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Inhibition of RNA polymerase II transcription in human cells by synthetic DNA-binding ligands

(HIV type 1/viral replication/hairpin polyamide/DNA recognition)

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ABSTRACT **Sequence-specific DNA-binding small molecules that can permeate human cells potentially could regulate transcription of specific genes. Multiple cellular DNA-binding transcription factors are required by HIV type 1 for RNA synthesis. Two pyrrole–imidazole polyamides were designed to bind DNA sequences immediately adjacent to binding sites for the transcription factors Ets-1, lymphoid-enhancer binding factor 1, and TATA-box binding protein. These synthetic ligands specifically inhibit DNA-binding of each transcription factor and HIV type 1 transcription in cell-free assays. When used in combination, the polyamides inhibit virus replication by >99% in isolated human peripheral blood lymphocytes, with no detectable cell toxicity. The ability of small molecules to target predetermined DNA sequences located within RNA polymerase II promoters suggests a general approach for regulation of gene expression, as well as a mechanism for the inhibition of viral replication.**

Simple rules have been developed to rationally control the sequence specificity of minor-groove binding polyamides containing pyrrole (Py) and imidazole (Im) amino acids (1–6). DNA recognition depends on a code of side-by-side aromatic amino acid pairings in the minor groove. A pyrrole opposite an imidazole (Py/Im pairing) targets a C·G bp whereas Im/Py targets a G·C bp. A Py/Py pair binds both A·T and T·A pairs. These compounds represent the only class of synthetic small molecules that can bind predetermined DNA sequences with affinities and specificities comparable to DNA-binding proteins (7). The DNA-binding activity of the 5S RNA gene-specific transcription factor TFIIIA was inhibited by an eight-ring hairpin polyamide that bound within the recognition site of zinc finger four in the DNA minor groove. As a result, transcription of 5S RNA genes by RNA polymerase III was suppressed *in vitro* and in cultured Xenopus cells (8). The question arises whether polyamides can permeate human cells and specifically regulate genes transcribed by pol II (RNA polymerase II). As a first case study, we examined the ability of polyamides to inhibit HIV type 1 (HIV-1) transcription in cell-free assays and viral replication in human lymphocytes.

The HIV-1 enhancer/promoter element contains binding sites for the cell-encoded proteins upstream stimulatory factor, Ets-1, lymphoid-enhancer binding factor 1 (LEF-1) the nuclear factors NF-κB, Sp1, and TATA-box binding protein (TBP) (Fig. 1*A*) (9). To shut-down the promoter, polyamides were designed to target the transcription factors TBP, LEF-1, and Ets-1 simultaneously. TBP is indispensable for initiation of HIV-1 transcription, and LEF-1, considered to be an architectural protein, plays a central role in coordinating activities of multiple transcription factors (10). Both TBP and LEF-1 bind the minor groove of DNA and are likely to be inhibited by the minor groove binding polyamides. Ets-1 predominantly contacts the major groove in the center of its binding site, with additional flanking contacts that are possibly in the minor groove (11, 12).

The DNA-recognition sites of these transcription factors are not optimal polyamide target sequences because they are found in the promoters of many cellular protein-coding genes. However, the sequences immediately flanking these transcription factor binding sites often are conserved for a particular gene, providing an address for gene-specific targeting. This study demonstrates how gene-specific polyamide inhibition can be achieved by targeting sequences that are located adjacent to, but do not coincide with, binding sites for transcriptional regulators and how strategic targeting of multiple transcription factors can result in effective inhibition of a pol II promoter and viral replication in human cells.

METHODS

Polyamides. Polyamides **1–4** were synthesized by solid phase methods as described (13).

DNA-Binding Assays. Recombinant human TBP (Promega) was used at 0.14 nM in gel mobility shift experiments with 100 ng of poly dG-poly dC per 10 μl of reaction as recommended. A 50-bp kinase-labeled oligonucleotide, corresponding to positions −46 to +4 of the HIV-1 promoter (9), was used at 1.5 nM final concentration. The adenovirus major late promoter TATA region oligonucleotide had the top-strand sequence 5′-GATCGGGGGCTATAAAAGGGGGTGGG-3′ and the complementary bottom-strand sequence. Six percent nondenaturing polyacrylamide gels containing 44 mM Tris-borate (pH 8.3), 1 mM EDTA, 4 mM $MgCl_2$, and 0.02% (vol/vol) Nonidet P-40 were used. Recombinant LEF-1 protein containing the 86-aa DNA-binding domain was the generous gift of J. Love (Scripps Research Institute, La Jolla, CA) (14). DNA binding reactions, containing 8 nM LEF-1, were performed in 10 mM Hepes-OH (pH 7.5), 100 mM KCl, 1 mM DTT, 1 mM $MgCl_2$, 10% (vol/vol) glycerol, 250 ng of poly dG-poly dC, and 50 pmol of a singly end-labeled 400-bp restriction fragment isolated from pHIV long terminal repeat–chloramphenicol acetyl transferase plasmid DNA (obtained from K. A. Jones, Salk Institute, La Jolla, CA) (15). DNase I footprinting reactions were as described (7). Recombinant Ets-1 DNA binding domain (ΔN331) kindly was provided by B. Graves, (University of Utah) and was used at 12 nM in gel

