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Real-Time Study of Interactions between a Composite DNA Regulatory Region (HIV-1 LTR NRE) and Several Transcription Factors of Nuclear Extracts

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Here we describe the first real-time study of nuclear protein interaction with a composite DNA regulatory region. We studied the interplay between the three target sites of the negative regulatory element (NRE) of HIV-1 LTR, comprising a noncanonical GATA site overlapping two negative regulatory regions, USF and NFIL-6, and their corresponding transcription factors in nuclear extracts. By bandshift analysis, no GATA binding activity could be detected between LTR NRE and different nuclear extracts, although evidenced by *in vitro* footprinting. Additionally, the LTR NRE and a USF oligonucleotide showed identical retarded complexes. BIAcore study of these interactions revealed the binding of huGATA-3, as well as USF, to the immobilized LTR NRE oligonucleotide. Competition analyses, performed with GATA, USF, and NFIL-6 oligonucleotides, clearly showed that this regulatory region could bind both huGATA-3 and USF factors. Finally, the presence of USF and huGATA-3 proteins in the complexes formed with LTR NRE was ascertained using specific anti-huGATA-3 and anti-USF2 polyclonal antibodies. © 1999 Academic Press

The 5′ long terminal repeat (LTR), and in particular the U3 region of the HIV-1 provirus, has been shown to play a major role in HIV-1 gene expression and thus in the virus cycle (1). Indeed, HIV-1 gene expression is controlled by multiple cellular transcription factors (2). In the LTR, as for any eukaryotic promoter, the organisation of target sequences and their capacity to interact with various transcription factors may be crucial in the regulation of HIV transcription. In the LTR, a negative regulatory element (NRE) is able to down-regulate the HIV transcription (3–5). In this NRE region, a binding site for the human nuclear protein called USF (6), MLTF (7) or UEF (8) has been shown to play a negative role in transcription (9) both in the presence or absence of Tat transactivation. USF is a dimer-forming family of transcription factors characterized by a highly conserved basic-helix-loop-helix-leucine zipper (bHLH-zip) DNA-binding domain. Two different USF proteins, termed USF1 (44 kDa) and USF2 (43 kDa), are ubiquitously expressed in both humans and mice. A binding site for NFIL-6 (10, 11) has also been described in the NRE region (12). This transcription factor, belonging to the CCAAT/enhancer binding protein (C/EBP) family (13) as well as C/EBPβ (14) or CRP2 (13) was shown to play a dual (positive/negative) role in HIV transcription in T-cells (12). Indeed, the same RNA messenger was able to encode, by alternative initiation of translation, three forms of protein (two activators and one inhibitor), described as LIP/LAP proteins (liver inhibitor/activator protein) (15, 16). Finally, huGATA-3 (17–21) regulatory elements have also been described in the LTR of HIV-1. Indeed, Yang and collaborators (22) have reported that the six huGATA-3 binding sites were able to enhance transcription and that it was dependent on the huGATA-3 transcription transactivation domain. One of these sites (site 2), a non-canonical huGATA3 target site is located in the overlapping region of USF and NFIL-6 binding sites (see Fig. 1A).

The DNA region LTR NRE which comprises potential binding sites for three nuclear factors, NFIL-6, USF and huGATA-3, was used as a model to study the interactions between multiple factors and a complex regulatory region. The interactions of these different factors with LTR NRE DNA composite region were investigated using two technical approaches: electrophoretic mobility shift assays (EMSA) and surface plasmon resonance (SPR). The SPR approach, which reveals interactions under real-time conditions, showed that both USF and GATA-3 are able to bind LTR NRE whereas the EMSA approach only detected a USF/LTR NRE interaction.

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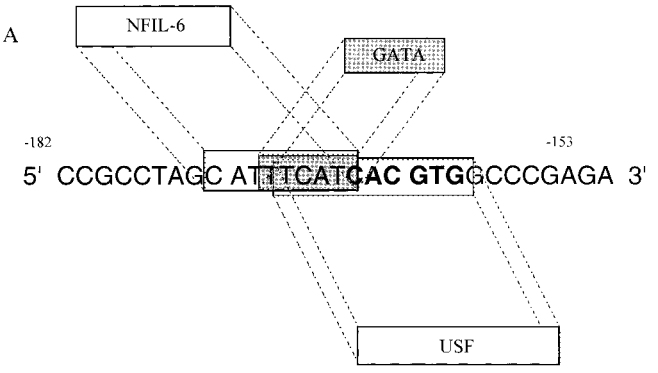
MATERIALS AND METHODS

Oligonucleotides and antibodies. The LTR NRE oligonucleotide was synthesized and biotinylated at its 5′ end by Genosys (Cambridge, UK). It was annealed with the antisense DNA strand and then immobilized on the streptavidin-coated sensor chip, for BIAcore assays. USF, NFIL-6, δE4 and site 3 (mutated or not) oligonucleotides were also synthesized (Gibco BRL Life Technologies, Eragny, France) for SPR competitive analyses and EMSA studies. Oligonucleotides are described in Fig. 1B. Anti-USF2 polyclonal antibodies (Santa cruz, Santa Cruz, California, USA), anti-HIV-1 Vpr polyclonal antibodies (NAIDS) and anti-huGATA-3 monoclonal antibody (MAb31, see (23), gift of P. H. Roméo), were used in upshift assays. Anti-USF2, anti-rat C/EBPβ, anti-human NFIL-6, anti-fra 1, anti-c jun and anti-STAT3 polyclonal antibodies (Santa Cruz, Santa Cruz, California, USA), and anti-GST (Sigma, L'Isle d'Abeau Chesnes, France) and anti-huGATA-3 (gift of P. H. Roméo, see 23) polyclonal antibodies were used for SPR immunocharacterization.

