Retinoic Acid Inhibition of Chromatin Remodeling at the Human Immunodeficiency Virus Type 1 Promoter

UNCOUPLING OF HISTONE ACETYLATION AND CHROMATIN REMODELING*

Received for publication, July 16, 2004, and in revised form, August 4, 2004
Published, JBC Papers in Press, August 6, 2004, DOI 10.1074/jbc.M408069200

Heather L. B. Kiefer‡§§, Timothy M. Hanley‡§§, Jennifer E. Marcell°¶, A. G. Karthik‡, and Gregory A. Viglianti‡**

From the ‡Department of Microbiology, Boston University School of Medicine, Boston, Massachusetts 02118

All-trans retinoic acid (RA) represses HIV-1 transcription and replication in cultured monocyte-derived macrophages. Here we examine the role of histone acetylation and chromatin remodeling in RA-mediated repression. RA pretreatment of latently infected U1 promonocytes inhibits HIV-1 expression in response to the histone deacetylase (HDAC) inhibitor, trichostatin A (TSA). TSA is thought to activate HIV-1 transcription by inducing histone hyperacetylation within a regulatory nucleosome, nuc-1, positioned immediately downstream from the transcription start site. Acetylation of nuc-1 is thought to be a critical step in activation that precedes nuc-1 remodeling and, subsequently, transcriptional initiation. Here we demonstrate that TSA treatment induces H3 and H4 hyperacetylation and nuc-1 remodeling. Although RA pretreatment inhibits nuc-1 remodeling and HIV-1 transcription, it has no effect on histone acetylation. This suggests that acetylation and remodeling are not obligatorily coupled. We also show that growth of U1 cells in retinoid-deficient medium induces nuc-1 remodeling and HIV-1 expression but does not induce histone hyperacetylation. These findings suggest that remodeling, not histone hyperacetylation, is the limiting step in transcriptional activation in these cells. Together, these data suggest that RA signaling maintains the chromatin structure of the HIV-1 promoter in a transcriptionally non-permissive state that may contribute to the establishment of latency in monocyte/macrophages.

Vitamin A and its bioactive metabolites (retinoids) are required for a wide variety of normal cellular processes including, but not limited to, growth and differentiation (1). These metabolites, including all-trans retinoic acid (RA)† and 9-cis retinonic acid, are potent modulators of gene transcription that bind to and activate nuclear receptor transcription factors (2). Two families of retinoid-dependent transcription factors have been identified: the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). These nuclear receptors bind to specific DNA sequences, retinoic acid response elements, as either heterodimers (RAR/RXR) or homodimers (RXR/RXR) to function as transcriptional activators or repressors. When bound by ligand, these receptors associate with co-activator complexes to activate transcription (3). In the absence of ligand, they associate instead with co-repressor complexes to repress transcription (4–6).

Epidemiological and molecular studies indicate that vitamin A can modulate human immunodeficiency virus type-1 (HIV-1) expression and pathobiology. Serum vitamin A levels have been inversely correlated with several properties of HIV-1-induced disease, including the degree of morbidity and mortality (7–16). Moreover, vitamin A supplementation can reduce HIV-1 associated morbidity and mortality in children (12–14, 16–19). We, and others (20–27), have also shown that retinoids can modulate HIV-1 long terminal repeat (LTR)-directed expression in cultured myeloid cells. Importantly, we recently reported (21, 22) that physiological concentrations of RA consistently repress HIV-1 replication in primary monocyte-derived macrophages grown in the presence of concentrations of interleukin (IL)-1β and IL-6 that are expected in vivo, during HIV-1 infection. Based on these results, we hypothesized that retinoids are natural repressors of HIV-1 transcription. However, their mechanism of action is not fully understood. Three lines of evidence indicate that retinoids induce the expression of a cellular factor(s) that restricts HIV-1 transcription in monocyte/macrophages: first, repression is blocked by protein synthesis inhibitors; second, HIV-1 expression is activated when latently infected U1 cells are grown in a synthetic, retinoid-free medium; and, third, a synthetic antagonist of RAR-mediated transcriptional transactivation strongly activates HIV-1 expression in U1 cells (21). Importantly, protein-DNA cross-linking experiments demonstrated that RA induces the binding of four cellular factors to nucleotides −51 through +12 of the core promoter, which includes the cis-acting sequences required for RA-mediated repression (23).