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Abbreviations: Py, pyrrole; Im, imidazole; pol II, RNA polymerase II; LEF-1, lymphoid-enhancer-binding factor 1; TBP, TATA-box binding protein; HIV-1, HIV type 1; PBMC, peripheral blood mononuclear cells; CMV, cytomegalovirus.

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mobility shift experiments (16) along with 50 pmol of a 43-bp HIV-1 oligonucleotide (positions −160 to −117).

***In Vitro* Transcription.** Transcription was monitored with a nuclear extract from HeLa cells (Promega). The human lymphoid cell line H9 (ATCC HTB 176) was grown as described (17), and whole-cell extracts were prepared by hypotonic lysis (18). Run-off RNA transcripts of ≈300 bases [cytomegalovirus (CMV) major immediate early promoter linked to a guanineless cassette, pE1B-GL (19)] and ≈500 bases (pHIV long terminal repeat–chloramphenicol aceytyl transferase) were obtained with *Eco*RI-digested plasmid DNA (100 ng per 25 μl reaction). Polyamide-DNA complexes were allowed to form at ambient temperature for 15–30 min before addition of extracts. Transcription reactions were carried out as described (17). The H9 cell extract was immunodepleted with antibody to LEF-1 (provided by K. Jones) prebound to protein A Sepharose beads as described (17). The efficiency of immunodepletion was determined by subjecting depleted and mock-depleted extracts to SDS/PAGE and Western blotting and was found to be >95%. The blot was probed with LEF-1 antibody (diluted 1:2500) and was detected by enhanced chemiluminescence (Amersham).

Virus Replication. Human peripheral blood mononuclear cells (PBMC) were separated from whole blood collected from normal adult volunteers by density gradient centrifugation as described (20, 21). Donors were provided by the General Clinical Research Center of The Scripps Research Institute, which is supported by National Institutes of Health Grant MO1 RR00833. PBMC were activated with 2 μg/ml phytohemagglutinin and 20 units/ml of interleukin 2 for 2–3 days before HIV-1 infection. Each culture of 5×10^5 PBMC was infected with 10^3 tissue culture infectious doses of HIV-1 for 24 hours; free virus was removed by washing the cells in medium, and polyamides were added to the culture. Virus replication in culture was measured by HIV-1 p24 viral capsid antigen ELISA (Dupont). Assays of HIV-1 replication were performed five times with five human PBMC donors.

RESULTS AND DISCUSSION

Polyamide Design. The sequence 5′-(A,T)GC(A,T)GC(A,T)-3′ is present on both sides of the TATA box and immediately upstream of the Ets-1 binding site. The sequence 5′-AGTACT-3′ is found between the recognition sites for Ets-1 and LEF-1 (Fig. 1*A*). These sequences are conserved for most reported strains of HIV-1 (22, 23). Although the propensity for mutation at these sites is unknown, allowed sequence changes in the promoter could be targeted with new polyamides designed by the pairing rules. According to these rules, 5′-(A,T)GC(A,T)GC(A,T)-3′ sequences may be targeted by hairpin polyamide **1** having sequence composition ImPy-β-ImPy-γ-ImPy-β-ImPy-β-Dp (Fig. 1*B*). Because the β/β pairing recognizes both A·T and T·A bp (24), polyamide **1** is expected to bind to all three sites. Quantitative footprint titration experiments revealed that polyamide **1** binds to each of these sites with an equilibrium dissociation constant (K_d) of 0.05 nM. A mismatch control polyamide ImIm-β-ImIm-γ-PyPy-β-PyPy-β-Dp (**2**), which differs only in the placement of the Im and Py amino acids, binds the 5′-(A, T)GC(A, T)GC(A, T)-3′ sites with 100-fold reduced affinity relative to polyamide **1**. According to the pairing rules, the sequence 5′-AGTACT-3′, between the LEF-1 and Ets-1 binding sites, will be bound by polyamide **3** of sequence composition ImPyPyPy-γ-ImPyPyPy-β-Dp (Fig. 1*B*) (7). Quantitative footprint titration experiments revealed that polyamide **3** binds this site with $K_d = 0.06$ nM. Mismatch polyamide **4** binds this sequence with >100-fold reduced affinity.

