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Transcriptional Regulation of Human JC Polyomavirus Promoters by Cellular Proteins YB-1 and Pur α in Glial Cells

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Transcription of the human polyomavirus (JCV) genome is regulated by host cell proteins and the viral early protein, T antigen. A region called the lytic control element (LCE), located within the enhancer of JCV, is important for transcription of JCV early and late promoters. Earlier studies have led to the identification of two single-stranded DNA-binding proteins, YB-1 and Pur α, with the ability to interact with nucleotides on the early and late strands of LCE, respectively. Of particular interest is the notion that the unique interplay between these two cellular proteins and JCV T antigen determines their binding activities with the LCE. In this study, we employed a series of cotransfection experiments to evaluate the levels of transcription from JCV early and late promoters in the presence of YB-1, Pur α, and T antigen. Results from these studies indicated that Pur α stimulates JCV early and has little effect on the late promoter. Moreover, T antigen was able to decrease the induced level of early gene transcription by Pur α. On the other hand, the extent of transactivation of the viral late promoter by T antigen was reduced upon overexpression of Pur α in the transfected cells. These observations suggest that Pur α and T antigen exert an antagonistic effect on each other's regulatory action upon their responsive promoters. Of particular interest was the observation that YB-1 liberated T-antigen-induced late promoter activity from repression imposed by overexpression of Pur α. Under similar conditions, overexpression of YB-1 showed no effect on the transcriptional activity of the early promoter in cells transfected with T-antigen- and Pur α-producing plasmids. On the basis of the data presented here and the previous binding results, a model is proposed which describes the potential role of Pur α, YB-1, and T antigen in differential expression of the viral genome during the lytic cycle.

The human polyomavirus (JCV) is the etiologic agent of a neurodegenerative disorder of the central nervous system, progressive multifocal leukoencephalopathy (for review, see references 10 and 18). This virus is closely related to two other polyomaviruses (25), the simian vacuolating virus 40 and BK virus of humans, which share greater than 70% homology in their protein coding regions (9). However, unlike BK virus and simian virus 40, JCV has a narrow host range and tissue tropism that restrict its replication to primary human fetal glial cells in tissue culture and oligodendrocytes, the myelin-producing cells of the brain, in affected individuals. The greatest divergence between JCV and the other polyomaviruses is in the viral control region, which in JCV contains the origin of DNA replication, promoters for early and late gene transcription, and tandem 98-bp direct repeats (for review, see reference 10). Several lines of study, including in vitro transcription of the viral promoters in extracts from glial and nonglial cells (1, 2) and transient transfection of various cell types with the reporter constructs, have indicated that JCV early and late promoters are more active in cells derived from the central nervous system (8, 13, 17, 24). Furthermore, JCV-T transgenic animals which experienced dysmyelination in the central nervous system exhibited preferential expression of the T antigen in the brain (21). Thus, these data together strongly suggest that the tissue tropism of the JCV, at least in large part, rests on tissue-specific expression of the viral promoters. Studies in our and other laboratories have indicated that the cell-specific activation of the JCV promoter requires its association with multiple cellular proteins present in glial cells (15, 16, 23, 24). More recently, we have identified a novel DNA-binding protein that is rapidly induced by several immunoregulators and cytokines and that by binding to the GGA/C sequence of the JCV origin of DNA replication outside of the 98-bp repeat, down-regulates transcription of the viral late genome (19). Among the viral *cis*-regulatory elements within the 98-bp repeat are nuclear factor 1 binding sites located in the B regions, which are activated by the previously characterized glial factor 1 (15). Moreover, a region called the lytic control element (LCE), containing a pentanucleotide repeat motif, 5′-AGG GAAGGGA-3′, on the late strand and its complementary sequence, 3′-TCCCTTCCCT-5′ on the early strand, has been identified between the B domain and the poly(dA) tract in the vicinity of the origin of DNA replication (Fig. 1). Previous studies in our laboratory have suggested that the pentanucleotide repeat sequence of LCE plays an important role in transcriptional regulation of the viral early promoter (22). Results from band shift, dimethyl sulfide protection, and UV cross-linking analyses revealed sequence-specific interaction of the late strand of this repeat (5′-AGGGAAGGGA-3′) with a single-stranded cellular DNA-binding protein (22) which bears a remarkable similarity to a previously characterized single-stranded cellular protein named Pur α, which recognizes purine-rich sequences (5). In a different set of studies, screening of an expression library with the DNA probe containing the LCE sequence led to isolation of a cDNA clone for the YB-1 protein, which binds to the early strand of the LCE enriched in C+T nucleotides (14). More recently, with bacterially produced YB-1 and Pur α, we discovered an unusual ability of

