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Inhibition of Human Immunodeficiency Virus Type 1 Replication by A Cellular Transcriptional Factor MBP-1

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Abstract A cellular transcriptional factor initially identified as the c-myc promoter binding protein (MBP-1) was subsequently characterized as a cell regulatory protein with multifunctional activities. In this study, the role of MBP-1 on human immunodeficiency virus type-1 (HIV-1) transcriptional activity was investigated. MBP-1 showed inhibition of HIV-1 long terminal repeat (LTR)-directed chloramphenicol acetyl transferase (CAT) activity in a transient cotransfection assay. Deletion of upstream elements of the HIV-1 LTR, including the nuclear factor kappa B (NF-kB) and Sp1 binding sites, did not affect the MBP-1 mediated suppression of HIV-1 LTR. The core promoter of the HIV-1 appeared to be the primary sequence involved in MBP-1 mediated inhibition. In the presence of HIV-1 TAR sequence and Tat protein, MBP-1 did not inhibit the viral promoter activity. In addition, cotransfection experiments with HIV-1 LTR and deletion mutants of MBP-1 suggested that the carboxyl terminal half of MBP-1 suppresses the HIV-1 promoter activity. Exogenous expression of MBP-1 showed suppression of HIV-1 replication in acutely infected cells and in cells cotransfected with a molecular clone of HIV-1. These results suggest that exogenous expression of MBP-1 plays an important role in the regulation of HIV-1 replication in infected cells. J. Cell. Biochem. 64:565–572.

Key words: transcriptional regulation; HIV-1; replication

Acquired immunodeficiency syndrome (AIDS) caused by HIV-1 is characterized by long periods of clinical latency which varies in length from a few months to many years (Gallo et al., 1986; Simmonds et al., 1990; Embretson et al., 1993a). HIV-1 transcription is regulated by several cellular and viral factors (Gaynor, 1992). The long terminal repeat (LTR), the promoter of HIV-1, contains at least five distinct regions which function as the binding sites for various cellular and/or viral proteins, and these include the negative regulatory element (NRE), enhancer, Sp1, TATA, and TAR regions (Gaynor, 1992). A number of cellular factors modulate HIV-1 LTR expression (Patarca et al., 1988; Kato et al., 1991; Garcia et al., 1992; Margolis et al., 1994; Duan et al., 1995; Kundu et al., 1995). These factors either act directly on the basal activity of LTR, or in cohort with the HIV-1 transactivating protein Tat, which greatly enhances HIV-1 LTR expression (Arya et al., 1985). Cellular factors that bind to HIV-1 LTR and repress basal transcription, prior to the accumulation of threshold levels of Tat protein in virus infected cells, are likely to play a central role in the restriction of viral mRNA expression. The viral and cellular factors responsible for the restricted HIV-1 mRNA expression in latently infected cells are multifactorial (Margolis et al., 1994; Duan et al., 1995; Kundu et al., 1995), and are not completely understood.

We initially identified MBP-1 as a c-myc promoter binding protein from a human cervical carcinoma (HeLa) cell expression library (Ray and Miller, 1991). Computer search in the GENBANK failed to reveal any sequence homology of MBP-1 with other known motifs associated with DNA binding or growth suppression. However, this protein has ~70% homology with human α-enolase without a demonstrable enolase activity (Ray and Miller, 1991). Thus, the homology between MBP-1 and human α-enolase appears to constitute the preservation of structural rather than enzymatic features. MBP-1 binds to the TATA box sequences of the