Inhibition of TBP Binding. Binding of the TBP subunit of TFIID in the minor groove nucleates assembly of the pol II transcription machinery for TATA-containing genes (25–27).

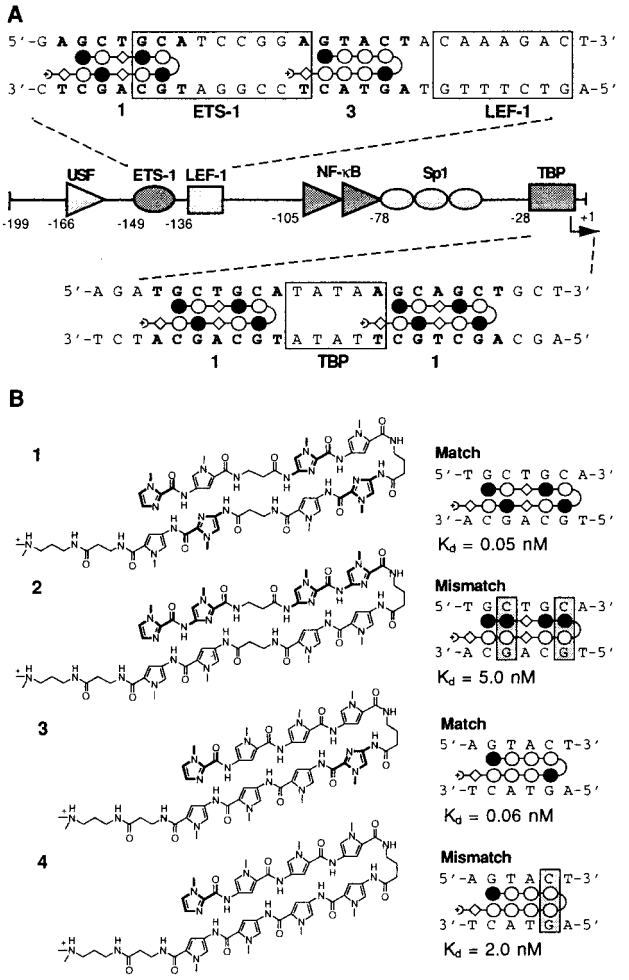

FIG. 1. Polyamide and transcription factor binding sites. (*A*) Schematic of the HIV-1 enhancer and promoter (nucleotide positions −199 to +1) showing binding sites for polyamides **1** and **3** and the transcription factors upstream stimulatory factor, Ets-1 (39, 40), LEF-1, NF-κB, Sp1, and TFIID (TBP). For polyamide binding models: shaded and unshaded circles, Im and Py rings, respectively; curved lines, γ-aminobutyric acid (γ); diamonds, β-alanine (β); and Dp, dimethylaminopropylamide. (*B*) Structures of polyamides ImPy-β-ImPy-γ-ImPy-β-ImPy-β-Dp (**1**), ImIm-β-ImIm-γ-PyPy-β-PyPy-β-Dp (**2**), ImPyPyPy-γ-ImPyPyPy-β-Dp (**3**), and ImPyPyPy-γ-PyPyPyPy-β-Dp (**4**). Binding models and measured dissociation constants are shown. Mismatches are highlighted.

TBP binds the HIV-1 TATA element with a K_d of ≈1–3 nM. A gel mobility shift assay revealed that polyamide **1** inhibits TBP binding to a double stranded oligonucleotide corresponding to the HIV-1 TATA box region (Fig. 2*A*, lanes 3–5) whereas no inhibition was observed for control polyamide **2** (Fig. 2*A*, lanes 13–15). Additionally, polyamide **1** does not inhibit TBP binding to the TATA box region of the adenovirus major late promoter (5′-GGGGGCTATAAAAGGGGGT-3′), which contains mismatch flanking sequences (Fig. 2*A*, lanes 8–10). The half-life of the polyamide **1**-DNA complex was determined by competition experiments to be in excess of 2.5 hours.