During HIV-1 infection, the provirus is integrated into cellular genomic DNA and packaged into chromatin. Verdin et al. (28, 29) have shown that the 5′-LTR is bound by two positioned nucleosomes, with a third positioned nucleosome located downstream. Nucleosome 0 (nuc-0) is located between nucleotides −415 and −255 (where +1 is the start site of transcription); nuc-1 is located between nucleotides +10 and +155; and nuc-2 is located between nucleotides +265 and +412 (29). Nuc-1 is thought to play a role in the restriction of HIV-1 transcription.
in latently infected cells (29–31). Moreover, it is thought that nuc-1 must be remodeled prior to transcription. This idea is supported by the findings that nuc-1 remodeling occurs rapidly in response to activators including TNF-α and tetradeoxyanopholrphol acetate (TPA) and is insensitive to the transcription inhibitor, α-amanitin (29, 30).

A number of studies have provided evidence that histone and/or transcription factor acetylation is involved in chromatin remodeling and the subsequent activation of HIV-1 transcription. In general, histone acetylation is correlated with transcriptional activation and is thought to both modify chromatin structure to allow greater access of the transcription machinery to promoters, and to provide binding sites for bromodomain-containing regulatory factors that recognize acetylated lysine residues (reviewed in Ref. 32). Van Lint et al. (30) first showed that histone deacetylase inhibitors induce chromatin remodeling and transcription from the HIV-1 promoter in latently infected cells. In agreement, studies of transcription from the HIV-1 promoter using in vitro reconstituted nucleosomes templates demonstrated that acetylation of either histones or another regulatory transcription factor plays a critical role in transcriptional activation (33). More recently, Lusic et al. (34) reported that the histone acetyltrasferases (HATs), CBP, hGCN5, and P/CAF are recruited to the LTR following activation by TPA and/or Tat, and this recruitment is associated with the acetylation of histones H3 and H4.

We recently reported that pretreatment of latently infected U1 cells with RA prevents activation of HIV-1 transcription by the histone deacetylase (HDAC) inhibitor trichostatin A (TSA) (21). Here, we show that TSA-mediated activation of HIV-1 transcription is associated with an increase in nuc-1 remodeling and the hyperacetylation of nuc-1 associated histones H3 and H4. RA pretreatment inhibits nuc-1 remodeling but has no effect on histone acetylation. Moreover, although activation of HIV-1 expression by retinoid depletion is associated with nuc-1 remodeling, there is no concomitant increase in histone acetylation above non-activated baseline levels. This suggests that remodeling, not histone hyperacetylation, is the limiting step in transcriptional activation in these cells. Together, these data indicate that retinoid signaling is required to maintain HIV-1 latency in monocyte/macrophages and acts by preventing nuc-1 remodeling at a step in transcriptional activation downstream from histone acetylation.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Reagents—**U1 cells (35) were grown in RPMI 1640 (Invitrogen Life Technologies) supplemented with 50 units of penicillin/ml, 50 μg of streptomycin/ml, and 10% FBS. For low retinoid experiments, U1 cells were grown in AIM V medium (Invitrogen Life Technologies, Inc.) supplemented with 0.5% FBS.

Stock solutions of all-trans RA were stored at −80°C at 5 × 10⁻² M in dimethyl sulfoxide with 10⁻⁵ M butylated hydroxytoluene added as an antioxidant. All reagents and media used for cell culture contained less than 0.3 endotoxin units/ml.

**p24 Antigen Enzyme-linked Immunosorbent Assays—**Release of HIV-1 into culture supernatants was measured by p24 antigen capture enzyme-linked immunosorbent assay (ELISA) (Beckman Coulter). Results were normalized for cell number.

**Semi-Quantitative RT-PCR—**Total cytoplasmic RNA was extracted from U1 cells using the RNeasy Mini Kit (Qiagen). RNA (100 ng) was analyzed by RT-PCR using the OneStep RT-PCR kit (Qiagen) as described previously (21).

**Analysis of HIV-1 Chromatin Structure—**Restriction enzyme digestion of purified nuclei with EcoR V, Scal, AflII, or Hinfl I was performed as described (29, 30). Purified DNA (30 μg) was digested to completion with PstI. The fragments were separated by electrophoresis on 1.5% or 1.8% agarose gels and analyzed by Southern blot as previously described using a radiolabeled HIV-1 probe including nucleotides +188 to +960 (29, 30). Radiolabeled 100-bp DNA ladder fragments (Invitrogen Life Technologies, Inc.) were used as size markers.