Preparation of nuclear extracts. CEM T-cells either uninfected (A3.01; 24) or chronically infected with HIV-1_{LAI} (8E5; 25), were maintained in RPMI medium with glutamax (Gibco BRL Life Technologies) with 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml). Cells were activated with 50 nM phorbol myristate acetate (PMA, Sigma Chemical, L'isle d'Abeau Quesnes, France) and 500 nM ionomycin D (Sigma) and aliquots of 2×10^8 cells were frozen 6 h after activation. The cells were thawed and lysed with two volumes of buffer I (10 mM Tris-HCl pH 7.8, 2 mM MgCl2, 10 mM KCl, 0.1 mM EGTA, 10% sucrose, 20% glycerol, 0.4 mM PMSF, 0.5 mM DTT, 0.02% NP40 and 1 μg/ml antipain, aprotinin, chymostatin ABC, pepstatin A and leupeptin), and nuclei were collected by centrifugation at 8,000 rpm (in an Eppendorf centrifuge) for 80 s at 4°C. Lysis of the nuclei was carried out with one volume of buffer II (20 mM Tris-HCl pH 7.8, 2 mM MgCl2, 300 mM KCl, 0.2 mM EGTA, 25% glycerol, 0.2 mM PMSF, 0.5 mM DTT and 1 μg/ml antipain and aprotinin) for 30 min at 4°C. After centrifugation at 17,000 g for 30 min at 4°C, the extracts were dialysed, using a Millipore (Saint-Quentin-en-Yvelines, France) dialysis membrane (VS type), against dialysis buffer (20 mM Tris-HCl pH 7.8, 100 mM KCl, 0.5 mM EDTA, 20% glycerol, 0.2 mM PMSF, 0.5 mM DTT and 1 μg/ml aprotinin), and stored at −80°C. Protein concentration was measured with the "BCA Protein Assay Reagent" kit (Pierce, Rockford, USA).

Electrophoretic mobility shift assays (EMSA). The binding sites for USF, huGATA-3 and NFIL-6 proteins were created by annealing sense and anti-sense oligonucleotides. ^{32}P end-labelled (γ-ATP, Amersham) ds oligonucleotide (30,000 cpm) was incubated with 2 μg nuclear extract and 200 μg/ml poly(dI-dC)-poly(dI-dC) 600 bp (Pharmacia Biotech, Saclay, France) in binding buffer (10 mM HEPES pH 7.4, 1.5 mM DTT, 150 mM NaCl, 1.6 mM EDTA, 4 mM spermidine, 5% glycerol, 0.5 mM ZnCl2) in a final volume of 11 μl, before loading on a 0.25 × Tris-borate EDTA native polyacrylamide gel (6%). For the super shift assays, the antibodies were pre-incubated with the proteins (under the conditions stated above) before adding the labelled probe. For competition experiments, the oligonucleotide competitors (50 fold excess) were also pre-incubated with the nuclear extract before adding the labelled probe.

Surface plasmon resonance (SPR): BIAcore (BIACORE AB, Saint-Quentin-en-Yvelines, France). The biotinylated (ds) oligonucleotide (LTR NRE) was immobilized on a streptavidin-coated sensor chip (600 RU). The running buffer (10 mM Hepes, 1.5 mM DTT, 150 mM NaCl, 1.6 mM EDTA, 0.5 mM ZnCl2) was applied at a flow rate of 10 μl/min. Proteins were injected in reaction buffer (10 mM Hepes, 1.5 mM DTT, 150 mM NaCl, 1.6 mM EDTA, 4 mM spermidine, 0.5 mM ZnCl2, 50 μg/ml poly (dIdC)/poly (dIdC), 1 mg/ml CM dextran, 2% horse serum). At the end of each cycle, the sensor chip was regenerated by injecting 6 μl running buffer plus 0.05% SDS. The binding response was determined by measurement of resonance units at 200 s after injection. Prior to numerical analysis, data were adjusted to zero baseline level by subtracting the response recorded immediately before the injection of analyte. For competition analyses, 6 μg (in 30 μl) of nuclear extracts (A3.01 or 8E5) were injected alone or with several competitors (2.5 μM): LTR GATA sites 1 and 3 (mutated or not), LTR NRE, δE4, NFIL-6 and USF oligonucleotides (see Fig. 1B). For antibody experiments, first the nuclear extract was injected to allow formation of the nucleoproteic complex, and then 30 μl (0.3 μg) of the various antibodies were injected to identify the proteins retained in the complexes.



B

Oligonucleotide	DNA sequences	promoter or enhancer
site3	CAGTTGAGCC **AGATAA**GGTA GAAGA	HIV-1 LTR
USF	GGTGTAGGC**C ACGTG**ACCGG GTGT	Adenovirus
NFIL-6	GGACGTCAC**A TTGCACAA**TC TTAATAA	huIL-6
site1	AGTACTTCAA GAACTGCT**GA TATC**GAGCTT	HIV-1 LTR
LTR NRE (site2)	CCGCCTAGC**A TTTCATCACG TGGCC**CGACA	HIV-1 LTR
δE4	TAATGCTAGA **GTTATCA**CTT TCT**GTTATCA** AGTGG	human TcR-δ
site3*	CAGTTGAGCC **cGActA**GGTA GAAGA	mutated

FIG. 1. (A) LTR NRE and transcription factor target sites. The noncanonical GATA site (shaded box) is located within the overlapping region of two regulatory elements: NFIL-6 and USF. The bHLH motif of the USF site is represented in bold letters. (B) Oligonucleotides used in EMSA and BIAcore assays. The GATA site 3, USF, and NFIL-6 used as prototype target sites for the three corresponding factors as well as GATA site 1, LTR NRE (site 2), mutant site 3*, and δE4 (GATA tandem). Mutated nucleotides of site 3* are indicated in lowercase and binding sites are in bold letters.

