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Physical and functional interaction between viral and cellular proteins modulate JCV gene transcription

Mahmut Safak and Kamel Khalili

Center for Neurovirology and Cancer Biology, Temple University, Philadelphia, Pennsylvania, USA

The lytic phase of JC virus (JCV) appears to be highly complex and remains elusive. A growing body of experimental evidence suggests that the regulation of JCV gene expression and replication requires, in addition to the presence of specific transcription factors, cooperativity between viral and cellular regulatory proteins. This cooperativity may be accomplished by physical interaction of the participant proteins on and/or off the viral DNA sequence. Here, we present evidence of specific physical and functional interaction between a cellular factor, YB-1, and the JCV early protein, T-antigen, and showed that both proteins play important roles in JCV gene transcription. Additionally, our data indicate that YB-1 also functionally interact with another viral protein, designated agnoprotein, which is expressed late during the course of infection, adding further complexity to the currently known picture on JCV gene regulation. *Journal of NeuroVirology* (2001) **7,** 288–292.

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Human polyoma virus, JC virus (JCV), is a small DNA virus with a double-stranded covalently linked circular genome. It is the causative agent of progressive multifocal leukoencephalopathy where oligodendrocytes, the myelin-producing cells of central nervous system, are selectively destroyed by this virus. JCV genome is composed of three functional regions including the viral early-, late-coding regions, and noncoding regulatory region (Frisque *et al*, 1984). The viral early- and late-coding regions encode regulatory (large and small t-antigens) and structural (VP-1, -2, and -3) proteins, respectively. The viral late genome also contains an open-reading frame for a small auxiliary protein called agnoprotein, whose function in the viral life cycle is virtually unknown. The viral noncoding regulatory region contains the origin of DNA replication, promoter elements for both early and late genes, and cis-acting enhancer elements, which are characterized by two 98-bp tandem repeats.

Transcriptional regulation of the JCV early and late promoters in the lytic cycle appears to be rather complex and requires both participation of viral and cellular factors. The JCV lytic cycle begins with the expression of early genes encoding the regulatory large and small t-antigens. Large T-antigen, the central regulator of the viral lytic cycle, initiates viral DNA replication and orchestrates the transition from early to late gene transcription by activating late gene expression and by suppressing its own early promoter (Khalili *et al*, 1987; Lashgari *et al*, 1989). Following the onset of DNA replication, the virus enters the late phase of infection during which capsid proteins are encoded, virions are matured, and finally the host cell is lysed (Raj and Khalili, 1995). Recently, we have presented evidence that, like large T-antigen, agnoprotein of JCV also participates in regulation of viral DNA replication and transcription (Safak *et al*, 2001).

Over the years, a number of cellular transcription factors have been identified and characterized for their involvement in JCV gene transcription including NF-κB (Ranganathan and Khalili, 1993; Safak *et al*, 1999b), Tst-1 (Wegner *et al*, 1993; Renner *et al*, 1994), NF-1 (Amemiya *et al*, 1989), Sp-1 (Henson *et al*, 1992), GBP-i (Raj and Khalili, 1994), YB-1 (Kerr *et al*, 1994; Safak *et al*, 1999a), and Purα (Chen and Khalili, 1995; Chen *et al*, 1995; Safak *et al*, 1999c). At the initial stages of infection, only host cellular transcription factors are responsible for

Address correspondence to Dr. Kamel Khalili, Center for Neurovirology and Cancer Biology, College of Science and Technology, Temple University, 1900 North 12th Street, 015-96, Room 203, Philadelphia, PA 19122, USA. E-mail: kkhalili@astro.temple.edu
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expression of early genes in the absence of T-antigen. At later stages of infection, particularly molecular interactions between host and viral proteins appears to take the central stage in governing both viral DNA replication and transcription. Among the previous cellular transcription factors, we have particularly focused our attention to the molecular mechanism(s) of the transcriptional regulation of JCV promoters by cellular factor, YB-1, through its interaction with viral early regulatory protein, large T-antigen, and with late regulatory protein, agnoprotein. YB-1 is a member of the large family of Y-box DNA binding proteins, whose (Wolffe, 1994) family members are conserved from bacteria to higher eukaryotes. All vertebrate Y-box binding proteins consist of three domains: a variable glycine-rich N-terminus, a highly conserved central nucleic acid recognition domain, and a hydrophilic C-terminus tail domain. Central nucleic acid recognition domain is also known as the cold-shock domain and shows 43% homology to the bacterial cold-shock protein (Lee *et al*, 1994). The tail domain, which is thought to stabilize protein-DNA interactions, contains alternating regions of predominantly basic or acidic amino acids. Compelling experimental evidence indicates that Y-box binding proteins are involved in a wide variety of biological functions including regulation of gene expression at both transcriptional (Wolffe, 1994; Mertens *et al*, 1997; Safak *et al*, 1999a, 1999c) and translational levels (Tafuri and Wolffe, 1993); DNA and RNA condensation, DNA repair, and stress-induction (Bargou *et al*, 1997; Koike *et al*, 1997).

