Interactions between c-Jun, Nuclear Factor 1, and JC Virus Promoter Sequences: Implications for Viral Tropism

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The infectious cycle of the human polyomavirus JC (JCV) is ultimately regulated in cellular nuclei at the level of viral protein expression and genomic replication. Such activity is prompted by interactions between variant nucleotide sequences within the JCV regulatory region (promoter) and cellular transcription factors that bind specific DNA consensus sites. In previous work we identified an NF-1 class member, NF-IX, as a critical transcription factor affecting the JCV cellular host range. Within variant JCV promoters, as well as other viral and cellular promoters, adjacent NF-1 and AP-1 consensus sites are often found. The close proximity of these two binding sites suggests the opportunity for interaction between NF-1 and AP-1 proteins. Here, by electrophoretic mobility shift assays, we show temporal and dose-dependent interference by an AP-1 family member, c-Jun, upon NF-1 proteins binding an NF-1 consensus site derived from JCV promoter sequence. Moreover, as demonstrated by protein-protein interaction assays, we identify specific binding affinity independent of DNA binding between NF-IX and c-Jun. Finally, to compare the binding profiles of NF-IX and c-Jun on JCV promoter sequence in parallel with in vivo detection of viral activity levels, we developed an anchored transcriptional promoter (ATP) assay. With use of extracts from JCV-infected cells transfected to overexpress either NF-IX or c-Jun, ATP assays showed concurrent increases in NF-IX binding and viral protein expression. Conversely, increased c-Jun binding accompanied decreases in both NF-IX binding and viral protein expression. Therefore, inhibition of NF-IX binding by c-Jun appears to play a role in regulating levels of JCV activity.

The human polyomavirus JC (JCV) is the etiologic agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy. During the course of trafficking to target oligodendrocytes sequestered in the central nervous system (CNS), JCV is known to also bind, enter, and to some extent infect peripheral cell types, including tonsillar stromal cells (stromal), B-lymphocytes, and kidney cells. While the presence of JCV in these varied cell types is well documented (22), a thorough understanding of how the inherent differences in cellular machinery affect viral activity has yet to be reached.

In part, cellular susceptibility to JCV is governed by events at the transcriptional level. Several cellular transcription factors implicated in the regulation of JCV gene expression include NF-κB (29), Tst-1 (35), Y-box binding protein 1 (16), and Pura (7), as well as members of transcription factor groupings, such as the nuclear factor 1 (NF-1) (2) and activator protein 1 (AP-1) families (1). Consensus binding sites for these and other DNA-binding proteins are centrally located within the JCV regulatory region (promoter) in an approximately 200-bp section of highly variable nucleotide sequence. This variable sequence offers distinct arrangements and assortments of transcription factor binding sites, which, in turn, lend to the diversity of viral activity seen between variant JCV genomes (15). Viral activity, however, is also affected by the unique expression pattern of transcription factors found within each cell type JCV enters (23, 26). Interestingly, cellular transcription factor expression patterns change as cells mature, and variations can be pronounced depending on the pathway of differentiation (26). Moreover, synergistic, competitive, and/or inhibitory interactions occurring between any number of cellular transcription factors (and cis-acting viral proteins) have the potential to further alter net levels of JCV activity. Such parameters suggest that JCV transcription and replication is controlled by multilayered interactions between cellular machinery, viral proteins, and infectious viral promoter sequences.

In humans, the NF-1 family of DNA-binding proteins (NF-1A, -B, -C, and -X) (X is also known as D) is encoded by four discrete genes. Differential splicing of the NF-1 mRNA transcripts results in multiple isoforms of each NF-1 class member (11). First isolated from HeLa cells and found to stimulate adenovirus DNA replication, NF-1 proteins have since been associated with transcriptional regulation of several cellular and viral genes. The variable C-terminal regions of NF-1 proteins convey differences in transcriptional activity and also serve to distinguish the NF-1 class types and splice variants. With highly conserved N-terminal domains shared between the NF-1 class types, homo- and/or heterodimerization bears a variety of protein structures, all capable of binding alpha-helical DNA representative of the consensus sequence 5’-TGG (A/C)N5GCCAA-3’ (6).

