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# CCAAT/Enhancer Binding Proteins Are Not Required for HIV-1 Entry but Regulate Proviral Transcription by Recruiting Coactivators to the Long-Terminal Repeat in Monocytic Cells

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CCAAT/enhancer binding proteins (C/EBP) have been shown to be required for HIV-1 transcription and replication in macrophages. However, whether these transcription factors influence the ability of virus to establish infection by altering cytokine or receptor expression or primarily regulate HIV-1 transcription has not been determined. By inhibiting endogenous C/EBP activity with a dominant-negative protein, we demonstrate that functional C/EBPs are not required for HIV-1 infection and that these factors influence replication by a transcriptional mechanism. C/EBP$\beta$ recruits coactivators to the HIV-1 long-terminal repeat (LTR) and physically interacts with histone acetyltransferase (HAT) complexes, suggesting that C/EBPs participate in remodeling the chromatin organization of the HIV-1 provirus. Furthermore, overexpression of a C/EBP dominant-negative inhibits displacement of nucleosomes located at the HIV-1 transcriptional start site. These results provide insight into the general mechanisms by which C/EBPs regulate macrophage-restricted HIV-1 transcription. 

*Key Words:* HIV; transcription; chromatin.

## INTRODUCTION

HIV-1 transcription is controlled by cellular and viral factors that bind the HIV-1 long-terminal repeat (LTR) (Gaynor, 1992). NF-$\kappa$B is one cellular factor that has been shown to be important in regulating HIV-1 expression. Recent experiments demonstrated that CCAAT/enhancer binding proteins (C/EBP) are also necessary for HIV-1 replication in macrophages and suggested that C/EBP$\beta$ is a macrophage-specific requirement for virus transcription (Henderson *et al.,* 1996; Henderson and Calame, 1997; Tesmer *et al.,* 1993). C/EBP family members are expressed in several tissues but have distinct expression patterns. For example, C/EBP$\beta$ is induced during monocytic cell differentiation and coordinates the expression of several genes during macrophage activation (Akira and Kishimoto, 1992; Poli, 1998). When infected macrophages are activated, HIV-1 transcription increases, partly due to induction of NF-$\kappa$B and C/EBP$\beta$ activities (Griffin *et al.,* 1989; Henderson *et al.,* 1996; Henderson and Calame, 1997; Jacque *et al.,* 1996). Whether the induction of transcription factors such as C/EBP$\beta$ solely regulate transcription or indirectly influence HIV-1 replication by modifying the expression of receptors or cytokines and thus the susceptibility of monocytes/macrophages to HIV-1 infection has not been examined.

The general mechanism by which C/EBPs regulate HIV-1 transcription is also not known. Although C/EBPs are necessary for HIV-1 expression and replication in macrophages (Henderson *et al.,* 1996; Henderson and Calame, 1997), overexpression of C/EBP$\beta$ is not sufficient to activate latent virus expression, indicating that posttranslation events are necessary for this factor to induce HIV-1 transcription (Henderson *et al.,* 1996). One explanation for this observation is that the ability of C/EBP$\beta$ to activate HIV-1 transcription is dependent on functional interactions with other factors. C/EBP$\beta$ has been shown to interact with several classes of transcription factors including transcriptional coactivators Swi/Snf and CBP/p300. Furthermore, it has been suggested that by interacting with these coactivators C/EBP$\beta$ potentially influences chromatin organization (Kowenz-Leutz and Leutz, 1999; Mink *et al.,* 1997; Oelgeschlager *et al.,* 1996).

HIV-1 expression is associated with displacement of nucleosomes and establishment of an open chromatin structure (El Kharroubi *et al.,* 1998; Sheridan *et al.,* 1997; Van Lint *et al.,* 1996; Verdin, 1991; Verdin *et al.,* 1993). CBP/p300 has been shown to functionally and physically interact with multiple factors that bind the LTR including NF-$\kappa$B, Sp1, and Tat (Benkirane *et al.,* 1998; Hottiger *et al.,* 1998; Kiernan *et al.,* 1999; Sheridan *et al.,* 1997; Vanden Berghe *et al.,* 1999). Chromatin-dependent *in vitro* tran-

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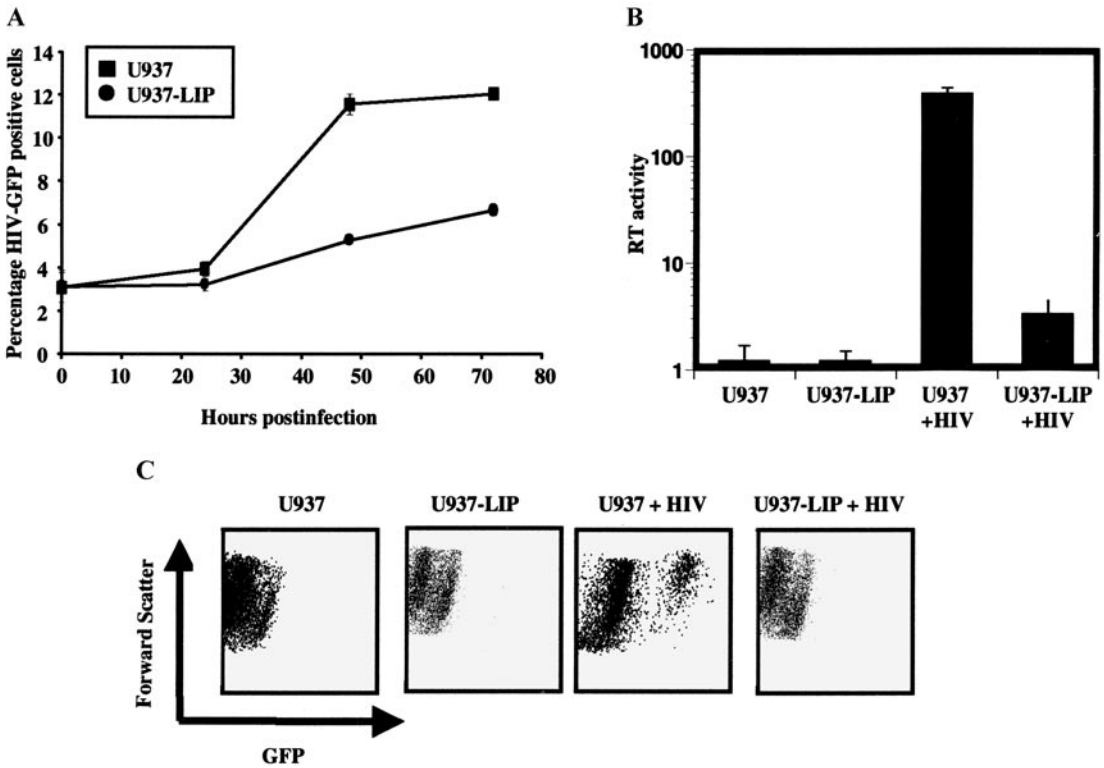


FIG. 1. C/EBP factors are necessary for HIV-1 replication in monocytes. (A) U937 and U937-LIP cells were infected with HIV-GFP and analyzed for infected cells at 24, 48, and 72 h postinfection by flow cytometry. (B) U937 and U937-LIP cells were infected with HIV-GFP and supernatants were assayed for RT activity 3 weeks postinfection. Each data point represents three independent infections. Error bars show one standard deviation. These results are representative of three separate experiments. (C) U937 and U937-LIP cells were infected with HIV-GFP and analyzed by flow cytometry for infected cells 3 weeks postinfection.

scription systems have also demonstrated a critical role for T cell-restricted factors TCF-1/LEF-1 and Ets-1 in remodeling the HIV-1 LTR (Pazin *et al.,* 1996; Steger and Workman, 1997). Factors that recruit chromatin remodeling complexes to the LTR in cells other than T cells have not been examined.

In this study we were interested in characterizing in greater detail the mechanisms by which C/EBPs regulate HIV-1 replication. We utilize monocytic cell lines that ectopically express a dominate-negative protein to inhibit endogenous functional C/EBPs. Our data show that C/EBP factors are not necessary for establishing HIV-1 infection in monocytic cells but primarily influence HIV-1 replication at a transcriptional level in part by reorganizing the chromatin structure of the HIV-1 proviral LTR.