RESULTS AND DISCUSSION

The technical approaches commonly used to study DNA/transcription factor interactions are EMSA and *in vitro* (and less frequently *in vivo*) footprinting analyses. EMSA shows direct visualisation of the different molecular complexes obtained with a regulatory region comprising adjacent and overlapping sites, whereas footprinting experiments, albeit laborious, may give useful information, on the DNA sequences within a promoter that interact with transcription factors. Nevertheless, since the proteins that have interacted with a complex DNA sequence are somehow difficult to iden-

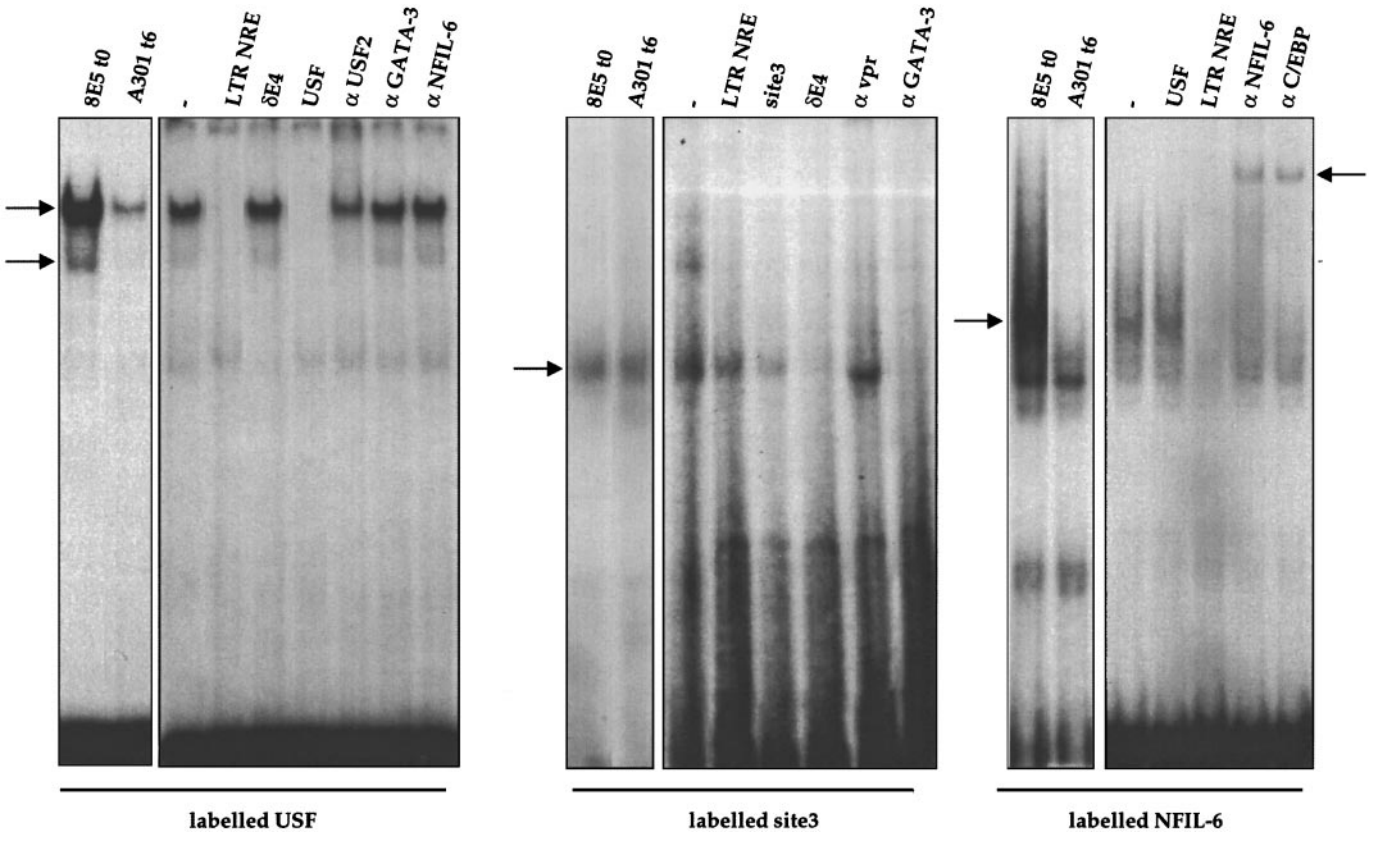


FIG. 2. EMSA determination of the level of expression of USF, huGATA-3, and NFIL-6 transcription factors in two different nuclear extracts. Labelled USF, site 3 (to detect GATA-3 binding activity) and NFIL-6 oligonucleotides were incubated with either 8E5 t0 (HIV-1 infected) or A3.01 t6 (noninfected) nuclear extracts, prior to or 6 h postcellular activation, respectively. The specificity of the DNA/protein complexes was verified using specific or irrelevant competitors and antibodies: LTR NRE, δE4, and USF oligonucleotides and anti-USF2, anti-GATA-3, and anti-NFIL-6 antibodies for USF; LTR NRE, site 3, and δE4 oligonucleotides and anti-HIV-1 vpr and anti-huGATA-3 antibodies for site 3; LTR NRE and USF oligonucleotides and anti-human-NFIL-6 and anti-rat C/EBPβ antibodies for NFIL-6. Arrows on the left represent the DNA/factors complexes and on the right the supershift DNA complexes.

tify, we employed a SPR method for real-time dynamic analysis of the factors interacting with a composite DNA sequence.

The oligonucleotides used for the study of transcription factor interaction with LTR NRE regulatory elements (Fig. 1A) are listed in Fig. 1B. We decided to study, in real time, the interactions between the LTR NRE and these 3 transcriptional factors: USF, GATA-3 and NFIL-6. For this purpose, we took advantage of the transcriptional factor diversity observed during T-cell activation and HIV-1 infection. Among nuclear extracts of several T-cell lines infected or not with HIV-1, two extracts were selected (8E5 t0 and A3.01 t6) showing different levels of the three transcription factors as determined by EMSA (Fig. 2) in the presence of three prototype oligonucleotides (USF, site 3 for GATA-3 and NFIL-6; Fig. 1B). Figure 2 shows that the level of USF and NFIL-6 factors varies in the 8E5 and A3.01 nuclear extracts: USF is higher in 8E5 t0 than in A3.01 t6 extract and NFIL-6 seems only expressed in 8E5 t0 extract. In contrast, the level of huGATA-3 is quite similar in both extracts. For the three factors, the specificity of DNA/protein interactions was verified after addition of relevant and irrelevant competitors and antibodies.

