ABSTRACT Human JC polyomavirus (JCV) is the etiologic agent of the neurodegenerative disease progressive multifocal leukoencephalopathy. By using JCV as a model, we investigated the role of the viral early protein tumor antigen (TAg) in the binding of two cellular proteins, Purα and YB-1, to JCV regulatory sequences. Results from band-shift assays with purified YB-1, Purα, and TAg indicated that efficient binding of Purα, a strong activator of early gene transcription, to a single-stranded target sequence corresponding to the viral lytic control element, is diminished in the presence of the late gene activator YB-1, which recognizes the opposite strand of the Purα binding site. Of particular interest was the ability of Purα and TAg to enhance binding of YB-1 to DNA molecules without being associated with this complex. Binding studies using a mutant peptide encompassing the N terminus of YB-1 indicated that the C terminus of YB-1 is important for its DNA binding activity. The ability of Purα and TAg to increase binding of YB-1 to DNA is independent of the YB-1 C terminus. Similarly, results from band-shift assays using Purα variants indicated that two distinct regions of this protein contribute either to its ability to bind DNA or to its ability to enhance YB-1 DNA binding activity. Based on the interaction of Purα, YB-1, and TAg, and their binding to DNA, a model is proposed for the role of these proteins in transcription of viral early and late genes during the lytic cycle.

Studies of the small DNA tumor viruses have provided insights into a number of regulatory pathways used in the synthesis and processing of eukaryotic mRNA. In particular, work on papovaviruses including simian virus 40 (SV40) has led to the identification of several regulatory factors that mediate transcription of eukaryotic genes (1, 2). This group of viruses utilizes the host cell machinery to promote viral transcription bidirectionally during lytic infection, leading to the production of regulatory tumor antigen (TAg) and structural capsid proteins at early and late phases of infection, respectively (3, 4). Accumulation of TAg at the early phase of infection is critical for the transcriptional switch from early to late promoters and the progression of subsequent events during the lytic cycle, including DNA replication in permissive cells (3, 4).

The human JC polyomavirus (JCV) replicates efficiently in glial cells and has been repeatedly isolated from demyelinated plaques within the brain of patients with progressive multifocal leukoencephalopathy, a neurodegenerative disease (for reviews, see refs. 5–7). Several studies have established that the restricted host range of JCV to glial cells is determined at the level of viral gene transcription that is mediated by glial-enriched DNA-binding regulatory proteins (8–12). In addition to cell-specific transcriptional factors, other regulatory proteins found in various cells and tissues are believed to be critical in facilitating viral gene expression during the lytic cycle (13–18). Results from this (10–12, 17, 19) and other (9, 20) laboratories have indicated that the 98-bp enhancer/promoter sequence of JCV encompasses multiple cis-regulatory elements that interact specifically with nuclear proteins and modulate viral early and late gene transcription. A region designated the lytic control element (LCE) in the 98-bp repeat proximal to the origin of DNA replication contains a pentanucleotide repeat sequence, AGGGAAGGGA, juxtaposed to a poly(dt-dA) tract that displays a single-stranded configuration (21). This motif differentially affects viral gene expression by positively and negatively modulating early and late promoter transcription (17, 19) and plays an important role in viral DNA replication (22, 23). In this study we demonstrate that two distinct cellular proteins, Purα and YB-1, activators for the early and late gene transcription of JCV, respectively, modulate each other’s binding to the LCE. Moreover, we show that the viral early protein TAg is capable of enhancing binding of YB-1 to DNA molecules. We propose a working model for the potential role of LCE-binding proteins and the viral TAg in programming the early-to-late shift of the viral gene transcription during the lytic cycle.

MATERIALS AND METHODS

Expression and Purification of Purα and YB-1 from Bacteria. Maltese-binding protein (MBP)–Purα and MBP–YB-1 fusion proteins were produced in bacteria using the plasmids pMAL-Purα and pMAL-YB-1, respectively. The expression plasmids were constructed by inserting cDNA sequences for Purα (24) and YB-1 (25) in-frame into pMAL-cR1 vectors. Proteins were expressed in Escherichia coli and purified on amylose affinity columns (New England Biolabs). Purα deletion mutants were expressed as glutathione S-transferase fusion proteins in E. coli BL21 Lys.S and purified with glutathione-agarose (Sigma) (26). Protein concentration was measured by the Bradford assay (Bio-Rad), and the purity of proteins was examined by SDS/PAGE.