## RESULTS

### C/EBP factors do not influence HIV entry but are necessary for efficient HIV-1 transcription in monocytic cells

Previous studies have shown that HIV-1 fails to productively infect monocytic cells overexpressing the C/EBP dominant-negative protein LIP (Henderson *et al.,* 1996; Henderson and Calame, 1997). LIP, which lacks a strong transcriptional activation domain, inhibits C/EBPβ by forming antagonistic homodimers or nonfunctional C/EBPβ-LIP heterodimers (Descombes and Schibler, 1990). To assess the importance of functional C/EBP factors during HIV-1 replication in monocytic cells, we infected U937 monocytic cells and U937 cells overexpressing LIP (U937-LIP) with a replication-competent GFP-tagged NL43 virus (HIV-GFP) to quantify the number of infected cells. Infection was monitored by flow cytometry and RT assays. The difference in the number of U937 and U937-LIP cells infected is not significant at the earlier times postinfection but the effects of LIP overexpression on HIV-1 infection become increasingly evident with time (Fig. 1). Twenty-four hours postinfection, equivalent numbers of U937 and U937-LIP cells were infected with HIV-GFP and by 2–3 days postinfection, twofold less U937-LIP cells were infected with HIV-GFP compared to U937 cells (Fig. 1A). Three weeks postinfection, U937 cells produced a significant amount of virus as indicated by RT activity (Fig. 1B) and 15–20% of the cells were infected as determined by GFP expression (Fig. 1C) whereas, consistent with previous studies (Henderson and Calame, 1997), U937-LIP cells expressed 100-fold less virus compared to U937 cells and very few cells were HIV-1 positive (Figs. 1B and 1C). These data sug-

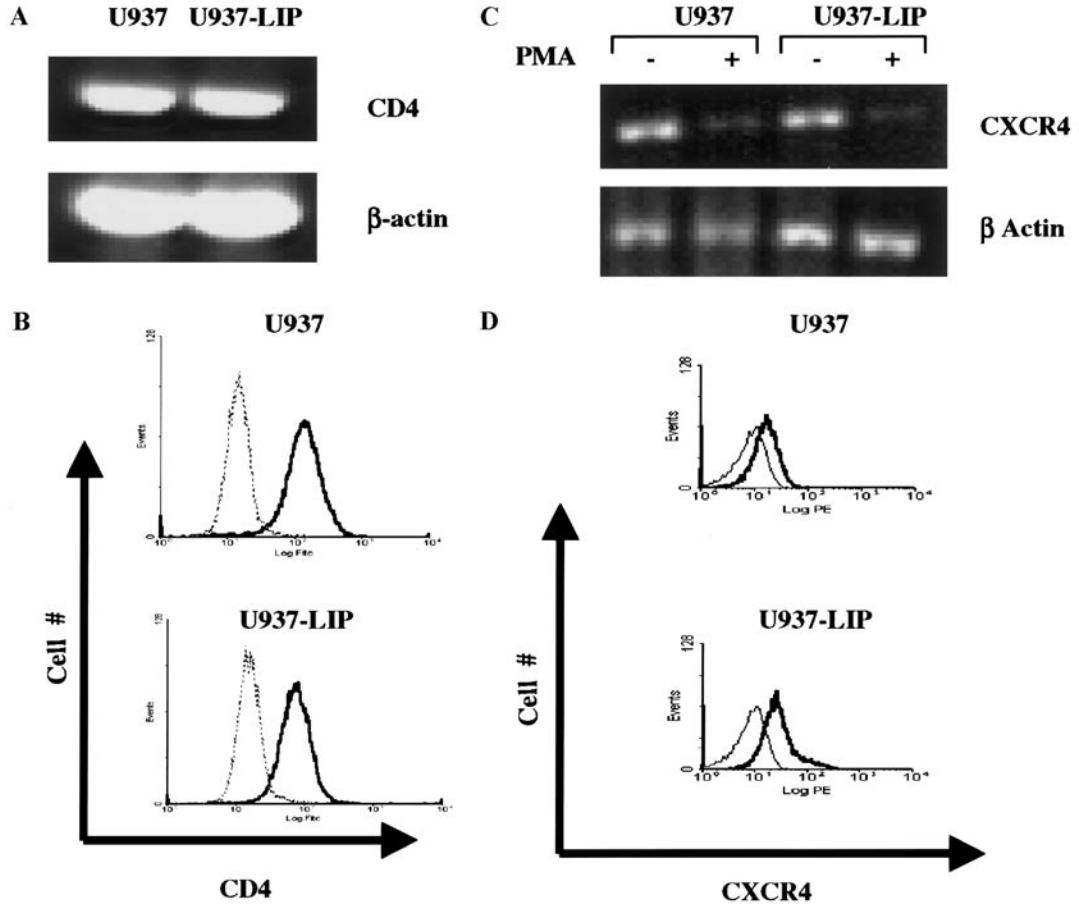


FIG. 2. C/EBP factors do not regulate expression of coreceptors necessary for HIV-1 infection. (A) U937 and U937-LIP cells were analyzed for CD4 expression by RT-PCR on total RNA or (B) by flow cytometry following staining with anti-CD4 antibody (solid line) or IgG isotype matched control (broken line). (C) Total RNA was prepared from U937 and U937-LIP cells cultured alone or with 10 ng/ml PMA for 6–12 h and analyzed by RT-PCR for CXCR4 expression. (D) Cells were stained with anti-human CXCR4 antibody (solid line) or IgG isotype matched control (broken line) and analyzed by flow cytometry.

gest that functional C/EBP factors are required for productive replication of HIV-1 in monocytic cells.

The inability of the virus to replicate in cells overexpressing LIP could be due to a block in virus entry, possibly at the level of coreceptor usage. HIV-1 requires CD4 and a chemokine receptor, in particular CXCR4 or CCR5, to infect the host cell. Changes in the levels of these receptors have been shown to alter susceptibility to HIV-1 infection (Basta *et al.,* 1999; Berger *et al.,* 1999; Franchin *et al.,* 2000; Moriuchi *et al.,* 1998; Tuttle *et al.,* 1998). To assure that C/EBP factors were not adversely influencing HIV-1 infection by altering the expression of CD4 or chemokine receptors, we used flow cytometry and semiquantitative RT-PCR to analyze U937 and U937-LIP cells for the expression of these receptors. When U937 and U937-LIP cells were analyzed for CD4, no significant difference in mRNA levels or surface expression was observed (Figs. 2A and 2B). Similarly, CXCR4 expression was unchanged between U937 and U937-LIP cells (Figs. 2C and 2D). Although there are slight shifts in the fluorescence intensity in CD4 and CXCR4 between U937 and U937-LIP cells, these changes represent less than a twofold difference in mean fluorescence intensity (mean fluorescence intensity for CD4, U937 = 128, U937-LIP = 72; mean fluorescence intensity for CXCR4, U937 = 15.1, U937-LIP = 27.7). Both cell types downregulated CXCR4 expression in response to PMA treatment, which is consistent with previous reports (Fig. 2C) (Moriuchi *et al.,* 1998). Neither unstimulated nor stimulated U937 and U937-LIP cells expressed detectable levels of CCR5 as determined by RT-PCR and flow cytometry (data not shown). Therefore, LIP overexpression does not affect CD4 and CXCR4 expression, suggesting that endogenous C/EBPs are not required for HIV-1 infection of monocytic cells.

To confirm that U937 and U937-LIP cells were equally susceptible to HIV-1 infection, cells were infected with a replication-incompetent virus in which the envelope gene (Env) was replaced with a luciferase reporter gene (HIV-luc). Genomic DNA was prepared 48 h postinfection and assayed for proviral sequences using PCR. As shown in Fig. 3A, U937 and U937-LIP cells had similar levels of HIV-1 provirus, demonstrating that LIP did not block viral entry and that functional C/EBP proteins are not required for establishing HIV-1 infection.

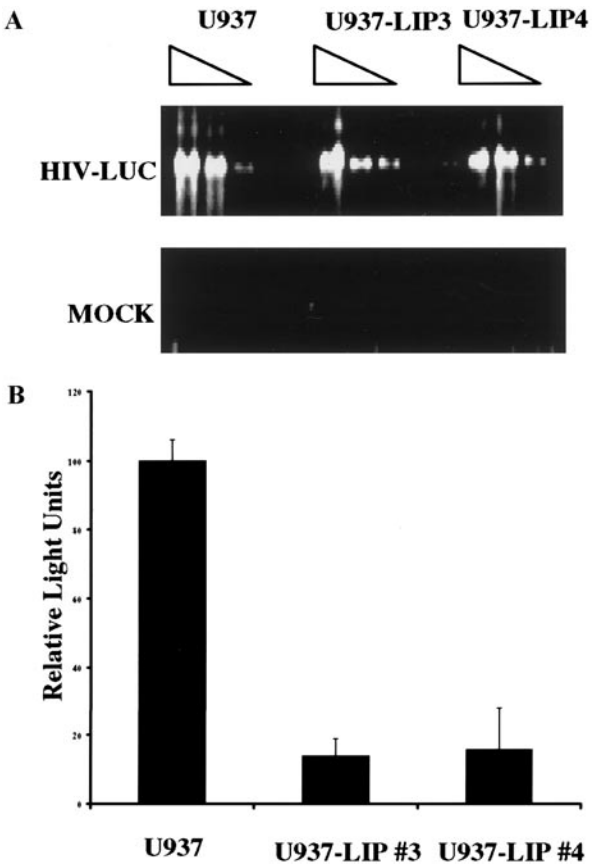


FIG. 3. C/EBP factors regulate HIV-1 replication in monocytes directly through a transcriptional mechanism. (A) U937 and U937-LIP cells were infected with HIV-luc and genomic DNA was prepared 48 h postinfection. Measures of 1, 0.1, or 0.01 μg genomic DNA were analyzed by PCR for proviral sequences. U937-LIP3 and U937-LIP4 refer to different clonal populations of cells. (B) Forty-eight hours postinfection with HIV-luc, U937 and U937-LIP cells were lysed and assayed for luciferase activity. These results are from a single experiment performed in triplicate and represent three independent experiments. Error bars show one standard deviation.

The lack of a role for C/EBP factors in establishing infection suggests that the inhibition of HIV-1 replication in monocytic cells overexpressing LIP is through a direct effect on virus transcription. To investigate this possibility, HIV-luc was used to infect U937 and U937-LIP cells and virus transcription was measured by luciferase activity 48 h postinfection. As shown in Fig. 3B, a sixfold decrease in luciferase activity was observed in U937-LIP cells compared to U937 cells. These data support the conclusion that C/EBP factors are influencing HIV-1 replication in monocytic cells through a transcriptional mechanism.