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c-myc P2 promoter and negatively regulates both human and mouse c-myc transcription (Ray, 1995). c-myc protooncogene is an important player in cell cycle regulation, proliferation, and differentiation (Cole, 1986). The binding site for MBP-1 in the c-myc P2 promoter includes the transcription factor TATA box binding protein (TBP) interacting site. Recent studies have shown that MBP-1 and TBP bind simultaneously in the minor groove of the c-myc P2 promoter (Chaudhary and Miller, 1995). Thus, it is possible that MBP-1 negatively regulates c-myc expression by preventing transcription initiation complex with general transcriptional factor(s). MBP-1 is ubiquitously expressed in different human tissues (Ray et al., 1994). Since exogenous expression of MBP-1 acts as a negative regulator in cell growth (Ray, 1995; Ray et al., 1995a) and HIV-1 has a long latency in infected cells (Simmonds et al., 1990; Embretson et al., 1993a), we investigated the role of MBP-1 on HIV-1 transcription and replication. We report here that exogenous expression of MBP-1 plays an important role in the regulation of HIV-1 LTR expression and virus replication in infected cells.

MATERIALS AND METHODS

Plasmids

The plasmid MuLV/MBP-1 containing MBP-1 coding sequences under the control of MuLV LTR promoter for expression in eukaryotic cells and puromycin resistance gene as a selection marker has been described earlier (Ray, 1995). The full length MBP-1 (CMV/MBP-1), as well as the deletion mutants of MBP-1 encoding the amino-terminal (dl_{1-178}) or the carboxyl-terminal ($dl_{190-335}$) domains were also cloned into pcDNA3 expression vector under the control of a CMV promoter. Deletion mutants were constructed by polymerase chain reaction amplification using specific primers (Ray et al., 1995a) containing appropriate start or stop codon using CMV/MBP-1 as a template. The stability of these mutants were verified after introduction into human breast carcinoma cells by Western blot analysis using specific antibody (Ray and Steele, 1996). A panel of HIV-1 LTR CAT reporter plasmids where the expression of the bacterial chloramphenicol acetyltransferase (CAT) gene is driven by either the full length HIV-1 LTR (Ray et al., 1995b) or a series of LTR deletion mutants (Duan et al., 1995) (a gift from Roger Pomerantz) were used in this study to determine the role of MBP-1 on the viral promoter. The plasmid pNL4-3 which contains a full length infectious molecular clone of HIV-1 (Adachi et al., 1986) was obtained from NIH AIDS Research and Reference Reagent Repository.

Cells

Various CD4+ cell lines; MT-2,and HeLa-T4, were obtained from the AIDS Reagent Repository. The WIL-2 cells, a human B-lymphocytes, were obtained from the American Type Culture Collection (Rockville, MD). HeLa-T4 cells were maintained in Dulbecco's medium supplemented with 10% fetal calf serum. All other cells were maintained in RPMI medium supplemented with 10% fetal bovine serum. HeLa-T4 cells were transfected with MuLV/MBP-1 or vector DNA alone and selected with puromycin for 2 weeks. Puromycin resistant cells were pooled and designated as HeLa-MuLV/MBP (MBP-1 transfected) and HeLa-pBabe/puro (vector transfected). WIL-2 cells do not express CD4 and not infectable with HIV.

CAT Assay

HeLa-T4 cells (5×10^5) were cotransfected with the effector and the reporter plasmid DNAs and 50–60% cells were transfected in our hands. Cell extracts were prepared at 48 h post-transfection and CAT assay was performed following the procedure described previously (Ray, 1995). The acetylated and nonacetylated forms of chloramphenicol were separated by thin-layer chromatography and scanned by Molecular Dynamics PhosphorImager. The level of CAT activity was calculated as the percentage of the two acetylated forms of chloramphenicol relative to the total amount of [^{14}C]chloramphenicol. Transfection efficiencies were normalized to an internal β-galactosidase control. Experiments were repeated at least three times to estimate the variations of transfection efficiency, which ranged from 10 to 25% as measured by CAT activity.

Virus Infections

Cell free infection with HIV-1 IIIB (obtained from the AIDS Reagent Repository) or transfections with pNL4-3 were performed. Virus replication was monitored by HIV-1 p24 antigen capture assay (Coulter, Hialieh, FL) as described earlier (Srinivas et al., 1994, 1995).