**Ligation-mediated PCR (LM-PCR)—**Purified nuclei were resuspended at 10⁷ nuclei/ml in 10 mM Tris (pH 7.4) 10 mM NaCl, 3 mM MgCl₂, 10 mM CaCl₂, and 0.3 mM sucrose and digested with the indicated concentrations of micrococcal nuclease for 20 min at 37°C. DNA was purified and treated with T4 polynucleotide kinase to phosphorylate the 5’-ends (36). LM-PCR was carried out as described previously (37) using primers specific for the HIV-1 5′-LTR (29). First strand synthesis was performed with primer A (5′-CTCGCTACTGTCGAATTTTGCG-3′). Subsequent double-stranded products were ligated with a linker formed by annealing primers LP1 (5′-GATCTGATCGGTGGAGATCTGAAC-3′) and LP2 (GAATTCGATC-3′). PCR amplification was performed with primers LP1 and B (5′-TACTACGCTACACAGTC-3′) for 18–22 cycles. Amplification products were then labeled for 2 cycles with 32P-labeled primer C (5′-TACGCAGCGGCGCGCTCCGCTTCGTTG-3′). Labeled products were separated on 8% denaturing polyacrylamide gels.

**Chromatin Immunoprecipitation (ChIP) Assay—**Immunoprecipitations for acetyl H3- or acetyl H4-containing chromatin were performed using an antibody against non-acetylated H4 N-terminal tails (Serotec). Cellular lysates were sonicated using a cup horn (550 Sonic Disembrator, Fisher Scientific) either at a power setting of 5, with 25 30-s pulses on ice (this fragmented the chromatin to an average length of 150–350 bp; protocol A) or at a power setting of 3, with four 10-s pulses on ice (this fragmented the chromatin to an average length of 900–1200 bp; protocol B).

Purified DNA samples from both ChIPs and controls were resuspended in distilled H₂O and analyzed by semi-quantitative PCR. PCR reactions contained 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 100 pmol of each primer, 200 μM each dATP, dGTP, dCTP, and dTTP, 5 μCi α³²P-dATP, and 1.25 units of AmpliTaq (Applied Biosystems) for nuc-1 and gng primers or 2.5 units of AmpliTaq Gold for nuc-0 primers in 50-μl total reaction volume. Following an initial denaturation step at 95°C for 15 min, DNAs were amplified for 30 cycles, each consisting of a 30-s denaturing step at 94°C, a 45-s annealing step at 65°C, and a 1-min extension step at 72°C. Samples were electrophoresed on 5% non-denaturing polyacrylamide gels, visualized by autoradiography, and quantified using a PhosphorImager SI (Molecular Dynamics). Control amplifications of a serial dilution of purified U1 genomic DNA were performed with each primer set to ensure that all amplifications were within the linear range of the reaction. To calculate the relative acetylation levels, PhosphorImager data of the amounts of PCR product obtained for immunoprecipitated chromatin samples were normalized against the amounts of PCR product obtained for input DNA. All values represent the average of at least three independent experiments.