### C/EBPβ requires coactivators to transactivate the HIV-1 LTR

Functional C/EBPs are necessary but not sufficient for HIV-1 transcription in macrophages (Henderson *et al.,* 1996; Henderson and Calame, 1997) and may require interactions with other transcription factors or coactivators. To determine whether functional interactions between coactivators and C/EBPβ were relevant for HIV-1 LTR activity, we performed cotransfection experiments using a HIV-1 LTR-luciferase reporter and different combinations of expression constructs encoding C/EBPβ and CBP. Consistent with previous studies C/EBPβ was able to strongly transactivate the HIV-1 LTR (Henderson *et al.,* 1995; Tesmer *et al.,* 1993). Ectopic expression of CBP was also able to activate the HIV-1 LTR in a dose-dependent manner. However, cotransfecting with both C/EBPβ and CBP led to synergistic activation of the HIV-1 LTR. As shown in Fig. 4 cotransfections with both CBP and C/EBPβ resulted in a 56-fold increase in HIV-1 LTR activity, whereas approximately 10-fold increases in transcriptional activity were observed with C/EBPβ or CBP alone (Fig. 4A). These results are not due to CBP increasing C/EBPβ expression since immunoblots demonstrate that cells have approximately equivalent levels of C/EBPβ in the absence or presence of exogenous CBP (data not shown). Furthermore, C/EBPβ-binding activity is unaffected by CBP overexpression (Fig. 4D). These data would suggest that C/EBPβ functionally interacts with CBP and that CBP is a limiting factor for this activity.

Although CBP has histone acetyltransferase (HAT) activity, this coactivator functions in part by recruiting other HAT coactivators including PCAF to the promoter (Yang *et al.,* 1996). Therefore, additional cotransfection experiments were performed to determine whether C/EBPβ and PCAF were able to cooperatively activate the HIV-1 LTR. Similar to the previous experiment, PCAF enhanced the ability of C/EBPβ to transactivate the HIV-1 LTR (Fig. 4B). This enhancement is not due to PCAF altering C/EBPβ binding activity, which remains unchanged in the absence or presence of PCAF (Fig. 4E). These data indicate that coactivators can interact with C/EBPβ to influence HIV-1 expression and are limiting the ability of C/EBPβ to fully induce HIV-1 transcription. Furthermore, ectopic expression of E1a, which binds directly to CBP/p300 and PCAF thereby inhibiting their activity (Chakravarti *et al.,* 1999; Hamamori *et al.,* 1999), decreases C/EBPβ-dependent HIV-1 LTR activity. The ability of C/EBPβ to transactivate the HIV-1 LTR was reduced by greater than 80% and this inhibition could be overcome by overexpression of either CBP or PCAF (Fig. 4C and data not shown), indicating that E1a inhibits the HIV-1 LTR by competing with C/EBPβ for coactivators. These data demonstrate that C/EBPβ activates HIV-1 transcription by recruiting HAT coactivators to the promoter.

### C/EBPβ interacts with chromatin remodeling complexes

PCAF participates in large multi-subunit HAT complexes that are analogous to SAGA and Ada in yeast.

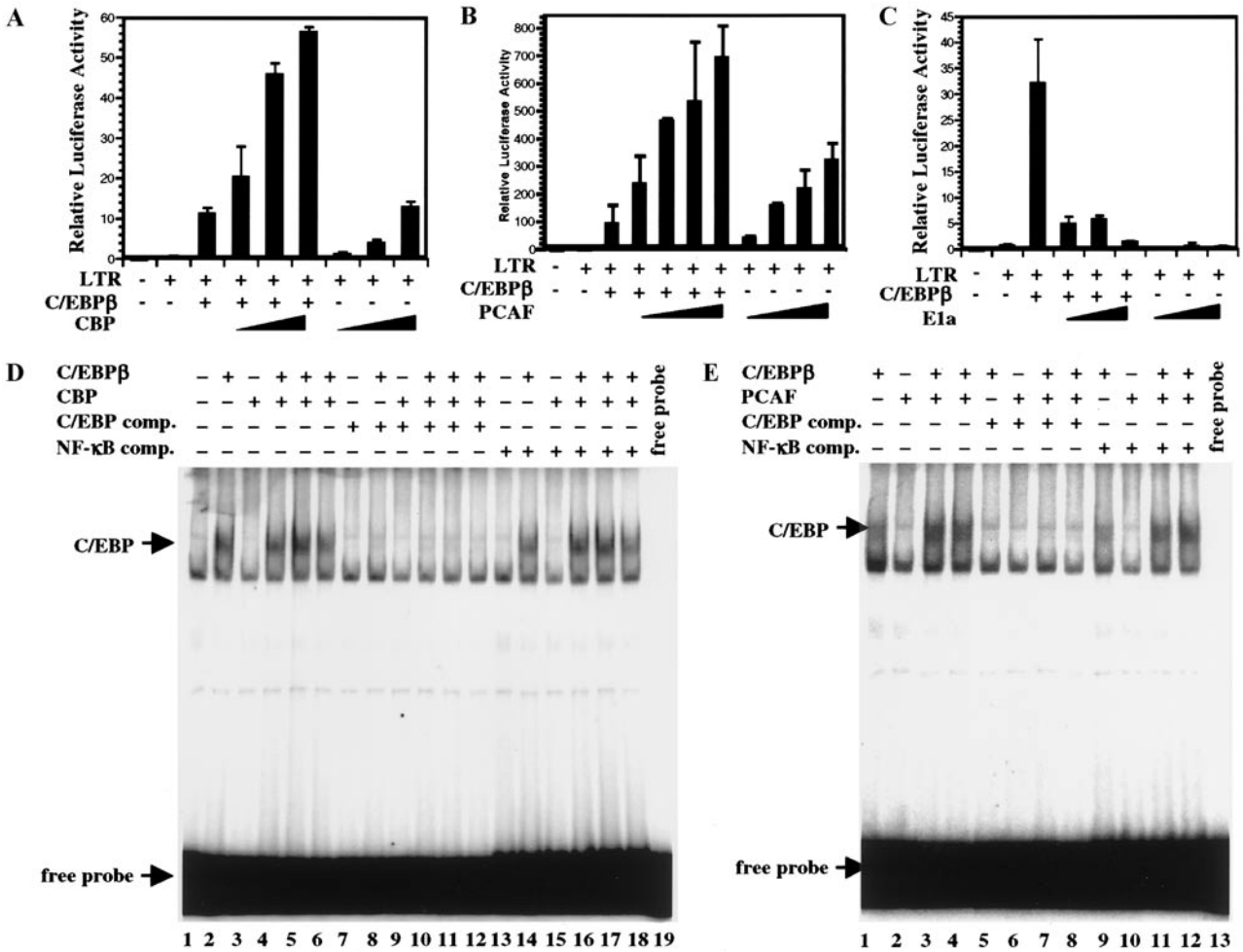


FIG. 4. C/EBP$\beta$ cooperates with CBP to activate the HIV-1 LTR. (A) Five micrograms of LTR-LUC reporter was cotransfected into 293T cells with 0 or 5 $\mu$g of C/EBP$\beta$ and 0, 1, 2, and 5 $\mu$g of CBP or (B) 0, 1, 3, 5, or 7 $\mu$g of PCAF or (C) 0, 1, 2, and 5 $\mu$g of 12S E1a as indicated. Forty-eight hours posttransfection, cells were collected and luciferase activity was measured. Each bar represents three independent transfections. These data are from single experiments that are representative of four independent experiments. (D) EMSA were performed with nuclear extracts prepared from 293T cells cotransfected with 0 or 5 $\mu$g of C/EBP$\beta$ and 0, 1, 2, or 5 $\mu$g of CBP (Lanes 2, 4–6) and a C/EBP binding site oligonucleotide probe. Extracts were incubated with no competitor (Lanes 1–6), a 50-fold excess of specific C/EBP binding site competitor (Lanes 7–12), or a 50-fold excess of nonspecific NF-$\kappa$B binding site competitor (Lanes 13–18). (E) EMSA were performed with nuclear extracts prepared from 293T cells cotransfected with 0 or 5 $\mu$g of C/EBP$\beta$ and 0, 3, or 5 $\mu$g of PCAF (Lanes 1, 3, 4) and a C/EBP binding site oligonucleotide probe. Extracts were incubated with no competitor (Lanes 1–4), a 50-fold excess of specific C/EBP binding site competitor (Lanes 5–8), or a 50-fold excess of nonspecific NF-$\kappa$B binding site competitor (Lanes 9–12).

Native HATs purified from yeast have been well characterized and demonstrated to interact with transcription factors from higher eukaryotes (Grant *et al.,* 1997; Howe *et al.,* 1999; Sterner and Berger, 2000; Wallberg *et al.,* 1999). Furthermore, yeast HATs have been shown to reorganize preassembled HIV-1 chromatin templates and stimulate transcription *in vitro* (Steger *et al.,* 1998). Since the yeast HATs are structurally and functionally similar to the mammalian complexes (Howe *et al.,* 1999; Ogryzko *et al.,* 1998; Sterner and Berger, 2000; Vassilev *et al.,* 1998), we examined the ability of C/EBP$\beta$ to bind SAGA using a GST pull-down assay. After incubating purified SAGA with glutathione-Sepharose beads and GST-C/EBP$\beta$ fusion protein, both beads and supernatant were assayed for HAT activity. Figure 5A shows HAT activity was retained on the GST-C/EBP$\beta$-bound beads while no significant HAT activity was observed with beads alone. These data indicate that C/EBP$\beta$ directly interacts with HAT complexes and suggest that C/EBP$\beta$ in part regulates HIV-1 transcription by recruiting nucleosome-acetylating native complexes to the LTR.

