# The HIV-1 Virion-associated Protein Vpr Is a Coactivator of the Human Glucocorticoid Receptor

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## Summary

The HIV-1 virion-associated accessory protein Vpr affects both viral replication and cellular transcription, proliferation, and differentiation. We report that Vpr enhances the activity of glucocorticoids in lymphoid and muscle-derived cell lines by interacting directly with the glucocorticoid receptor and general transcription factors, acting as a coactivator. Vpr contains the signature motif LXXLL also present in cellular nuclear receptor coactivators, such as steroid receptor coactivator 1 and p300/CREB-binding protein, which mediates their interaction with the glucocorticoid and other nuclear hormone receptors. A mutant Vpr molecule with disruption of this coactivator signature motif lost its ability to influence transcription of glucocorticoid-responsive genes and became a dominant-negative inhibitor of Vpr, possibly by retaining its general transcription factor–binding activities. The glucocorticoid coactivator activity of Vpr may contribute to increased tissue glucocorticoid sensitivity in the absence of hypercortisolism and to the pathogenesis of AIDS.

Key words: nuclear receptors • AIDS • mouse mammary tumor virus • p300/CBP • steroid receptor coactivator 1

The HIV-1 protein Vpr, a 96–amino acid virion-associated accessory protein, has multiple functions (for reviews, see references 1–4). Vpr enhances the replication of HIV-1 virus in lymphocyte- and monocyte-derived cell lines (5), is a weak transcriptional activator of several viral promoters (6), causes host cell arrest in the G2/M phase of the cell cycle (7–10), and induces terminal differentiation in some cell lines (11). Vpr has been proposed to increase the translocation of the HIV-1 preintegration complex into the nucleus, and promotes efficient infection of nondividing macrophages (12–15). Vpr was also reported to bind to a host 41-kD cytosolic Vpr interacting protein (Rip-1) and to associate with the activated glucocorticoid receptor (GR)[1] (16).

Since Vpr has been shown to circulate at detectable levels in HIV-1–infected individuals, its effects may be extended to cells not infected by HIV-1 (17, 18). To explore the potential involvement of Vpr in the pathogenesis of AIDS, we examined the effect of Vpr on glucocorticoid-responsive promoters and the interactions of Vpr with GR and components of the GR-induced transcription complex in lymphoid and rhabdomyosarcoma cell lines. We show that Vpr is a virus-encoded coactivator of the GR, suggesting that it may contribute to the development of symptoms in patients with AIDS such as muscle wasting in the absence of increased glucocorticoid levels.

Glucocorticoids play major roles in maintaining resting and stress-related homeostasis (19) and also exert antiinflammatory and immunosuppressive effects, which have made them invaluable therapeutic agents in numerous diseases (20). Host tissue sensitivity to glucocorticoids may be altered in several disease states, becoming one of the determinants of disease outcome; both glucocorticoid hypersensitivity and resistance have been reported (21). Glucocorticoid hypersensitivity could be involved in the immunosuppression and myopathy and muscle wasting observed in patients with AIDS, even in the presence of normal plasma cortisol concentrations. Glucocorticoids exert their ubiquitous and

[1]*Abbreviations used in this paper:* CAT, chloramphenicol acetyltransferase; CBP, CREB-binding protein; CREB, cAMP-response element binding protein; GFP, green fluorescence protein; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GST, glutathione *S*-transferase; h, human; MMTV, mouse mammary tumor virus; NF, nuclear factor; PKA, protein kinase A; RSV, Rous sarcoma virus; SRC, steroid receptor coactivator; SV40, simian virus 40.

pleiotropic effects through the GR, a ligand-dependent transcription factor. Binding of the hormone to the receptor causes it to dissociate from a heterooligomer of heat shock proteins and to translocate into the nucleus, where it binds as a homodimer to specific DNA enhancer elements, the glucocorticoid response elements (GREs), or to other transcription factors, such as AP-1 and nuclear factor (NF)-κB (22).

Several host coactivators of the GR have been described that directly interact with the GR and components of the transcription initiation complex to enhance the glucocorticoid signal to the transcription machinery (23, 24). Different signal transduction systems share several of these newly described coactivators, which may act not only in a synergistic but also in an inhibitory fashion (25–27). It recently became known that some coactivators possess histone acetyltransferase activity, which helps loosen promoter DNA from the tightly bound histone octamers by acetylating the free $NH_2$ termini of lysine residues, facilitating the access of components of the transcriptional initiation complex to the promoter region (28–30). Here we show that Vpr enhances the activation of a glucocorticoid-inducible gene. We studied the mechanism of this effect and especially the interactions of Vpr with other components of the transcription machinery. It was determined that Vpr interacts directly with components of the RNA polymerase holoenzyme and contributes to the formation of a large complex competent for transcription.