**RESULTS**

**All-trans Retinoic Acid Prevents the Remodeling of a Regulatory Nucleosome Positioned at the Start Site of HIV-1 Transcription—**We recently reported that pretreatment of latently infected U1 cells with RA prevents TSA-mediated activation of HIV-1 transcription (21). Because TSA is thought to activate HIV-1 expression by inducing histone hyperacetylation and chromatin remodeling of the LTR (30), we were interested in determining whether RA pretreatment would also repress these steps in transcriptional activation. In agreement with our previous studies, we found that HIV-1 expression in U1 cells was induced 11-fold by TSA, and that RA pretreatment for 48 h prevented this activation (Fig. 1A). We next determined whether RA pretreatment affected the accessibility of the HIV-1 LTR to restriction enzymes that have recognition sites within or between the known positioned nucleosomes of the LTR (29) (Fig. 1B). Nuclei were prepared from U1 cells digested with EcoRV, Scal, AflII, or Hinfl I. DNA was purified from the nuclei and digested to completion with PstI, which has a recognition site downstream of nuc-2 at position +960 (30). The digested products were then analyzed by Southern blot, using a ³²P-labeled probe encompassing nucleotides +188 to +960 (29). We found that the Scal and Hinfl I sites within the internucleosomal regions between either nuc-0 and nuc-1 or nuc-1 and nuc-2 were accessible in nuclei from untreated cells (Fig. 1, C and D). The accessibility of these sites was not affected by a 3-h TSA treatment. However, in agreement with previous reports (30), TSA increased the accessibility of nuc-1
Fig. 1. RA prevents remodeling of a nucleosome positioned at the start site of HIV-1 transcription. A, RA signaling inhibits TSA-mediated activation of HIV-1. U1 cells were grown in the presence or absence of 10^{-6} M RA for 48 h and then treated with 150 nM TSA for 18 h. Cell-free supernatants were collected from the treated cultures and the levels of HIV-1 expression were measured by p24-antigen capture ELISA. The data are the average (± S.E.) of at least three independent experiments. B, diagram shows the positions of nucleosomes bound to the HIV-1 LTR and the location of restriction enzyme recognition sites (29). The location of the DNA probe used for Southern blot analyses of nucleosome remodeling is indicated. C, RA signaling inhibits TSA-mediated remodeling of nuc-1. U1 cells were grown in the presence or absence
DNA to both AflI and Hinf I. Pretreatment of the cells with RA for 48 h prior to TSA treatment not only decreased the accessibility of nuc-1 DNA to AflI or Hinf I in nuclei from untreated cells, but also inhibited by ~2.5-fold the increase in accessibility induced by TSA (Fig. 1, C and D). This RA-mediated decrease in accessibility appears to be specific for nuc-1. RA pretreatment did not inhibit restriction enzyme accessibility to inter-nucleosomal regions or to nuc-0 DNA; instead, it induced a slight increase in accessibility of these regions.

To confirm that RA inhibits nuc-1 remodeling, we examined this region using ligation-mediated PCR amplification of DNA purified from micrococcal nuclease digested nuclei (29) (Fig. 1E). Micrococcal nuclease preferentially cleaves DNA in inter-nucleosomal regions and can therefore be used to map the location of positioned nucleosomes. We found that TSA treatment increased accessibility of the LTR to micrococcal nuclease in a region located immediately downstream from the transcription start site. This region corresponds to the previously reported position of nuc-1 (29). Importantly, TSA treatment did not affect accessibility of the sequences flanking nuc-1. In agreement with our restriction enzyme accessibility studies, RA pretreatment inhibited the TSA-induced increase in nuc-1 DNA accessibility. Together, these results indicate that RA signaling prevents remodeling of nuc-1.

We initially examined the effects of RA pretreatment on nuc-1 remodeling 3 h after TSA treatment. However, it is possible that TSA might induce a transient increase in nuc-1 remodeling in RA-treated cells that was missed in our analysis. We therefore examined the accessibility of nuc-1 DNA to Hinf I over a 24-h period after TSA treatment (Fig. 2, A and B). Increased accessibility was detected within 10 min after TSA treatment and was greatest between 2 and 3 h after treatment. Between 18 and 24 h, accessibility began to decrease, suggesting that a nucleosome structure similar to that observed in non-activated cells was reestablished for the LTR. Importantly, RA pretreatment of the cells inhibited the TSA-induced remodeling of nuc-1 at all time points examined (Fig. 2, A and B), indicating that the repressive effects of RA are robust and long lasting. We also measured HIV-1 mRNA expression and p24 release at various times after TSA treatment. HIV-1 mRNA was detected until ~2 h after TSA treatment indicating that there is no lag between nuc-1 remodeling and transcriptional activation of the LTR (Fig. 2B). A lag was also seen between HIV-1 transcription and virion release from the infected cells; p24 was not detected in the supernatant until 12 h after TSA treatment. As expected, RA pretreatment of the cells strongly inhibited HIV-1 mRNA accumulation and p24 release at all time points examined. These findings are consistent with the hypotheses that nuc-1 remodeling is a limiting step in HIV-1 transcriptional activation and that RA signaling maintains HIV-1 latency in U1 cells by preventing remodeling.

All trans-RA Does Not Affect TSA-induced H3 or H4 Acetylation at nuc-1—Our findings that RA inhibited TSA-induced nuc-1 remodeling and HIV-1 transcriptional activation raised the possibility that RA may prevent histone acetylation within nuc-1. We used a ChIP assay to examine the levels of H3 and H4 acetylation. The antibodies used in these experiments recognize either acetylated histone H3 (lysines 9 and 14) or acetylated histone H4 (lysines 5, 8, 12, and 16). The PCR primers and the sizes of chromatin DNA generated for our ChIP experiments are shown in Table I and Fig. 3.