Enhanced expression of NF-1 class X has been correlated with the support of JCV activity. Previously, we showed that JCV-susceptible stromal cells express elevated levels of NF-1X compared to nonsusceptible cell types (26). The same relationship between cellular NF-1X expression and JCV susceptibility...
was observed in permissive SH-EP and nonpermissive SH-SY5Y neuroblastoma cell lines (32). Moreover, we demonstrated that when a JCV-susceptible human hematopoietic progenitor cell line, KG-1, was treated with the mitogen phorbol 12-myristate 13-acetate to induce differentiation to a macrophage-like phenotype, it no longer supported JCV activity. However, transfecting the macrophage-like phenotype with an expression vector encoding NF-1X restored JCV susceptibility. A similar observation was made using nonsusceptible human neuronal cells derived from a human CNS progenitor cell culture system (23). In both cases, the conveyance of JCV susceptibility was specific to NF-1X, as demonstrated by the failure of parallel transfections with expression vectors encoding the other NF-1 class members.

The AP-1 family of transcription factors has also been associated with JC viral activity (1, 17). Many members of this transcription factor family, like the NF-1 proteins, dimerize to facilitate DNA binding. AP-1 dimers are composed of Jun (c-Jun, JunB, and JunD) as well as Fos (c-Fos and FosB) proteins, and have affinity for DNA containing the consensus sequence 5′-TGA(G/C)TCA-3′. AP-1 proteins comprise three domains: a C-terminal leucine zipper necessary for dimerization (19, 34), a central basic region responsible for DNA binding, and an N terminus involved in transcriptional activation (4). It has been demonstrated that c-Jun can modulate transcriptional activity of JC virus, as well as the unwinding of DNA mediated by the early T antigen of mouse polyomavirus (12, 13, 17).

Adjacent NF-1 and AP-1 consensus sites are encountered in JCV and BK virus promoters, as well as several brain-specific genes, including those encoding myelin basic protein, neurofilament, human glial fibrillary acidic protein (GFAP), and proenkephalin (1, 18, 33). This juxtaposition of NF-1 and AP-1 consensus sites in various genes associated with the human CNS suggests that interactions between NF-1 and AP-1 proteins play a role in tissue-specific host and viral gene expression. In addition to juxtaposed binding sites, physical interaction between NF-1 and AP-1 proteins has recently been observed in regard to GFAP expression in an astrocytic cell line (10); however, the nature of this interaction is unclear.

Here, we demonstrate interference by c-Jun upon binding of NF-1 proteins to an NF-1 consensus site derived from JCV promoter sequence. We also demonstrate specific protein-protein binding affinity, independent of DNA binding, between NF-1X and c-Jun using a number of techniques, including immunoprecipitation assays, Western blots, in vitro transcription/translation, and glutathione S-transferase (GST) fusion protein capture. Moreover, utilization of a JCV-anchored transcriptional promoter (ATP) assay we have developed reveals differentials of viral activity concurrent with varied profiles of NF-1X and c-Jun binding to JCV promoter sequence. The interactions described here between NF-1X, c-Jun, and JCV promoter sequence likely play a role in the regulation of JCV activity.

**Stromal protein extract.** Stromal cells were washed three times with ice-cold phosphate-buffered saline (PBS), and whole-cell extracts were prepared by a modification of the procedure of Andrews and Faller (3). Cells were scraped from tissue culture flasks, pelleted, and then disrupted by three freeze-thaw cycles on dry ice. Cell pellets were resuspended in two volumes of ice-cold buffer C (20 mM Tris-HCl [pH 7.9], 1.5 mM MgCl₂, 420 mM NaCl, 0.2 M EDTA, 25% glycerol) including protease inhibitors (0.5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 5 μg/ml antipain, 5 μg/ml leupeptin, 5 μg/ml chymostatin, 5 μg/ml aprotinin, 5 μg/ml pepstatin A). Lysates were then centrifuged at 9,000 × g for 5 min at 4°C. Supernatant protein fractions were divided into aliquots and stored at −80°C. Protein concentrations were determined by Bradford assay (5).