EMSA Only Reveals LTR NRE/USF Interaction

As shown in Fig. 3, the profiles obtained with nuclear extracts of several CEM T-cell lines (here prior to activation: 8E5 t0) did not provide evidence for GATA-3 binding to the LTR NRE DNA sequence. Indeed, formation of DNA/protein complexes with labelled LTR NRE (Fig. 3A, lane 1) was not abrogated with any of the GATA oligonucleotides used as competitors: i.e. with LTR GATA site 1 (lane 2), site 3 (lane 4) or δE4 (lane 5). In addition, an anti-huGATA-3 monoclonal antibody (lane 10) did not later the complexes and do not reveal any upshift. Moreover, the EMSA profiles obtained with labelled LTR NRE (Fig. 3A, lane 1) and labelled USF (Fig. 3B, lane 1) regulatory element (from Adenovirus late promoter) are very similar. Indeed, these DNA/protein complexes, observed with the two labelled oligonucleotides, were totally abolished by

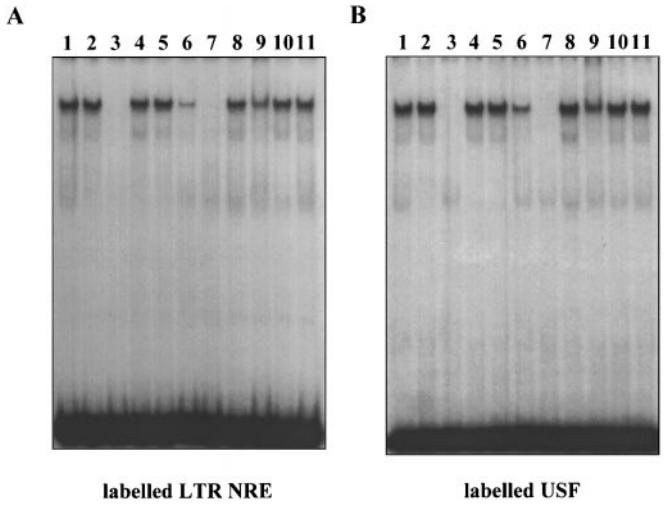


FIG. 3. EMSA profiles of either LTR NRE or USF oligonucleotides with 8E5 t0 nuclear extract. Labelled LTR NRE (A) or USF (B) oligonucleotides were incubated with 2 μg of 8E5 t0 nuclear extract either alone (lane 1) or with 50-fold excess of LTR GATA site 1 (lane 2), LTR NRE (lane 3), site 3 (lane 4), δE4 (lane 5), NFIL-6 (lane 6), USF (lane 7) oligonucleotides as well as irrelevant anti-HIV-1 vpr protein (lane 8), anti-USF2 protein (lane 9), anti-huGATA-3 monoclonal antibody (lane 10), and anti-human NFIL-6 polyclonal antibody (lane 11).

LTR NRE and USF oligonucleotides (lanes 3 and 7) as well as partially inhibited by the anti-USF2 polyclonal antibodies (both lanes 9), but were unaltered by an irrelevant anti-vpr polyclonal antibody (lanes 8). Finally, the protein complex formation of both labelled LTR NRE and USF oligonucleotides (lanes 6) was partially inhibited by the NFIL-6 oligonucleotide, used as competitor although no alteration and no upshift was observed with anti-NFIL-6 antibody (lanes 11). Therefore, huGATA-3 does not appear to interact with LTR NRE oligonucleotide under EMSA conditions.

An Interaction between LTR NRE and huGATA-3, as Well as USF, Is Observed by SPR

EMSA profiles of LTR NRE and USF oligonucleotides are very similar and no GATA-3/LTR NRE interaction was revealed by EMSA although previously reported by *in vitro* footprinting (22). For SPR assays, the biotinylated LTR NRE oligonucleotide was immobilized (600 RU) onto a BIAcore streptavidin covered sensor chip surface. Thirty microliters (6 μg) of each nuclear extract were first injected alone on the LTR NRE, immobilized on the sensor chip. The binding observed 200 s after injection was higher with 8E5 t0 (236 RU), expressing higher level of factors, than that observed with A3.01 t6 (186 RU, see Fig. 4).

After injection of nuclear extracts, the proteins interacting with the oligonucleotide were recovered by elution and subjected to SDS PAGE, Western-blotting and EMSA, as we previously described (26). After binding to immobilized LTR NRE, neither USF nor