Inhibition of Ets-1 Binding. The Ets-1 recognition site in the HIV-1 enhancer is flanked by binding sites for polyamides **1** and **3** (Fig. 1). The isolated Ets-1 DNA-binding domain, ΔN331 (amino acids 331 to 416) (16) bound to the HIV-1 enhancer with a K_d of ≈0.5 nM. When polyamides were preincubated with the labeled, double-stranded HIV-1 oligonucleotide before adding ΔN331, polyamide **3** had no effect on Ets-1 DNA-binding (Fig. 2*B*, lanes 3–5). Polyamide **1**, how-

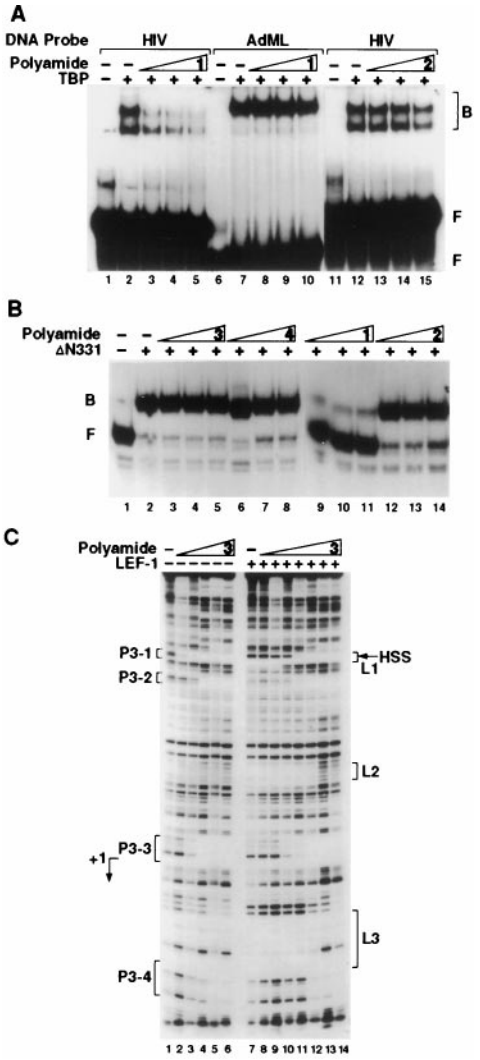

FIG. 2. Inhibition of transcription factor binding to the HIV-1 promoter and enhancer. (*A*) Gel mobility shift assay with TBP and polyamides **1** and **2**. Oligonucleotides corresponding to the HIV-1 TATA box (lanes 1–5 and 11–15) or adenovirus major late promoter TATA box (lanes 6–10) were incubated with no polyamide (lanes 1, 2, 6, 7, 11, and 12), 50 nM polyamide (lanes 3, 8, and 13), 100 nM polyamide (lanes 4, 9, and 14), and 200 nM polyamide (lanes 5, 10, and 15) before the addition of recombinant human TBP, as indicated (+). The positions of free probes (F) and the monomer and dimer TBP-DNA complexes (B) are indicated. (*B*) Differential effect of polyamide **1** and **3** on Ets-1 DNA-binding. Polyamide concentrations were 100 nM (lanes 3, 6, 9, 12), 200 nM (lanes 4, 7, 10, 13), and 400 nM (lanes 5, 8, 11, 14). (*C*) DNase I footprint experiment with LEF-1 (lanes 7 to 14) and polyamide **3** used at the following concentrations: no polyamide (lanes 1 and 7), 10 pM (lanes 2 and 8), 30 pM (lane 9), 0.1 nM (lanes 3 and 10), 0.3 nM (lane 11), 1 nM (lanes 4 and 12), 3 nM (lanes 5 and 13), and 10 nM (lanes 6 and 14). Polyamides and DNA were incubated before the addition of transcription factors.

ever, prevented the Ets-1/DNA complex formation (Fig. 2*B*, lanes 9–11). The two mismatch polyamides **2** and **4** did not prevent complex formation (lanes Fig. 2*B*, lanes 6–8 and 12–14). DNase I footprinting revealed that polyamide **1** prevents Ets-1 DNA-binding whereas polyamide **3** cooccupies the DNA with Ets-1. Polyamide **1** likely inhibits Ets-1 binding by steric interaction with protein contacts in the minor groove whereas polyamide **3**, which does not inhibit Ets-1 binding, must occupy the minor groove beyond the Ets-1 contacts.

Inhibition of LEF-1 Binding. LEF-1 is a member of the high mobility group family of minor-groove binding proteins (14) and has been shown to be essential for HIV-1 transcription and replication in lymphoid cells (28). Three sites on the HIV-1 promoter/enhancer restriction fragment are bound by the 86-aa LEF-1 DNA binding domain, sites L1, L2, and L3 (Fig. 2*C*), with K_d = 1.4, 5.8, and 4.9 nM, respectively. The LEF-1 footprint at site L1, characterized by a marked DNase I hypersensitive site, changes to the polyamide footprint in the presence of match polyamide **3** (Fig. 2*C*, lanes 8–14). LEF-1 binding is inhibited 50% at a polyamide **3** concentration of ≈60 pM. Thus, polyamide **3**, located in the minor groove immediately adjacent to site L1, inhibits LEF-1 binding to this site. Polyamide **3** inhibits LEF-1 binding to the sites L2 and L3 at markedly higher polyamide concentrations (3 nM and above; Fig. 2*C*, lanes 13–14) because there are no high affinity polyamide recognition sites adjacent to sites L2 and L3. Inhibition was observed either by adding the polyamide to the DNA before LEF-1 or after preincubation of the DNA with LEF-1, consistent with the relative dissociation constants for the two binding reactions. Mismatch polyamide **4** failed to inhibit LEF-1 binding.