**Radiolabeled oligonucleotide probes.** Oligonucleotides derived from JCV Mad-1 promoter sequence (nucleotides [nt] 38 to 53) (9) containing either an intact NF-1 binding site (5′-ATGCGTACCAGCCAAAG-3′) or a mutated NF-1 site (mutated sites underlined; 5′-ATATCCTGACCTGAG-3′) were synthesized (Invitrogen). Complementary strands were produced and annealed to each corresponding sequence above to form double-stranded oligonucleotides.

**Electrophoretic mobility shift assay (EMSA).** Unless otherwise noted, radiolabeled oligonucleotide probes (200,000 cpm) were end labeled with [γ-32P]ATP for 2 h at 37°C, centrifuged through a Microspin G-25 column (Pharmacia/GE Healthcare-Amersham Biosciences) at 735 × g for 2 min, and brought to a working concentration of 0.2 pM with buffer D (10 mM HEPES, 50 mM KCl, 10% glycerol).

**Human progenitor-derived astrocytes (PDA).** Human CNS progenitor cells (progenitors) were isolated from the telencephalon of a human fetal brain at 8 weeks of gestation, obtained in accordance to NIH guidelines and differentiated into an astrocytic lineage (PDA) according to a previously described protocol (26).

**Immunoprecipitation (IP) and Western blot.** In these assays we utilized whole-cell extracts. Stromal and PDA cell cultures were lysed in ice-cold lysis buffer E (20 mM HEPES [pH 7.5], 150 mM NaCl, 3% glycerol, 5 mM MgCl₂, 1 mM CaCl₂, 1 mM EDTA, 1.0% NP-40, and one Complete Mini protease/phosphatase inhibitor tablet [Roche] per 20 ml). Lysates were sonicated on ice three times, 5 s each. Lysates were centrifuged to remove cellular debris, supernatants were precleared by incubation at 4°C for 1 h with 100 μg of protein G slurry (50% protein G agarose beads [Santa Cruz Biotechnology]) suspended in 50% lysis buffer E and 1 μg of a nonspecific rabbit polyclonal antibody (anti-14-3-3; Santa Cruz Biotechnology). Non specific complexes were pelleted by centrifugation at 10,000 × g at 4°C for 10 min, and the precleared supernatants were rotated at 4°C with either 1 μg of preimmune rabbit sera (Santa Cruz Biotechnology), anti-NF-1X (Geneka/Active Motif), or anti-c-Jun (Santa Cruz Biotechnology). After 1 h of incubation with these antibodies, a fresh 100 μl of protein G slurry was added for another 1 h of rotation at 4°C. The protein G beads were pelleted by centrifugation at 4°C and washed three times in ice-cold lysis buffer E, one time in ice-cold PBS. Beads were again pelleted by centrifugation at 4°C but then resuspended in 50 μl Laemmli protein loading buffer (PLB) and boiled for 5 min. Supernatants (the immunoprecipitates) from the pelleted beads were resolved on a 7.5% SDS-PAGE gel and transferred to a PVDF membrane (Millipore). The transferred membranes were probed with primary antibodies (anti-c-Jun [Santa Cruz Biotechnology] or anti-NF-1X [Geneka/Active Motif]) at 4°C overnight in blocking buffer (25 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20, and 5% nonfat dry milk). Bound primary antibodies were detected with an antirabbit, horseradish peroxidase-conjugated secondary antibody and the Chemiluminescence Luminol reagent kit (Santa Cruz Biotechnology), according to the manufacturer’s protocol.