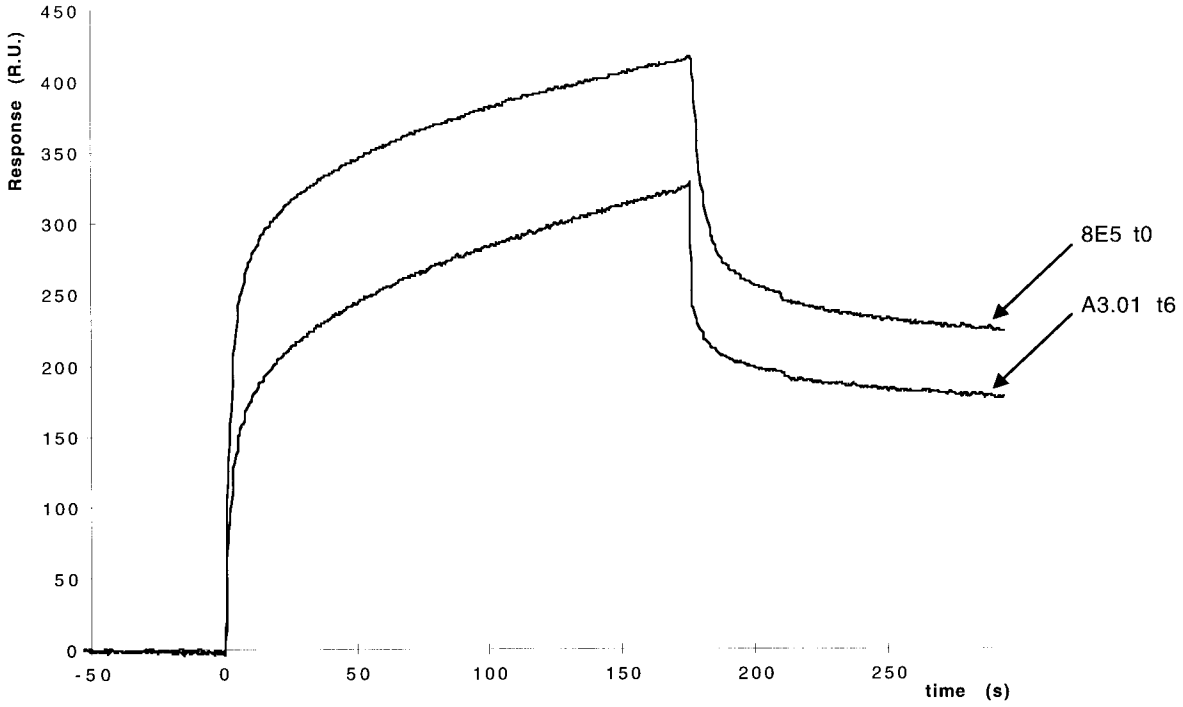


FIG. 4. Sensorgram depicting in real-time the interaction of A3.01 t6 or 8E5 t0 nuclear extract proteins on immobilized LTR NRE oligonucleotide. Six micrograms (in 30 μl) of both nuclear extracts (A3.01 or 8E5) was injected at a flow rate of 10 μl/min onto immobilized LTR NRE oligonucleotide (600 RU).

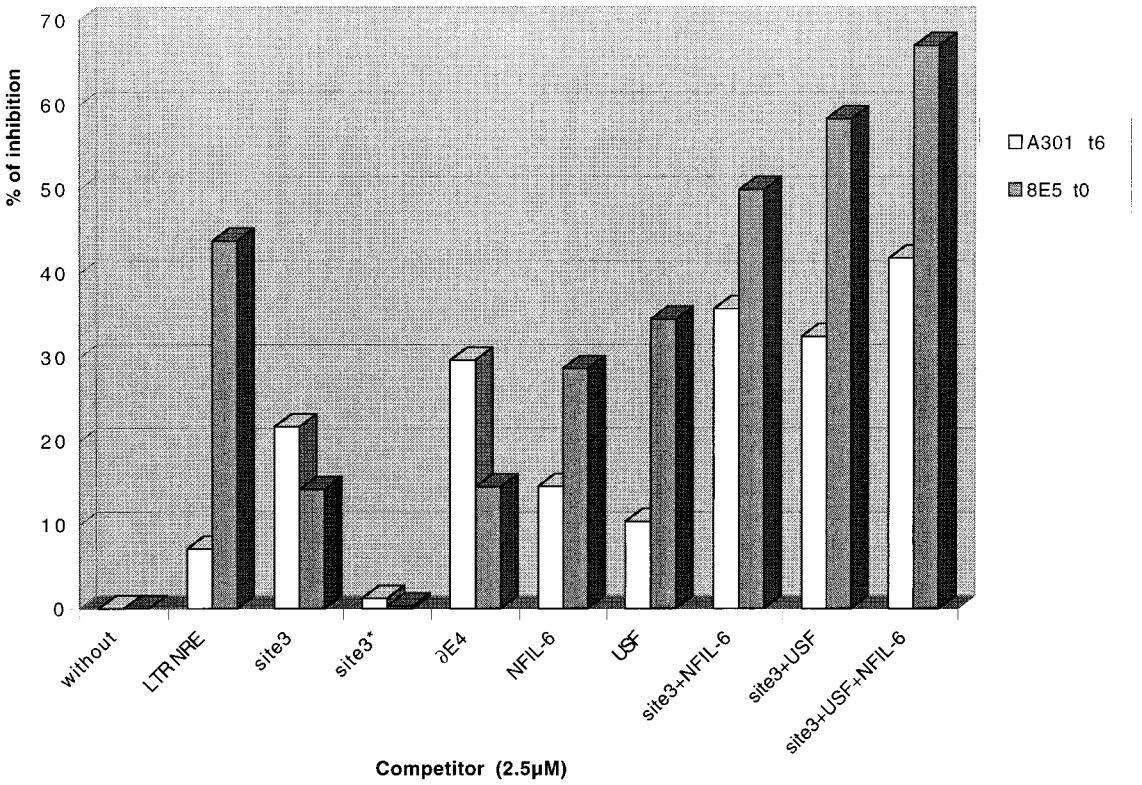


FIG. 5. SPR assays with either A3.01 t6 or 8E5 t0 nuclear extracts alone or in the presence of several oligonucleotides used as competitors: single or in combination. Several GATA sites, LTR NRE (site 2), LTR GATA site 3, its mutated counterpart (site 3*) and δE4 as well as NFIL-6 and USF oligonucleotides were used as competitor (2.5 μM) and injected alone or in combination with either A3.01 t6 or 8E5 t0 (6 μg in 30 μl) onto the immobilized LTR NRE oligonucleotide (600 RU). The results are expressed as the percentages of inhibition measured at 200 s of the binding without competitor (A3.01 t6: 186 RU; 8E5 t0: 236 RU of Fig. 4).

huGATA-3 could be detected in the eluted fractions. SPR competition assays were therefore employed to identify the protein(s) interacting with LTR NRE. The two nuclear extracts were injected alone (Fig. 4) or with specific competitors (2.5 μM). In Fig. 5, the results are expressed, for both nuclear extracts, as an inhibition percentage of interaction observed without competitor.