To further explore the significance of the above interaction, we tested whether PCAF, the mammalian homolog of the GCN5 subunit of yeast SAGA, specifically interacts with C/EBP$\beta$ in a cellular system. 293T cells were transiently transfected with PCAF and C/EBP$\beta$ and nuclear extracts were analyzed for protein interactions by coimmunoprecipitation. As shown in Fig. 5B, immunoprecipitation with anti-C/EBP$\beta$ antibody pulled-down PCAF, demonstrating a physical interaction between

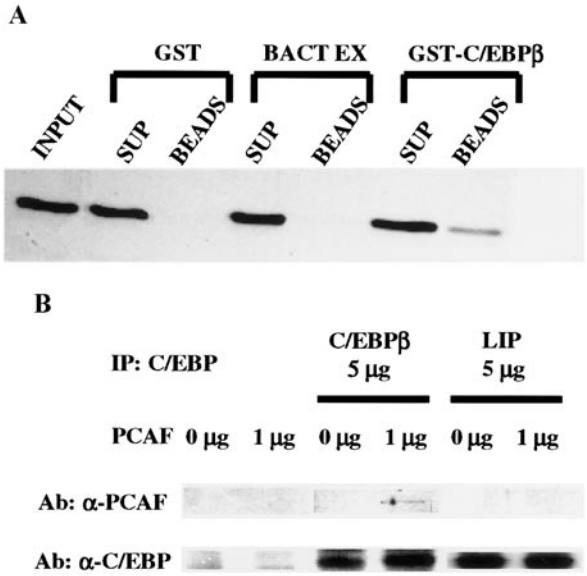


FIG. 5. C/EBPβ interacts with chromatin remodeling complex SAGA. (A) GST pull-down assays were performed with either GST-C/EBPβ, mock-transformed bacterial extracts, or GST bound to glutathione-Sepharose beads. SAGA purified from yeast was incubated with the protein–bead complexes as described under Materials and Methods. Supernatants and beads were assayed for their ability to acetylate core histones as described under Materials and Methods. (B) 293T cells were transfected with 0 or 1 μg of PCAF and 0 or 5 μg of C/EBPβ or LIP. Nuclear extracts were immunoprecipitated with anti-C/EBPβ antibody and Western blotting was performed to detect PCAF and C/EBP proteins.

PCAF and C/EBPβ. However, we were unable to coimmunoprecipitate PCAF from cell extracts with transfected LIP, which lacks the N-terminal transactivation domain (Fig. 5B). These results demonstrate that C/EBPβ physically interacts with components of SAGA in a cellular context and that the transactivation domain of C/EBPβ mediate this interaction.

### Inhibiting endogenous C/EBPs prevents chromatin remodeling of the HIV-1 LTR

We have used transient transfections to suggest a functional interaction between C/EBPβ and HATs; however, the majority of the transfected DNA is unintegrated and, although associated with histones, would be expected to have a simpler chromatin structure. Therefore, it is critical that we employ a relevant cell type containing integrated HIV-1 provirus. U1 monocytic cells harbor latent provirus and C/EBPβ has been demonstrated to be required for induction of virus expression (Henderson *et al.,* 1996). In addition, the induction of HIV-1 transcription in these cells is associated with displacement of a nucleosome positioned at the transcriptional start site (Verdin *et al.,* 1993). To determine whether functional C/EBPβ is required for remodeling the chromatin structure of the HIV-1 proviral LTR, we took advantage of U1 lines that ectopically express the C/EBP dominant-negative protein, LIP (Henderson *et al.,* 1996). Transcriptional induction is associated with an open chromatin structure, which can be detected by increased sensitivity to restriction enzymes (El Kharroubi *et al.,* 1998; Verdin *et al.,* 1993). Upon activation with PMA, which induces U1 HIV-1 transcription, the nucleosome positioned at −2 to +142 is displaced, making this region susceptible to *Hin*dIII and *Pst*I digestion (Fig. 6B and data not shown) (Verdin *et al.,* 1993). Other restriction sites that are adjacent to the positioned nucleosome, such as *Pvu*II, generate fragments regardless of whether U1 cells have been activated (Fig. 6B) (Verdin *et al.,* 1993). When U1-LIP cells were treated with PMA, consistent with previous studies, virus replication was significantly reduced (Fig. 6A) (Henderson *et al.,* 1996). More importantly, despite detecting a *Pvu*II-specific restriction fragment, *Hin*dIII did not efficiently digest DNA from nuclei obtained from activated U1-LIP cells (Figs. 6C and 6D). Therefore, inhibiting endogenous C/EBPs prevents the displacement of the nucleosome at the HIV-1 transcriptional start site. Since C/EBPβ is the primary C/EBP family member induced following activation of U1 cells (Natsuka *et al.,* 1992), these results suggest that by recruiting chromatin remodeling complexes to the LTR, C/EBPβ regulates HIV-1 transcription.

## DISCUSSION

Previous studies have shown that the transcriptional activator C/EBPβ activates the HIV-1 LTR and is necessary for HIV-1 replication in monocytes (Henderson *et al.,* 1996; Henderson and Calame, 1997; Tesmer *et al.,* 1993). However, the role of C/EBPβ in establishing HIV-1 infection has not been determined. We demonstrate that while C/EBP factors are not necessary for establishing HIV-1 infection of monocytes they influence HIV-1 replication by regulating the chromatin structure of the LTR and thus virus transcription.

C/EBPβ has been shown to control the expression of a number of genes during macrophage activation including CD14, ICAM-1, Mac-1, and IL-6, which are expressed at significantly lower levels in cell lines that ectopically express the C/EBP dominant-negative protein LIP (Henderson *et al.,* 1996; Henderson, A. J., Lee, E. S., and Zhou, H., unpublished data). However, as shown in this study, the expression of receptors essential for HIV-1 infection, CD4 and CXCR4, does not require functional endogenous C/EBPs. Furthermore, inhibition of C/EBP activity in monocytic cells does not adversely affect HIV-1 infection. We were unable to detect CCR5 expression in U937 cells and therefore could not evaluate whether this chemokine receptor is potentially regulated by C/EBPs in monocytes/macrophages, although the promoter for CCR5 has been recently characterized and does have potential C/EBP binding sites (Moriuchi *et al.,* 1997; Rosati *et al.,* 2001). While these data suggest that C/EBP factors do

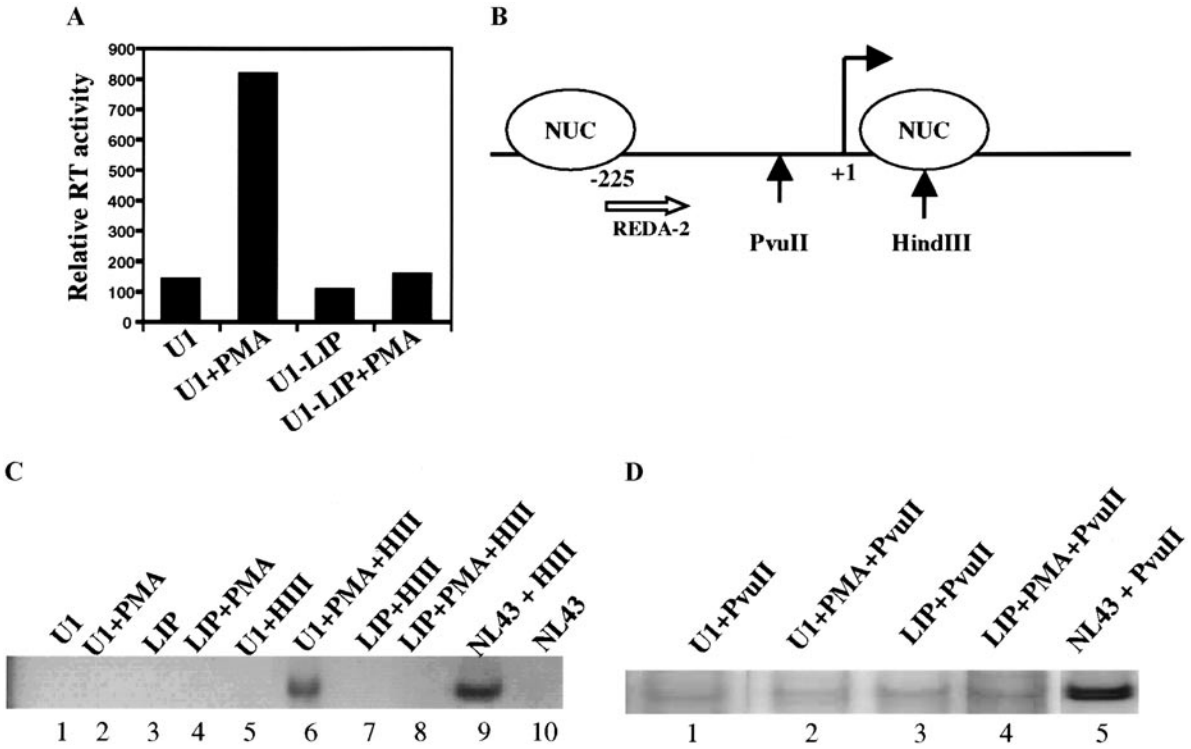


FIG. 6. LIP inhibits accessibility of restriction enzymes at the HIV-1 LTR. (A) U1 and UI-LIP cells were cultured in the absence or presence of PMA for 12 h. Supernatants were collected and reverse transcriptase assays (RT) were performed to determine virus levels. (B) Schematic of HIV-1 LTR showing positioned nucleosomes and relevant restriction enzyme sites. (C) Nuclei from U1 and U1-LIP cells were isolated following culture in the absence (Lanes 1, 3, 5, 7) or presence of PMA (Lanes 2, 4, 6, 8) for 12 h. Genomic DNA was purified from nuclei following no restriction digest (Lanes 1–4) or *Hin*dIII digestion (Lanes 5–8). Restriction fragments were detected by LM-PCR as described under Materials and Methods. Lane 9 is a positive control using NL43 cDNA digested with *Hin*dIII and Lane 10 is undigested NL43 DNA negative control. (D) Nuclei from U1 and U1-LIP cells digested with *Pvu*II. Lane 5 is NL43 cDNA digested with *Pvu*II.