To investigate the effect of YB-1 on basal and T-antigen-induced transactivation of the JCV promoters, we performed transient transfection experiments in the human glial cell line, U-87MG, using reporter constructs containing the JCV early and late gene promoters. As shown in Figure 1A, cotransfection of the reporter construct with a T-antigen expression plasmid resulted in a substantial increase in the transcriptional activity from the late promoter (8- to 9-fold increase, compare lanes 1 and 2). However, when a constant amount of T-antigen was cotransfected with an increasing amount of YB-1, we observed a synergistic effect on the transcriptional activity of the late promoter (14- to 18-fold increase, compare lane 2 to 3 and 4). At the highest YB-1 concentration alone, we observed only a 3-fold increase in transcription (Figure 1A, lane 5), demonstrating a transcriptional synergy between these two transactivators on transcription from the late promoter. Of note, expression of T-antigen or YB-1 remains unaltered upon expression of either transactivator (data not shown), suggesting that transcriptional cooperativity observed between T-antigen and YB-1 is not due to the effect of one transactivator on the expression of the other.

Similarly, we also carried out cotransfection assays to evaluate the transcriptional cooperation between YB-1 and T-antigen on the viral early promoter. As shown in Figure 1B, a CAT reporter construct containing the JCV early promoter showed a notable basal expression in the absence of transactivators (lane 1). However, as expected, cotransfection of the reporter construct with the T-antigen expression plasmid resulted in a substantial decrease in transcriptional activity, demonstrating the T-antigen-mediated transcriptional suppression of the early promoter (compare lanes 1 and 2). In contrast, when a constant amount of T-antigen was cotransfected with an increasing amount of YB-1, we observed a dose-dependent alleviation in the T-antigen-mediated transcriptional suppression of the early promoter (compare lane 2 to 3 and 4), again indicating the presence of a functional interaction between YB-1 and T-antigen. As expected, YB-1 alone significantly activates transcription from early promoter (lane 5).

In addition to functional interaction observed between YB-1 and T antigen, we also investigated direct physical interaction between these two proteins utilizing *in vitro* GST (glutathione-*S*-transferase)-pulldown assays and localized the interaction domain of YB-1 with large T-antigen (Safak *et al*, 1999b). A series of amino- and carboxy-terminal deletion mutants of YB-1 was created as GST fusion proteins and incubated with whole cell extracts prepared from hamster glial cells (HJC) constitutively expressing JCV large T-antigen. Unbound proteins were washed extensively with binding buffer and bound proteins were analyzed by Western blot analysis with antibodies specific for T-antigen. As shown in Figure 1C, results from these assays showed that YB-1 and T-antigen physically interacts, and T-antigen interaction domain of YB-1 is localized to its cold shock domain and immediate surrounding residues.

We have recently presented evidence that JCV agnoprotein can physically and functionally interact with the viral early protein, T-antigen and downregulate both T-antigen-mediated viral gene expression and DNA replication (Safak *et al*, 2001). Because cellular factor YB-1 participates in JCV gene regulation through its interaction with both cellular factors (Chen *et al*, 1995; Safak *et al*, 1999c) and JCV T-antigen (Safak *et al*, 1999a), we sought to investigate whether this factor is also involved in JCV gene regulation through its interaction with viral late regulatory protein, agnoprotein. We performed cotransfection experiments in U-87MG cells, using a reporter construct containing the JCV early gene promoter and expression plasmids for YB-1 and agnoprotein. As shown in Figure 2A, YB-1-induced expression of viral early promoter is substantially suppressed by agnoprotein in a dose-dependent manner (lanes 3 and 4), indicating the functional interaction between these two proteins. As previously observed for viral late promoter (Safak *et al*, 2001), agnoprotein also suppressed the basal expression of viral early