**Plasmid constructs.** A GST fusion protein plasmid, pGEX-Jun (21), which encodes GST-c-Jun, was generously provided by James A. Goodrich (University of Colorado). Additionally, pGEX2T, which encodes GST alone, was pur-
NF-1X cDNA was subcloned into the pCHA vector to form a fusion protein with cases where monoclonal primary antibodies were used, a horseradish peroxidase-actin (Sigma) and anti-GFAP (Chemicon) primaries. In blot section but with anti-NF-1A, anti-NF-1B, and anti-NF-1X (Geneka/Active Motif), 12% gradient bis-Tris gels (Invitrogen), transferred to a PVDF membrane (Millipore), and analyzed by anti-NFX-1 (Geneka/Active Motif), anti-c-Jun (Santa Cruz Biotechnology), the polyclonal rabbit anti-Vp-1 developed in our lab, and anti-β-actin (Sigma).

Overexpression experiments. PDA were transfected with pCDNA3, pCDNA3NF-1X, or pET-c-Jun (a generous gift from James A. Goodrich, University of Colorado) using 5 μg of DNA/106 cells with Amaxa (Amaxa Inc.) astrocyte nucleo-fector reagent. At 12 h posttransfection, the media were replaced with fresh medium containing JCV at 200 hemagglutination units/106 cells. After 12 h, medium containing virus was aspirated, cells were washed once, and fresh medium was added. Nuclear fractions of transfected/infected PDA were prepared 4 days post-JCV exposure, resolved by SDS-PAGE, and analyzed by Western blotting as described in the “PDA nuclear extract” section but with anti-NF-1X (Geneka/Active Motif), anti-c-Jun (Santa Cruz Biotechnology), the polyclonal rabbit anti-Vp-1 developed in our lab, and anti-β-actin (Sigma).

ATP binding assay. The Mad-1 JCV promoter was PCR amplified from pMITC (9) using a modified forward primer (JCV Mad-1) (nt 4992 to 5011) containing a biotinylated PCR product coupled to streptavidin agarose beads, as previously described (30), to generate ATP-Mad-1. For preclearing of PDA extracts prior to ATP-Mad-1 exposure, an ATP pull-down assay. GST and GST-c-Jun were generated, purified, and immobilized on glutathione-agarose as described above. Cultures of PDA were transiently transfected with pCDNA3NF-1X (2 μg/2 × 106 cells) using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. To generate whole-cell extracts, the PDA were disrupted 36 h posttransfection with ice-cold lysis buffer E (20 mM HEPES [pH 7.5], 150 mM NaCl, 3% glycerol, 5 mM MgCl2, 1 mM CaCl2, 1 mM EDTA, 1% NP-40, and 1% Complete Mini protease/phosphatase inhibitor tablet [Roche] per 20 ml). Lysates were sonicated on ice for 20 min and incubated with immobilized GST or GST-c-Jun at 4°C, rotating for 2 h. Resulting complexes were washed four times with 100-bed volumes of binding buffer containing 0.1% NP-40 and once with PBS. Bound proteins were eluted by boiling the complexes for 3 min in 2× PBS, resolved on 12% precast SDS-polyacrylamide ReadyGels (Bio-Rad), transferred to PVDF membranes (Millipore), and analyzed by anti-NFX-1 (Geneka/Active Motif), anti-c-Jun (Santa Cruz Biotechnology). In vitro binding. GST and GST-c-Jun were generated and purified as described above. Employing pCDNA3NF-1X as a template, [35S]methionine-labeled NF-1X (Fig. 1, lane 5). A primary 30-min incubation of stromal cell nuclear extract, containing nuclear proteins, was added. Nuclear fractions of transfected/infected PDA were prepared 4 days post-JCV exposure, resolved by SDS-PAGE, and analyzed by Western blotting as described in the “PDA nuclear extract” section but with anti-NF-1X (Geneka/Active Motif), anti-c-Jun (Santa Cruz Biotechnology), the polyclonal rabbit anti-Vp-1 developed in our lab, and anti-β-actin (Sigma).