not directly affect HIV-1 infection in monocytes, C/EBPs may indirectly influence disease progression by contributing to aberrant macrophage function, inappropriate expression of inflammatory cytokines, and a more permissive microenvironment for disease progression (Akira and Kishimoto, 1992; Honda *et al.,* 1998; Lee *et al.,* 2001).

Although C/EBP$\beta$ does not influence HIV-1 infection, this factor impacts HIV-1 replication by regulating virus transcription in part through the recruitment of coactivators to the LTR. It has been demonstrated that activation of latent HIV-1 proviral expression in monocytic and T cell lines is associated with specific chromatin changes within the 5′ LTR, including displacement of a nucleosome positioned at the transcriptional start site (El Kharroubi *et al.,* 1998; Verdin, 1991; Verdin *et al.,* 1993). Furthermore, treatment of cell lines harboring latent HIV-1 provirus with trichostatin A, trapoxin, or sodium butyrate, specific inhibitors of histone deacetylases, induces virus expression indicating that HIV-1 transcription is dependent on histone acetylation (Laughlin *et al.,* 1993; Van Lint *et al.,* 1996). The importance of histone acetylation and chromatin remodeling by HATs has been confirmed using nucleosome-associated HIV-1 templates with *in vitro* transcription systems (Pazin *et al.,* 1996; Sheridan *et al.,* 1995, 1997; Steger *et al.,* 1997, 1998; Widak *et al.,* 1997). Transcription factors such as NF-$\kappa$B, SP1, Ets-1, and LEF-1, which bind the proviral enhancer/promoter located in the site upstream of the nucleosome core particle, participate in reorganizing chromatin by facilitating the recruitment of HAT coactivators to the LTR (Pazin *et al.,* 1996; Sheridan *et al.,* 1995, 1997; Steger *et al.,* 1997, 1998; Widak *et al.,* 1997). However, the transcription factors Ets-1 and LEF-1 are restricted to T cells and it would be expected that macrophage-restricted factors may also interact with chromatin-remodeling complexes. Our results show that C/EBP$\beta$ functionally interacts with HAT coactivators and physically interacts with chromatin remodeling complexes in monocytic cells, demonstrating that coactivators are critical for the ability of C/EBP$\beta$ to induce HIV-1 transcription. However, it is unclear the exact role chromatin remodeling plays during *de novo* infection since the number of cells with latent provirus is estimated to be low, although reactivation of virus expression in these cells may be a major source of HIV-1 at late stages of infection or upon removal of anti-retroviral treatments (McCune, 1995).

Previous work by Mink *et al.* demonstrates that the amino-terminal transcriptional activation domain of C/EBP$\beta$ binds regions of CBP/p300 and PCAF through the E1a binding domain (Mink *et al.,* 1997). E1a com-

pletely inhibits the ability of C/EBPβ to transactivate the HIV-1 LTR, suggesting that E1a is competing with C/EBPβ for binding to CBP/p300 or PCAF (Chakravarti *et al.,* 1999; Hamamori *et al.,* 1999). Furthermore, the LIP dominant-negative protein, which lacks this domain (Mink *et al.,* 1997), does not physically interact with PCAF, fails to activate HIV-1 transcription, and is unable to mediate the displacement of nucleosomes that repress HIV-1 transcriptional initiation. Therefore, the HIV-1 LTR is similar to other myeloid-specific promoters requiring C/EBPβ and coactivators for differential activity (Kowenz-Leutz and Leutz, 1999; Mink *et al.,* 1997; Oelgeschlager *et al.,* 1996).

C/EBPβ has also been demonstrated to interact with components of the basal transcriptional machinery such as TBP and TFIIB (Nerlov and Ziff, 1995) and other transcription factors, including NF-κB and CREB (Hohaus *et al.,* 1995; LeClair *et al.,* 1992; Ross *et al.,* 2001; Stein *et al.,* 1993; Westendorf *et al.,* 1998; Zhang *et al.,* 1996). These proteins may cooperate in the recruitment of coactivators to the HIV-1 LTR in monocytic cells. Furthermore, the recruitment of coactivators by transcription factors, including C/EBPβ, may functionally impact Tat, which physically interacts with HATs including CBP/p300, PCAF, $TAF_{II}250$, and Tip60 (Benkirane *et al.,* 1998; Hottiger *et al.,* 1998; Kamine *et al.,* 1996; Kiernan *et al.,* 1999; Marzio *et al.,* 1998; Weissman *et al.,* 1998). In addition to recruiting HATs, C/EBPβ physically interacts with the ATP-dependent chromatin remodeling complex Swi-Snf (Kowenz-Leutz and Leutz, 1999) and therefore may coordinate multiple factors that participate in establishing a transcriptionally accessible HIV-1 LTR.

## MATERIALS AND METHODS

### Cell lines and expression constructs

U937, U937-LIP, U1, and U1-LIP pro-monocytic cell lines were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. U937 and U1 cells overexpressing LIP have been previously described (Henderson *et al.,* 1996; Henderson and Calame, 1997). 293T human embryonic kidney cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine.

The LTR-luciferase reporter and the GST-C/EBPβ constructs have been previously described (Artandi and Calame, 1993; Henderson *et al.,* 1995). The C/EBPβ expression construct was generated by subcloning C/EBPβ cDNA from pSCT-LAP (Descombes and Schibler, 1991) into the *Eco*RI site of the MSCV-IRES-GFP retroviral expression construct (generously provided by G. Nolan, Stanford University) (Anderson *et al.,* 1996). The CMV-LIP construct has been previously described (Henderson *et al.,* 1996). The CBP expression construct was previously described (Kwok *et al.,* 1996) and was provided by Dr. R. Goodman (Oregon Health Sciences University). Dr. D. Livingston (Dana-Farber Cancer Institute, Harvard University) generously provided the 12s E1a expression construct (White *et al.,* 1991), and PCAF expression construct (Yang *et al.,* 1996) was obtained from Dr. Y. Nakatani (National Institute of Child Health and Human Development, Bethesda, MD).

### Transient transfections and luciferase assays

293T cells were transfected with different DNA constructs as indicated in the figure legends by calcium phosphate. Forty-eight hours posttransfection cells were harvested, lysed, and assayed for luciferase activity using the Promega Reporter Assay System (Madison, WI). Transfection efficiencies were determined by monitoring GFP expression from either the MCSV-C/EBPβ-IRES-GFP or the cotransfected MCSV-IRES-GFP. Protein expression was monitored by immunoblot as previously described (data not shown) (Cooper *et al.,* 1995).

### Generation of HIV-1 infectious titers and infections

Replication-competent virus was generated by transfecting 293T cells with 15 μg T-tropic pHIV-EGFP $R^+E^+$ DNA and 3 μg RSV-Rev DNA by $CaPO_4$ transfection (Pear *et al.,* 1993). Production of viral particles was monitored by RT activity 48 h posttransfection. Infectious virus stocks were added to $1.0 \times 10^6$ cells/ml U937 and U937-LIP cells for 24 h. The infection medium was then removed and replaced with fresh RPMI supplemented with 10% FCS, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine. At various times postinfection, infected cells were fixed with 2% paraformaldehyde and quantitated using a Coulter flow cytometer at the Penn State flow cytometry core facility.

Replication-incompetent virus was also generated by transient transfection using 10 μg of pNL43-Luc(+)Env(−) (HIV-luc) DNA (Connor *et al.,* 1995), 2 μg HXB2-Env DNA, and 2 μg RSV-Rev DNA. Transfection efficiency was assessed by luciferase activity. Two hundred microliters of undiluted viral stocks was added to $1.0 \times 10^6$ cells/ml U937 and U937-LIP cells for 24 h. Cells were harvested 48 h postinfection and assayed for viral transcription by luciferase assays. $1.0 \times 10^5$ cells were lysed in 1× Reporter Lysis Buffer (Promega) and supernatants were collected. Twenty microliters of cell extract was added to 100 μl luciferase substrate (Promega) and activity was measured using a luminometer.