RESULTS

Inhibition of HIV-1 LTR Promoter Activity by MBP-1

The upstream region of HIV-1 LTR possesses the regulatory sequences necessary for viral transcription. To examine the role of MBP-1 on HIV-1 LTR-directed transcriptional regulation, varying amounts of MBP-1 (CMV/MBP-1) and a constant amount of HIV-1 LTR CAT were cotransfected in HeLa-T4 cells. As demonstrated in Figure 1A, an approximately 85% reduction in CAT expression was observed with 0.5 µg MBP-1 plasmid. The vector DNA (2 ug), used as a negative control, did not show inhibition of CAT expression from HIV-1 LTR (not shown in figure). Cotransfection of HIV-1-LTR CAT reporter plasmids with a different MBP-1 expression vector MuLV/MBP-1 also resulted in an inhibition of CAT activity (Fig. 1B). Taken together, these results suggested that suppression of the HIV-1 LTR promoter activity is due to the exogenous expression of MBP-1.

To identify the sequences in HIV-1 LTR required for MBP-1 mediated inhibition, reporter constructs containing a panel of HIV-1 LTR deletion mutants were tested in the cotransfection assay (Fig. 2). CAT expression from HIV-1 LTR reporter constructs encompassing -65 to +3 nucleotides was still responsive to MBP-1 mediated inhibition. These results suggested that MBP-1 affects basal transcription by HIV-1 core promoter and MBP-1-mediated suppression does not result by interference with either the upstream negative regulatory or enhancer elements. However, CAT expression was not inhibited by MBP-1 when cells were cotransfected in HeLa-T4 cells with full length HIV-1 LTR containing TAR sequences, Tat expression plasmid, and CMV/MBP-1 (data not shown). Tat is a viral regulatory protein which dramatically alters the HIV-1 LTR activity through Tat-binding site, TAR (Arya et al., 1985). Thus, a repression of basal transcription; prior to the accumulation of threshold levels of Tat protein

