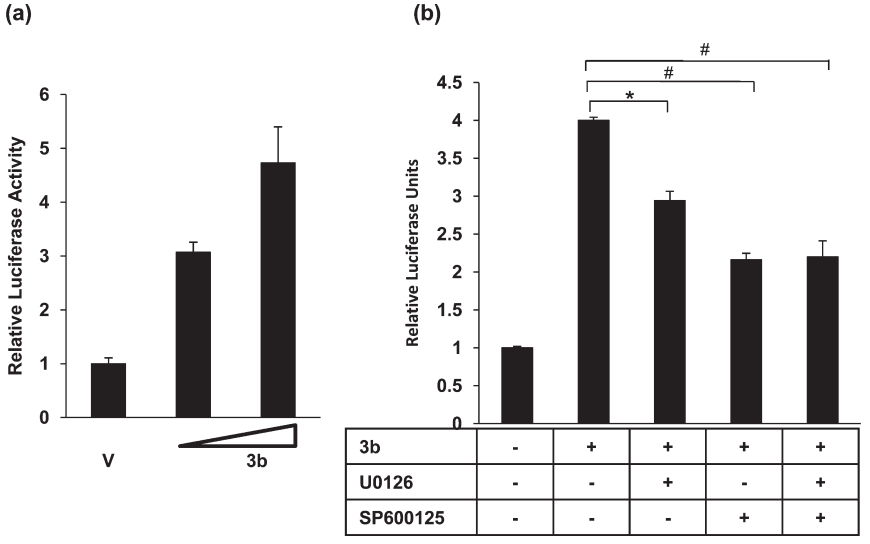

Figure 6. Activation of AP-1-dependent MCP-1 promoter activity by 3b. Huh7 cells were transfected with MCP-1-Luc and increasing amounts of 3b expression plasmid (500 and 900 ng) (a) or 3b construct and treated with SP600125 and U0126 (b). The relative luciferase activity was calculated and is shown as the mean ± SD of three independent experiments performed in triplicate. $^{*}p < 0.05$, and $^{\#}p < 0.005$.

reduced to nearly 70 and 50% when treated with ERK and JNK inhibitors, U0126 and SP600125, respectively (Figure 6b). Treatment of 3b-expressing cells with U0126 and SP600125 simultaneously led to a 2-fold decrease in the MCP-1 promoter activity, suggesting that 3b-dependent activation of the MCP-1 promoter occurs partially by activation of ERK and JNK pathways, involved in regulating AP-1 transcription factors.

DISCUSSION

Consistent with all viral infections, SARS coronavirus infection also entails activation of various cellular signaling pathways. These include pathways sensitive to external stimuli like MAPKs and PI3K/Akt pathways that turn "on" or potentiate activity of various transcription factors, like AP-1 and NF-κB family members. AP-1 transcription factors have been known to contribute in diverse biological processes, including the cell cycle, cell growth, oncogenesis, and many more.[29] Besides, its role in viral infection, replication, and pathogenesis has been studied well. Accumulation of viral RNA in influenza A virus infection induces JNK and AP-1 and as a result triggers expression of antiviral cytokines.[26] Hepatitis B virus-encoded X protein (HBx) stimulates ERK and JNK, leading to the activation of AP-1 transcription factors, and thus might play a role in virus-induced transformation and pathogenesis.[28] Epstein-Barr virus (EBV)-encoded latent membrane protein-1 (LMP-1) and EBV nuclear antigen-1 (EBNA-1) also stimulate AP-1 activity. LMP-1 triggers the SEK/JNK/c-Jun/AP-1 pathway that contributes to its immortalizing and transforming potential.[25] EBNA-1-induced AP-1 activity contributes to its angiogenic potential.[27] A recent study by Yoshikawa et al. showed activation of AP-1 transcription factors 24 h after SARS-CoV infection in human bronchial epithelial cells.[30] Other groups have reported the involvement of SARS-CoV nucleocapsid and spike proteins in activation of the AP-1 family of transcription factors.[31,32] Our study is mainly focused on the activation of AP-1 due to the transient expression of SARS-CoV accessory

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protein 3b. This study reports that transient expression of 3b in SARS coronavirus permissive cell lines Huh7 and Vero potentiates AP-1 transcriptional activity and also improves its DNA binding ability on the AP-1 consensus element.