Inhibition of NF-1 binding by rhAP-1(c-Jun). We previously identified the juxtaposition of the NF-1 and c-Jun binding sites in JCV promoters using DNase footprinting analysis (1, 2) and have posed the hypothesis that there is direct protein-protein interaction between NF-1 and c-Jun. To investigate this hypothesis and the effect c-Jun may have on NF-1 binding to a JCV promoter, EMSA assays were performed using a radio-labeled oligonucleotide probe containing the NF-1 consensus site derived from the JCV Mad-1 sequence (NF-1 probe) (Fig. 1). A gel-shifted band specific for binding of NF-1 proteins was observed when stromal cell extract was incubated with the NF-1 probe (Fig. 1, lane 3). The presence of a molar excess of unlabeled NF-1 probe (cold) competed out binding to the NF-1 probe (Fig. 1, lane 4), while an excess of an altered unlabeled probe containing a mutated NF-1 consensus site (mutant) did not reduce binding, demonstrating specificity (Fig. 1, lane 5). A primary 30-min incubation of stromal cell

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rhAP-1(c-Jun) is not recognized by the NF-1 probe, had no affect on binding to the NF-1 probe (Fig. 1, lanes 11 and 12). These results demonstrate that c-Jun can inhibit binding of the NF-1 protein to the consensus NF-1 site of the JCV Mad-1 promoter if introduced prior to and/or in sufficient quantity with an NF-1 source (e.g., stromal cell extract). Interestingly, the inhibition of NF-1 binding occurs independently of c-Jun binding the NF-1 probe, as demonstrated by the failure of rhAP-1(c-Jun) to shift the NF-1 probe by itself (Fig. 1, lane 2). Free NF-1 probe was routinely run off the bottom the EMSA gels to avoid interference with the clear identification of entire NF-1 smears. In EMSAs where the NF-1 probe was not run off the bottom of the gel (data not shown), addition of rhAP-1 showed no reduction in the free NF-1 probe band compared with lanes having no rhAP-1, ruling out rhAP-1-mediated isotope cleavage from the probe. Therefore, the observed inhibition may be the result of a physical interaction between NF-1 and c-Jun that prevents NF-1 from binding to the JCV promoter and/or an effect of free c-Jun on the NF-1 consensus site, such as electrostatic interactions that have been described by others (27, 28).

**IP and Western blot analysis for NF-1 and c-Jun.** To identify specific interactions between NF-1 class members and c-Jun, we first examined whether these proteins physically bind each other. Western blot analyses were conducted with stromal and PDA total cell lysates subjected to immunoprecipitation (IP) with either anti-NF-1X or an antirabbit immunoglobulin G (IgG) control and then resolved by PAGE and probed with anti-c-Jun. As shown in Fig. 2, c-Jun coprecipitated with NF-1X in both stromal and astrocyte lysates (Fig. 2A, lanes 1 and 3). No binding from the cell lysates was seen with the antirabbit IgG control (Fig. 2A, lanes 2 and 4). These data demonstrate that NF-1 and c-Jun were physically associated in the lysates of both stromal cells and PDA. The detection of NF-1X in reciprocal co-IPs (anti-c-Jun precipitation, followed by anti-NF-1X Western blotting) was problematic, because NF-1X resolves near the 55-kDa size of IgG heavy chains.

**GST pull-down assays.** GST pull-down assays were utilized in which recombinant GST and GST-c-Jun were separately incubated with the total cell lysates of either nontransfected PDA or pdDNA3NF-1X-transfected PDA (Fig. 2B). Proteins dissociated from the isolated (pulled-down) GST and GST-c-Jun were resolved on gels and analyzed in Western blots versus anti-NF-1X. While the constitutively expressed NF-1X in nontransfected PDA lysate showed an association with GST-c-Jun, the overexpression of NF-1X in the transfected PDA more clearly demonstrated this association. These experiments substantiate our findings of a direct binding interaction between NF-1X and c-Jun.

**In vitro translation of NF-1X.** An additional GST pull-down assay was performed using the recombinant GST and GST-c-Jun described above, along with in vitro-transcribed/translated 35S-labeled-NF-1X protein (35S[NF-1X]) to rule out possible effects of other cellular proteins on the observed association of NF-1X and c-Jun (Fig. 2C). 35S[NF-1X] showed specific association with GST-c-Jun, further demonstrating the specific interaction between NF-1X and c-Jun.