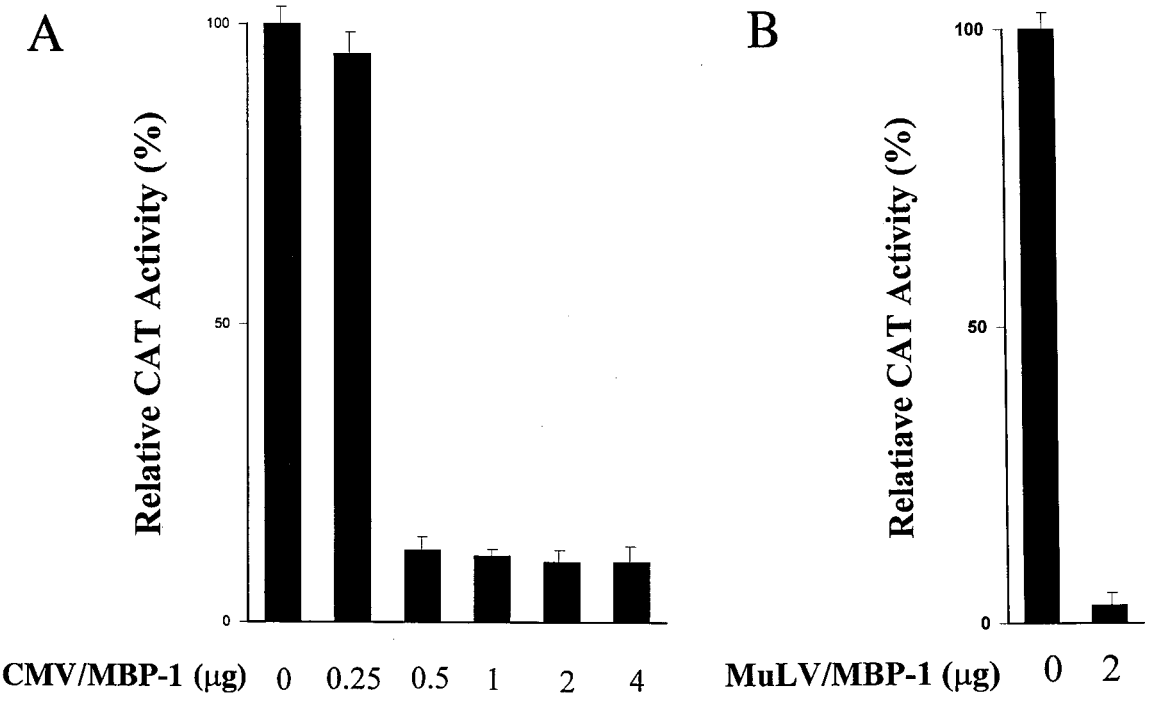

Fig. 1. Transcriptional repression of HIV-1 LTR promoter by MBP-1. The results are shown as representative of at least three separate experiments and presented as relative CAT activity with HIV-1 LTR given a value 100%. **A**: Transcriptional repression of HIV-1 LTR promoter by the MBP-1 in a dose dependent manner. HeLa-T4 cells were transfected with 2 ug of HIV-1 LTR CAT plasmid DNA and increasing concentration (0, .25, .5, 1, 2, and 4 ug) of MBP-1 plasmid DNA (CMV/MBP-1). The total amount of DNA was kept constant by adding pcDNA3 vector in each transfection. CAT assay was performed after 48 h of transfection. The MBP-1 mediated decreased CAT activity was observed with 0.5 ug of the effector DNA. **B**: HeLa-T4 cells were cotransfected with 2 ug of HIV-1 LTR CAT and MuLV/MBP-1 plasmid DNA (0, 2 ug), and CAT assay was performed after 48 h of transfection. HIV-1 LTR promoter activity was suppressed by MBP-1.

appears to restrict HIV-1 transcription, and modulate viral replication.

MBP-1 Domain Responsible for the Inhibition of HIV-1-LTR Basal Transcription

HeLa-T4 cells were cotransfected with HIV-1 LTR CAT reporter plasmid and effector plasmid expressing either full-length MBP-1 (CMV/MBP), or its deletion mutants (Fig. 3A) dl_{1-178} (containing amino terminal half) or $dl_{190-335}$ (containing carboxy terminal half). As shown in Figure 3B, the carboxy-terminal domain of MBP-1 was sufficient for the inhibition of HIV-1-LTR basal transcription. Deletion mutant dl_{1-178} and the vector DNA (negative control) showed a similar level of activity on the HIV-1 LTR promoter. However, suppression of HIV-1 LTR promoter activity was observed by the full length MBP-1 (CMV/MBP-1) and the carboxy terminal half of MBP-1($dl_{190-335}$).

Effect of MBP-1 Overexpression on HIV-1 Replication

HIV-1 p24 antigen production was investigated in HeLa-T4 cells transfected with either plasmid DNA encoding full length MBP-1 (HeLa-MuLV/MBP) or vector DNA control (HeLa-pBabe/puro) and infected HIV-1 virus. As shown in Figure 4A, little or no reduction in p24 antigen production was noted in cultures infected at a higher multiplicity of infection (~0.05), but an appreciable reduction in p24 yields were observed in cultures infected at a lower multiplicity of infection (0.005). HIV-1-replication in cells of T-lymphocytic lineage, the primary targets of the virus, after cotransfection with the plasmids expressing MBP-1 and HIV-1 was also investigated. Since we were unable to establish a MT-2 stable cell line expressing exogenous MBP-1, cells (5×10^5)were transfected with HIV-1 proviral DNA. Exponential cultures of MT-2 cells were transfected with 10 ug of pNL4-3; and 0, 10, or 25 ug of MBP-1, and assayed for HIV-1 p24 antigen production after 5 days. As shown in Figure 4B, there was a dose-dependent inhibition of HIV-1 replication by MBP-1, and a near complete inhibition of HIV-1 replication was noted at higher concentration (~25 ug) of MBP-1. We also examined the cultures for longer periods of time to determine whether co-expression of MBP-1 confers a lasting protection against HIV-1-infections. All cultures eventually succumbed to HIV-1-induced cytopathology, and the cell viability was completely abrogated after 7, 12, and 19 days in cultures receiving 0, 10, and 25 ug MBP-1 DNA, respectively (data not shown). Extensive cell fusion, and ballooning syncytia were detected prior to cell death in all the cultures. A likely reason for the cell death, which were initially protected by MBP-1 co-expression, may involve syncytium formation with non-MBP-1 express-