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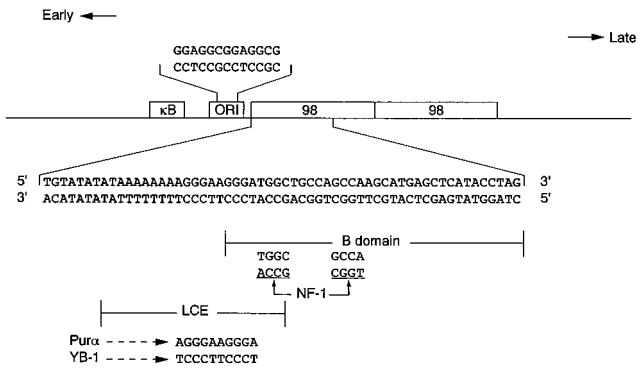

FIG. 1. JCV regulatory region. The two 98-bp enhancer/promoter repeats, the origin of replication (ORI), and the κB motif for binding of NF-κB (20) protein are depicted. The origin of replication contains the GGA/C sequence, which binds to the cytokine-induced protein GBPi (19). The B domain (16) contains the target sequence for nuclear factor 1 (NF-1 [3, 4]), and the LCE (22) has a pentanucleotide repeat motif, 5′-AGGGAAGGGA-3′, on the late coding strand, which binds to Pur α, and 3′-TCCCTTCCCT-5′, on the early coding strand, which binds to YB-1 (7). The arrows on the top indicate the direction of the viral early and late genes.

these two cellular proteins to modulate each other's binding to their respective target nucleotides on the LCE early and late strands, respectively (7). Whereas Pur α enhanced binding of YB-1 to the LCE early strand, association of this protein with the pentanucleotide repeat in the LCE late strand was decreased in the presence of YB-1 protein. Of interest was the notion that similar to Pur α, the viral early protein, T antigen, exhibits the ability to stimulate binding of YB-1 to DNA molecules. These observations suggest that the interplay between YB-1, Pur α, and T antigen is important for binding of these cellular regulators to the JCV sequence and, therefore, for their regulatory function on JCV gene transcription. In this study, we have employed a transfection assay to examine the action of Pur α on transcription of viral early and late promoters in the absence or presence of YB-1, T antigen, and YB-1 plus T antigen in glial cells. On the basis of data from this study and the previous DNA-binding observations, we propose a model for the involvement of Pur α and YB-1 in T-antigen-mediated transition of the early-to-late phase of JCV gene transcription during the viral life cycle.

We began our studies by comparing the activities of the JCV early and late promoters in the presence of Pur α, YB-1 and JCV T-antigen as produced by pCMV-Pur α, pCMV-YB-1, and pCMV-T, respectively, in transient transfection studies. The plasmids pJC_E-CAT and pJC_L-CAT, which contain 286 bp of DNA sequence of the JCV control region at the 5′ position of the reporter chloramphenicol acetyltransferase (CAT) gene in early and late orientations, respectively (13), were introduced into the U-87MG human glial cell line singly or in combination with pCMV-Pur α, pCMV-YB-1, or pCMV-T. As shown in Fig. 2A, the activity of the JCV early promoter was increased when cells were cotransfected with the Pur α-expressing plasmid. Under similar conditions, expression of Pur α showed a minor effect on the level of transcription from the JCV late promoter. As anticipated, transcriptional activity of the JCV late promoter was significantly increased when cells were cotransfected with pJC_L-CAT and the plasmid encoding JCV T antigen (Fig. 2B). The stimulating effect of T antigen may not be attributed to the amplification of the template DNAs, since the auxiliary sequences required for JCV DNA replication located on the early side of the origin are deleted in the JCV reporter CAT constructs (6). Cotransfection of glial cells with YB-1 expresser plasmid led to an elevation in basal transcriptional activity of the viral late and early promoters. The extent of activation of the early promoter, however, was less than that of the late promoter in the transfected cells (Fig. 2C). Together, the ability of Pur α to augment transcription of the JCV early promoter, but not the late promoter, and the capacity of YB-1 and T antigen to enhance JCV late promoter activity in glial cells set the stage for subsequent studies to examine the interplay of these proteins in modulating transcription of the JCV genome.