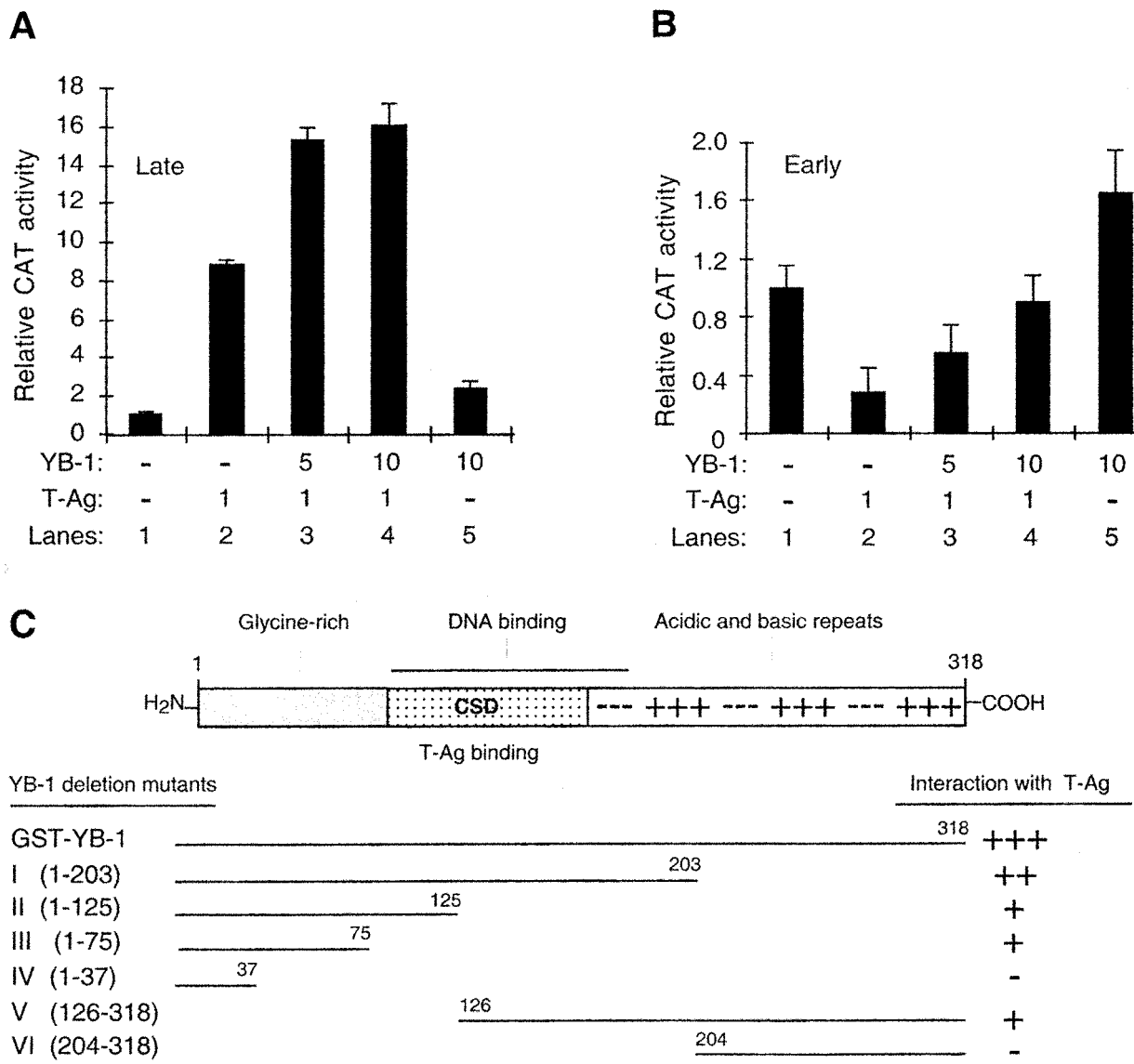

Figure 1 Functional and physical interaction between YB-1 and T-antigen. (**A**) A reporter plasmid (pBLCAT$_3$-Mad-L) (7.5 μg) containing the JCV late gene promoter (late) was transfected into U-87MG cells alone or together with EBV-YB-1 and CMV-T antigen expression plasmids. Expression plasmid concentrations were normalized with appropriate empty vectors. At 36 h posttransfection, cells were harvested and cloramphenicol acetyl transferase (CAT) activity of extracts were determined using equal amount of protein from each transfectant. CAT activities were presented as CAT activity relative to the basal activity of the promoter. (**B**) Experiments similar to those detailed for panel A were also performed with a reporter plasmid containing the JCV early gene promoter (pBLCAT$_3$-Mad-E). Expression plasmid DNA concentrations used in each transfection are indicated at the bottom of the each panel in micrograms. Bars indicate standard deviation. (**C**) A summary of results obtained from GST-pulldown assays. A schematic representation of full-length YB-1 is shown at the top. The ability of YB-1 and its deletion mutants to interact with T-antigen is depicted on the right as +++, specific interaction; ++ or +, reduced interaction; and –, no interaction.

promoter (lane 5). Additionally, our preliminary co-transfection experiments indicate that agnoprotein also appears to suppress YB-1-mediated transactivation from JCV late promoter (data not shown). These observations suggested that YB-1 may physically interact with JCV agnoprotein. To test this possibility, we performed affinity chromatography (GST pulldown) experiments. GST or GST-Agno protein was immobilized on glutathione-sepharose beads and incubated with whole cell extracts from HJC-15b cells, transfected with histidine-tagged YB-1. Proteins bound to beads were extensively washed and analyzed by Western blot analysis using antibodies specific for histidine-tagged YB-1. As demonstrated in Figure 2B, YB-1 was specifically retained by the sepharose column containing GST-Agno (compare lane 4 with 3).