In order to look specifically at nuc-1, we developed a sonication protocol (protocol A) to generate chromatin fragments that contain, on average, 150–350 bp and that are less than 500 bp (Fig. 3B). These fragments are therefore approximately the size of an average nucleosome including linker DNA. Because nuc-1 is a positioned nucleosome that is separated from neighboring nucleosomes by about 265 bp (5') and 110 bp (3') (28, 29), DNA fragments that contain nuc-1 and are less than 500 base pairs should not contain adjacent nucleosomes. To ensure that we were examining acetylation of nucleosomes bound to the 5'-LTR, we used a nuc-1 reverse primer positioned within the gag leader sequence (Table I and Fig. 3A). Using this sonication protocol, we found that TSA treatment for 20 min increased the acetylation of H3 (2.0-fold) and H4 (5.2-fold) (Fig. 4A). Surprisingly, RA pretreatment, which inhibited both TSA-induced nuc-1 remodeling and HIV-1 DNA accumulation, did not inhibit these increases in histone acetylation (Fig. 4A). To eliminate the possibility that differences in the levels of acetylated histones are masked by nucleosome displacement from the LTR, we examined the levels of non-acetylated H4 by ChIP and found no change within nuc-1 because of either TSA or RA (Fig. 4B). Together, these data suggest that RA does not repress transcription by preventing histone acetylation at nuc-1.

Although RA signaling had no effect on histone acetylation within nuc-1, it was possible that it might affect other nucleosomes within the 5'-LTR. To address this question, we used a sonication protocol (protocol B) that generates chromatin fragments between 500 and 1200 bp (Fig. 3B). Fragments in this size range can contain more than one nucleosome and therefore allowed us to examine changes in histone acetylation over a larger region of the LTR. Using this sonication protocol, we found that a 20-min TSA treatment increased the acetylation of both H3 (3.3-fold) and H4 (11.5-fold) within nuc-1 (Fig. 4C; nuc-1 primers). As with our ChIP analysis specific for nuc-1, RA pretreatment did not prevent TSA-induced H3 and H4 acetylation within the 5'-LTR (Fig. 4C; nuc-1 primers). We also found that the levels of TSA-induced acetylation within a broader 5'-LTR region are greater than the levels seen specifically within nuc-1. This suggests that TSA induces higher levels of acetylation of the histones that are included within nuc-0 and/or nuc-2 than of the histones included within nuc-1. We next performed ChIP analyses using PCR primers to amplify HIV-1 nuc-0 and gag regions (Table I and Fig. 3A); however, it is important to note that the primers used for nuc-0 do not distinguish between the 5'- and 3'-LTRs. TSA treatment increased H3 and H4 acetylation in both the nuc-0 (4.8-fold and 15.1-fold) and the gag regions (4.7 fold and 9.3-fold); moreover, RA did not prevent TSA-induced acetylation in these regions (Fig. 4C). Although these data are consistent with a role of histone acetylation in HIV-1 transcriptional activation, they indicate that remodeling of nuc-1 is not obligatorily coupled to
hyperacetylation. They further indicate that RA signaling represses nuc-1 remodeling and transcriptional activation at a step downstream from acetylation.

Growth of U1 cells in Retinoid-free Medium Activates HIV-1 Expression and nuc-1 Remodeling—We recently reported that the low concentrations of retinoids present in growth medium containing 10% FBS restrict HIV-1 expression under normal cell culture conditions (21). HIV-1 expression is activated either when infected cells are grown in retinoid-free media or when RA signaling is blocked by RAR antagonists. For exam-
When U1 cells are grown for 72 h in a retinoid-free synthetic medium (AIM V) supplemented with 0.5% FBS, HIV-1 expression is activated ~30-fold (Fig. 5A). These findings and our studies described above suggest that RA signaling maintains the chromatin structure of the HIV-1 LTR in a transcriptionally repressive state. To test this hypothesis, we examined nuc-1 remodeling in U1 cells grown for 48 h in AIM V medium containing either 10 or 0.5% FBS. We found that nuc-1 DNA became accessible to Hinf I when the cells were grown under retinoid-depleted conditions (Fig. 5B). In contrast, these retinoid-depleted growth conditions did not change the accessibility of internucleosomal DNA to Hinf I or ScaI (Fig. 5B). We next examined the kinetics of nuc-1 remodeling after transferring U1 cells to retinoid-depleted culture medium. Nuc-1 remodeling was first detected at 18 h and continued to increase over time (Fig. 5, C and D). To confirm that RA could prevent the remodeling of nuc-1, we also examined U1 cells grown in AIM V medium containing 0.5% FBS and supplemented with 10⁻⁶ M RA. As expected, RA treatment inhibited the accessibility of nuc-1 DNA to Hinf I. Similarly, supplementation of AIM V with 10% FBS also prevented nuc-1 remodeling, indicating that the endogenous retinoids present in normal cell culture conditions restrict HIV-1 transcription (Fig. 5, C and D).