## Materials and Methods

*Plasmids.* The pCDNA3-VPR and pGEX-4T3-VPR vectors for expression of Vpr and GST-Vpr, respectively, were constructed using PCR-amplified Vpr sequence in pNLA1 (a gift from Dr. K. Strebel, National Institutes of Health, Bethesda, MD), which contains the full coding sequence of the wild-type Vpr (31). The plasmids used were pCDNA3 (Invitrogen) and PGEX-4T3 (Amersham Pharmacia Biotech), respectively. The pCMV-FLAG-VPR and pCMV-FLAG-VPR(36–96) vectors were generated by in-frame insertion of PCR-amplified Vpr cDNA corresponding to amino acids 1–96 or 36–96 between the HindIII and XbaI sites of pFLAG-CMV-2 (Eastman Kodak Co.). These plasmids express Vpr fragments tagged at the $NH_2$ terminus by the FLAG epitope under the control of the CMV promoter. The 64 leucine to alanine–substituted mutant Vpr cDNAs were prepared by PCR-assisted in vitro mutagenesis of the Vpr expression vectors and named pCDNA3-VPRL64A, PGEX-4T3-VPRL64A, and pCMV-FLAG-VPRL64A, respectively. Similarly, a vector expressing Vpr with an arginine to alanine substitution at position 80 was named pCDNA3-VPRR80A. For the yeast two hybrid assay, DNA-binding LexA fusion vector pEG202, B42-activation domain fusion vector pJG4-5, and *lacZ* reporter plasmid pSH18-34 were used (32). Plasmids LexA-Vpr and LexA-Tat express Vpr or Tat fused to LexA DNA-binding domain, respectively (33). LexA-VprL64A and LexA-VprR80A expression vectors were constructed by PCR-assisted in vitro mutagenesis. The full-length human (h)GRα protein fused to B42-activation domain expression vector was constructed by in-frame insertion of human GRα cDNA into pJG4-5 (pJG45-GRα). The other plasmids used were as follows: pRc/RSV, pCR3.1, and pHook™-1 (Invitrogen); pSV40-β-Gal (Promega Corp.); β-actin-luc, containing −472 to +49 of human β-actin promoter, pRShGRα and pGR107, containing the full-length coding region of hGRα, and pRS-erbA$^{-1}$, containing a thyroid receptor cDNA in inverse orientation but otherwise similar to pRShGRα (all gifts from Dr. R. Evans, Salk Institute, La Jolla, CA); pF25-GFP-hGRα, expressing green fluorescent protein (GFP) tagged at the $NH_2$ terminus of hGRα; pSVLPRA, containing the full-length coding region of human progesterone receptor A (a gift from S.S. Simons, Jr., National Institutes of Health); pRc/RSV-CREB341, containing the full-length coding region of cAMP-responsive element binding protein (CREB); RSV-PKA, which expresses the constitutive active form of protein kinase A (PKA); p(−71)SRIF-CAT, containing nucleotides −71 to +53 of rat somatostatin 5′ flanking region (all gifts from Dr. R.H. Goodman, Vollum Institute, Portland, OR); CMVβ-p300-CHA expression vector (a gift from Dr. D. Livingston, Dana-Farber Cancer Institute, Boston, MA); pCR-SRC-1a (a gift from Dr. B.W. O'Malley, Baylor College of Medicine, Houston, TX); HE0, containing the full-length coding region of human estrogen receptor α (a gift from Dr. P. Chambon, University of Strasbourg, Strasbourg, France); pRSV-β-Gal and ERE-tk-luc, containing synthetic vitellogenin A2 ERE sequence from −336 to −310 (gifts from Dr. J. Segars, National Institutes of Health), pMMTV-luc (a gift from Dr. G. Hager, National Institutes of Health), pHH-luc and pM-luc (American Type Cell Collection), containing either full-length MMTV-LTR, −223 to +105, or −109 to +105 of the MMTV-LTR, respectively; and the synthetic GRE-containing plasmids, pGRE$_2$-NF1-E1B-CAT, pNF1-E1B-CAT, pGRE$_2$-E1B-CAT, and pE1B-CAT (gifts from Dr. J.A. Cidlowski, National Institutes of Health, Research Triangle Park, NC).

*Cell Transfections.* A204, HS729, and CV-1 cells were transfected using lipofectin (Life Technologies); CEM and Jurkat cells were transfected using electroporation (960 μF, 250 mV [34]). The cells were treated with dexamethasone 24 h after transfection, and cell lysates were collected after a 24-h incubation with the steroid. Luciferase activity, chloramphenicol acetyltransferase (CAT) activity, and protein concentrations were determined as described (35–37). All measurements of the reporter gene activity were conducted in triplicate transfections and averaged. Cells from the same transfection were used with and without dexamethasone whenever the effects of the steroid were studied.

