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Trans-activation of the human immunodeficiency virus long terminal repeat sequences, expressed in an adenovirus vector, by the adenovirus E1A 13S protein

(acquired immune deficiency syndrome virus/gene expression/latency)

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ABSTRACT **The human immunodeficiency virus 1 (HIV-1) long terminal repeat (LTR) sequences were inserted into adenovirus in place of the E1 region. The HIV-1 LTR contained in this recombinant adenovirus responds to trans-activation by *tatIII* in a HeLa cell line constitutively expressing that HIV-1 gene product. In addition, the HIV-1 LTR is activated by the adenovirus E1A 13S, but not 12S or 9S, gene product when it is supplied in trans by a coinfecting wild-type adenovirus. The Rous sarcoma virus LTR, in a similar recombinant adenovirus, is insensitive to *tatIII* but is also trans-activated by the E1A 13S protein. The action of the 13S E1A and *tatIII* proteins are additive for the HIV-1 LTR in the context of adenovirus and they appear to act at the transcriptional level. As in HeLa cells, the adenovirus-borne HIV-1 LTR is inactive in the absence of a trans-activator in H9 and Jurkat cells, two human leukemic T-cell lines. This suggests that recombinant adenoviruses have diagnostic potential for the detection of trans-activators of the HIV-1 LTR that are present in circulating human lymphocytes.**

Transcription of human immunodeficiency virus 1 (HIV-1), the causative agent of acquired immune deficiency syndrome (AIDS) (1, 2), is directed by the HIV-1 long terminal repeat (LTR) sequences. The HIV-1 LTR is composed of several regulatory elements: a negative regulatory element (located between nucleotides −340 and −185, relative to the cap site), a core enhancer (−105 to −80), three SP1 binding sites (−76 to −48), a "TATA box" (−26 to −22), and an element termed TAR (−17 to +54) (3–8). The TAR element confers responsiveness to *tatIII*, a HIV-1-encoded trans-acting protein. The mechanism of action of *tatIII* is unclear; experiments involving transfection of plasmid DNAs have suggested transcriptional regulation (7, 9, 10), posttranscriptional regulation (11), or a combination of the two (12–15). The course of AIDS, from initial infection with HIV-1 to manifestation of clinical symptoms, may be influenced by superinfection with other viruses capable of activating latent HIV-1. Several DNA viruses, most notably herpes simplex virus 1, have been shown to stimulate HIV-1 LTR-directed gene expression (16–18). The herpes gene products involved in this trans-activation have been identified as ICP0 and ICP4 (19, 20).

To study regulation of HIV-1 LTR-directed gene expression, we have inserted the HIV-1 LTR, fused to the chloramphenicol acetyltransferase (CAT) gene, into adenovirus (Ad) in place of the E1 region. This recombinant Ad, HIV-1CAT-Ad, contains nucleotides −453 to +80 from the HIV-1 LTR. We also constructed a similar recombinant adenovirus, RSVCAT-Ad, containing the Rous sarcoma virus (RSV) LTR fused to the CAT gene. These two viruses allow efficient introduction of the retroviral promoters into cells containing various trans-acting control factors.

Transcription of wild-type Ad is under strict temporal regulation during the infectious cycle. The *E1A* gene is the first transcription unit expressed after infection and produces, by differential splicing, two major mRNAs whose sizes are 12S and 13S. They encode polypeptides that differ only by an additional internal 46 amino acids contained in the larger protein (21–24). Transcriptional activation of all other early Ad promoters is due primarily to the action of the 13S E1A protein (25). The 13S protein also activates some resident cellular promoters, notably the major heat shock protein hsp 70 and β-tubulin genes, as well as a wide variety of both RNA polymerase II and polymerase III promoters introduced into cells by transfection of plasmid DNAs or by infection with recombinant Ad (reviewed in ref. 26). The E1A proteins, particularly the 12S protein, are also able to repress enhancer-mediated expression of some viral and cellular genes (27, 28).