### Reverse transcriptase (RT) assays

Virus replication was measured by RT assays at various times postinfection (Henderson *et al.,* 1996). Briefly, 10 μl of supernatant was added to a mixture containing 60 mM Tris, 24 mM DTT, 7 mM $MgCl_2$, 75 mM NaCl, 6 μg/ml poly(dG), 12 μg/ml poly(rC), 0.06% NP-40, and 10

$\mu$Ci [$\alpha$-$^{32}$P]dGTP in a final volume of 50 $\mu$l and incubated at 37°C for 1 h. Ten microliters of this reaction mixture was then transferred to DEAE paper and washed twice in 2× SSC (0.3 M NaCl, 0.03 M Na citrate) for 15 min at 25°C. RT activity was quantitated using a phosphorimager.

### Immunofluorescent staining

To detect cell-surface receptors CD4 or CXCR4, 1.0 × $10^6$ U937 or U937-LIP cells were incubated in staining media (1% FCS in PBS) with 1 $\mu$g $IgG_1$ isotype FITC-conjugated mouse anti-human CD4 mAb (Pharmingen, San Diego, CA) or 1 $\mu$g $IgG_{2a}$ isotype PE-conjugated mouse anti-human CXCR4 mAb (Pharmingen) for 30 min at 4°C. Cells were washed three times in staining media and fixed with 2% paraformaldehyde. Fluorescence was measured using a Coulter flow cytometer at the Penn State flow cytometry core facility.

### RNA extraction and RT-PCR

Total RNA was prepared by lysing cells with 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarkosyl, and 0.1 M 2-mercaptoethanol followed by a phenol-chloroform extraction (Chomczynski and Sacchi, 1987). cDNA was prepared from 2 $\mu$g of RNA using murine leukemia virus reverse transcriptase and random primers. To amplify the cDNA, a 30-cycle polymerase chain reaction of 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min was performed with 0.2 $\mu$g of cDNA using various primers. CD4 cDNA was amplified using the upstream primer 5′-TGGTAGTAGCCCCTCAGTGC-3′ and downstream primer 5′-CACCACCAGGTTCACTTCCT-3′. CXCR4 cDNA was amplified using the upstream primer 5′-TTCCTGCCCACCATCTACTC-3′ and downstream primer 5′-CCATGATGTGCTGAAACTGG-3′. $\beta$-actin cDNA was amplified using the upstream primer 5′-CCTAAGGCCAACCGTGAAAAG-3′ and downstream primer 5′-TCTTCATGGTGCTAGGAGCCA-3′.

### PCR for proviral sequences

To detect proviral sequences in genomic DNA, U937 and U937-LIP cells were lysed 48 h postinfection with TES buffer (10 mM Tris, pH 8.0, 400 mM NaCl, 2 mM EDTA, 1% SDS) and treated with 0.5 mg/ml proteinase K at 55°C for 5 h. Measures of 1, 0.1, or 0.01 $\mu$g genomic DNA were amplified by a 30-cycle PCR of 94°C for 1 min, 53°C for 2 min, 72°C for 2 min using the upstream primer 5′-GCCTGCATGGGATGGA-3′ and downstream primer 5′-CCACTGCTAGAGATTTTCCAC-3′ (Henderson *et al.,* 1996). PCR products were resolved on a 1% agarose gel.

### Nuclear extract preparations and electromobility shift assays (EMSA)

Nuclear extracts from 2.0 × $10^6$ 293T cells were prepared as described previously (Schreiber *et al.,* 1989) by lysing cells with 10% Nonidet P-40 in buffer A [10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF]. Extracts were recovered in buffer C [20 mM HEPES (pH 7.9), 0.4 M NaCl, 1mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF]. Fifty nanograms of annealed C/EBP-binding site DNA (5′-GATCGCCTAGCATTTCATCACACGT-3′ and 5′-AGCTCCTGGAAAGTCCCCAG CGGAAAGTCCCTT 3′) was end-filled with [$\alpha$-$^{32}$P]-dCTP using bacterial Klenow fragment (Promega). Four micrograms of nuclear extract was incubated in a reaction mixture containing the DNA probe, 3 $\mu$g of poly(dIdC) (Amersham Pharmacia Biotech, Arlington Heights, IL), 0.25 M HEPES (pH 7.5), 0.6 M KCl, 50 mM $MgCl^2$, 1 mM EDTA, 7.5 mM DTT, and 9% glycerol for 20 min at 25°C. Fiftyfold excess of unlabeled C/EBP or NF-$\kappa$B binding site DNA was used as specific and nonspecific competitors, respectively. The samples were run on a 6% polyacrylamide gel and visualized by autoradiography.

### Coimmunoprecipitation and Western blotting

Nuclear extracts from 293T cells transfected with 0 or 1 $\mu$g PCAF and 0 or 5 $\mu$g C/EBP$\beta$ or LIP were immunoprecipitated with 1 $\mu$g rabbit-anti-C/EBP$\beta$ polyclonal antibody and 10 $\mu$L protein A/G-agarose beads (Santa Cruz). Complexes were washed four times with PBS and resuspended in 50 $\mu$L 1× SDS buffer [50 mM Tris–HCl (pH 6.8), 2% SDS, 0.1% bromphenol blue, 10% glycerol, 100 mM DTT]. Ten microliters of each sample was run on a 10% SDS–polyacrylamide gel and transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, NH). Membrane was probed for PCAF using mouse-anti-Flag monoclonal antibody (Sigma) for 1 h at 25°C and horseradish peroxidase conjugated sheep anti-mouse antibody (Chemicon, Temecula, CA) for 1 h at 25°C. For detection of C/EBP proteins, membrane was stripped with 100 mM $\beta$-mercaptoethanol, 62.5 mM Tris–HCl (pH 6.7), 2% SDS for 1 h at 55°C and probed with rabbit anti-C/EBP$\beta$ antibody (Santa Cruz) for 1 h at 25°C and horseradish peroxidase conjugated goat anti-rabbit antibody (Gibco) for 1 h at 25°C. Proteins were detected using ECL Plus Western Blotting Detection system (Amersham Pharmacia Biotech).

### GST pull-down assay

GST and GST-C/EBP$\beta$ fusion products were purified from bacterial extracts by binding to glutathione-Sepharose 4B beads (Pharmacia) as described (Barlev *et al.,* 1995). SAGA complexes were obtained from Dr. J. Workman's laboratory (Penn State) (Grant *et al.,* 1997). Equivalent amounts of SAGA, precleared with glutathione-Sepharose beads, were incubated with immobilized GST fusion proteins for 2 h at 4°C in binding buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 0.1% Tween 20, 0.5 mM DTT, 1 mM PMSF, 2 $\mu$g/ml leupeptin, 2 $\mu$g/ml pepstatin A]. The supernatants were removed and the

beads were washed three times with binding buffer. Input SAGA, supernatants, and beads were directly assayed for HAT activity on histones (Grant *et al.,* 1997). Briefly, 2 μg of core histones was incubated with 10 μl of sample to be tested and $^3$H-labeled acetyl-CoA (0.125 μCi) in HAT buffer [50 mM Tris–HCl (pH 8.0), 50 mM HCl, 5% glycerol, 0.1 mM EDTA, 1 mM DTT, 1 mM PMSF, 10 mM sodium butyrate] at 30°C for 30 min. The reactions were then run on 18% SDS–polyacrylamide gel electrophoresis (SDS–PAGE) (Grant *et al.,* 1997). Gels were Coomassie stained to determine the amounts of histone loaded in each lane and destained before determining acetylation by fluorography using EN$^3$HANCE autoradiography enhancer (NEN, Boston, MA).

### Restriction enzyme accessibility assay and ligation-mediated PCR

U1 and U1-LIP cells were stimulated with phorbol myristate acetate (PMA) for 48 h and HIV-1 transcription was monitored by RT activity in the cell culture supernatant. Cells were harvested and resuspended at $2.5 \times 10^7$ cells/ml in cold buffer A [10 mM Tris–HCl (pH 7.4), 10 mM NaCl, 3 mM $MgCl_2$, and 0.3 M sucrose] for 5 min and then lysed in 0.3% NP-40 for 30 s. Nuclei were collected by centrifugation and resuspended in Buffer B [10 mM Tris–HCl (pH 7.9), 10 mM $MgCl_2$, 50 mM NaCl, 1 mM DTT, 100 μg/ml BSA, and 0.1 mM PMSF]. Nuclei were digested with 400 U of *Hin*dIII, *Pvu*II, *Pst*I, and *Hin*fI (New England Biolabs) for 8–16 h. Genomic DNA was prepared by lysing nuclei with TES buffer [10 mM Tris (pH 8.0), 400 mM NaCl, 2 mM EDTA, and 1% SDS] and treating with 200 μg/ml proteinase K at 55°C for 5 h. DNA was extracted once with 1× volume phenol, followed by a 1:1 phenol:chloroform:isoamyl alcohol extraction, and two extractions with 24:1 chloroform:isoamyl.