*Magnetic Cell Sorting and Immunoblots for the GR and Vpr.* The transfection-positive cells ($0.5–1 \times 10^7$ cells) were enriched by the pHook™-1 plasmid method following the company's recommendations, and homogenized and centrifuged at 300 *g* for 5 min in Taps buffer (25 mM Taps [pH 8.0], 2 mM dithiothreitol, Complete™ tablets 1 Tab/50 ml [Boehringer Mannheim], 10% glycerol). The supernatants were used in Western blot analyses, with affinity-purified polyclonal rabbit anti–human GR antibodies (Affinity Bioreagents), anti-GRα antibody (36), HIV-1$_{NL4-3}$ Vpr antiserum (National Institutes of Health AIDS Research and Reference Reagent Program), or an anti-FLAG (M2) antibody (Eastman Kodak Co.).

*In Vitro Binding Assay.* $^{35}$S-labeled human GRα were generated by in vitro translation and tested for interaction with GST-Vpr, GST-VprL64A, or glutathione *S*-transferase (GST) protein, immobilized on glutathione-sepharose beads in the presence or absence of $10^{-6}$ M dexamethasone and/or $10^{-5}$ M RU 486 in buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM NaCl, 1 mM EDTA, 0.1% NP-40, 10% glycerol, and 0.1 mg/ml BSA at 4°C for 1 h. After vigorous washing with the buffer, proteins were eluted and separated on a 4–20% SDS-PAGE gel. Gels were fixed and exposed on film.

*Yeast Two Hybrid Assay*. Yeast strain EGY48 (Clontech) was transformed with the *lacZ* reporter plasmid pSH18-34, pJG45-GRα coding sequence fused to B42-activation domain under the control of the galactose-inducible promoter, and pLexA-Vpr coding for wild-type or mutant Vpr-LexA DNA-binding domain fusion in pEG202 vector (32). The cells were grown to the early stationary phase in a selective medium with galactose to induce GRα fusion expression, and permeabilized with $CHCl_3$-SDS treatment, and the β-galactosidase activity was measured in the cell suspension using a colorimetric assay with ONPG as a substrate.

*Coimmunoprecipitation of Vpr*. 3 h before cell lysis, cells were exposed to $10^{-6}$ M dexamethasone and/or $10^{-5}$ M RU 486. Cell lysis and coimmunoprecipitation were carried out using lysis buffer (50 mM Tris-HCl [pH 7.4], 400 mM NaCl, 0.2% NP-40, Complete™ tablets 1 Tab/50 ml). Proteins were precipitated by anti–human TFIID (TBP) antibody, anti–human TFIIB antibody (Santa Cruz Biotechnology, Inc.), or by anti-GRα antibody bound to protein A Trisacryl (Pierce Chemical Co.). After blotting on nitrocellulose membrane, FLAG-tagged Vprs were detected by anti-FLAG (M2) antibody.

*Statistical Analyses*. Statistical analysis was carried out by analysis of variance, followed by Student's *t* test with Bonferroni correction for multiple comparisons.

## Results

*Vpr Enhances Glucocorticoid Response*. We first examined the effect of Vpr on the LTR promoter of mouse mammary tumor virus (MMTV)-driven luciferase activity in human T lymphoblastoma–derived CEM and Jurkat cells, and human rhabdomyosarcoma–derived A204 and HS729 cells. We cotransfected different amounts of a Vpr expression vector, pCDNA3-VPR, with the dexamethasone-responsive plasmid pMMTV-luc (Fig. 1, A–D). Vpr induced a 3.4-, 4.4-, 20-, and 4.5-fold increase of luciferase activity in the dexamethasone-stimulated cell lines, respectively, whereas it had minimal effects in the absence of dexamethasone. As A204 cells showed the highest increase of luciferase activity, we conducted a dexamethasone titration experiment in the presence or absence of pCDNA3-VPR in this cell line (Fig. 1 E). The dose–response curve of the MMTV promoter to dexamethasone was potently shifted in the presence of Vpr, indicating that this protein potentiates the glucocorticoid signal transduction pathway in a fashion reminiscent of a classic coactivator (23).

To examine the dependence of the coactivator effect of Vpr on the glucocorticoid ligand–receptor interaction, we conducted several experiments in A204 cells using the GR antagonist RU 486. The effect of Vpr on the MMTV promoter was antagonized by RU 486 in a dose-dependent fashion (Fig. 1 F). Vpr-enhanced luciferase activity was completely abolished by $10^{-5}$ M RU 486, and returned to the levels seen in the absence of dexamethasone.