**Growth of U1 Cells in Retinoid-free Medium Does Not Increase Histone Acetylation within nuc-1**—We were interested in determining whether nuc-1 remodeling that is induced when cells are grown in retinoid-free medium is, like TSA-induced nuc-1 remodeling, associated with increases in H3 and/or H4 acetylation. Using sonication protocol A in a ChIP analysis, we did not detect any increases in acetylated H3 (1.2-fold) or acetylated H4 (1.2-fold) within nuc-1 above non-activated baseline levels (Fig. 6A). This finding suggests that activation of HIV-1 transcription by retinoid depletion does not require histone hyperacetylation of nuc-1. However, we also examined histone acetylation within the broader LTR using sonication protocol B (Fig. 6B). We found, for nuc-1, that retinoid depletion had no effect on H3 acetylation (1.3-fold) but increased H4 acetylation 2.2-fold. For nuc-0, we found no increase in H3 acetylation (1.3-fold) but a 4.6-fold increase in H4 acetylation. However, we must emphasize again that the PCR primers used to examine nuc-0 do not distinguish between the 5'- and 3'-LTRs. Finally, for gag, we found that retinoid depletion had no effect on H3 acetylation (1.3-fold) but increased H4 acetylation 1.9-fold. Whether these small increases in acetylation are biologically relevant remains to be determined. Importantly, we found that RA treatment had no effect on histone acetylation, except that it decreased the levels of acetylated H4 within nuc-0 ~2-fold (Fig. 6B).

**FIG. 3.** Schematic representation of chromatin immunoprecipitation strategies. A, diagram shows the positions of the primers used in the ChIP analyses and the average sizes of the DNA fragments generated by the two sonication protocols. The antisense nuc-1 primer extends into the gag region ensuring that only the 5'-LTR is amplified by PCR. The fragments highlighted in bold are representative of the sonicated chromatin that can be amplified with these nuc-1 primers. B and C, agarose gel electrophoresis of DNAs purified from nuclei sonicated using either protocol A (B) or protocol B (C).
DISCUSSION

The nucleosomal organization of the HIV-1 LTR is conserved between three chronically infected cell lines, including the monocytic cell line, U1, and the T-cell lines, ACH-2 and 8E5 (28, 29). It is thought that nuc-1, located at the start site of transcription, plays a key role in the repression of HIV-1 transcription in unactivated and latently infected cells (29, 30).