The recombinant viruses HIV-1CAT-Ad and RSVCAT-Ad do not contain the Ad E1 region and, therefore, do not produce any of the multiple protein products of the *E1A* and *E1B* genes, including the 13S E1A protein. Consequently, expression of Ad genes is extremely inefficient in HIV-1CAT-Ad and RSVCAT-Ad infections. We have found that low levels of CAT activity are expressed in HeLa cells infected with HIV-1CAT-Ad. In contrast, several hundred-fold higher levels of CAT expression occur after infection in a HeLa cell line, called HeLa/*tatIII* (11), which constitutively expresses the tatIII protein. RSVCAT-Ad expresses equivalent levels of CAT activity in HeLa and HeLa/*tatIII* cells. Thus, the HIV-1 LTR inserted in Ad responds specifically to trans-activation by *tatIII*. This trans-activation was investigated in detail in a separate report, (29) and was found to occur largely, if not entirely, by a transcriptional control mechanism. We present here further analysis of the trans-activation of the HIV-1 LTR contained in Ad. We show that the Ad 13S E1A gene product can activate the HIV-1 LTR, and we extend our studies to human T cells.

MATERIALS AND METHODS

Cell Lines. HeLa and HeLa/*tatIII* cells (11), obtained from C. Rosen and W. Haseltine (Harvard Medical School), were grown as monolayer cultures in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, streptomycin (100 μg/ml), and penicillin (100 μg/ml). 293 cells (30) were grown as monolayer cultures in DMEM supplemented with 10% calf serum, streptomycin, and pen-

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Abbreviations: HIV, human immunodeficiency virus; LTR, long terminal repeat; CAT, chloramphenicol acetyltransferase; RSV, Rous sarcoma virus; Ad, adenovirus(es); moi, multiplicity of infection; pfu, plaque-forming unit(s); araC, cytosine arabinonucleoside.

icillin. H9 and Jurkat cells, generously provided by R. Franza (Cold Spring Harbor Laboratory), were grown in RPMI medium with 20% fetal calf serum.

Construction of Recombinant Ad. To construct RSVCAT-Ad, the *Nde* I/*Bam*HI fragment, containing the RSVCAT sequences, was removed from plasmid pRSVCAT (31) and inserted into Ad by *in vivo* recombination in 293 cells by the method of Gluzman *et al.* (32). HIV-1CAT-Ad was constructed identically using the *Xho* I/*Bam*HI fragment from pU3RIII (33). Recombinant viruses were propagated and titered in 293 cells. Their genome structures are shown in Fig. 1.

Conditions of Infection and CAT assays. Infections in 293 cells were carried out at a multiplicity of infection (moi) of 10 plaque forming units (pfu) per cell. Infections and coinfections in HeLa, HeLa/*tatIII*, H9, and Jurkat cells were carried out at a moi of 50 pfu per cell for each virus. To inhibit DNA synthesis, some infections were done in the presence of cytosine arabinoside (araC) (20 μg/ml), added 4 hr postinfection; at 12-hr intervals thereafter, the medium was removed and replaced with fresh medium with or without the drug. CAT assays were performed by the standard procedure (31). After thin-layer chromatography, CAT assays were quantitated with an Ambis Beta Scan System (Automated Microbiology Systems, San Diego, CA). CAT activity is presented as relative units, with 1 unit defined as 1% conversion of chloramphenicol substrate to its acetylated forms by an extract from 5×10^5 cells during a 60-min incubation. Extracts with high CAT activity were diluted and/or incubated for short periods so that <50% of the chloramphenicol substrate was acetylated in the assay. All experiments were repeated a number of times; the data presented are from a single experiment.

Plasmid Transfections. HeLa cells were transfected by the calcium phosphate precipitation method. Cell extracts were prepared 48 hr after transfection, and assays were performed as described above. Units of activity in plasmid transfections are also as defined above.

RESULTS

Expression of CAT Activity from Recombinant Viruses in 293 Cells. The HIV-1CAT-Ad and RSVCAT-Ad viruses contain the respective retroviral LTR, fused to the CAT gene, inserted in place of the adenovirus E1 region (Fig. 1). These viruses are propagated in human 293 cells, which supply the *E1* gene products in trans. To monitor CAT expression by HIV-1CAT-Ad and RSVCAT-Ad in 293 cells, cells were infected with each recombinant Ad, and CAT expression was measured (Fig. 2). Only low levels of CAT activity (<10 units) were expressed by both viruses up to 8 hr postinfection. However, from 12 to 36 hr postinfection, CAT activity accumulated exponentially for both viruses, to a level of 150,000 units for RSVCAT-Ad and 16,000 units for HIV-1CAT-Ad. When a higher moi was used for RSVCAT-Ad (moi, 100), CAT expression was 14, 5, and 10 units of activity at 2, 4, and 8 hr postinfection, respectively, and it increased to 2000 units at 12 hr postinfection and to 41,000 units at 24 hr postinfection. Similarly, when a higher moi was used for HIV-1CAT (moi, 1000), CAT expression was <10 units of activity through 8 hr postinfection and increased exponentially thereafter. Both recombinant Ad expressed much higher levels of CAT activity at late times after infection of 293 cells than at comparable times after infection of HeLa cells (see below). This may, in part, reflect the fact that Ad infection proceeds more rapidly in 293 cells; higher levels of expression in HeLa cells might have been observed if later time points had been analyzed.