In conclusion, we have presented evidence for physical and functional interactions between a cellular factor, YB-1, and two viral proteins, viral early

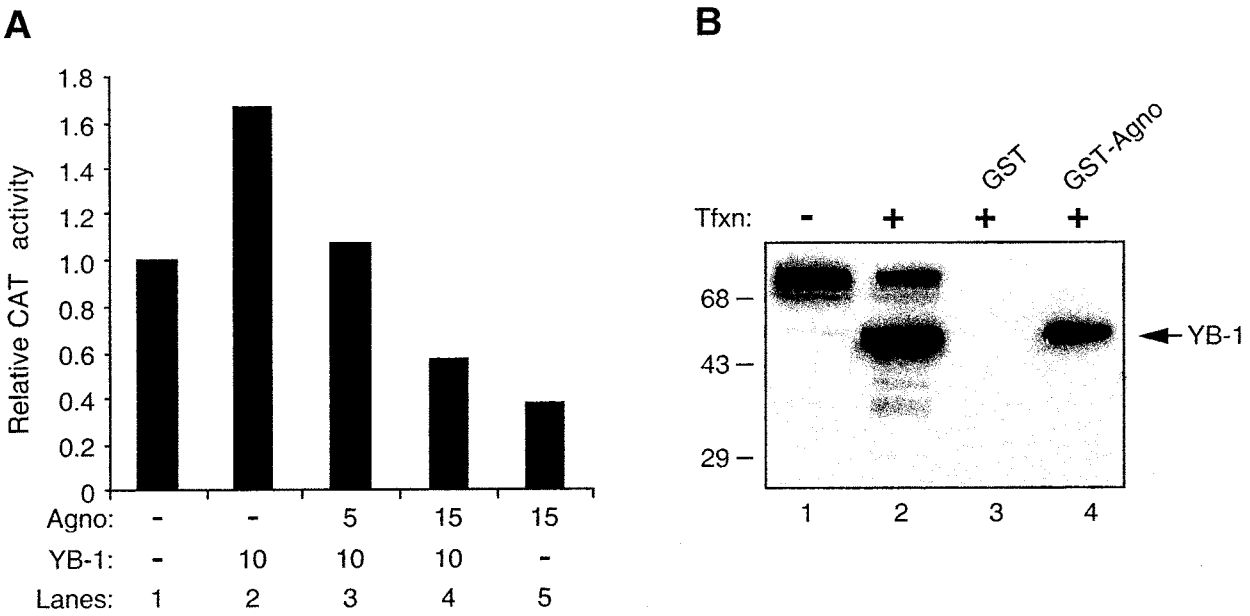

Figure 2 (**A**) Agnoprotein suppresses YB-1-induced expression of viral early promoter. A reporter plasmid (pBLCAT$_3$-Mad-E) (5 μg) was transfected into U-87MG cells alone or together with CMV-Agno and EBV-YB-1 expression plasmids. CAT activities for each transfectant was determined as detailed for Figure 1A. (**B**) *In vitro* interaction of YB-1 with agnoprotein. Whole-cell extracts prepared from HJC-15b cells transfected with a histidine-tagged YB-1 expression plasmid (pEBV-YB-1) (lanes 3 and 4) were incubated with either GST alone (lane 3) or GST-Agno (lane 4) as indicated. Beads were washed extensively and proteins interacting with GST or GST-Agno were analyzed by Western blot analysis using anti-T7 antibody for detection of His-tagged YB-1. Whole cell extract from HJC-15b cells either untransfected (lane 1) or transfected with pEBV-His-YB-1 expression plasmid (lane 2) were loaded as negative and positive migration controls respectively. Agno designates agnoprotein. Tfxn indicates transfection.

regulatory protein, large T-antigen, and viral late regulatory agnoprotein. Characterization of such delicate interactions at the molecular level will further our understanding of molecular mechanism(s) governing the JC virus lytic cycle and may eventually help us to design effective molecular therapeutics against JC virus infections before the virus enters its lytic cycle.

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