Inhibition of Transcription. The effects of polyamides **1** and **2** on HIV-1 transcription were tested in an *in vitro* transcription assay with a HeLa cell nuclear extract. Polyamide **1** inhibited basal transcription from the HIV-1 promoter (Fig. 3*A*, lanes 2–4) but not from the CMV major immediate early promoter, which contains a mismatched TATA-flanking sequence (5′-GAGGTCTATATAAGCAGA-3′) (Fig. 3*A*, lanes 6–8). The mismatch polyamide **2** did not inhibit transcription from either promoter (Fig. 3*A*, lanes 10–12 and 14–16). We performed titrations of polyamide **1** over a wide range of concentrations (1 to 200 nM) with both of the HIV-1 and CMV templates in the same reaction. Under these conditions, we observed 50% inhibition of HIV-1 transcription at 60 nM polyamide **1**, which corresponds to a 6- to 10-fold excess of polyamide over binding sites (Fig. 3*B*, closed triangles). No significant inhibition of CMV transcription was observed (Fig. 3*B*, open circles).

To test the effects of polyamides **3** and **4** on LEF-1-activated HIV-1 transcription, we supplemented the HeLa nuclear extract with a human lymphoid H9 cell extract, which contains high levels of LEF-1 protein (as determined by Western blot analysis). The mixture of H9 and HeLa cell extracts stimulated HIV-1 transcription 2.5- to 3-fold over the level of transcription observed with the HeLa extract alone (Fig. 3*C*, lane 1). Immunodepletion of LEF-1 protein from the H9 extract abolished this activated transcription (Fig. 3*C*, compare lanes 1 and 6). Polyamide **3** inhibited HIV-1 transcription in this system (Fig. 3*C*, lanes 2–5) with a 50% reduction of transcription observed at 10–30 nM polyamide. Polyamide **3** failed to inhibit HIV-1 transcription in the LEF-1-depleted extract (Fig. 3*C*, lanes 7–10). We also monitored the activity of the CMV major immediate early promoter in both the mock-depleted and LEF-1-depleted H9 cell extract, with the result that LEF-1 depletion had no effect on CMV transcription. As additional controls, we tested the effect of the mismatch polyamide **4** on HIV-1 transcription (Fig. 3*D*, lanes 4 and 5) and polyamides **3** and **4** on CMV transcription (Fig. 3*D*, lanes 6–10). No potential binding sites for either polyamide **3** or **4** are present in the CMV major immediate early promoter sequence. As expected, polyamide **3** fails to inhibit CMV transcription (Fig. 3*D*, lanes 7–8), and polyamide **4** fails to inhibit either HIV-1 or CMV transcription (Fig. 3*D*, lanes 4–5 and 9–10).

Notably, polyamide **3** does not inhibit basal transcription, although binding sites for this polyamide are present at the start site for transcription and within the HIV-1 RNA coding sequence (Fig. 2*C*). These observations suggest that pol II can transcribe DNA with a polyamide bound in the minor groove and that polyamides are only inhibitory to transcription when these compounds interfere with the DNA binding activity of a required transcription factor.

Inhibition of Virus Replication. Because there are multiple spliced and unspliced species of HIV-1 RNA with different