Expression and Purification of JCV and SV40 Tag from Insect Cells. A JCV DNA fragment (nt 5130–4771/4426–2473) containing exons 1 and 2 of the TAg gene was inserted into the baculovirus cloning vector pVL1392 (Invitrogen) downstream from the polyhedrin promoter. This construct, pVL1392-JCT (Int–), was used to generate the virus B-JCT by recombination with wild-type baculovirus Autographa californica.

Abbreviations: JCV, JC polyomavirus; TAg, tumor antigen; SV40, simian virus 40; LCE, lytic control element; MBP, maltose-binding protein; CSD, cold-shock domain.

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nica nuclear polyhedrosis virus, AcMNPV. The recombinant baculovirus 941T that expresses the SV40 TAg was a gift from M. Summers (Texas A&M University) and R. Lanford (Southwest Foundation for Biomedical Research). To prepare purified JCV and SV40 TAg, Sf9 insect cells were infected with B-JCT or 941T virus and lysed 48 h later. Viral proteins were purified by binding to immunoaffinity columns containing monoclonal antibody PAB2000 (anti-JCV TAg) or PAB901 (anti-SV40 TAg) and eluting with triethylamine buffer containing 20 mM triethylamine (pH 11.3), 10% glycerol, and leupeptin (10 µg/ml).

**Band-Shift Assay.** Binding reactions with purified MBP-YB-1 and MBP-Pura were performed in 12 mM Hepes, pH 7.9/4 mM Tris-HCl, pH 7.5/60 mM KCI/5 mM MgCl2/0.8 mM dithiothreitol/0.5 µg of poly(dI-dC). DNA–protein complexes were allowed to form during a 20- to 30-min incubation of the protein sample with 50,000 cpm of probe DNA on ice. Reaction products were analyzed on a 9% polyacrylamide/0.5 x TBE gel (27) and detected by autoradiography. Binding activities were quantified by laser densitometry of autoradiograms.

**RESULTS**

**Interaction of YB-1 and Pura with LCE.** The pentanucleotide repeat sequence of LCE (Fig. 1A) is a potential target for the binding of Pura, a single-stranded DNA-binding protein that exhibits specific affinity for a purine-rich sequence element present in several gene flanking regions and origins of DNA replication (24, 28). Pura preferentially binds to closely spaced repeats of the triplet GGN. The cDNA encoding Pura was cloned into a prokaryotic expression system (pMAL-cR), and the bacterially expressed Pura protein exhibits binding affinity to the pentanucleotide repeat located on the late strand of the LCE (Pp), but not to that on the early strand (Pp). Pura also binds, although weakly, to the duplex LCE (PpL) (Fig. 1B).

By using the DNA fragment derived from the central region of the JCV 98-bp repeat as a probe, we isolated a recombinant YB-1 clone with the ability to bind to a C + T-rich sequence of LCE (D.A.K., C.F.C., N.N.C., G.L.G., C. Raj, B. Schwartz, and K.K., unpublished data). YB-1 is a member of a large family of DNA-binding proteins that was initially isolated from a human B-cell expression library by its capacity to interact with the Y-box region found in class II major histocompatibility complex genes (25). Evidently, YB-1 plays a role in transcriptional activity of genes containing a Y box and may influence transport of the nascent RNAs from nuclei to cytoplasm (for review, see ref. 29). The bacterially produced YB-1 protein showed an affinity to the early strand of the LCE (Pp) and, like Pura, had a reduced ability for association with the double-stranded PpL probe (Fig. 1B). Addition of Pura to the YB-1 binding reaction mixture increased the intensity of the band corresponding to the YB-1–Pp complex (Fig. 2A) or the YB-1–PpL complex (Fig. 2C, lanes 1–3) *in vitro*. Even under these conditions, Pura did not interact with PpL either alone or complexed with YB-1. Apparently, Pura enhances YB-1 binding to its target sequence without direct interaction with the DNA molecule. Addition of YB-1 to the Pura binding reaction mixture diminished formation of the Pura–PpL complex (Fig. 2B). Again, YB-1 showed a similar inability to interact with the PpL sequence either directly or indirectly through Pura. Furthermore, YB-1 inhibited the binding of Pura to the PpL probe (Fig. 2C, lanes 5–7). These observations indicate that cross-communication between the cellular proteins YB-1 and Pura determines their association with an important regulatory sequence of JCV; this may determine their regulatory function on the viral genome.