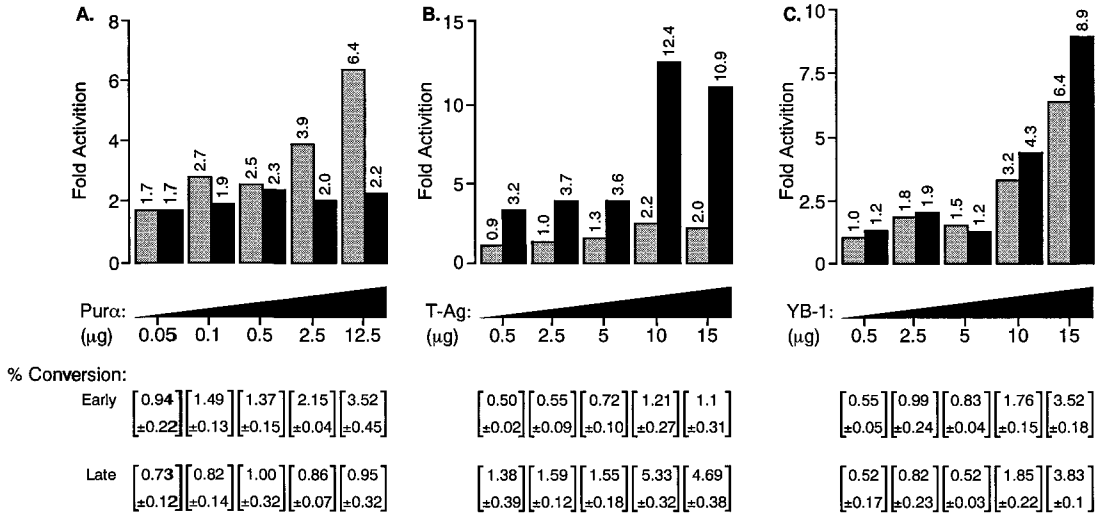

FIG. 2. Activity of the JCV early (shaded bars) and late (solid bars) promoters in the presence of Pur α, T antigen (T-Ag), and YB-1. Various amounts of pCMV-Pur α (A), pCMV-T (B), and pCMV-YB-1 (C) were introduced into glial cells with 3 μg of pJC_E-CAT by the Ca phosphate precipitation method (12). At 36 h posttransfection, extracts were prepared and CAT enzyme activity was determined (11). The percents conversion for JCV early and late promoters at the basal levels were 0.55 ± 0.09 and 0.43 ± 0.07, respectively. These values were used as the base level to estimate fold activation of the JCV early and late promoters by Pur α (A), T antigen (B), and YB-1 (C).