Digested DNA fragments were detected using a ligation-mediated PCR (LM-PCR) (Mueller and Wold, 1989). The primer REDA (5′ CTAGCATTTCATCACATGG3′) (0.3 pmol/μl) was used in an initial primer extension reaction with 1–5 μg of digested genomic DNA as a template, 40 mM NaCl, 0.01 M Tris–HCl (pH 8.9), 5 mM $MgSO_4$, 0.01% gelatin, 0.2 mM dNTPs, and 1 unit of Vent DNA polymerase for one cycle of 95°C for 5 min, 49°C for 30 min, and 76°C for 15 min. Annealed linkers were prepared (LM1 primer, 5′ GCTGTGACAAGGAGATCTGAATTC 3′ and LM2 primer, 5′ GAATTCAGATC3′) and 100 pmol was ligated to extension products at 15°C using 1 unit T4 ligase in 3 mM ATP, 10 mM $MgCl_2$, 0.02 M DTT, and 1.25 μg BSA. DNA was precipitated before using in a PCR reaction consisting of 10 pmol REDA-2 primer (5′TGCATCCGGAGTACTTCAAGAAC3′) and LM-1, 0.2 mM dNTPs, 20 mM Tris–HCl (pH 8.9), 40 mM NaCl, 5 mM $MgSO_4$, 0.01% gelatin, and 0.1% Triton X-100, 1 unit Vent DNA polymerase for 15 cycles at 95°C for 1 min, 63°C for 2 min, and 76°C for 4 min. PCR products were detected by primer extension using 5 pmol of an end-labeled primer (REDA-3; 5′ GAGTACTTCAAGAACTGCTGACATCG3′), 0.2 mM dNTPs, 5 units of DNA Vent polymerase, and a five-cycle reaction of 94°C for 40 s and 71°C for 3 min. The reaction was stopped by adding 0.1 M EDTA. Products were precipitated with 0.3 M sodium acetate and 100% ethanol before resolving on an 8% polyacrylamide gel and visualizing by audioradiography.

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## REFERENCES

Akira, S., and Kishimoto, T. (1992). IL-6 and NF-IL6 in acute-phase response and viral infection. *Immunol. Rev.* **127,** 25–50.

Anderson, M., Tjioe, I., Lorincz, M., Parks, D., Herzenberg, L., Nolan, G., and Herzenberg, L. (1996). Simultaneous fluorescence-activated cell sorter analysis of two distinct transcriptional elements within a single cell using engineered green fluorescent proteins. *Proc. Natl. Acad. Sci. USA* **93,** 8508–8511.

Artandi, S., and Calame, K. (1993). Association of DNA-binding transcription activators in solution. *Methods Mol. Genet.* **1,** 267–279.

Barlev, N., Candau, R., Wang, L., Darpino, P., Silverman, N., and Berger, S. (1995). Characterization of physical interactions of the putative transcriptional adaptor, ADA2, with acidic activation domains and TATA-binding proteins. *J. Biol. Chem.* **270,** 19337–19344.

Basta, S., Knoetig, S., Spagnuolo-Weaver, M., Allan, G., and McCullough, K. (1999). Modulation of monocytic cell activity and virus susceptibility during differentiation into macrophages. *J. Immunol.* **162,** 3961–3969.

Benkirane, M., Chun, R., Xiao, H., Ogryzko, V., Howard, B., Nakatani, Y., and Jeang, K.-T. (1998). Activation of integrated provirus requires histone acetyltransferase: P300 and p/CAF are coactivators for HIV-1 Tat. *J. Biol. Chem.* **273,** 24898–24905.

Berger, E. A., Murphy, P. M., and Farber, J. M. (1999). Chemokine receptors as HIV-1 coreceptors: Roles in viral entry, tropism, and disease. *Annu. Rev. Immunol.* **17,** 657–700

Chakravarti, D., Ogryzko, V., Kao, H. Y., Nash, A., Chen, H., Nakatani, Y., and Evans, R. M. (1999). A viral mechanism for inhibition of p300 and PCAF acetyltransferase activity. *Cell* **96,** 393–403.

Chomczynski, P., and Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162,** 156–159, doi:10.1006/abio.1987.9999.

Connor, R., Chen, B., Choe, S., and Landau, N. (1995). Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology* **206,** 936–944, doi:10.1006/viro.1995.1016.

Cooper, C., Henderson, A., Artandi, S., Avitahl, N., and Calame, K. (1995). Ig/EBP (C/EBPγ) is a transdominant negative inhibitor of C/EBP family transcriptional activators. *Nucleic Acids Res.* **23,** 4371–4377.

Descombes, P., and Schibler, U. (1991). A liver-enriched transcriptional activator protein, LAP, and a transcriptional inhibitory protein, LIP, are translated from the same mRNA. *Cell* **67,** 569–579.

El Kharroubi, A., Piras, G., Zensen, R., and Martin, M. A. (1998). Transcriptional activation of the integrated chromatin-associated human immunodeficiency virus type 1 promoter. *Mol. Cell. Biol.* **18,** 2535–2544.

Franchin, G., Zybarth, G., Dai, W., Dubrovsky, L., Reiling, N., Schmidtmayerova, H., Bukrinsky, M., and Sherry, B. (2000). Lipopolysaccharide inhibits HIV-1 infection of monocyte-derived macrophages through direct and sustained down-regulation of CC chemokine receptor 5. *J. Immunol.* **164,** 2592–2601.

Gaynor, R. (1992). Cellular transcription factors involved in the regulation of HIV-1 gene expression. *AIDS* **6,** 347–363.

Grant, P. A., Duggan, L., Cote, J., Roberts, S. M., Brownell, J. E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C. D., Winston, F., Berger, S. L., and Workman, J. L. (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: Characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes Dev.* **11,** 1640–1650.

Griffin, G., Leung, K., Folks, T., Kunkel, S., and Nabel, G. (1989). Activation of HIV gene expression during monocyte differentiation by induction of NF-κB. *Nature* **339,** 70–73.

Hamamori, Y., Sartorelli, V., Ogryzko, V., Puri, P. L., Wu, H. Y., Wang, J. Y., Nakatani, Y., and Kedes, L. (1999). Regulation of histone acetyltransferases p300 and PCAF by the bHLH protein twist and adenoviral oncoprotein E1A. *Cell* **96,** 405–413.

Henderson, A. J., and Calame, K. L. (1997). CCAAT/enhancer binding protein (C/EBP) sites are required for HIV-1 replication in primary macrophages but not in CD4+ T cells. *Proc. Natl. Acad. Sci. USA* **94,** 8714–8719.

Henderson, A. J., Connor, R. I., and Calame, K. L. (1996). C/EBP activators are required for HIV-1 replication and proviral induction in monocytic cell lines. *Immunity* **5,** 91–101.

Henderson, A. J., Zou, X., and Calame, K. L. (1995). C/EBP proteins activate transcription from the human immunodeficiency virus type 1 long terminal repeat in macrophages/monocytes. *J. Virol.* **69,** 5337–5344.

Hohaus, S., Petrovick, M. S., Voso, M. T., Sun, Z., Zhang, D. E., and Tenen, D. G. (1995). Pu.1 (Spi-1) and C/EBP-alpha regulate expression of the granulocyte-macrophage colony-stimulating factor receptor alpha gene. *Mol. Cell. Biol.* **15,** 5830–5845.

Honda, Y., Rogers, L., Nakata, K., Zhao, B., Pine, R., Nakai, Y., Kurosu, K., Rom, W. N., and Weiden, M. (1998). Type I interferon induces inhibitory 16-kD CCAAT/enhancer binding protein (C/EBP), repressing the HIV-1 long terminal repeat in macrophages: Pulmonary tuberculosis alters C/EBP expression, enhancing HIV-1 replication. *J. Exp. Med.* **188,** 1255–1265.

Hottiger, M., Felzien, L., and Nabel, G. (1998). Modulation of cytokine-induced HIV gene expression by competitive binding of transcription factors to the coactivator p300. *EMBO J.* **17,** 3124–3134.

Howe, L., Brown, C. E., Lechner, T., and Workman, J. L. (1999). Histone acetyltransferase complexes and their link to transcription. *Crit. Rev. Eukaryot. Gene Expr.* **3–4,** 231–243.

Jacque, J. M., Fernandez, B., Arenzana-Seisdedos, F., Thomas, D., Baleux, F., Virelizier, J. L., and Bachelerie, F. (1996). Permanent occupancy of the human immunodeficiency virus type 1 enhancer by NF-κB is needed for persistent viral replication in monocytes. *J. Virol.* **70,** 2930–2938.

Kamine, J., Elangovan, B., Subramanian, T., Coleman, D., and Chinnadurai, G. (1996). Identification of a cellular protein that specifically interacts with the essential cysteine region of the HIV-1 Tat transactivator. *Virology* **216,** 357–366, doi:10.1006/viro.1996.0071.

Kiernan, R. E., Vanhulle, C., Schiltz, L., Adam, E., Xiao, H., Maudoux, F., Calomme, C., Burny, A., Nakatani, Y., Jeang, K. T., Benkirane, M., and Van Lint, C. (1999). HIV-1 Tat transcriptional activity is regulated by acetylation. *EMBO J.* **18,** 6106–6118.

Kowenz-Leutz, E., and Leutz, A. (1999). A C/EBP beta isoform recruits the SWI/SNF complex to activate myeloid genes. *Mol. Cell* **4,** 735–743.

Kwok, R. P., Laurance, M. E., Lundblad, J. R., Goldman, P. S., Shih, H., Connor, L. M., Marriott, S. J., and Goodman, R. H. (1996). Control of cAMP-regulated enhancers by the viral transactivator Tax through CREB and the co-activator CBP. *Nature* **380,** 642–646.

Laughlin, M. A., Zeichner, S., Kolson, D., Alwine, J. C., Seshamma, T., Pomerantz, R. J., and Gonzalez-Scarano, F. (1993). Sodium butyrate treatment of cells latently infected with HIV-1 results in the expression of unspliced viral RNA. *Virology* **196,** 496–505, doi:10.1006/viro.1993.1505.