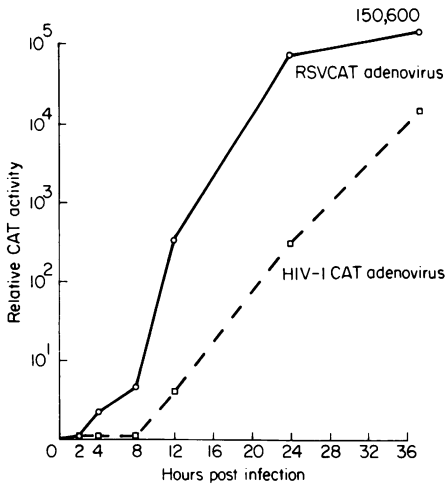

FIG. 2. Expression of CAT activity in 293 cells. Cells were infected at a moi of 10 pfu per cell with RSVCAT-Ad (solid line) or HIV-1CAT-Ad (dashed line), and cell extracts were prepared and assayed for CAT activity at the indicated time postinfection.

Since these recombinant Ad replicate in 293 cells, CAT expression could be affected by increased template concentrations. To monitor effects of DNA replication, infections with HIV-1CAT-Ad were also carried out in the presence of araC to prevent viral DNA replication. High levels of CAT activity (>10,000 units) were still observed when DNA replication was blocked but were slightly reduced relative to levels seen in the absence of the drug (data not shown). Control experiments in HeLa cells show that araC does not

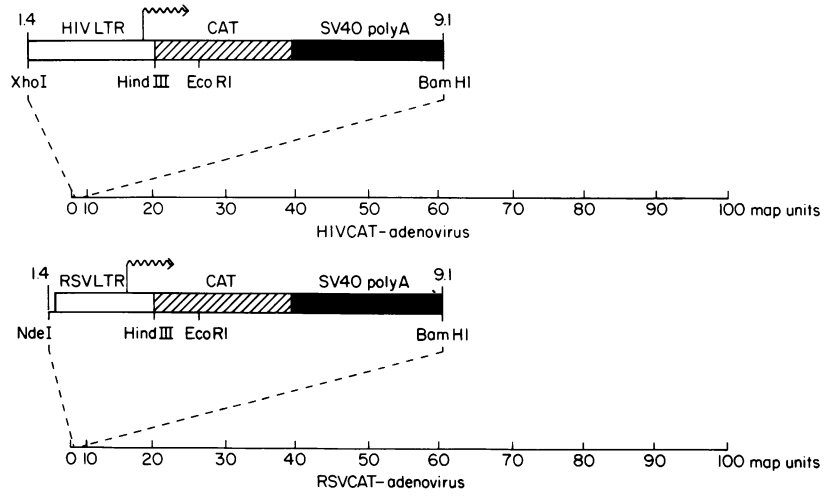

FIG. 1. Structure of recombinant Ad. Shown are HIV-1CAT-Ad and RSVCAT-Ad. These recombinant Ad contain a deletion of Ad sequences from 1.4 to 9.1 map units of wild-type Ad5 (*Pvu* II/*Bgl* II fragment, leaving 453 nucleotides of Ad left-flanking sequences; see ref. 32), comprising all of the coding region for the *E1A* genes and all but the last 60 amino acids of the *E1B* genes. The site of initiation and direction of transcription from the HIV-1 and RSV LTRs are indicated by arrows. Also shown are the RSV and HIV-1 LTRs (open box), CAT coding sequence (hatched box), and simian virus 40 (SV40) polyadenylylation sequences (solid box).

inhibit CAT expression (see below). This indicates that template concentration does affect CAT expression from the recombinant Ad. Nevertheless, the high level of expression of HIV-1CAT-Ad in the absence of *tatIII* suggests that 293 cells contain an activator of the HIV-1 LTR.