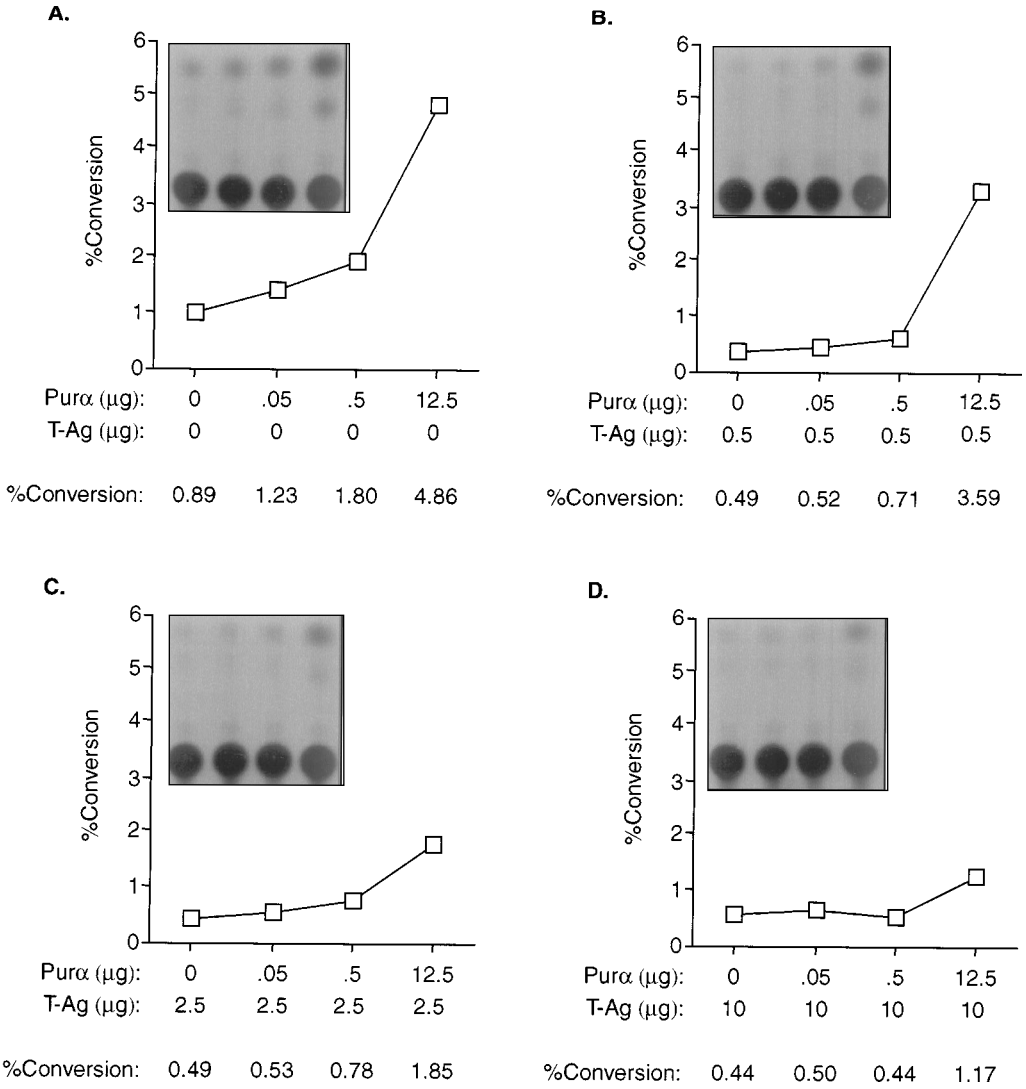

FIG. 3. Effect of Pur α and T antigen (T-Ag) on JCV early promoter activity. Three micrograms of pJC_E-CAT plasmid was mixed with increasing amounts of pCMV-Pur α in the absence (A) or presence (B to D) of 0.5 (B), 2.5 (C), and 10 (D) μg of pCMV-T and introduced into U-87MG cells. The final DNA amount was kept at 25.5 μg by the addition of pCMV plasmid. At 36 h posttransfection, extracts were prepared and analyzed for CAT enzyme activity. The average percent conversion of three independent experiments is shown for each panel.

First, we carried out a series of transfection experiments to examine the effect of increasing concentrations of T antigen on Pur α-mediated transcriptional activity of the JCV early promoter. Toward this end, the pJC_E-CAT construct was introduced into glial cells along with pCMV-Pur α in the absence or presence of 0.5, 2.5, and 10.0 μg of the pJC-T construct. Comparison of JCV early promoter activity in cells cotransfected with pCMV-Pur α in the absence (Fig. 3A) or presence (Fig. 3B, C, and D) of various amounts of pJC-T indicates that increasing concentrations of T antigen in cells result in a gradual decrease in Pur α-induced enhancement of JCV early promoter activity. These findings suggest that the interaction between Pur α and T antigen may play an important role in the negative feedback mechanism for down-regulation of JCV early promoter activity during the late phase of infection.

In parallel, a similar series of experiments were carried out to evaluate the cooperative action of Pur α and JCV T antigen on the transcription of the viral late promoter. We found that cells transfected with 0.05 and 0.5 μg of pCMV-Pur α plus 0.5 and 2.5 μg of pJC-T exhibit no significant effect on T-antigen-induced JCV late promoter activity (data not shown). However, at a higher amount (12.5 μg), inclusion of pCMV-Pur α in the transfection mixture reduced the level of CAT activity observed in cells receiving 0.5 or 2.5 μg of pJC-T plasmids (data not shown). In the presence of 10 μg of pJC-T, when the JCV late promoter is highly induced, expression of very low levels of Pur α by 0.05 and 2.5 μg of pCMV-Pur α showed inhibitory effects on viral late gene transcription (Fig. 4). These observations suggest that Pur α has the ability to interfere with the stimulatory action of T antigen on viral late gene transcription. These data, along with the results presented in Fig. 3, strongly suggest that Pur α and T antigen exert antagonistic effects on each others' transcriptional activity on the JCV promoters.