**Stimulation of YB-1–Pp Complex Formation by TAg.** Results from numerous studies on SV40 have established that TAg, the viral early protein, is crucial for the regulation of early and late promoter activities (30, 31). Apparently, SV40 TAg exerts its regulatory function indirectly through cellular intermediary factor(s). In our initial studies to evaluate the possible effect of JCV TAg on binding of YB-1 and Pura to their respective targets in the viral LCE, we found that a purified baculovirus-produced JCV TAg (B.B., P. M. MacKen, and R.J.F., unpublished data) has no binding activity to the single- or double-stranded LCE sequences in the absence or presence of the LCE binding proteins, including Pura and YB-1. Interestingly, the JCV TAg, like Pura, did enhance binding of YB-1 to its single-stranded (Pp) and double-stranded (PpL) target sequence (Fig. 3A, lanes 2 and 3). Addition of an optimal amount of TAg to this reaction showed no further enhancement of the binding activity of YB-1 to Pp or PpL DNAs (Fig. 3A, compare lanes 3 and 4). Inclusion of an additional concentration of TAg resulted in no significant alteration in the intensity of the band corresponding to the Pura–PpL complex (Fig. 3A, compare lanes 5 and 6). Furthermore, JCV TAg had no detectable effect on YB-1-mediated reduction of Pura binding (Fig. 3A, probe L, compare lane 5 with lanes 7 and 8). SV40 TAg, which...
exhibits 72% sequence homology with JCV TAg (32), behaved similarly to JCV TAg in the binding reactions containing P_E probe (Fig. 3B). However, in the presence of YB-1, SV40 TAg showed minor, if any, inhibitory activity on Purα binding to the pentanucleotide repeat sequence (Fig. 3B, probe L, compare lanes 5 and 8 with lanes 4, 7, and 9). Competition experiments using unlabeled P_E DNA revealed that preincubation of YB-1 with TAg or Purα drastically increased the stability of the YB-1-P_E complex (Fig. 4). Again, pretreatment of YB-1 with TAg or Purα did not result in alterations in the electrophoretic mobility of the YB-1-P_E complex (data not shown), ruling out direct involvement of these proteins in YB-1-P_E complexes.

**Fig. 2.** Influence of YB-1 and Purα interplay on DNA binding activity. (A) Binding reactions were carried out with a 15-nt end-labeled P_E (E) probe in the presence of 300 ng of YB-1 protein (lanes 1–3) and 300 (lane 2) and 600 (lane 3) ng of Purα in the reaction mixture. Lane 4 shows the binding reaction in the presence of 600 ng of Purα without YB-1. (B) P_L (L) probe was mixed with 300 ng of Purα alone (lane 1) or with 300 and 600 ng of YB-1 (lanes 2 and 3, respectively). In lane 4, P_L was mixed with 600 ng of YB-1 alone. (C) Binding reactions in a mixture of 5'-end-labeled double-stranded P_EL probe and 300 ng of YB-1 (lane 1) alone or with the addition of 300 and 600 ng of Purα (lanes 2 and 3, respectively). Binding of Purα to double-stranded DNA was evaluated in a reaction mixture containing 600 ng of Purα in the absence (lanes 4 and 5) or presence of 300 and 600 ng of YB-1 (lanes 6 and 7, respectively). Lane 8 illustrates binding of 600 ng of YB-1 to the duplex P_EL probe.