Expression of CAT Activity in HeLa and HeLa/*tatIII* Cells Coinfected with Recombinant Ad and Wild-Type Ad. To explore interactions between Ad gene product(s) and the HIV-1 LTR, coinfection experiments were performed in HeLa and HeLa/*tatIII* cells with wild-type Ad and HIV-1CAT-Ad and RSVCAT-Ad. Wild-type Ad supplies the *E1A* and *E1B* gene products in trans, allowing expression of Ad genes and replication of HIV-1CAT-Ad and RSVCAT-Ad in the coinfection. To monitor effects of viral DNA replication during the coinfection, infections were carried out in the presence or absence of araC (Tables 1 and 2). Little CAT expression was detected after HIV-1CAT-Ad infection of HeLa cells, and the presence of araC during single infection did not affect CAT expression (Table 1). In contrast, several hundred units of CAT activity were detected in HeLa/*tatIII* cells after infection with HIV-1CAT-Ad. Thus, the HIV-1 LTR contained in an Ad vector responds to trans-activation by *tatIII*.

When wild-type Ad was coinfected with HIV-1CAT-Ad in HeLa cells, CAT expression was detected, indicating activation of the HIV-1 LTR (Table 1). This activation did not require DNA replication, as it occurred in coinfection in the presence of araC, which prevents viral DNA replication and the expression of late viral gene products. When HeLa cells were coinfected with HIV-1CAT-Ad and Ad mutant dl312, deleted for the E1 region, no trans-activation of the HIV-1 LTR was seen (M. Laspia and A.P.R., unpublished results). It can be concluded that expression of an Ad early gene product acts to trans-activate the HIV-1 LTR. In coinfection of HeLa/*tatIII* cells, CAT activity was increased ≈10-fold, indicating "super"-trans-activation of the HIV-1 LTR. This activation also did not require DNA synthesis. The mechanisms of trans-activation by *tatIII* and coinfection by Ad were found to involve transcriptional regulation (29).

The RSV LTR is active during RSVCAT-Ad infection in both HeLa and HeLa/*tatIII* cells (Table 2), demonstrating the specificity of trans-activation by *tatIII* for HIV-1CAT-Ad. In the experiment shown in Table 2, CAT activity at 36 hr postinfection of RSVCAT-Ad alone in HeLa/*tatIII* cells was 1/10th that in HeLa cells. However, this low level in HeLa/*tatIII* cells was not reproducible, as in four independent experiments there was a negligible difference in expression between the two cell lines. Coinfection with wild-type Ad stimulated expression of the RSV LTR, ≈100-fold at 36 hr postinfection, in both cell lines. As with HIV-1CAT-Ad, this activation did not require viral DNA synthesis. These data show that expression of an Ad gene product acts to trans-activate these retroviral LTRs contained in vectors. AraC generally stimulated CAT expression at late times (36 hr postinfection) for HIV-1CAT-Ad and RSVCAT-Ad (Tables 1 and 2). This may be due to increased levels of Ad E1A proteins in cells treated with the drug (34) and could more than compensate for reduced template levels.

Trans-Activation of the HIV-1 LTR Requires Expression of the 13S E1A Protein. Coinfections, in the presence of araC to prevent DNA replication, were carried out with HIV-1CAT-Ad and Adenovirus mutants (35) capable of expressing only individual 13S, 12S, or 9S E1A proteins (36) (Table 3). Clearly, expression of the 13S protein is required for trans-activation in HeLa cells and augments trans-activation in HeLa/*tatIII* cells. Neither the 12S nor the 9S protein is sufficient for trans-activation.

In HeLa cells coinfected with this 13S virus, CAT expression was generally stimulated to 10-fold higher levels than that obtained by coinfection with wild-type virus. This difference could be attributed to repression by the 12S protein produced by wild-type virus but absent in the 13S virus. The difference was much less (a factor of <2) in HeLa/*tatIII* cells where the activity of the *tatIII* protein might obscure repression by the 12S protein.