To show the dependency of the Vpr effect on the presence of GREs, we used three MMTV deletion mutants, four GRE-containing promoter constructs, the simian virus 40 (SV40) and Rous sarcoma virus (RSV) promoters, and the human β-actin promoter, which contain no recognizable GREs. Because the NF1 site is important for the full activation of the MMTV promoter, we used promoter constructs containing a synthetic NF1 site (Fig. 2 A). As shown in Fig. 2, B and C, the coactivator effect of Vpr depended on the presence of GREs. Decreasing the numbers of GREs in the MMTV or in synthetic GRE promoters was associated with diminishing Vpr coactivator activity. Vpr had no or minimal effect on the synthetic promoters not containing GRE sites and on the SV40, RSV, and β-actin promoters (data not shown). We also used CV-1 cells, which contain no functional GR, to show the requirement of the GR for the coactivator effect of Vpr on the MMTV promoter. Vpr-dependent activation could be observed only when CV-1 cells were cotransfected with the GRα expression vector pRShGRα (Fig. 2 D).

To rule out the possibility that Vpr might cause activation of the MMTV promoter by changing the levels of the GR or the ratio of the GR α and β isoforms, we examined the protein levels of the two isoforms of the GR in A204 cells (38). We enriched the transfected cell population up to 90% by using Capture-Tec™ beads and examined the effect of Vpr on the levels of the GR isoforms by Western blot. As shown in Fig. 2 E, Vpr affected neither the levels of these isoforms nor the isoform ratio in A204 cells.

We also examined the possibility that Vpr influenced the translocation of the GR induced by dexamethasone in A204 cells by using GFP-tagged hGRα (GFP-GRα [39, 40]). Vpr did not change the translocation rate or efficiency of dexamethasone-activated GFP-GRα (data not shown). In addition, dexamethasone did not affect localization of GFP-tagged Vpr at concentrations sufficient to translocate the GFP-GRα from the cytosol into the nucleus (data not shown).

*Vpr Interacts Directly with GR through the Coactivator Motif LXXLL*. The recent discovery that cellular nuclear receptor coregulators contain one or more signature motifs (LXXLL) through which they interact with nuclear hormone receptors and exert their coregulator effects (41) prompted examination of the Vpr sequence for such motifs. Since Vpr contains the sequence LQQLL at amino acids 64–68, a region of α-helical secondary structure (42), we examined the functional importance of this motif by generating mutant Vpr proteins with disrupted sequences. A mutant Vpr containing a leucine to alanine substitution at amino acid 64 (VprL64A) failed to exert any coactivator effect in our assays, and showed a concentration-dependent dominant-negative effect on the wild-type Vpr (Fig. 3 A). In contrast, VprL64A was fully functional in arresting cells in G2/M phase of the cell cycle. A second point mutant, VprR80A, showed the opposite phenotype; it had coactivation function similar to Vpr, but did not arrest cells in G2/M (33). These results show that the glucocorticoid coactivator activity of Vpr is distinct from its cell cycle arrest function and requires an intact LXXLL domain.

To determine the specificity of the Vpr coactivator effect on the glucocorticoid and other nuclear receptor signal transduction pathways, we examined the effect of this protein on the progesterone, estrogen, and cAMP signal transduction pathways in A204 cells. In the first two systems, using the MMTV and the vitellogenin ERE-containing