Although the mechanism by which these two regulators influence each other's activities remains unclear, our recent DNA binding studies have suggested that T antigen may indi-

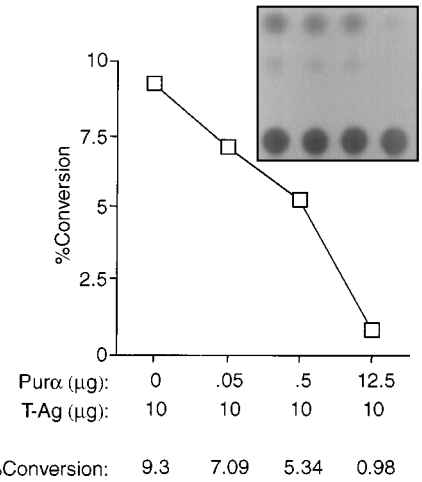

FIG. 4. Effect of Pur α and T antigen (T-Ag) on JCV late promoter activity. Three micrograms of pJC_L-CAT was mixed with 10 μg of pCMV-T plasmid DNAs in the absence and presence of 0.05, 0.5, and 12.5 μg of pCMV-Pur α, and after adjustment of the total amount of DNA to 25.5 by the pCMV vector, the mixture was introduced into U-87MG cells by the Ca phosphate precipitation method. At 36 h posttransfection, CAT enzyme activity was determined. The results represent the average percent conversion of three independent experiments.

rectly destabilize the association of Pur α with the pentanucleotide repeat sequence (7). In this respect, T antigen, by increasing the rate of YB-1 binding to its target nucleotides, could cause dissociation of Pur α from the LCE motif. To examine this hypothesis and evaluate the importance of our binding data on transcriptional regulation of the viral early and late promoters, a series of transfection assays were performed with an optimal amount of pJC-T (10 μg), small (0.05 μg) and large (5.0 μg) amounts of pCMV-Pur α, and increasing amounts of pYB-1. The results shown in Fig. 5A indicate that with the small amount (0.05 μg) of Pur α, the reduced transcriptional activity of the viral early promoter in the presence of T antigen is partially recovered upon expression of YB-1 in the transfected cells. Under similar conditions, in the presence of the larger amount of pCMV-Pur α (5.0 μg), YB-1 was unable to improve the overall transcriptional activity of the early promoter. With regard to the viral late promoter under conditions in which no antagonistic activity by T antigen and Pur α was detected, expression of YB-1 led to an elevation in viral late gene transcription. Of particular interest was the ability of YB-1, at a larger amount, to relieve the suppression of the T-antigen-induced late promoter activity seen upon overexpression of Pur α. Therefore, it appears that in the presence of T antigen and high levels of Pur α, YB-1 could play an important role in reactivation of the viral late, but not early, promoter in glial cells. Next, we directly examined expression of the transgenes, i.e., Pur α, YB-1, and T antigen, in the transfected cells. Toward this end, cells were transfected with various combinations of pCMV-YB-1, pCMV-T, and pCMV-Pur α as described above, and after 36 h, nuclear proteins or total RNAs were isolated and used in Western (immunoblot) and Northern (RNA) blot assays, respectively. As shown in Fig. 6A, expression of JCV T antigen remained unaltered upon expression of Pur α and YB-1 in the transfected cells (compare lane 6 with lanes 7, 8, and 9). The level of endogenous Pur α was extremely low, and we were not able to detect this protein in the untransfected cells. However, cells transfected with pCMV-Pur α showed a 39-kDa band corresponding to Pur α, which remained constant in the presence of T antigen and YB-1 (compare lane 5 with lanes 7, 8, and 9). Expression of YB-1 from the transfected genes was evaluated by Northern blot analysis because of the complexity associated with the large family of YB-1 proteins and the lack of a monoclonal antibody which specifically recognizes the YB-1 family members. As shown in Fig. 6B, in the presence of pCMV-YB-1 the signal corresponding to YB-1 mRNA was increased in the transfected cells (compare lane 1 with lanes 2 and 3). It appears that expression of Pur α by pCMV-Pur α (lanes 4 and 5) and T antigen by pCMV-T (lane 6) had an insignificant effect on the endogenous level of YB-1 RNA in the cells. Furthermore, the levels of YB-1 transcription by the exogenous pCMV-YB-1 remained unchanged in the presence of a high level of Pur α (compare lanes 2 and 3 with lanes 8 and 9). From these observations, we have concluded that YB-1, Pur α, and T antigen do not significantly influence each other's promoter