We also used cotransfection of plasmid DNAs to determine whether the HIV-1 LTR responded to trans-activation by a plasmid-borne E1A 13S protein. A plasmid expressing CAT under control of the HIV-1 LTR was cotransfected into HeLa cells with plasmids expressing *tatIII*, E1A 13S, or E1A 12S

Table 1. HIV-1CAT-Ad coinfections in the presence of araC

Time post-infection, hr	Relative CAT activity			
	HIV-1-CAT-Ad	HIV-1-CAT-Ad + araC	HIV-1-CAT-Ad + wt Ad2	HIV-1CAT-Ad + wt Ad2 + araC
	HeLa cells			
12	<1	<1	<1	<1
24	<1	<1	7	5
36	<1	<1	12	96
	HeLa/*tatIII* cells			
12	29	44	<1	41
24	67	90	3360	720
36	202	341	2430	4860

Cells were infected as indicated. All viruses were used at a moi of 50 pfu per cell, and araC was used at 20 μg/ml. Extracts were prepared at 12, 24, and 36 hr postinfection and assayed for CAT activity. After thin-layer chromatography, relative CAT activity was quantitated with an Ambis Beta Scan System. wt, Wild type.

Table 2. RSVCAT-Ad coinfections in the presence of araC

Time post-infection, hr	Relative CAT activity			
	RSVCAT-Ad	RSVCAT-Ad + araC	RSVCAT-Ad + wt Ad2	RSVCAT-Ad + wt Ad2 + araC
	HeLa cells			
12	2	4	1,640	136
24	11	14	6,900	3,030
36	264	336	21,600	27,900
	HeLa/*tatIII* cells			
12	3	2	840	80
24	9	9	15,300	1,660
36	12	19	17,100	4,500

Cells were infected as indicated. All viruses were used at a moi of 50 pfu per cell. Extracts were prepared at 12, 24, and 36 hr postinfection and assayed for CAT activity. After thin-layer chromatography, relative CAT activity was quantitated with an Ambis Beta Scan System. wt, Wild type.

Table 3. HIV-1CAT-Ad coinfections

Time post-infection, hr	Relative CAT activity				
	HIV-1-CAT-Ad	HIV-1-CAT-Ad + wt Ad2	HIV-1-CAT-Ad + 13S Ad	HIV-1-CAT-Ad + 12S Ad	HIV-1-CAT-Ad + 9S Ad
	HeLa cells				
12	<1	<1	<1	<1	<1
24	<1	15	33	1	1
36	2	18	194	2	5
	HeLa/*tatIII* cells				
12	8	16	10	5	9
24	36	510	750	23	37
36	525	2160	3030	114	129

Cells were infected as indicated. All viruses were used at a moi of 50 pfu per cell. Extracts were prepared at 12, 24, and 36 hr postinfection and assayed for CAT activity. After thin-layer chromatography, relative CAT activity was quantitated with an Ambis Beta Scan System.

proteins (Table 4). As in coinfection experiments, cotransfection of plasmid DNAs showed that the HIV-1 LTR was trans-activated by the E1A 13S, but not 12S, protein. The magnitude of trans-activation by the 13S E1A protein was considerably higher in coinfection of virus than in cotransfection of plasmids (100-fold vs. 4-fold; Table 3), perhaps reflecting a more efficient delivery of both the HIV-1 LTR and 13S *E1A* gene into cells.

Trans-Activation May Require a cis-Modification of the HIV-1 LTR. The kinetics of CAT expression in coinfections of HeLa cells with HIV-1CAT-Ad and wild-type or 13S Ad, or in single infection of 293 cells, are noteworthy. There is little expression up to 12 hr postinfection, and then it increases exponentially to 36 hr postinfection (Tables 1–3; Fig. 2). Because this activation occurs in the presence of araC, we infer that expression of an early Ad gene product is involved in transcriptional activation of the HIV-1 LTR, either directly or indirectly through a second viral or cellular trans-acting factor, and that it may require 12 hr for the appearance or activation of this factor(s).

We carried out a superinfection experiment, similar to those of Thomas and Mathews (37) and Gaynor and Berk (38), to address this possibility. The superinfection was carried out in the presence of araC to prevent possible complications due to viral DNA synthesis. HeLa and HeLa/*tatIII* cells were first infected with wild-type Ad for 14 hr and then superinfected with HIV-1CAT-Ad. CAT activity was assayed in cell extracts prepared throughout superinfection (Table 5). If the lag in expression of the HIV-1 LTR in coinfection is due to the time required for the appearance of an adenovirus-induced transcription factor, then in the superinfection experiment the LTR should be expressed with a greatly decreased lag. It is clear from Table 5 that expression of the HIV-1 LTR still undergoes a long lag period, although preinfected with wild-type Ad for 14 hr. We conclude that the 12-hr lag before expression cannot be simply explained by the time required for the build up of a trans-acting factor.