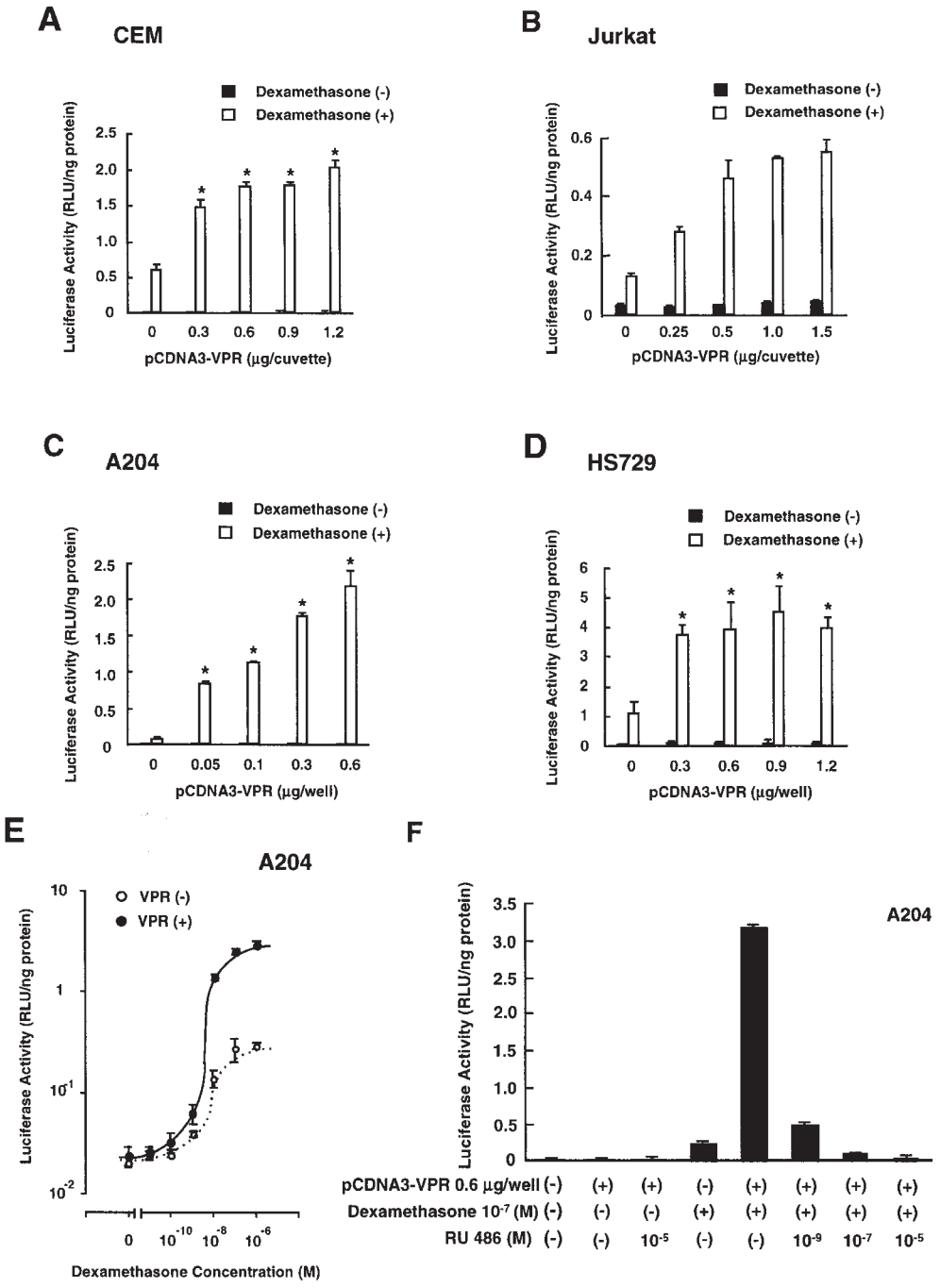


**Figure 1.** (A–D) Vpr potentiates the transactivating effects of dexamethasone on an MMTV-LTR–driven luciferase reporter gene in CEM (A), Jurkat (B), A204 (C), and HS729 (D) cells in a dose-dependent fashion. Cells were transfected with different amounts of pCDNA3-VPR and pMMTV-luc. Black and white bars, mean ± SEM values obtained in the absence or presence of $10^{-7}$ M dexamethasone, respectively. $^{*}P < 0.001$. (E) Vpr produces a typical coactivator shift of the dexamethasone dose–response curve. A204 cells were transfected with pCDNA3-VPR or pCDNA3, and pMMTV-luc. Each point shows the mean ± SEM value. (F) The coactivator effect of Vpr can be antagonized by the glucocorticoid antagonist RU 486 in a dose-dependent fashion. A204 cells were transfected with different amounts of pCDNA3-VPR and pMMTV-luc. The indicated amounts of dexamethasone and/or RU 486 were added into the culture medium 24 h after transfection. Black bars, mean ± SEM values.

promoters, respectively, we detected a small but significant potentiation effect of Vpr by two- and fivefold, respectively. In the third system, using a cAMP-responsive promoter, Vpr showed no coactivator effect, even though the GR and cAMP signal transduction pathways share p300/CBP as a coactivator (25; Fig. 3 B). These findings suggest that the coactivator effect of Vpr is exerted on the glucocorticoid as well as other nuclear receptor signal transduction pathways, and are in agreement with the effects reported for other coactivators of steroid nuclear receptors (25, 26, 41). We also tested the coactivator activity of HIV-2 and SIVmac239 Vpr and Vpx, since these proteins are evolutionary related to HIV-1 Vpr (43), but do not have any LXXLL coactivator signature motifs. None of these proteins showed GR coactivator activity (data not shown).

To test the direct interaction of Vpr and GR, we used in vitro–translated hGRα and bacterially expressed GST-tagged Vpr. Some binding of GR to GST-Vpr was detected in the absence of dexamethasone, whereas binding was increased in the presence of the steroid. In contrast, GR did not bind to the mutant GST-VprL64A (Fig. 4 A). RU 486 antagonized the dexamethasone-induced interac-