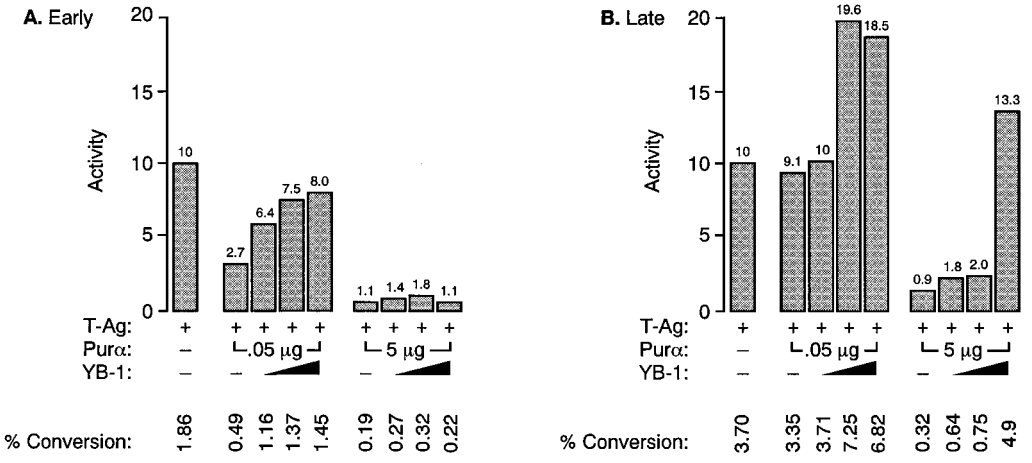

FIG. 5. Regulation of JCV early and late promoters by T antigen (T-Ag), Pur α, and YB-1 in glial cells. Three micrograms of pJC_E-CAT (A) or pJC_L-CAT (B) was cointroduced into glial cells with 10 μg of pCMV-T in the presence of 0.05 or 5.0 μg of pCMV-Pur α and increasing concentrations (0.5, 2.5, and 10 μg) of pCMV-YB-1. In all cases, the final amount of DNA in the transfection mixture was brought to 28 μg with pCMV-DNA. At 36 h posttransfection, extracts were prepared and analyzed for CAT enzyme activity. The average percent conversion of each CAT assay is shown below. The percents conversion of the JCV early and late promoters in the absence of T antigen were 0.42 and 0.31, respectively. The levels of CAT activity of the JCV early (A) and late (B) promoters in the presence of T antigen were set at 10 arbitrary units, and the effects of Pur α and YB-1 on the T-antigen-induced levels are presented.