**Comparative NF-1 expression in progenitors and PDA.** To uncover additional details regarding the control of JCV activity by NF-1X, we analyzed NF-1 protein expression in our model extract with the NF-1 probe, followed by a secondary 30-min incubation after the addition of rhAP-1(c-Jun), reduced binding to the NF-1 probe to some extent (Fig. 1, lane 6). However, reversing the order by first incubating with rhAP-1(c-Jun) and then adding stromal cell nuclear extracts eliminated NF-1 protein binding to the consensus sequence altogether, suggesting a significant inhibitory interaction (Fig. 1, lane 7). This inhibition of NF-1 binding through primary c-Jun incubation was dose dependent. Reducing the concentration of rhAP-1(c-Jun) by 4-, 16-, or 64-fold decreased the inhibitory effect proportionally (Fig. 1, lanes 8 to 10). As a negative control, NF-κB, which like
cell culture system of multipotential human CNS progenitors. Progenitors support only low levels of JCV activity compared with that for PDA (23). Our studies here revealed differential protein expression patterns of NF-1 class members, with elevated expression of NF-1X in PDA (Fig. 3A). β-Actin detection served as the control for equivalent total protein loading between progenitor and PDA nuclear extracts. The cytosolic fraction probed with anti-GFAP (a cytoskeletal marker that
JCV infections in the multipotent human CNS progenitor-cell culture system. JCV activity in human CNS progenitors and PDA was analyzed at 4 days post-JCV exposure (the time interval used for all JCV infection studies presented here) by Western blot assays of nuclear extracts (Fig. 3B). At this time point, PDA showed significant expression of JCV early (T) and late (Vp-1) proteins compared with levels for progenitor cells. In subsequent experiments, levels of Vp-1 expression were used as the measure of JCV activity. This experiment demonstrates the positive role of NF-1X on JCV activity.

Interference of c-Jun on NF-1 binding to JCV promoter. Since some AP-1 binding sites are found adjacent to NF-1 binding sites in JCV promoters, we explored how increased protein expression of either c-Jun or NF-1X might affect protein interactions with JCV promoter sequence (specifically Mad-1) and if interference with NF-1 binding alters JC viral activity. We used ATP binding experiments to compare the binding levels of NF-1X and c-Jun from nuclear extracts of JCV-infected PDA transfected to overexpress NF-1X or c-Jun. Proteins that bound ATP–Mad-1 were eluted and analyzed by Western blotting using anti-NF-1X or anti-c-Jun. PDA transfected to express NF-1X showed a corresponding increase in NF-1X association to ATP–Mad-1 and an increased Vp-1 level compared with control PDA (Fig. 4). On the other hand, the increased association of c-Jun with ATP–Mad-1 in PDA transfected to overexpress c-Jun was partnered with a decrease in Vp-1 expression. This finding suggests that increased c-Jun association with AP-1 binding sites could be responsible for interference with NF-1X binding to adjacent NF-1 sites. Therefore, the inability of NF-1X to bind efficiently to particular NF-1 consensus binding sites in JCV promoters as a result of c-Jun interaction is, in the same measure, playing a role in the observed decrease of JCV activity (Fig. 4 and 5).