The reciprocal experiment using Purα and P_L probe indicated that preincubation of Purα with YB-1 had no effect on the stability of Purα-P_L complex. These data describe an activity

**Fig. 3.** Effect of TAg on binding of YB-1 and/or Purα to their targets. (A) Reaction mixtures containing single-stranded Pur or P_E probes or double-stranded P_EL probes were incubated with YB-1 (300 ng), Purα (600 ng), and JCV TAg (100 ng) in the combinations indicated. Probes were used in large excess (3 × 10^5 cpm per lane) in each reaction mixture to ensure detection of all possible complexes. No major bands other than those corresponding to the DNA–protein complexes were detected. (B) SV40 TAg (100 ng) was mixed with Purα and YB-1 as described in A, and the complexes were resolved by band-shift assay.

**Fig. 4.** Effect of Purα and TAg on the stability of the YB-1–P_E complex. Reaction mixtures containing 300 ng of YB-1 were incubated with P_E probe alone or in the presence of 5, 25, and 125 ng of unlabeled P_E competitor and analyzed by band-shift assay. In the experiments, YB-1 (300 ng) was preincubated with either Purα (300 ng) or TAg (100 ng) for 15 min on ice before addition of the probe and competitors. Complexes were analyzed by band-shift assay and the intensities of the bands corresponding to the YB-1–P_E complex were determined by scanning densitometry and are presented in arbitrary units (ADU) from 1 to 10.
 Fig. 5. Contribution of the N-terminal region of YB-1 to DNA binding activity. (A) Schematic representation of YB-1 and its truncated variant, HF-1 (D.A.K., et al., unpublished data). The 80 amino acids of the CSD are conserved between HF-1 and YB-1. The arginine/proline-rich domain (+ + +) and the alternating acidic domain (− − −) are noted. (B) Approximately 300 ng of highly purified MBP-HF-1 protein was incubated alone or with JCV TAg (100 ng) or Pura (600 ng) in a reaction mixture containing P₆, P₇, or duplex (P₆/P₇) DNA probes, as indicated. Y and P indicate the positions of HF-1- and Pura-associated complexes, respectively.

Identification of the Regions Within YB-1 and Pura That Are Important for Their DNA-Binding Activities. YB-1 is a member of an emerging family of DNA-binding proteins that share common structural motifs, including the highly conserved 80-amino acid cold-shock domain (CSD) at the N terminus, a central arginine/proline-rich domain, and alternating acidic domains at the C terminus (Fig. 5A). To identify the region(s) of YB-1 that determines its binding to the P₂ and its perturbation of the Purα–P₁ interaction, a mutant variant of YB-1 possessing the N terminus of the protein (HF-1) was used in the band-shift DNA-binding experiment. Like YB-1, mutant HF-1 formed a complex, designated Y, with Pₓ and Pₓ DNA but not with P₁ DNA (Fig. 5B, lane 2). Furthermore, HF-1 decreased, in trans, binding of Purα to its target pentanucleotide repeat sequence (Fig. 5B, compare lanes 3 and 4). Unlike YB-1, association of HF-1 and Pₓ was not increased upon addition of TAg and Pura at optimum concentrations (Fig. 5B, compare lane 5 with lanes 6 and 8). Both the DNA-binding activity of HF-1 and its inhibitory action on Purα DNA binding remained unchanged upon the addition of JCV TAg (Fig. 5B). These observations suggest that the N terminus of YB-1, which includes CSD, is sufficient for its ability to bind DNA and to inhibit the Purα DNA-binding activity. These data also indicate that the C terminus of YB-1 is important for Purα and JCV TAg to exert their positive action on YB-1 DNA-binding activity.