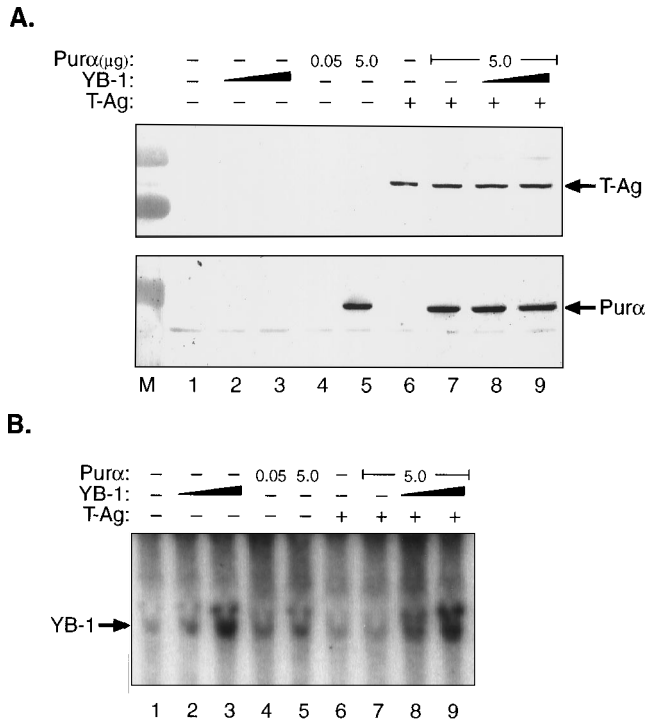

FIG. 6. Expression of T antigen (T-Ag), Pur α, and YB-1 from the transfected plasmids. U-87MG cells were transfected with 0.5 and 10.0 μg of pCMV-YB-1 (lanes 2 and 3), 0.05 and 5.0 μg of pCMV-Pur α (lanes 4 and 5), 10 μg of T antigen (lane 6), and 10 μg of T antigen plus 5.0 μg of pCMV-YB-1 in the absence (lane 6) or presence (lanes 7 and 8, respectively) of 0.5 and 10 μg of pCMV-YB-1. The total amount of plasmid DNA in the transfection mixture was kept constant (25 μg) and normalized by pCMV plasmid at 36 h posttransfection. Nuclear proteins (A, top and bottom) or RNAs (B) were prepared and analyzed by Western blotting with monoclonal antibodies against T antigen (A, top) or against Pur α (A, bottom). (B) Northern blot of RNAs hybridized to YB-1 cDNA. The arrow indicates the position of YB-1 RNAs.

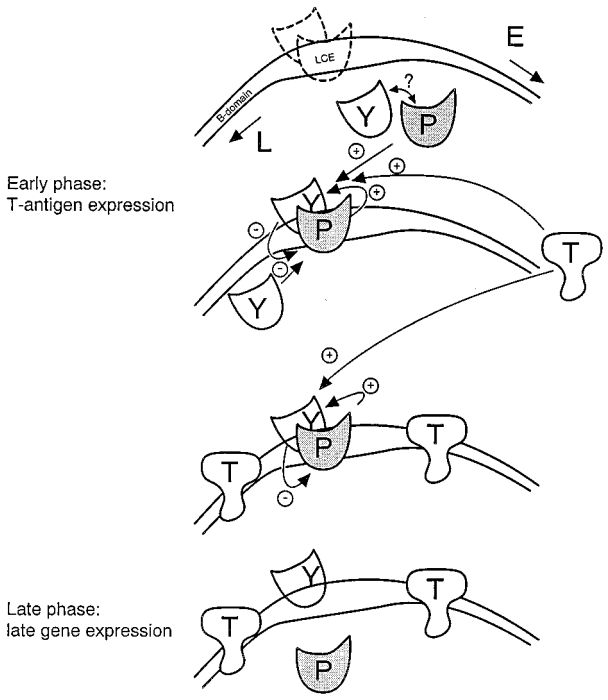

FIG. 7. Proposed model for the involvement of Pur α (P), YB-1 (Y), and T antigen (T) in transition of early-to-late gene transcription. Efficient binding of Pur α to the LCE late strand (L) stimulates early gene transcription and facilitates binding of YB-1 to its target position on the LCE early strand (E). Binding of YB-1 to the DNA, which is concurrent with T-antigen production, and its binding to the origin of DNA replication and the B region results in dissociation of Pur α from the LCE late strand. This alteration in the pattern of DNA-protein complexes results in a decrease in the level of early gene transcription and an increase in late promoter activity at the late phase of infection.

activities; hence, the observation described in the legend to Fig. 5 is likely due to the effect of these proteins on JCV promoter activity. On the basis of the data presented here and the results from DNA-protein interaction studies (7), a working model which includes a role for T antigen in the transition of viral promoter activity during the lytic cycle is proposed. As shown in Fig. 7, it is likely that at the initial phase of infection, strong binding of Pur α to the LCE preferentially stimulates transcription of the viral early genome. As lytic infection progresses and the level of T-antigen in the cells increases, the pattern of DNA-protein complexes in the LCE may be altered, since T antigen, by stabilizing the association of YB-1 with DNA, facilitates YB-1-mediated dissociation of Pur α from the pentanucleotide repeat of the LCE motif. At this phase, removal of Pur α from the LCE results in a substantial decrease in early promoter activity, and the ability of YB-1 to moderately enhance transcriptional activity of the early promoter may be blocked by T antigen. At the same time, YB-1, by releasing the late promoter from constraints mediated by overexpression of Pur α, allows T antigen to enhance the expression of the late gene during this phase of infection. Thus, this model suggests that the interplay between two cellular proteins at the early phase of lytic infection and the participation of the viral early protein at the late phase of infection differentially regulate expression of the bidirectional viral promoter during the lytic cycle.

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