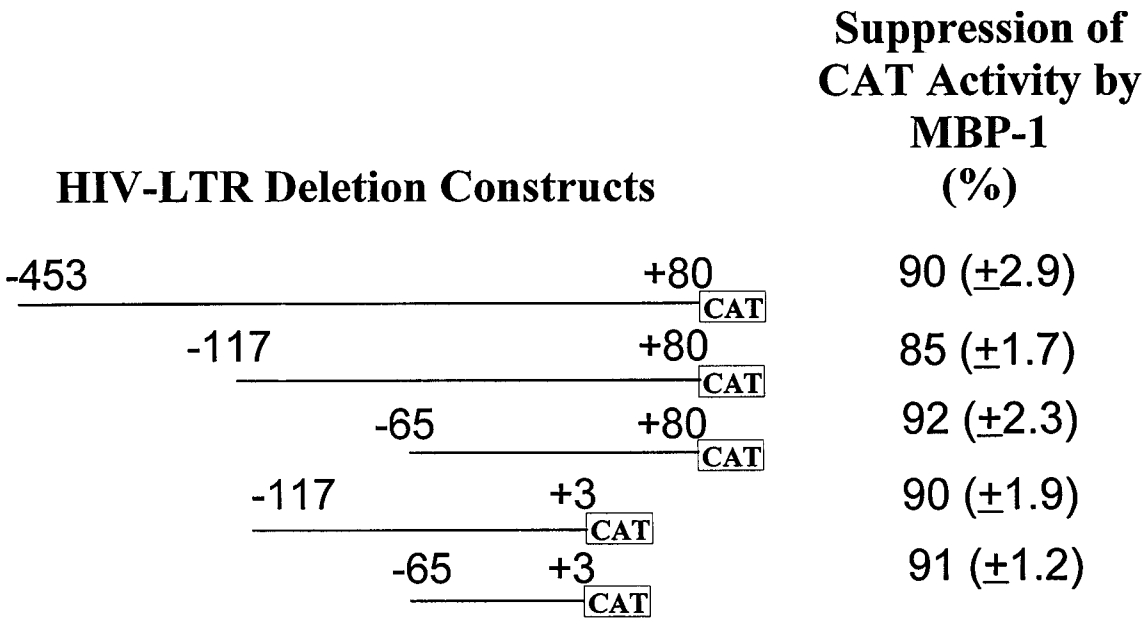

Fig 2. Schematic presentation of the HIV-1 LTR promoter and deletion mutants showing CAT activity in presence of MBP-1. Results are shown as percent of suppression.