Discussion

JCV transcription and replication depends on a complex and organized sequence of molecular events involving both the virus and the host cell (17). The variant JCV promoter region contains consensus binding sites for a number of cellular transcription factors, including c-Jun (1), NF-1 (2), NF-κB (25), Y-box binding protein 1 (17), Tst-1 (35), and Purα (8). We have previously demonstrated that of the NF-1 family of transcription factors, NF-1X has a significant role in the regulation of JCV expression (26). NF-1X is highly expressed in cell types known to support JCV expression. These include human glial cells, B lymphocytes, tonsillar stromal cells, some established permissive human cell lines, and the astrocytic lineage (PDA) derived from a model cell culture system of human CNS progenitor cells. Neuronal cultures derived from this multipotent cell culture system, which are not susceptible to JCV infection, have been rendered susceptible by transfection-induced overexpression of NF-1X (23). In the same manner, differentiated KG-1 cells, which have lost JCV susceptibility present in the undifferentiated state, have had susceptibility...
restored due to NF-1X overexpression (26). HeLa cells, which normally do not support activation of the JCV early promoter, also demonstrate the ability to do so after transfection of an NF-1X-expressing plasmid (32). Our EMSA experiments suggest that interactions between NF-1 and c-Jun may have functional consequences for JCV activity. The interactions observed in our EMSAs are modulated by concentrations of each transcription factor, as well as the order and timing of transcription factor addition to the binding complex. Furthermore, our EMSA strongly supports the idea that NF-1 and c-Jun can physically bind one another (protein to protein) either in the absence of DNA or in the presence of JCV promoter sequence.

In our studies here, the range of susceptibility to JCV infection observed between progenitor and PDA cultures was again linked to differential levels of NF-1X protein expression. Our examinations of NF-1A, NF-1B, and NF-1X proteins in various cell types lead us to believe that expression levels of each NF-1 family member play a distinct role in the control of JCV cellular tropism.

The close proximity of the NF-1 and AP-1 binding sites in the JCV promoter suggest that interactions between NF-1X and the AP-1 proteins are important in the modulation of JCV expression (14). Recently, interaction between NF-1 and AP-1 proteins has been proposed to have an effect on GFAP expression in an astrocytic cell line (10), with the conclusion that the basal expression of GFAP specific to astrocytes depends on both AP-1 and NF-1. Our experiments here show that NF-1X and c-Jun undergo direct, physical interactions, independently of DNA binding, that alter associations of these transcription factors with JCV promoters. Here we used immunoprecipitation, in vitro transcription/translation, and GST–c-Jun pull-down assays to demonstrate binding between the c-Jun and NF-1X proteins. Our in vitro binding experiments demonstrate that only c-Jun and NF-1 are required for such interaction, free of DNA and/or other cellular proteins. The experiments reported here provide evidence that DNA-independent mechanisms affecting interactions between nuclear transcription factors have relevance to aspects of viral activity.

Having established that NF-1X is a critical cellular component conveying transcriptional support of JCV infection and that the association of NF-1X and c-Jun occurs with or without DNA binding, we have set out to better understand what role c-Jun and NF-1X play in affecting viral activity. Our experiments utilizing overexpression of NF-1X showed concurrent increases in expression of JC Vp-1 and binding of NF-1X to ATP–Mad-1, without affecting c-Jun binding. However, overexpression of c-Jun showed increases in c-Jun binding, along with decreases in not only the association of NF-1X to ATP–Mad-1 but also the expression of JC Vp-1 (Fig. 5A). These observations strongly suggest that increased binding of c-Jun on JCV promoters interferes with NF-1X binding by masking the binding site, which may be, in part, responsible for decreased JCV activity (Fig. 5B).

In addition to c-Jun masking NF-1X binding on JCV promoters, the decreased viral activity in cells that overexpress c-Jun may be partly due to “quenching” of NF-1X through protein-protein interaction. The in vivo interaction between NF-1X and c-Jun described here could be functionally significant for JCV propagation and for the regulation of cellular gene expression.

The positive regulation of JCV expression is complex and is limited to cell types that support virus replication (31). Nuclear transcription factors certainly have a significant role in JCV virus expression and host cell restriction (14, 16, 24–26). The experiments described here suggest that interactions between transcription factors from different families (NF-1 and AP-1) not only occur independently of DNA binding (protein to protein) but also in the context of associating with JCV promoters (protein to promoter), offering multilevel regulation of JCV expression. These findings support the hypothesis that binding of NF-1X to JCV promoters is a critical mechanism supporting JCV propagation, while c-Jun may also play a role in the regulation of JCV activity by the inhibition of NF-1X binding.

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