When the different GATA oligonucleotides were used as single competitors, binding between the immobilized LTR NRE and the factors present in the nuclear extracts was inhibited by 7 to 42%. In contrast, site 3* oligonucleotide, mutated in the GATA sequence (Fig. 1B), was unable to out compete the complex. These results suggest that, under real-time conditions, huGATA-3 probably interacts with the LTR NRE. Surprisingly, with the A3.01 t6 extract, the most efficient competitor was the canonical δE4 oligonucleotide (30% of inhibition), followed by site 3 (20%) and finally by LTR NRE itself (7%). This result is in good agreement with the higher level of huGATA-3 compared to that of USF in this extract (USF being practically absent, Fig. 2). Moreover, the magnitude of inhibition appeared to be correlated with the affinity of the GATA-3 transcription factor for the different oligonucleotides; i.e. a greater affinity for δE4 than for site 3, which was in turn greater than for LTR NRE (site 2) as determined by EMSA and BIAcore experiments (26). The very low inhibition driven by the LTR NRE site 2 might be due to the non-canonical GATA sequence since this sequence was reported to be protected in DNAse I protection assays (22). With the 8E5 t0 extract and the same competitors, the magnitude of inhibition was similar (approximately 12%) for the two canonical GATA oligonucleotides (site 3 and δE4) and higher for LTR NRE (over 40%). This is in accordance with the high level of both USF and huGATA-3 proteins in this extract suggesting that LTR NRE would be able to compete for both USF and huGATA-3. In addition, several antibodies were injected over the proteins of the 8E5 t0 nuclear extract bound to the immobilized LTR NRE (600 RU), to confirm the interaction with the GATA-3. An enlarged view of the post-injection phase, following antibody injection, is presented in Fig. 6. The baseline has been adjusted to zero after injection (300 s) of the 8E5 t0 extract in order to visualize the additional binding response induced by the antibodies. Anti-huGATA-3 showed an additional binding response, when compared to irrelevant sera (anti-GST, anti-fra-1 and anti-c-jun polyclonal antibodies) and

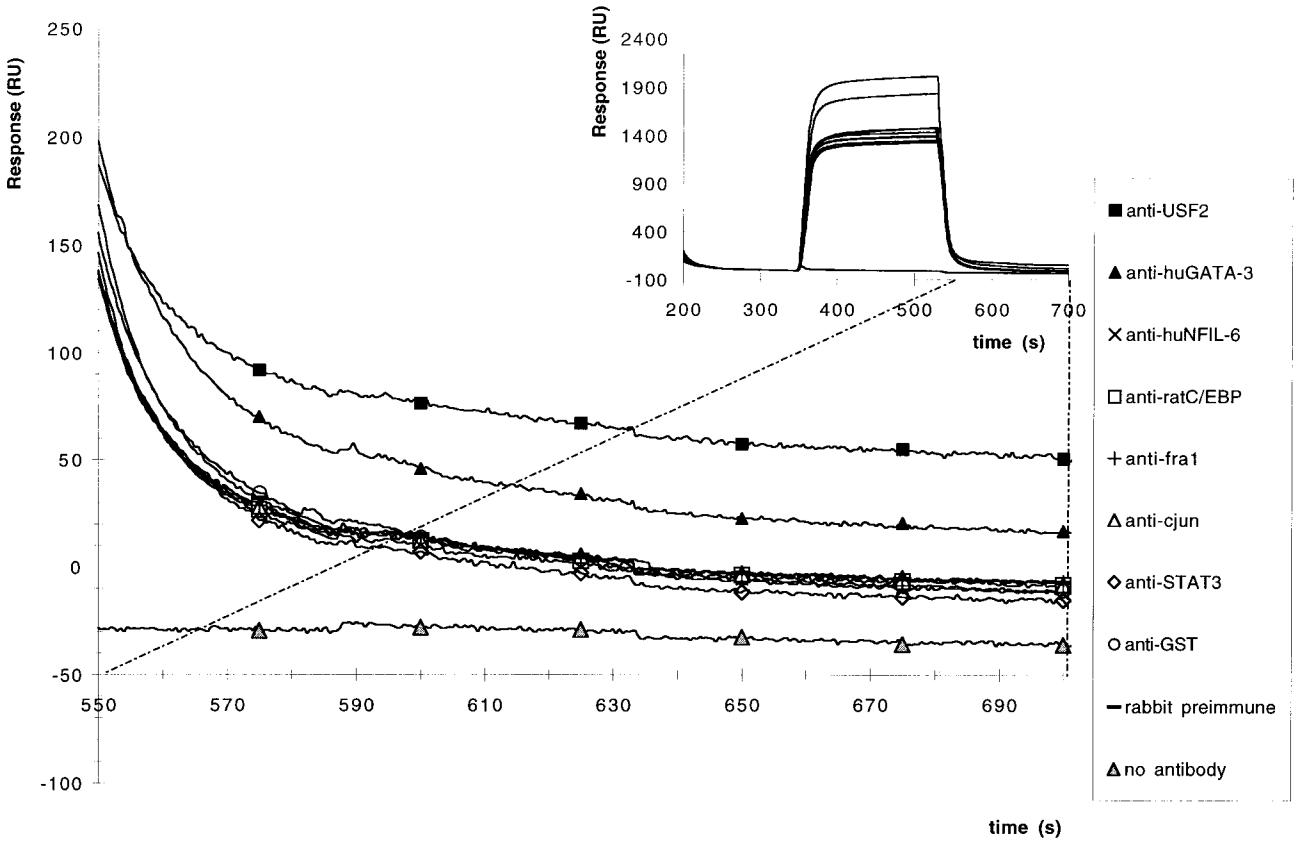


FIG. 6. SPR assays with several antibodies injected onto the 8E5 t0/LTR NRE complexes. Six micrograms (in 30 μl) of 8E5 t0 nuclear extract was previously injected at a flow rate of 10 μl/min onto immobilized LTR NRE oligonucleotide (600 RU). The baseline was adjusted to zero before antibody injection. Thirty microliters (0.3 μg) of several antibodies—anti-USF2, anti-huGATA-3, anti-huNFIL-6, anti-ratC/EBP, anti-transcription factors (i.e., anti-fra-1, anti-cjun and anti-STAT3), anti-GST polyclonal antibodies, and rabbit preimmune serum as control antibodies—was then injected onto complexes formed with LTR NRE. Here is an enlarged view of the postinjection phases of the sensorgram is presented in the inset.