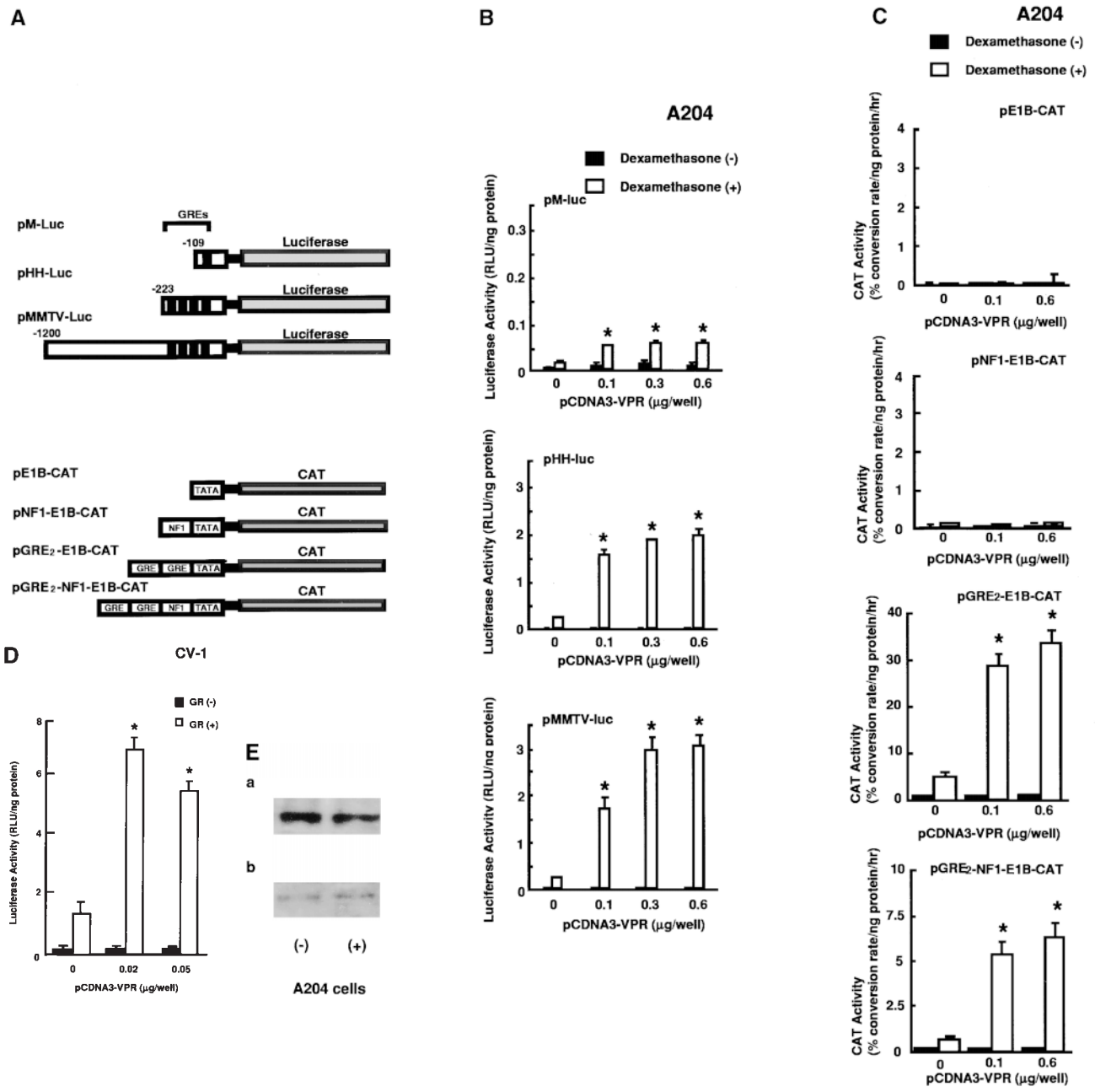


**Figure 2.** (A) MMTV (top) and synthetic GRE-containing (bottom) promoter constructs used to demonstrate dependence of Vpr effect on the presence of GREs. (B and C) Decreasing numbers of GREs in the MMTV (B) or synthetic GRE-containing (C) promoter were associated with decreasing Vpr glucocorticoid coactivator activity. A204 cells were transfected with different amounts of pCDNA3-VPR and pMMTV-luc, pHH-luc, pM-luc, pGRE2-E1B-CAT, pGRE2-NF1-E1B-CAT, pNF1-E1B-CAT, or pE1B-CAT. Black and white bars, mean ± SEM values obtained in the absence or presence of $10^{-7}$ M dexamethasone, respectively. $*P < 0.001$. (D) The coactivator effect of Vpr depends on the presence of functional hGR in CV-1 cells. Cells were transfected with different amounts of pCDNA3-VPR and pMMTV-luc in the presence (white bars) or absence (black bars) of pRShGRα. For control, pRS-erbA$^{-1}$ was transfected instead of pRShGRα. Bars, mean ± SEM values obtained in the presence of $10^{-7}$ M dexamethasone. $*P < 0.001$. (E) Detection of the GR isoforms α and β (a) or only α (b) in A204 cells transfected with pHook™-1 and either pCDNA3 (−) or pCDNA3-VPR (+). Transfection-positive cells were collected by Capture-Tec™ beads. The GR (α and β) and the GRα were detected after blotting by using antibodies that can recognize both the GR α and β (a), and specific antibodies for the GRα (b).

tion of GR to GST-Vpr. We also detected interaction of GR with Vpr in the yeast two hybrid system (Fig. 4 B). Wild-type Vpr and VprR80A, which retains coactivator activity, interacted with the GR and induced β-galactosidase activity, whereas the dominant-negative mutant VprL64A produced no such effect, further supporting a direct interaction between the two molecules in the cell.