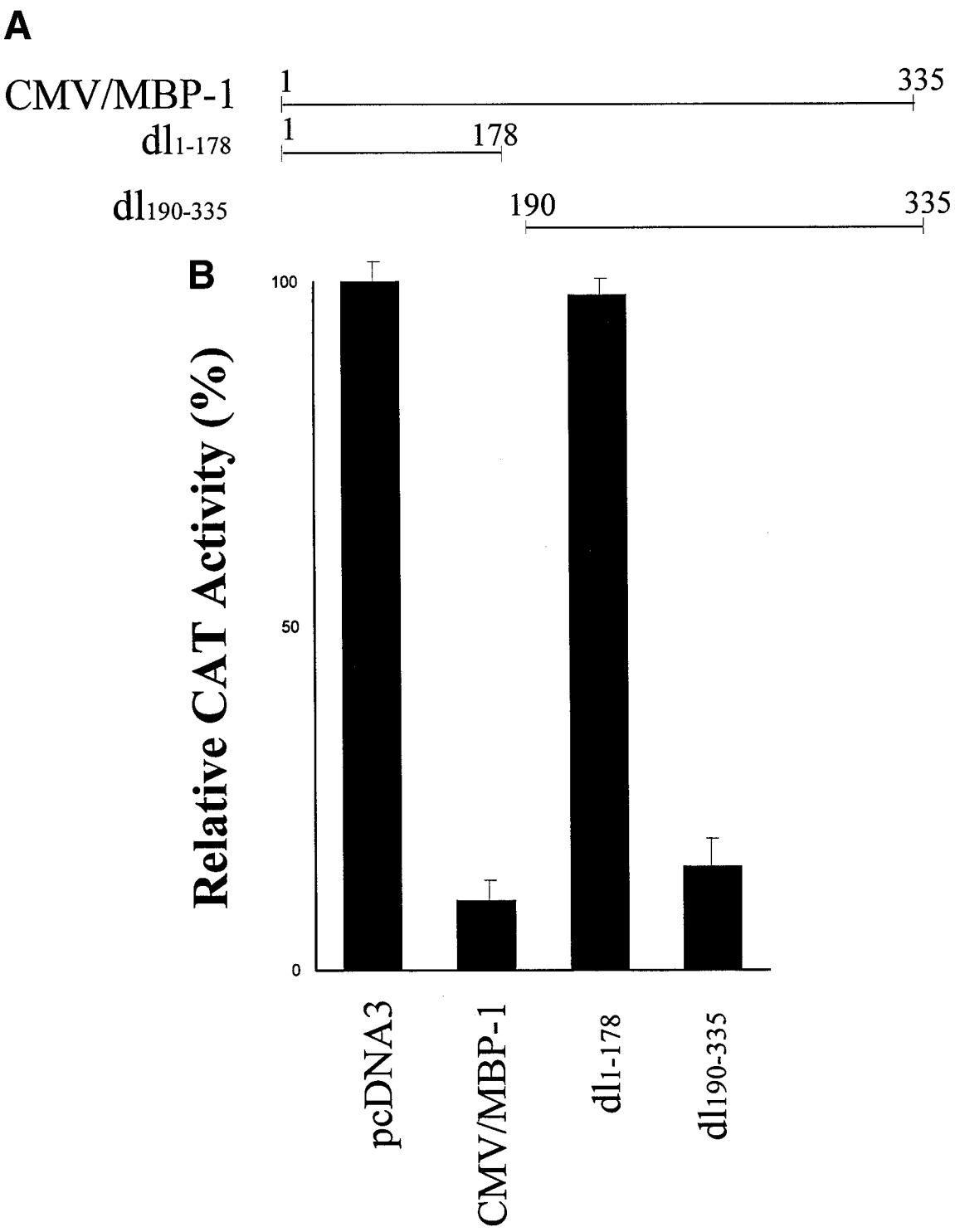

Fig. 3. Localization of transcriptional repressor domain of the MBP-1. **A**: A schematic diagram showing the deletion mutants of MBP-1 (number represents amino acid residues). **B**: 2 ug of HIV-1 LTR CAT reporter plasmid and 2 ug of effector plasmid were cotransfected into HeLa-T4 cells and CAT assay was performed 48 h after transfection. The results are shown from three separate experiments and presented as relative CAT activity with HIV-1 LTR given a value 100%.

ing HIV-1-infected cells within the culture. To further investigate this possibility, we tested the effects of pNL4-3 and MBP-1 co-expression in CD4-negative WIL-2 cell line which is refractory to HIV-1 infection and HIV-1-induced cell fusion. Low levels of HIV-1 were produced from WIL-2 cells transfected with pNL4-3, and a near complete inhibition of HIV-1 production was observed in cells cotransfected with proviral DNA and MBP-1 (Fig. 4B). No cytopathology was detected either in cells transfected with pNL4-3 alone or cells cotransfected with MBP-1. HIV-1 production was not detected from the cotransfected cultures even after 3 weeks. Together these results underscore the effect of MBP-1 on HIV-1 replication.