control pre-immune rabbit anti-serum. Thus, taken together, the results of competition and antibody experiments indicate that huGATA-3 binds to the LTR NRE oligonucleotide under SPR conditions. These data are in agreement with the report of Yang *et al.* (22) suggesting the involvement of the different LTR GATA sites in the regulation of HIV-1 transcription. Indeed, Yang *et al.* have shown that: 1) *in vitro* footprinting analysis revealed 6 GATA target sequences in the LTR U3 region, 2) in Hela cells, huGATA-3 stimulated the HIV LTR driven transcription as much as 10 fold and 3) mutation of all GATA sites led to the abrogation of the GATA-3 mediated stimulation and, to a marked reduction of transcription in Jurkat cells.

Naturally, as previously shown by EMSA (Fig. 3), SPR competition experiments revealed interaction between USF and the LTR NRE. Indeed, the USF oligonucleotide (2.5 μM) can competitively inhibit LTR NRE/protein interactions (Fig. 5). This competitor, however, is more efficient in inhibiting the interaction of LTR NRE with the 8E5 t0 extract (35%) than with the A3.01 t6 extract (10%), in agreement with the higher level of USF proteins in the 8E5 t0 extract. Additionally, the anti-USF2 antibody increased the binding response of the LTR NRE/8E5 t0 interaction (Fig. 6).

Analysis of LTR NRE/NFIL-6 Interaction by SPR

In contrast to USF and huGATA-3, NFIL-6 appeared to be present only in the 8E5 t0 extract (Fig. 2). The specificity of interaction was verified, as for both USF and GATA-3, by competition experiments with LTR NRE and several oligonucleotides, and with two antibodies against human NFIL-6 and rat C/EBP. Moreover, both anti-human NFIL-6 and anti-rat C/EBPβ were unable to bind substantially to the complexes in BIAcore assays (Fig. 6). Hence, neither EMSA nor SPR assays can demonstrate a conclusive interaction between NFIL-6 factor(s) and LTR NRE.

However, NFIL-6 has been shown to play a significant role in the fine tuning of HIV-1 regulation of transcription, either positive in U937 promonocytic cell line (27, 28) or negative in brain-derived cells) (29). The

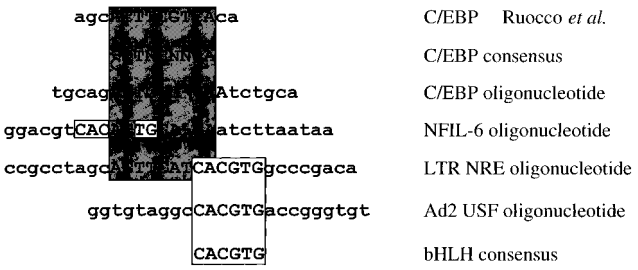


FIG. 7. Binding sites shared by C/EBP, USF, and NF-IL6 oligonucleotides. The sequence of each oligonucleotide is presented with its C/EBP binding site (shaded square) and the bHLH site (open square). The C/EBP and bHLH consensus motifs are represented at second and bottom line, respectively. The oligonucleotide used by Ruocco *et al.* (34) is also seen at the top.

biological relevance of HIV-1 C/EBP is suggested by the fact that the C/EBP sites appear to be conserved in primary isolates from AIDS patients over several years (30). Moreover, these sites are functional in the production of negative strand RNA transcripts from a novel HIV-1 promoter (3′ LTR, Ref. 31). Furthermore, HIV-1 isolates carrying linker mutations of the HIV-1 C/EBP at −174/−166 have an enhanced infectivity (32), suggesting a negative role for the HIV-1 C/EBP sequence in vivo. It is likely that, in spite of our results, NFIL-6 interacts with the LTR NRE region.

In our experiments, the partial inhibition mediated by the NFIL-6 oligonucleotide in EMSA (Fig. 3, lane 6) although less efficient than with USF oligonucleotide (lane 7) and in SPR competition experiments (Fig. 5) might be due to shared interaction of USF and NFIL-6 regulatory elements for USF protein. In SPR experiment, under real-time conditions (Fig. 5), NFIL-6 oligonucleotide was capable, as USF oligonucleotide, of inhibiting LTR NRE/8E5 t0 or A301 t6 interactions. The maximal level (over 50%) was reached when USF or NFIL-6 oligonucleotides were used in combination with the canonical LTR GATA site 3 and the 8E5 t0 extract, where both GATA-3 and USF transcription factors are efficiently expressed when compared to 30–35% inhibition observed with A3.01 t6 extract. Finally, the CACPuNTG site present in the NFIL-6 oligonucleotide (Fig. 7) could mimic a bHLH motif, as described in yeast (33), and bind USF protein, thus competing out USF binding to the LTR NRE or USF oligonucleotides (Fig. 3). In contrast, the Ad2 USF oligonucleotide although a strong inhibitor of LTR NRE/USF interaction as it does not possess a C/EBP-like sequence (Fig. 7) did not succeed in out-competing the binding of the NFIL-6 protein to labelled NFIL-6 oligonucleotide (Fig. 2).

Finally, the lack of substantial interaction between LTR NRE oligonucleotide and NFIL-6 protein of the 8E5 t0 extract could be explained by the hindrance of this interaction by the USF factor. Indeed, the 12 nucleotide long USF binding element of the LTR NRE, comprising the CACGTG central helix-loop-helix leucine zipper (bHLH) consensus motif, target for USF, overlaps the (A/C)TTNCNN(A/C)A consensus sequence of the C/EBP family (Fig. 1A). A similar DNA sequence (shaded box of Fig. 7) lacking the 3′ end of the USF site present in the LTR NRE was shown by Ruocco *et al.* (34) to be capable of interacting with over expressed C/EBPβ or C/EBPδ proteins in non T-cell lines (i.e. without GATA-3 expression).