*Vpr Facilitates Formation of GR-containing Transcription Complex through Multiple Contacts.* It has been shown that Vpr interacts with the RNA polymerase II ancillary factor

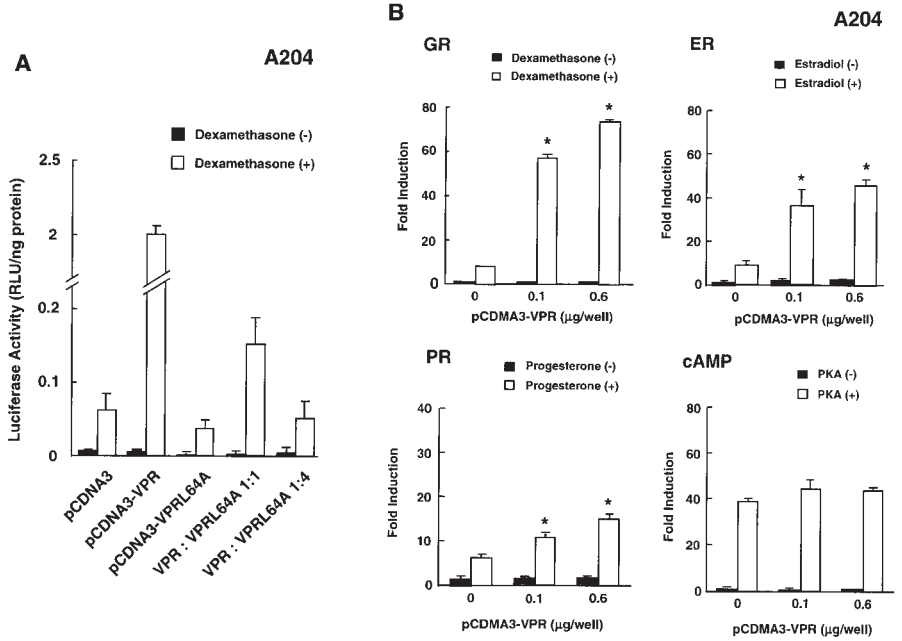


**Figure 3.** (A) The coactivator effect of Vpr depends on the presence of its LXXLL signature motif. A mutant Vpr with a disrupted coactivator motif lost its transcription enhancing effect and gained dominant-negative function. Black and white bars, mean ± SEM values obtained in the absence or presence of $10^{-7}$ M dexamethasone, respectively. (B) The coactivator effect of Vpr is seen on nuclear receptor signal pathways but not on the cAMP signal pathway. A204 cells were transfected with GR, ER, or PR expression vector and pMMTV-luc (for GR and PR) or ERE-tk-luc (for ER) and treated with the corresponding ligands. For analysis of the cAMP-induced pathway, A204 cells were transfected with pRc/RSV-CREB341 and p(−71)SRIF-CAT. To activate CREB, RSV-PKA (white bars) or pRc/RSV (black bars, control) was also transfected. Bars, mean ± SEM values obtained in the absence or presence of $10^{-7}$ M dexamethasone, progesterone, estradiol, and 0.5 μg/well of RSV-PKA, respectively. $^*P < 0.001$.

TFIIB (44). The sequence necessary for this interaction was mapped between amino acids 15 and 77. We examined the coactivator function of an $NH_2$-terminal deletion mutant of Vpr using FLAG-Vpr(36–96), which contains a deletion of the first 35 amino acids of Vpr and does not bind to TFIIB. FLAG-Vpr(36–96) did not show any coactivator effect on the MMTV promoter in A204 cells (Fig. 4 C). In contrast, Vpr and FLAG-Vpr showed similar coactivator