DISCUSSION

Our study suggested that MBP-1 acts as a repressor for HIV-1 transcription and replication. A significant number of CD4-positive lymphocytes show integrated proviral DNA during all stages of HIV-1 infection. However, viral mRNA is expressed only in a fraction of these cells (Emberston et al., 1993a,b; Pantaleo et al., 1993). HIV-1 is dependent on its host cell for the synthesis and processing of viral RNA and number of cellular factors modulate this process. For example, the TATA element modulatory factor (Garcia et al., 1992) inhibits HIV-1 transcription by binding to the transcription factor TF IID. The cellular factor USF-1 represses HIV-1-transcription through a binding site in the U3 region of HIV-1 LTR (Giacca et al., 1992), but can activate transcription by binding to a site near the initiator (Du et al., 1993). However, the in vivo role of these cellular factors was not investigated. The present study shows that MBP-1 is a new member of this group of cellular factors that inhibit HIV-1 basal transcription, although the underlying mechanism remains to be understood. MBP-1 and TBP simultaneously bind to the c-myc P2 promoter and thus, MBP-1-mediated inhibition may not involve the interaction between these two transcription factors. Studies with LTR deletion mutants suggested that core promoter is sufficient for repression of HIV-1 basal transcription by MBP-1. Neither the upstream NRE or enhancer sequences (including NF-kB), nor the down-stream TAR elements were required for repression by MBP-1. Therefore, MBP-1-mediated repression may involve interactions