Homodimers of Jun family members or heterodimers of Jun and Fos family members constitute the AP-1 transcription factor complex. Two MAPKs signaling are centrally involved in regulating its activity, namely, ERK and JNK. While the stimulation of ERK induces sequential activation of the Elk-1−TCF complex and c-Fos transcription, the stimulation of the JNK pathway induces c-Jun activation by enhancing phosphorylation at the N-terminus. We demonstrated that 3b expression increases AP-1 activity by triggering ERK and JNK cascades, consequently increasing levels of c-Fos and N-terminal c-Jun phosphorylation. Because cross-talk between PI3K/Akt and Raf/Ras pathways can lead to ERK activation, the involvement of the PI3K/Akt pathway was ruled out by using its specific inhibitor, LY294002.

AP-1 regulates transcription of many genes involved in viral pathogenesis, including pro-inflammatory and antiviral cytokines like IL-6,[33] IL-8,[34] RANTES,[35] MCP-1,[19] interferons,[9] etc., that are characteristic of an infection. SARS pathology is the result of an exacerbated pro-inflammatory immune response by cytokines in the lungs of patients and in infected animal models.[30,36] A recent study reported induction of IL-8 levels by baculovirus-expressed spike protein via activation of MAPKs and AP-1.[31] In this study, we found escalated MCP-1 promoter activity in 3b-expressing cells by virtue of augmented AP-1 activity. This led us to the hypothesis that 3b might serve as another virulence factor for SARS-CoV.

AP-1 transcription factors, in conjunction with IRF3 and NF-κB, regulate transcription of interferons. Virus infection is followed by activation of these factors that consequently leads to upregulation of IFN transcription.[9] To counteract this host defense strategy, virus-encoded interferon antagonists employ different mechanisms to inhibit induction of IFN and interferon inducible genes and build an environment for persistent replication. For example, influenza virus NS1 protein prevents the IFN response by sequestering viral double-stranded RNA (a byproduct of the virus replication cycle), thereby preventing activation of cytosolic sensor RIG-I.[37,38] Rabies virus phosphoprotein P interferes with phosphorylation of IRF3.[39] SARS-CoV ORF3b, nucleocapsid, and SARS6 are reported to inhibit expression of type I interferon by preventing IRF3 phosphorylation.[5] However, the NS1 variant of highly pathogenic H5N1 is also the key determinant of cytokine release in the case of infection.[40] Thus, we argue that 3b, despite acting as an interferon antagonist, might also be responsible for the cytokine storm generated in SARS coronavirus infection by regulating AP-1 activity.

The massive pro-inflammatory cytokine response and acute lung injury in SARS-CoV-infected humans are believed to be the result of transmission of the virus across species from a natural to a non-natural host.[41] Natural host bats, though persistently infected, do not display any clinical symptoms. This might be attributed to the architecture of their immune system.[42] However, in humans, the precise mechanism by which SARS-CoV proteins cause pathology is still an enigma. Our study has unveiled 3b as an inducer of AP-1 activity and once again has introduced it as a crucial accessory protein that might play a role in virus pathogenesis by triggering levels of AP-1-dependent proinflammatory cytokines like MCP-1, levels of which were found to be elevated in SARS-CoV-infected patients.

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ABBREVIATIONS

MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; MCP-1, monocyte chemoattractant protein-1; EBV, Epstein-Barr virus; LMP-1, late membrane protein-1; EBNA-1, EBV nuclear antigen-1.

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