Although CAT mRNA levels and transcription rates of the HIV-1 LTR promoted CAT gene were not measured directly in the superinfection experiment shown in Table 5, CAT assays appear to be a valid measure of relative transcription rates in the Ad vector system (29). We feel that the most likely explanation for the result shown in Table 5 is that the HIV-1 LTR must undergo some cis modification—for instance, assembly of a transcription complex and this requires ≈12 hr—before it can be expressed from the Ad chromosome. Similar data and conclusions were reached by Gaynor and Berk in a study, using superinfection experiments, that analyzed transcription of Ad early region 3 (38).

Expression of HIV-1CAT-Ad and RSVCAT-Ad in Human T-Cell Lines. Expression of HIV-1CAT-Ad and RSVCAT-Ad was monitored in two human T-cell leukemic lines, H9 and Jurkat. After single or coinfection of both cell lines, extracts were prepared 48 hr postinfection and assayed for CAT activity (Fig. 3). HIV-1CAT-Ad expressed no detectable CAT activity in single infection in either H9 or Jurkat cells, a result similar to that obtained in HeLa cells. However, coinfection with wild-type Ad activated the HIV-1 LTR, again as in HeLa cells. RSVCAT-Ad expressed CAT activity when infected alone in both H9 and Jurkat cells, as in HeLa and HeLa/*tatIII* cells, and coinfection with wild-type Ad also stimulated the RSV LTR in H9 and Jurkat cells. Thus, it seems likely that in lymphoid cells trans-activators that are not normally present are required for HIV-1, but not for RSV LTR expression.

Table 4. Plasmid cotransfections in HeLa cells

Relative CAT activity			
HIV LTR	*tatIII*	E1A 13S	E1A 12S
4	>70	16	2

HeLa cells were transfected with a plasmid expressing CAT under control of the HIV-1 LTR (pU3RIII; ref. 37) along with plasmids expressing *tatIII* under control of the HIV-1 LTR (ref. 37), E1A 13S protein, or E1A 12S protein (both under control of the metallothionein-1 promoter; ref. 36). Cell extracts were prepared 48 hr after transfections and assayed for CAT activity.

Table 5. HIV-1CAT-Ad superinfection

Time postinfection, hr	Relative CAT activity		
	HIV-1CAT-Ad	Coinfection with wt Ad2	Preinfection with wt Ad2
	HeLa cells		
2	<1	<1	<1
4	<1	<1	<1
7	<1	<1	<1
12	<1	<1	<1
27	2	22	300
31	3	810	610
	HeLa/*tatIII* cells		
2	<1	<1	<1
4	<1	<1	<1
7	<1	<1	<1
12	42	3	7
27	466	1260	3930
31	260	1980	4950

Cells were infected with HIV-1CAT-Ad alone, coinfected with HIV-1CAT-Ad and wild-type (wt) Ad2, or preinfected with wt Ad2 for 14 hr prior to superinfection with HIV-1CAT-Ad. Infections were carried out in the presence of araC, and all viruses were used at a moi of 50 pfu per cell. Cell extracts were prepared 2, 4, 7, 12, 27, and 31 hr postinfection, and relative CAT activity was determined as described in Tables 1–4.

DISCUSSION

We have used a recombinant Ad containing the HIV-1 LTR to study regulation of HIV-1 promoter activity by homologous and heterologous trans-activators—namely, the HIV-1