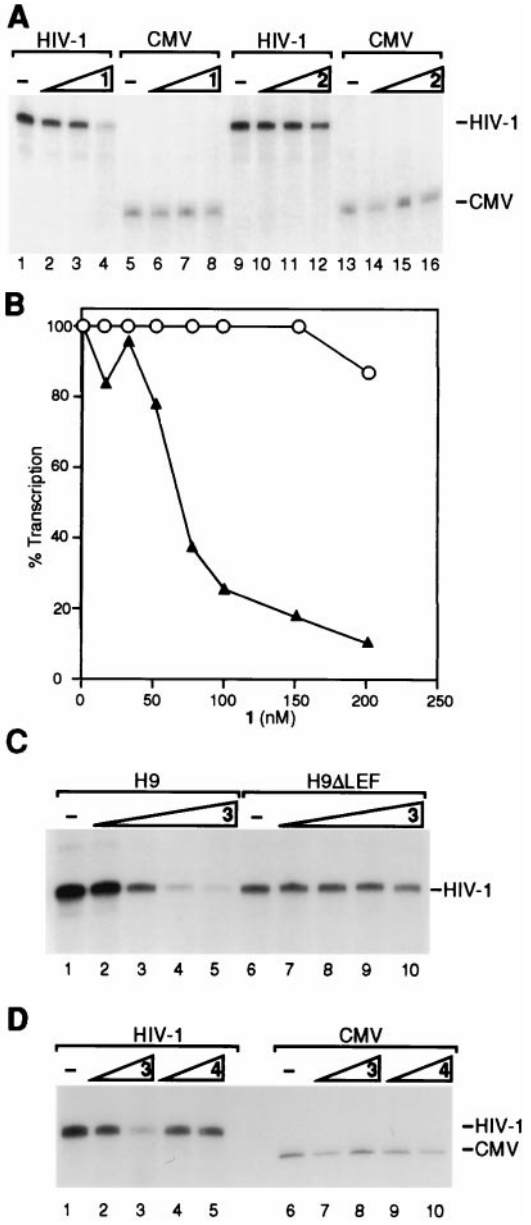

FIG. 3. Polyamide inhibition of HIV-1 transcription *in vitro*. (*A*) DNA templates containing the HIV-1 promoter (lanes 1–4 and 9–12) and the CMV major immediate early promoter (lanes 5–8 and 13–16) were incubated with the following concentrations of polyamide **1** or **2** (as indicated) before the addition of HeLa nuclear extract: no polyamide (lanes 1, 5, 9, 13) 50 nM polyamide (lanes 2, 6, 10, 14), 100 nM (lanes 3, 7, 11, 15), and 200 nM (lanes 4, 8, 12, 16). (*B*) Relative levels of HIV-1 transcription (closed triangles) compared with CMV transcription (open circles) are plotted as a function of polyamide **1** concentration, from mixed template reactions with HeLa nuclear extract. (*C*) Polyamide **3** inhibits LEF-1-activated transcription. Transcription reactions were performed with HeLa nuclear extract supplemented with H9 whole cell extract (lanes 1–5) or LEF-depleted H9 extract (lanes 6–10). The final concentrations of polyamide were no polyamide (lanes 1 and 6), 10 nM (lanes 2 and 7), 30 nM (lanes 3 and 8), 100 nM (lanes 4 and 9), and 300 nM (lanes 5 and 10). (*D*) Transcription with mismatch control polyamide **4** and control CMV template. Plasmid DNA was incubated with polyamide **3** (10 nM, lanes 2 and 7; 100 nM, lanes 3 and 8) or polyamide **4** (10 nM, lanes 4 and 9; 100 nM, lanes 5 and 10).

turn-over kinetics (29), we examined HIV-1 transcription indirectly by measuring the levels and kinetics of HIV-1 replication in human PBMC in culture. PBMC were infected with the T cell-tropic HIV-1 strain WEAU1.6 (30, 31) or with the macrophage-tropic strain SF162 (32, 33). Polyamides were added to the culture medium, and the levels of HIV-1 p24 viral capsid protein released into the culture media (primarily as virions) were determined on subsequent days after infection by using a standard ELISA assay. In control PBMC cultures with no added polyamide, viral replication of both strains resulted in increasing p24 levels between 4 and 10 days of culture (Fig. 5*A* and *C*). Polyamide **1** at 1 μM concentration caused an 80% reduction in virus whereas polyamide **3** at 1 μM concentration caused a 60% reduction after 6 to 8 days (Fig. 4*A*). The combination of polyamides **1** + **3** inhibited HIV-1 replication at 10 nM to 1 μM concentration, but the closely related polyamides **2** + **4** did not (Fig. 4*B*). The combination of polyamides **1** and **3** at 1 μM each acted in synergy to reduce viral p24 levels to below the threshold of detection after 6–8 days for WEAU (<10 pg/ml; >99.9% inhibition of viral replication) (Figs. 4*A* and 5*C*) and were as effective as 1 μM azidothymidine in blocking HIV-1 replication. The macrophage-tropic SF162 isolate, which replicates in both macrophages and $CD4^+$ T-lymphocytes, was not inhibited efficiently by either polyamide alone, but the combination of 1 μM each polyamide **1** and **3** reduced and eventually blocked its replication after 10 days (Fig. 5*A*). Addition of mismatch polyamides **2** and **4**, which differ by a single atomic substitution (**4**), or a rearrangement of the Im and Py amino acids (**2**), from the match polyamides, had no effect on the level of virus in the medium, either alone or in combination (Fig. 4*B*).