Activation of HIV-1 transcription by TPA or TNF-α is associated with the rapid remodeling of nuc-1, suggesting that remodeling is a prerequisite for, and not a consequence of, transcription. Supporting this idea is the finding that both TSA and TPA induce nuc-1 remodeling in cells in the presence of the transcription inhibitor, α-amanatin (29, 30). Furthermore, HIV-1 transcription from in vitro assembled nucleosomal tem-
plates can be activated by purified yeast HATs (38). In this system, α-amanitin prevented transcription but did not prevent nucleosome remodeling. Here we show that retinoid signaling inhibits both HIV-1 transcription and nuc-1 remodeling. Because nuc-1 remodeling occurs prior to, and is independent of, transcription, it is un-
likely that RA prevents transcription by targeting post-remodeling events such as initiation or elongation. Moreover, RA does not appear to inhibit transcription by targeting histone acetylation since RA treatment did not affect the levels of H3 and H4 acetylation within nuc-1 in either TSA-treated or retinoid-depleted U1 cells. Therefore the prevention of nuc-1 remodeling appears to be uncoupled from histone acetylation. This may be explained if acetylation and remodeling are ordered and discrete steps in the transcriptional activation of the HIV-1 LTR. It appears that the recruitment of HATs to the HIV-1 LTR is an early event in transcriptional activation. Lasic et al. (34) showed that the HATs, CBP, hGCN5 protein, and P/CAF are recruited to the LTR within 1 h of TPA treatment and that this recruitment is associated with an increase in H3 and H4 acetylation. In this system, HAT recruitment and histone acetylation preceded transcription. HATs, including p300/CBP and P/CAF, can also be recruited to the LTR by HIV-1 Tat (39–41). These interactions are most likely biologically important since overexpression of p300 in cells can enhance Tat-mediated transcription (41). In addition, Marzio et al. (41) used ChIP assays to show that Tat can increase the association of p300/CBP with the LTR of integrated proviruses. One consequence of histone acetylation appears to be the recruitment of the ATPase-dependent chromatin remodeling complex, hSWI/SNF, to the LTR. Recently, Henderson et al. (42) showed that BRG-1, an ATPase subunit of hSWI/SNF, is recruited to the 3′-end of nuc-1 through its interactions with ATP-3 and the non-histone chromosomal protein, HMGA1. Importantly, the recruitment of BRG-1 is facilitated by histone acetylation. Together, these studies suggest that during HIV-1 transcriptional activation histone acetylation acts as a signal for the recruitment of chromatin remodeling complexes. This model for HIV-1 transcriptional activation is similar to that worked out for the human interferon-β promoter. Activation of this promoter appears to occur in a series of ordered steps (43). Initially, the hGCN5 complex is recruited to the promoter, leading to histone acetylation and the subsequent recruitment of RNA polymerase II and CBP. The hSWI/SNF chromatin remodeling complex is then recruited through its interactions with CBP and acetylated histones. This recruitment scheme culminates in nucleosome remodeling and transcription initiation.

Despite the connection between histone acetylation and chromatin remodeling, it appears that these steps in transcriptional activation can be uncoupled. Recently, Hsia and Shi (44) reported that HIV-1 transcription from LTR minichromosomes is activated in Xenopus oocytes by either TSA treatment or by ligand-activated thyroid hormone receptor. Importantly, although TSA treatment activated transcription and increased histone acetylation, it did not induce a change in chromatin structure supporting the idea that acetylation and remodeling are not obligatorily coupled. Our results suggest that RA signaling blocks HIV-1 transcription at a step between histone acetylation and chromatin remodeling. We hypothesize that RA is inducing the expression of a cellular factor(s) that interferes with either the recruitment or activity of an HIV-1-associated chromatin remodeling complex. Remodeling complexes can be recruited to promoters by physically interacting either with transcriptional activators (reviewed in Ref. 45) or through interactions between the bromodomains of subunits of the complex and acetylated histones (46). In the case of HIV-1, it appears that hSWI/SNF is recruited through interactions with ATP-3, HMGA1, and, most likely, acetylated histones (42). One model to explain the repression we see is that RA induces the expression of a factor that either displaces ATP-3/HMGA1 from the LTR or interferes with the binding of hSWI/SNF to either these factors or acetylated histones. An alternative model is that an RA-induced factor interferes with the ability of hSWI/SNF to remodel chromatin. For example, the remodeling activity of SWI/SNF can be inhibited by histone H1 bound to the linker DNA between adjacent nucleosomes (47, 48). Whether H1 is bound to the HIV-1 LTR in RA treated cells remains to be determined. SWI/SNF remodeling activity may also be inhibited by factors that regulate the BRG1 and/or hBRM ATPase subunits (reviewed in Ref. 49). Experiments are currently underway to distinguish between these possibilities.

The levels of retinoids in normal cell culture media containing 10% FBS are sufficient to restrict HIV-1 transcription (Fig. 5) (21). However, when cells are grown in either reti-
oid-free media or when retinoid signaling is inhibited by a synthetic pan-RAR antagonist, HIV-1 transcription is activated (Fig. 5) (21). Here we have shown that the transcriptional activation induced by retinoid depletion is associated with nucle-1 remodeling but not with an increase in histone acetylation. This indicates that the level of constitutive acetylation in U1 cells is sufficient to allow the subsequent recruitment of factors required for chromatin remodeling and transcription. This further suggests that remodeling, but not acetylation, is the limiting step in transcriptional activation in these cells. Why then does TSA treatment induce remodeling and transcription in cells grown under standard culture conditions? One possibility is that the low endogenous concentrations of retinoids present in 10% FBS induce the expression of a limiting amount of the repressor factor whose action is overcome by either histone hyperacetylation or the acetylation of a transcriptional activator.