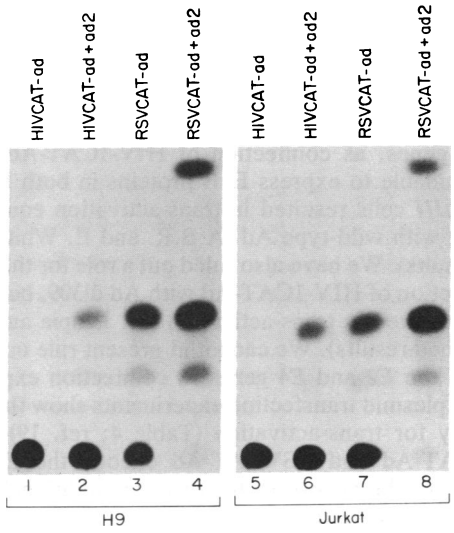

FIG. 3. Expression of CAT activity in human T-cell lines. H9 and Jurkat cells were infected with HIV-1CAT-Ad (lanes 1 and 5), HIV-1CAT-Ad and wild-type Ad (ad2) (lanes 2 and 6), RSVCAT-Ad (lanes 3 and 7), or RSVCAT-Ad and wild-type Ad (lanes 4 and 8), and 48 hr postinfection cell extracts were prepared and assayed for CAT activity. All viruses were used at a moi of 50 pfu per cell. Levels of CAT activity expressed were: 1, 5, 42, >90, <1, 9, 28, and >90 units (lanes 1–8, respectively).

tatIII protein and Ad E1A proteins. In this system, the HIV-1 LTR contained in Ad is trans-activated by *tatIII* largely, if not entirely, at the transcriptional level (29). Transcriptional regulation of the HIV-1 LTR by *tatIII* has also been observed in plasmid transfection experiments in COS cells (15) and is reported to operate through suppression of a termination of transcription that normally occurs ≈55 nucleotides 3′ to the cap site, rather than by increasing the rate of initiation of transcription from the HIV-1 LTR (10).

The HIV-1 LTR contained in Ad is inactive in HeLa, H9, and Jurkat cells in the absence of either the tatIII or Ad 13S E1A proteins (Table 1; Fig. 3). In HeLa cells, these trans-activators can act separately or additively to stimulate HIV-1 LTR-directed transcription. While the action of *tatIII* is specific to the HIV-1 LTR, the 13S E1A protein also trans-activates the RSV LTR contained in Ad. A wide variety of both RNA polymerase II and III genes introduced into cells as plasmid DNAs or recombinant Ad is activated by the 13S protein, but in no case is the mechanism of action of the 13S protein clear. No DNA consensus sequence involved in 13S activity has been identified, and the 13S E1A protein is not thought to interact directly with DNA (39). Rather, the 13S protein may stimulate the activity of cellular transcription factors, and the precise nature of the interaction between E1A and such factors may be promoter specific (40, 41).

When the 13S protein, supplied by a coinfecting wild-type Ad, activates the HIV-1 LTR contained in the recombinant Ad, there is an ≈12-hr lag period prior to expression. This lag cannot be reduced by 14-hr preinfection with wild-type virus prior to infection with the recombinant Ad (Table 5). The lag in expression is also observed in 293 cells, which constitutively express the 13S protein. These results support the model that the 13S protein acts to facilitate a cis modification of the HIV-1 LTR, such as assembly of a transcription complex, prior to transcription. Interestingly, the HIV-1 LTR contained in Ad, when trans-activated by *tatIII*, also undergoes a similar 12-hr lag prior to expression (Tables 1 and 3).

Expression of the 13S E1A protein is required for Ad-mediated trans-activation of the HIV-1 and RSV LTRs contained in Ad; the 12S and 9S proteins do not suffice. This activation does not involve expression of Ad late genes (Tables 1–3), but since expression of the 13S protein activates all the other early Ad promoters, additional early gene products may be involved. We have ruled out a role for the Ad *E1B* genes, as coinfection of HIV-1CAT-Ad with Ad mutants unable to express E1B proteins in both HeLa and HeLa/*tatIII* cells resulted in trans-activation equivalent to that seen with wild-type Ad (A.B.R. and E. White, unpublished results). We have also ruled out a role for the *E3* gene, as coinfection of HIV-1CAT-Ad with Ad dl309, both deleted for E3, resulted in trans-activation (M. Laspia and A.P.R., unpublished results). We cannot at present rule out involvements of the *E2* and *E4* genes in coinfection experiments, although plasmid transfection experiments show they are not necessary for trans-activation (Table 4; ref. 19). Because HIV-1CAT-Ad and RSVCAT-Ad encode the *E2* and *E4* genes, it will be necessary to construct additional recombinant Ad to resolve this issue.

Because the expression of CAT activity from HIV-1CAT-Ad is dependent on the presence of a trans-activator in the infected cell, recombinant Ad such as those described here have diagnostic potential.

Note Added in Proof. Nabel *et al.* (42) have also shown that the Ad 135 E1A protein trans-activates the HIV-1 LTR.

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