The inhibitory effects of polyamides was not caused by obvious toxicity. No significant decrease in cell viability was apparent in PBMC cultures treated with polyamides **1** and **3** for 10 days, in contrast to 90% mortality observed for PBMC cells treated with 1 μM azidothymidine for the same period (Fig. 5 *B* and *D*). Cell viability was slightly higher in WEAU-infected cultures that were treated with polyamides **1** and **3** than in untreated cultures (Fig. 5*D*, filled circles versus open circles), probably because the cytopathic effect of HIV-1 infection was reversed completely. Cell recovery was not impacted by polyamide treatment but was reduced by azidothymidine treatment.

The observed polyamide inhibition of virus replication is likely caused by interference with the DNA-binding activities of TBP and Ets-1 by polyamide **1** and the binding activity of LEF-1 by polyamide **3**, but it is possible that inhibition of

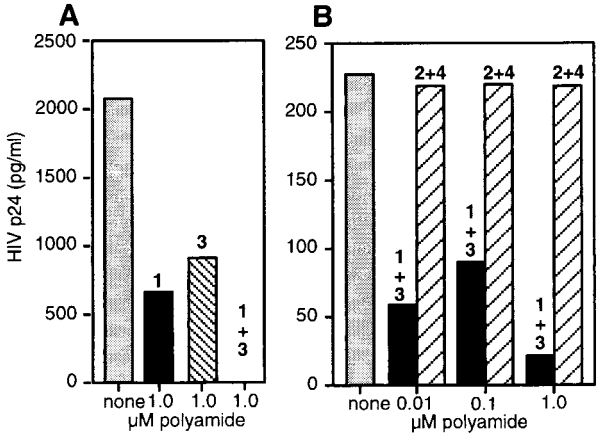

FIG. 4. Polyamide inhibition of HIV-1 replication. *A* and *B* depict two independent experiments in which polyamides alone or in combination were added to cultures of PBMCs, obtained from two separate donors, infected with the primary HIV-1 isolate WEAU 1.6 (kindly provided by G. Shaw). Replication was measured 6 or 8 days after infection by release of p24 capsid antigen into the medium. Assays were performed in duplicate and showed <5% variation from the mean. In *B*, polyamides were added 24 hours before virus exposure and were continuously present thereafter.

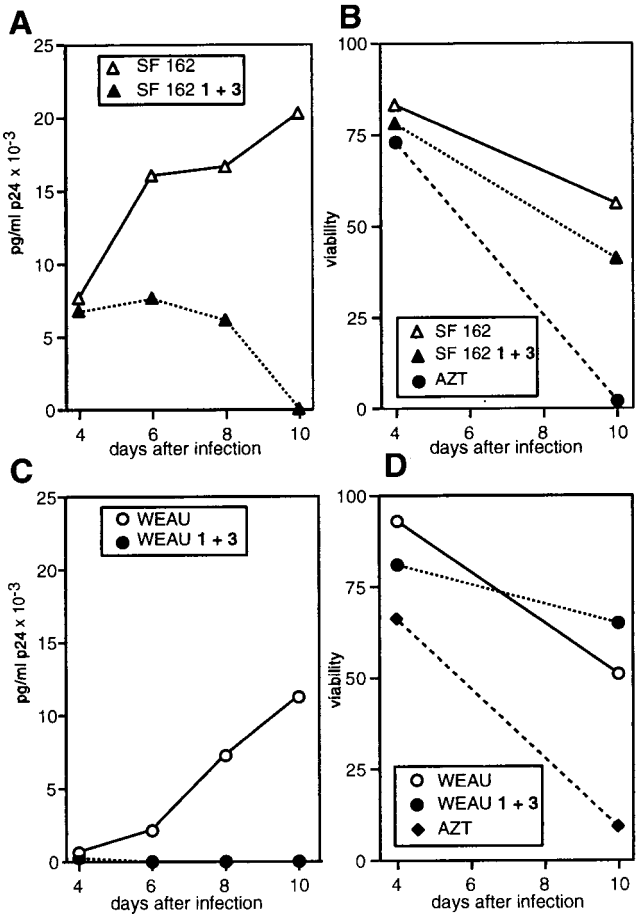

FIG. 5. Kinetics of polyamide inhibition of HIV-1 replication and effects on cell viability. PBMC were infected with the macrophage-tropic HIV-1 isolate SF162 (*A* and *B*) or the T-cell tropic isolate WEAU 1.6 (*C* and *D*). Polyamides **1** and **3** were added in combination at 1 μM each. Virus replication was monitored by p24 ELISA (*A* and *C*). Cell viability was determined by trypan blue exclusion in the presence of polyamides or azidothymidine (*B* and *D*).