Regardless of the mechanism of repression, we propose that RA signaling induces an intracellular environment that is restrictive for HIV-1 transcription and that contributes to the establishment of latent infections in monocyte/macrophages. Identification of the factors involved will not only provide important insights into the regulation of HIV-1 transcription, but will also help to clarify the potential therapeutic roles of retinoid agonists and antagonists in the treatment of AIDS.

Acknowledgments—We thank Lynn Denekamp, Barbara Nikolajczyk, and Vikram Suri for helpful discussions and for critical comments on the manuscript. We also thank Thomas Folks and the NIH AIDS Research and Reference Reagent Program for providing the U1 cell line.

REFERENCES

1. Linney, E. (1992) Curr. Top. Dev. Biol. 27, 309–350
2. Stunnenberg, H. G. (1993) Bioessays 15, 309–315
3. Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Chen, J. D., and Evans, R. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 6550–6554
4. Pfeifer, G. P., and Riggs, A. D. (1991) J. Biol. Chem. 266, 17905–17908
5. Folks, T. M., Justement, J., Kinter, A., Dinarello, C. A., and Fauci, A. S. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6790–6794
6. Fawzi, W. W., Mbise, R. L., Hertzmark, E., Fataki, M., Ndossi, G., and Spiegelman, D. (1999) Pediatr. Infect. Dis. J. 18, 127–133
7. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Glass, C. K., and Rosenfeld, M. G. (1995) EMBO J. 14, 2535–2544
8. Neely, K. E., and Workman, J. L. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 13519–13524
9. Carrozza, M. J., Coniglio, S. J., Talmage, D. A., and Viglianti, G. A. (1998) J. Virol. 72, 5862–5869
10. Karter, D. L., Karter, A. J., Yarrish, R., Patterson, C., Kass, P. H., Nord, J., and Mullen, T. M., Glass, C. K., and Rosenfeld, M. G. (1998) Mol. Cell. Biol. 18, 2535–2544
11. Basu, S., LaBella, G. A., and Hoover, D. R. (1994) Lancet 343, 1593–1597
12. Benkirane, M., Chun, R. F., Ogryzko, V. V., Nakatani, Y., and Howard, B. H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 3699–3703
13. Fawzi, W. W., Mbise, R. L., Hertzmark, E., Fataki, M., Ndossi, G., and Spiegelman, D. (1999) Pediatr. Infect. Dis. J. 18, 127–133
14. Fawzi, W. W., Mbise, R., Spiegelman, D., Fataki, M., Hertzmark, E., and Ndossi, G. (2000) J. Pediatr. 137, 660–667
15. Melkian, G., Mmire, F., Ndguwa, C., Perry, R., Jackson, J. E., Garrett, E., Tielensch, J., and Sembra, R. D. (2001) J. Nutrition 131, 567–572
16. Fawzi, W. W., Chalmers, T. C., Herrera, M. G., and Mosteller, F. (1993) J. Am. Med. Assoc. 269, 886–893
17. Fawzi, W. W., Chalmers, T. C., Herrera, M. G., and Mosteller, F. (1993) J. Am. Med. Assoc. 269, 886–893
18. Villamor, E., Mbise, R., Spiegelman, D., Hertzmark, E., Fataki, M., Peterson, K. E., Ndossi, G., and Fawzi, W. W. (2002) Pediatrics 109, 86
19. Villamor, E., and Fawzi, W. W. (2000) J. Infect. Dis. 182, Suppl. 1, S122–S133
20. Poli, G., Kinter, A. L., Justement, J. S., Breslax, P., Kehrl, J. H., and Fauci, A. S. (1992) Proc. Natl. Acad. Sci. U. S. A. 91, 2689–2693
21. Mullen, T. M., Glass, C. K., and Rosenfeld, M. G. (1998) J. Virol. 72, 5862–5869
22. Karter, D. L., Karter, A. J., Yarrish, R., Patterson, C., Kass, P. H., Nord, J., and Mullen, T. M., Glass, C. K., and Rosenfeld, M. G. (1998) Mol. Cell. Biol. 18, 2535–2544
23. Sheridan, P. L., Mayall, T. P., Verdin, E., and Jones, K. A. (1997) Genes Dev. 11, 3527–3540
24. Maciaszek, J. W., Coniglio, S. J., Talmage, D. A., and Viglianti, G. A. (1998) J. Virol. 72, 6598–6604
25. Maciaszek, J. W., Coniglio, S. J., Talmage, D. A., and Viglianti, G. A. (1998) J. Virol. 72, 6598–6604