LeClair, K. P., Blanar, M. A., and Sharp, P. A. (1992). The p50 subunit of NF-κB associates with the NF-IL6 transcription factor. *Proc. Natl. Acad. Sci. USA* **89,** 8145–8149.

Lee, E. S., Zhou, H., and Henderson, A. J. (2001). Endothelial cells enhance HIV-1 replication in macrophages through a C/EBP-dependent mechanism. *J. Virol.* **75,** 9703–9712.

Marzio, G., Tyagi, M., Gutierrez, M., and Giacca, M. (1998). HIV-1 Tat transactivator recruits p300 and CREB-binding protein histone acetyltransferase to the viral promoter. *Proc. Natl. Acad. Sci. USA* **95,** 13519–13524.

McCune, J. M. (1995). Viral latency in HIV disease. *Cell* **82,** 183–188.

Mink, S., Haenig, B., and Klempnauer, K. H. (1997). Interaction and functional collaboration of p300 and C/EBPβ. *Mol. Cell. Biol.* **17,** 6609–6617.

Moriuchi, H., Moriuchi, M., and Fauci, A. S. (1998). Differentiation of promonocytic U937 subclones into macrophagelike phenotypes regulates a cellular factor(s) which modulates fusion/entry of macrophage-tropic human immunodeficiency virus type 1. *J. Virol.* **72,** 3394–3400.

Moriuchi, M., Moriuchi, H., Turner, W., and Fauci, A. S. (1997). Cloning and analysis of the promoter region of CXCR4, a coreceptor for HIV-1 entry. *J. Immunol.* **159,** 4322–4329.

Mueller, P. R., and Wold, B. (1989). In vivo footprinting of a muscle specific enhancer by ligation mediated PCR. *Science* **246,** 780–786.

Natsuka, S., Akira, S., Nishio, Y., Hashimoto, S., Sugita, T., Isshiki, H., and Kishimoto, T. (1992). Macrophage differentiation-specific expression of NF-IL6, a transcription factor for interleukin-6. *Blood* **79,** 460–466.

Nerlov, C., and Ziff, E. B. (1995). CCAAT/enhancer binding protein-alpha amino acid motifs with dual TBP and TFIIB binding ability co-operate to activate transcription in both yeast and mammalian cells. *EMBO J.* **14,** 4318–4328.

Oelgeschlager, M., Janknecht, R., Krieg, J., Schreek, S., and Luscher, B. (1996). Interaction of the co-activator CBP with Myb proteins: Effects on Myb-specific transactivation and on the cooperativity with NF-M. *EMBO J.* **15,** 2771–2780.

Ogryzko, V., Kotani, T., Zhang, X., Schiltz, R., Howard, T., Yng, X., Howard, B., Qin, J., and Nakatani, Y. (1998). Histone-like TAFs within the PCAF histone acetylase complex. *Cell* **94,** 35–44.

Pazin, M., Sheridan, P., Cannon, K., Cao, Z., Keck, J., Kadonaga, J., and Jones, K. (1996). NF-κB-mediated chromatin reconfiguration and transcriptional activation of the HIV-1 enhancer in vitro. *Genes Dev.* **10,** 37–49.

Pear, W. S., Nolan, G. P., Scott, M. L., and Baltimore, D. (1993). Production of high-titer helper-free retroviruses by transient transfection. *Proc. Natl. Acad. Sci. USA* **90,** 8392–8396.

Poli, V. (1998). The role of C/EBP isoforms in the control of inflammatory and native immunity functions. *J. Biol. Chem.* **273,** 29279–29282.

Rosati, M., Valentin, A., Patenaude, D., and Pavlakis, G. (2001). CCAAT-enhancer-binding protein β (C/EBPβ) activates CCR5 promoter: Increased C/EBPβ and CCR5 in T lymphocytes from HIV-1 infected individuals. *J. Immunol.* **167,** 1654–1662.

Ross, H., Nonnemacher, M., Hogan, T., Quiterio, S., Henderson, A., McAllister, J., Krebs, F., and Wigdahl, B. (2001). Interaction between CCAAT/enhancer binding protein and cyclic AMP response element binding protein 1 regulates human immunodeficiency virus type 1 transcription in cells of the monocyte/macrophage lineage. *J. Virol.* **75,** 1842–1856.

Schreiber, E., Matthias, P., Muller, M. M., and Schaffner, W. (1989). Rapid

detection of octamer binding proteins with "miniextracts" prepared from a small number of cells. *Nucleic Acids Res.* **17,** 6419.

Sheridan, P., Mayall, T., Verdin, E., and Jones, K. (1997). Histone acetyltransferases regulate HIV-1 enhancer activity in vitro. *Genes Dev.* **11,** 3327–3340.

Sheridan, P., Sheline, C., Cannon, K., Voz, M., Pazin, M., Kadonaga, J., and Jones, K. (1995). Activation of the HIV-1 enhancer by the LEF-1 HMG protein on nucleosome-assembled DNA in vitro. *Genes Dev.* **9,** 2090–2104.

Steger, D., Eberharter, A., John, S., Grant, P., and Workman, J. (1998). Purified histone acetyltransferase complexes stimulate HIV-1 transcription from preassembled nucleosome arrays. *Proc. Natl. Acad. Sci. USA* **95,** 12924–12929.

Steger, D. J., and Workman, J. L. (1997). Stable co-occupancy of transcription factors and histones at the HIV-1 enhancer. *EMBO J.* **16,** 2463–2472.

Stein, B., Cogswell, P. C., and Baldwin, A. S. (1993). Functional and physical associations between NF-κB and C/EBP family members: A Rel domain-bZIP interaction. *Mol. Cell. Biol.* **13,** 3964–3974.

Sterner, D., and Berger, S. (2000). Acetylation of histones and transcription-related factors. *Microbiol. Mol. Biol. Rev.* **64,** 435–459.

Tesmer, V. M., Rajadhyaksha, A., Babin, J., and Bina, M. (1993). NF-IL6-mediated transcriptional activation of the long terminal repeat of the human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **90,** 7298–7302.

Tuttle, D., Harrison, J. K., Anders, C., Sleasman, J., and Goodenow, M. (1998). Expression of CCR5 increases during monocyte differentiation and directly mediates macrophage susceptibility to infection by human immunodeficiency virus type 1. *J. Virol.* **72,** 4962–4969.

Van Lint, C., Emiliani, S., Ott, M., and Verdin, E. (1996). Transcriptional activation and chromatin remodeling of the HIV-1 promoter in response to histone acetylation. *EMBO J.* **15,** 1112–1120.

Vanden Berghe, W., De Bosscher, K., Boone, E., Plaisance, S., and Haegeman, G. (1999). The nuclear factor-κB engages CBP/p300 and histone acetyltransferase activity for transcriptional activation of the interleukin-6 gene promoter. *J. Biol. Chem.* **274,** 32091–32098.

Vassilev, A., Yamauchi, J., Kotani, T., Prives, C., Avantaggiati, M. L., Qin, J., and Nakatani, Y. (1998). The 400 kDa subunit of the PCAF histone acetylase complex belongs to the ATM superfamily. *Mol. Cell* **2,** 869–875.

Verdin, E. (1991). DNase I-hypersensitive sites are associated with both long terminal repeats and with the intragenic enhancer of integrated human immunodeficiency virus type 1. *J. Virol.* **65,** 6790–6799.

Verdin, E., Paras, P., and Van Lint, C. (1993). Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. *EMBO J.* **12,** 3249–3259.

Wallberg, A. E., Neely, K. E., Gustafsson, J. A., Workman, J. L., Wright, A. P., and Grant, P. A. (1999). Histone acetyltransferase complexes can mediate transcriptional activation by the major glucocorticoid receptor activation domain. *Mol. Cell. Biol.* **19,** 5952–5959.

Weissman, J., Brown, J., Howcroft, T., Hwang, J., Chawla, A., Roche, P., Schiltz, L., Nakatani, Y., and Singer, D. (1998). HIV-1 Tat binds $TAF_{II}250$ and represses $TAF_{II}250$-dependent transcription of major histocompatibility class I genes. *Proc. Natl. Acad. Sci. USA* **95,** 11601–11606.

Westendorf, J. J., Yamamoto, C. Y., Lenny, N., Downing, J. R., Selsted, M. E., and Hiebert, S. W. (1998). The t(8;21) fusion product, AML-1-ETO, associates with C/EBP-α, inhibits C/EBP-α-dependent transcription and blocks granulocyte differentiation. *Mol. Cell. Biol.* **18,** 322–333.

White, E., Cipriani, R., Sabbatini, P., and Denton, A. (1991). Adenovirus E1B 19-kilodalton protein overcomes the cytotoxicity of E1A proteins. *J. Virol.* **65,** 2968–2978.

Widak, P., Gaynor, R., and Garrard, W. (1997). In vitro chromatin assembly of the HIV-1 promoter. *J. Biol. Chem.* **272,** 17654–17661.

Yang, X. J., Ogryzko, V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996). A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* **382,** 319–324.

Zhang, D. E., Hetherington, C. J., Meyers, S., Rhoades, K. L., Larson, C. J., Chen, H. M., Hiebert, S. W., and Tenen, D. G. (1996). CCAAT enhancer-binding protein (C/EBP) and AML (CBFα2) synergistically activate the macrophage colony-stimulating factor receptor promoter. *Mol. Cell. Biol.* **16,** 1231–1240.