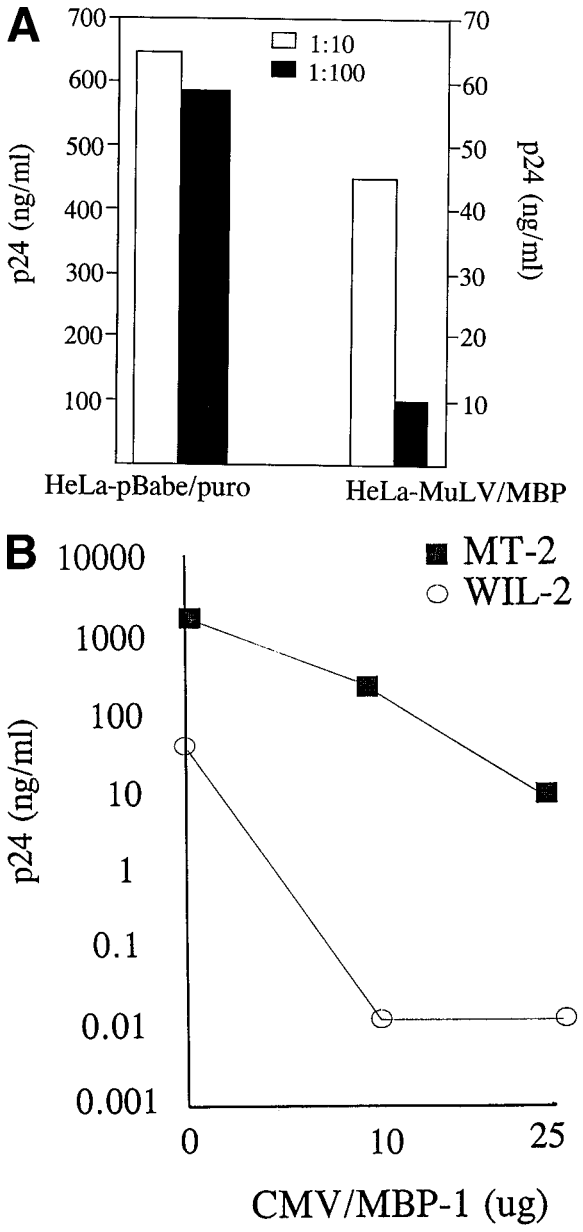

Fig. 4. Effect of MBP-1 expression on HIV-1 replication. **A**: HeLa-pBabe/puro or HeLa-MuLV/MBP cells were infected 24 h later with a 1:10 and 1:100 dilution of a virus stock. After 5 days, the culture supernatants were tested for HIV-1 replication by p24 antigen capture assay. The results show a reduced efficiency of HIV-1 p24 antigen production in cells expressing MBP-1. Values of p24 antigen production from control HeLa-pBabe/puro cells are presented in a higher scale than that of HeLa-MuLV/MBP cells. **B**: HIV-1 replication in MT-2 or WIL-2 cells cotransfected with pNL4-3 and MBP-1. Exponential cultures of MT-2 or WIL-2 cells were transfected with 10 ug of pNL4-3 and 0 ug, 10 ug, or 25 ug of CMV/MBP-1. The cultures were assayed for p24 antigen production after 5 days. The results show a dose-dependent inhibition of HIV-1 replication by MBP-1 plasmid in both MT-2 and WIL-2 cells.

with other cellular factor(s) for biological effect on HIV-1 LTR.

Recent studies have shown that the wild-type tumor suppressor gene p53 inhibits HIV-1 LTR expression by binding to the TBP subunit of the general transcription factor TF IID (Duan et al., 1995), while many mutant p53 molecules augment HIV-1 transcription (Subler et al., 1994; Duan et al., 1995). A mutifunctional transcriptional factor YY-1 (Margolis et al., 1994), also represses viral transcription by binding to the HIV-1 initiator region. In contrast, Rpt-1 (regulatory protein, T lymphocyte-1) inhibits HIV-1 transcription (Patarca et al., 1988) by down-regulation of IL2R α-chain expression and inhibition of T cell activation. Transcriptional factor E2F1 also inhibits HIV-1 transcription and replication (Kundu et al., 1995), although the mechanism of inhibition is unknown. Whether MBP-1 mediated regulation of HIV-1 basal transcription is responsible for inhibition of HIV-1 replication in cell culture is presently unclear, especially since HIV-1 transcription is not inhibited in the presence of Tat protein. Nevertheless, inhibition of HIV-1 basal transcription prior to the accumulation of threshold levels of Tat protein can result in a state of transcriptional latency at the cellular level and influence the extent of virus replication or spread. Essentially similar inhibitory effect on HIV-1 replication has been reported with other inhibitors of HIV-1 basal transcription (Duan et al., 1995). However, it should be pointed out that many of these cellular factors are multifunctional, and affect diverse cellular and/or viral promoters. Thus, it is possible that some of the observed effects of virus latency in infected cells may indeed be due to the activities of cellular rather than viral factors. HIV-1 infection is intimately associated with cell proliferation and any changes in the proliferative potential of the cells may affect the kinetics of viral replication. We did not observe any overt differences in the viability or proliferation of MBP-1 transfected HeLa cells, although it is possible that transcription from certain cellular promoters (e.g., c-myc) are likely to be inhibited under these conditions. HIV replicates primarily in actively proliferating lymphocytes. Nevertheless, HIV can infect many non dividing cells including monocytes, dendritic cells, and other various cell types that may form a reservoir for HIV. Moreover, a large number of HIV-infected cells are probably quiescent. The host-, tissue-, or cell cycle specific differences, if any, in levels of MBP-1 expression may affect the course or outcome of HIV infection. Further understanding on the mechanisms involved in the regulation of HIV-1 replication by cellular factors may help in the development of preventive measures against this important viral pathogen.

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