cellular genes involved in T-cell activation could have an indirect effect on HIV-1 replication. To assess this possibility, we performed an RNase protection assay for transcripts of a number of cytokine and growth factor genes, including interleukin 2, interleukin 5, and interleukin 13, which differ in the target sequences flanking the TATA box. Four other cytokine genes that lack binding sites for either polyamide **1** or **3** in their promoters also were examined (Fig. 6). The results show that exposure of activated human PBMC to a combination of either polyamides **1** + **3** or **2** + **4** (1 μM each) for 6 days failed to inhibit RNA expression of all of the genes examined (Fig. 6). There was no difference in the intensity of CD4 and CD8 RNA bands (data not shown), indicating equivalent recovery of CD4 and CD8 T cells in treated and untreated cells. This lack of inhibition of cytokine gene transcription suggests that the polyamides reduce virus replication in cells by a direct effect on HIV-1 RNA transcription.

Future Plans. Our present studies show that polyamides designed to target DNA sequences 6–7 bp in length are effective inhibitors of gene transcription in cell-free systems and viral replication in human cells. Because sequences of these lengths would be highly redundant in the human genome, it had seemed likely that these ligands would have deleterious effects on cell metabolism because of interference with the activity of cellular genes. However, the results described here indicate that a set of two polyamides that recognize 6- to 7-bp sequences can be sufficient for gene-specific regulation *in vivo*.

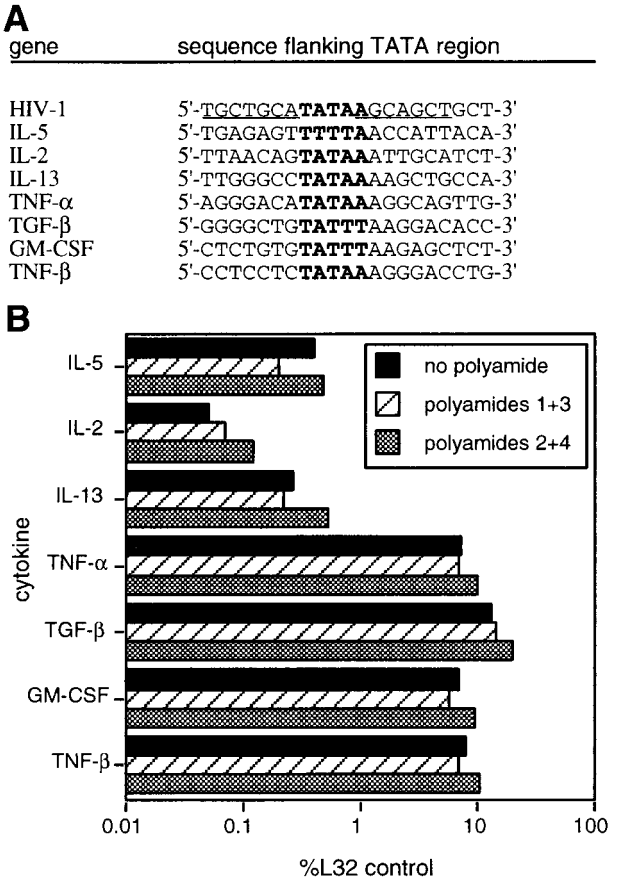

FIG. 6. Control gene expression. (*A*) The TATA box (bold) and flanking sequences (from GenBank listings) of each of the cytokine/growth factor genes examined are shown. The polyamide **1** binding site is underlined. (*B*) Ribonuclease protection assays for the indicated mRNAs (41, 42). PBMCs were cultured and either were left untreated or were treated with 10 μM polyamides **1** + **3** or **2** + **4** for six days. Data are expressed as the intensity of each RNA relative to the intensity of the ribosomal L32 RNA band to standardize for RNA loading.

It is interesting to compare these small molecule transcription repressors to eukaryotic transcriptional regulatory proteins that also recognize multiple short sequences to increase functional specificity (27, 34, 35). The observations that polyamides do not interfere with pol II elongation, and that polyamides can bind simultaneously with certain major groove proteins, should further enhance gene-specificity (36). In addition, polyamides are not limited to 6- to 7-bp recognition. For example, polyamides of similar size to those described here have been shown to bind as cooperative dimers to sites 10–16 bp in length (37, 38). The polyamide binding site size required to elicit optimal biological function should be reported in due course.

The specific inhibition of genes transcribed by pol II represents an important first step toward asking whether cell-permeable small molecule transcription antagonists might regulate gene expression in complex organisms (8). We have chosen TBP and two additional key regulators of HIV-1 transcription, Ets-1 and LEF-1, as targets for inhibition of pol II-driven transcription. Because most tissue-specific cellular genes and viral genes contain TATA elements as well as enhancer factor binding sites, this approach may be generally applicable for the inhibition of most target genes.

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