Actually, efficient binding of USF to the LTR NRE may inhibit NFIL-6 interaction. Therefore, in absence of USF, NFIL-6 might be able to interact with the LTR NRE, since expression of USF factor is decreased during T-cell activation, while GATA-3 expression is quite stable and NFIL-6 is induced. Hence the 3 overlapping regulatory targets might govern the regulation of transcription, according to the diversity of transcription factors (35) that varied with the cellular context (lymphocytes *versus* macrophages) or with the adaptive context during the differentiation and activation process of the cells.

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REFERENCES

1. Gaynor, R. (1992) *Aids* **6,** 347–63.
2. Trono, D. (1995) *Cell* **82,** 189–192.
3. Garcia, J. A., Wu, F. K., Mitsuyasu, R., and Gaynor, R. B. (1987) *Embo J.* **6,** 3761–3770.
4. Lu, Y., Stenzel, M., Sodroski, J. G., and Haseltine, W. A. (1989) *J. Virol.* **63,** 4115–4119.
5. Lu, Y. C., Touzjian, N., Stenzel, M., Dorfman, T., Sodroski, J. G., and Haseltine, W. A. (1990) *J. Virol.* **64,** 5226–5229.
6. Sawadogo, M., Van Dyke, M. W., Gregor, P. D., and Roeder, R. G. (1988) *J. Biol. Chem.* **263,** 11985–11993.
7. Carthew, R. W., Chodosh, L. A., and Sharp, P. A. (1987) *Genes Dev.* **1,** 973–980.
8. Moncollin, V., Stalder, R., Verdier, J. M., Sentenac, A., and Egly, J. M. (1990) *Nucleic Acids Res.* **18,** 4817–4823.
9. Giacca, M., Gutierrez, M. I., Menzo, S., Di Fagagna, F. D., and Falaschi, A. (1992) *Virology* **186,** 133–147.
10. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T., and Kishimoto, T. (1990) *Embo J.* **9,** 1897–1906.
11. Poli, V., Mancini, F. P., and Cortese, R. (1990) *Cell* **63,** 643–653.
12. Tesmer, V. M., Rajadhyaksha, A., Babin, J., and Bina, M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 7298–7302.
13. Williams, S. C., Cantwell, C. A., and Johnson, P. F. (1991) *Genes Dev.* **5,** 1553–1567.
14. Thomassin, H., Hamel, D., Bernier, D., Guertin, M., and Belanger, L. (1992) *Nucleic Acids Res.* **20,** 3091–3098.
15. Buck, M., Turler, H., and Chojkier, M. (1994) *Embo J.* **13,** 851–860.

16. Descombes, P., Chojkier, M., Lichtsteiner, S., Falvey, E., and Schibler, U. (1990) *Genes Dev.* **4,** 1541–1551.
17. Joulin, V., Bories, D., Eleouet, J. F., Labastie, M. C., Chretien, S., Mattei, M. G., and Romeo, P. H. (1991) *Embo J.* **10,** 1809–1816.
18. Ko, L. J., Yamamoto, M., Leonard, M. W., George, K. M., Ting, P., and Engel, J. D. (1991) *Mol. Cell. Biol.* **11,** 2778–2784.
19. Marine, J., and Winoto, A. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 7284–7288.
20. Ko, L. J., and Engel, J. D. (1993) *Mol. Cell. Biol.* **13,** 4011–4022.
21. Merika, M., and Orkin, S. H. (1993) *Mol. Cell. Biol.* **13,** 3999–4010.
22. Yang, Z., and Engel, J. D. (1993) *Nucleic Acids Res.* **21,** 2831–2836.
23. Yang, Z., Gu, L., Romeo, P. H., Bories, D., Motohashi, H., Yamamoto, M., and Engel, J. D. (1994) *Mol. Cell. Biol.* **14,** 2201–2212.
24. Folks, T., Benn, S., Rabson, A., Theodore, T., Hoggan, M. D., Martin, M., Lightfoote, M., and Sell, K. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4539–4543.
25. Folks, T. M., Powell, D., Lightfoote, M., Koenig, S., Fauci, A. S., Benn, S., Rabson, A., Daugherty, D., Gendelman, H. E., Hoggan, M. D., and *et al.* (1986) *J. Exp. Med.* **164,** 280–290.
26. Galio, L., Briquet, S., Cot, S., Guillet, J. G., and Vaquero, C. (1997) *Anal. Biochem.* **253,** 70–77.
27. Henderson, A. J., Zou, X., and Calame, K. L. (1995) *J. Virol.* **69,** 5337–5344.
28. Henderson, A. J., Connor, R. I., and Calame, K. L. (1996) *Immunity* **5,** 91–101.
29. Mondal, D., Alam, J., and Prakash, O. (1994) *J. Mol. Neurosci.* **5,** 241–258.
30. Michael, N. L., D'Arcy, L., Ehrenberg, P. K., and Redfield, R. R. (1994) *J. Virol.* **68,** 3163–3174.
31. Michael, N. L., Vahey, M. T., d'Arcy, L., Ehrenberg, P. K., Mosca, J. D., Rappaport, J., and Redfield, R. R. (1994) *J. Virol.* **68,** 979–987.
32. Kim, J. Y., Gonzalez-Scarano, F., Zeichner, S. L., and Alwine, J. C. (1993) *J. Virol.* **67,** 1658–1662.
33. Bram, R. J., and Kornberg, R. D. (1987) *Mol. Cell. Biol.* **7,** 403–409.
34. Ruocco, M. R., Chen, X., Ambrosino, C., Dragonetti, E., Liu, W., Mallardo, M., De Falco, G., Palmieri, C., Franzoso, G., Quinto, I., Venuta, S., and Scala, G. (1996) *J. Biol. Chem.* **271,** 22479–22486.
35. Paillard, F., Sterkers, G., and Vaquero, C. (1992) *Biochem. Biophys. Res. Commun.* **186,** 603–611.