In analogous studies, the importance of various regions of Purα in binding to DNA and stimulating the binding of YB-1 to its target sequence was evaluated by using a series of purified bacterially produced peptides encompassing various regions of Purα (Fig. 6). Structurally, Purα has several interesting features, including three 23-amino acid repeats separated by two 26-amino acid repeats thought to be important for its DNA-binding activity (28). The N terminus of Purα is highly glycine-rich, whereas the C terminus includes a region of a potential amphipathic helix. A classical Pro-Glu-Ser-Thr (PEST) domain in the central region of Purα is believed to accelerate its turnover in cells (33). Experiments to evaluate the ability of Purα variants (2B, 3B, NR, SR, 200, BN, 100, and B) to interact with the P₁ probe (Fig. 6) indicated that the central region of Purα, spanning amino acids 65–191 present in the B peptide, is sufficient for its interaction with the P₁ peptide. Note that Purα variants containing the N and/or C terminus potentiated YB-1 interaction with the Pₑ probe, whereas the B variant, which encompasses the Purα central region but not the N and C terminus, was unable to stimulate YB-1 DNA-binding activity. These observations differentiate between at least two distinct domains of Purα: a domain located between amino acids 65 and 274 that is essential for DNA binding and two domains, one from amino acids 1 to 85 and one from amino acids 274 and 322 that are essential for facilitating binding of YB-1 to its target DNA sequence.

**Discussion**

The data presented here demonstrate that the cellular proteins YB-1 and Purα differentially modulate their reciprocal binding
to the respective single- and double-stranded target sequences. YB-1 augments its own binding to P_E and P_EL at the expense of Purα binding to the P_E, in what appears to involve two distinct interactions: one is mediated by the N terminus of YB-1 and reduces binding of Purα to its DNA target and the other requires the C terminus of YB-1 and the glycine- or glutamine-rich domain of Purα, which increases YB-1 binding to the DNA. The enhancement in binding is unlikely to involve any topological modifications in the target DNA, given the small size of the pentanucleotide probes (15 nt). It should be noted that in addition to the LCE, PUR elements are also present in the origin of the JCV DNA replication. Recent studies in this laboratory have shown that the ori-PUR element mediates activation of the JCVI_P promoter by the human immunodeficiency virus 1 Tat protein in glial cells (34). It is conceivable that interaction of Purα and Tat and their cooperation may influence JCV gene regulation. Our present studies indicate that cellular proteins Purα and YB-1 mediate a broad spectrum of activities regulating viral life cycle.

These observations are likely to have a functional significance in regulation of viral gene expression. Recent preliminary results in our laboratory indicate that Purα is a potent activator of early gene transcription (N.N.C. and K.K., unpublished data), whereas YB-1 is a weak enhancer of early gene transcription that more effectively stimulates viral late gene transcription in glial cells (D.A.K., et al., unpublished data). Therefore, intercommunication between YB-1 and Purα, which influences their interaction with the JCV regulatory sequences, may be an important determinant for Tat synthesis at the early phase of infection. Perhaps it should be noted that some JCV isolates contain a 23-bp insert in the LCE motif that provides a potential target for binding of G+C-rich binding protein(s) (15). Future characterization of protein(s) that bind to the 23-bp insert, and their interaction with YB-1 and Purα in the presence and absence of Tat should shed light on the cooperative interaction of these regulatory proteins with the JCV control sequence.

The ability of two unrelated proteins, the cellular Purα and the viral Tat, to exert similar effects on the binding of YB-1 to DNA is intriguing, both mechanistically and functionally. It is possible that a shift occurs in the binding of YB-1 to its target DNA during the course of the viral lytic cycle. According to this model, YB-1 may have poor affinity for the viral DNA early in the lytic cycle. At this stage, binding of Purα to its target P_E, which is facilitated by the single-stranded configuration displayed by the LCE (21), enhances expression of the early genes and assists YB-1 in binding to the early strand of LCE. In the late phase of the viral lytic cycle, viral Tat increases the interaction of YB-1 with DNA and, thereby, stimulates late gene transcription, removes Purα from the viral DNA, and hence, suppresses early gene expression. Such a model of the interplay between Purα and YB-1 in the absence and the presence of Tat is reminiscent of the early and late phases of the viral lytic cycle. Thus the interaction of these two cellular proteins with the viral antigen could be an important determinant for the transition of the early-to-late cycle of infection and for the transactivation of the JCV late promoter.

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