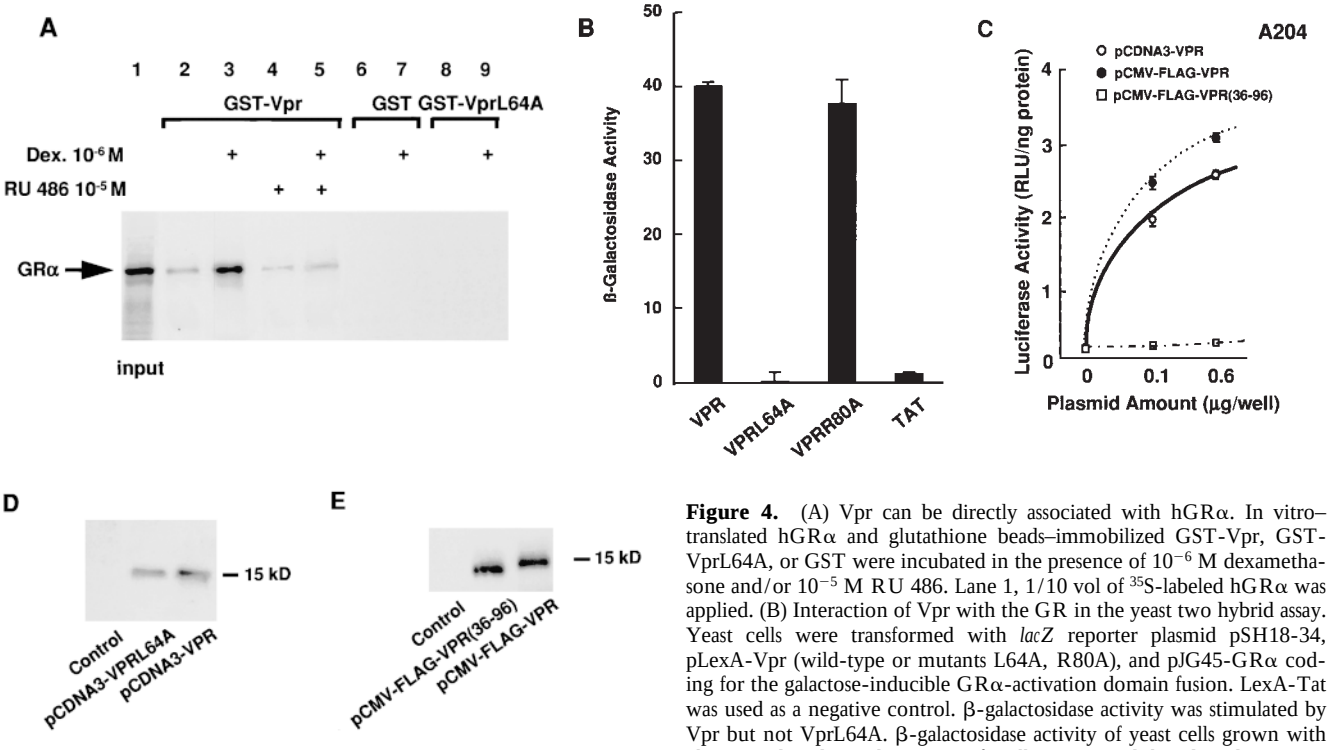


**Figure 4.** (A) Vpr can be directly associated with hGRα. In vitro–translated hGRα and glutathione beads–immobilized GST-Vpr, GST-VprL64A, or GST were incubated in the presence of $10^{-6}$ M dexamethasone and/or $10^{-5}$ M RU 486. Lane 1, 1/10 vol of $^{35}$S-labeled hGRα was applied. (B) Interaction of Vpr with the GR in the yeast two hybrid assay. Yeast cells were transformed with *lacZ* reporter plasmid pSH18-34, pLexA-Vpr (wild-type or mutants L64A, R80A), and pJG45-GRα coding for the galactose-inducible GRα-activation domain fusion. LexA-Tat was used as a negative control. β-galactosidase activity was stimulated by Vpr but not VprL64A. β-galactosidase activity of yeast cells grown with glucose rather than galactose was for all strains much less than the activity produced by GR-LexA-Tat interaction viewed by us as a background value. (C) Coactivator function of Vpr, FLAG-Vpr, and FLAG-Vpr(36–96). A204 cells were transfected with different amounts of pCDNA3-VPR, pCMV-FLAG-VPR, or pCMV-FLAG-VPR(36–96), and pMMTV-luc. Each point shows the mean ± SEM values obtained in the presence of $10^{-7}$ M dexamethasone. $^*P < 0.001$. (D and E) Vpr, VprL64A, FLAG-Vpr, and FLAG-Vpr(36–96) expressed and detected by Western blot in A204 cells using anti-Vpr or anti-FLAG (M2) antibody. A204 cells were transfected with pCDNA3 (control), pCDNA3-VPR, or pCDNA3-VPRL64A, and Vpr was detected after immunoblotting by using HIV-$1_{NL4-3}$ Vpr antiserum (D). FLAG-Vpr or FLAG-Vpr(36–96) was detected by using anti-FLAG antibody (E).

activities on this promoter. Vpr, VprL64A, FLAG-Vpr, and FLAG-Vpr(36–96) were expressed at similar levels and could be detected in Western blots of extracts from A204 cells transfected with the corresponding plasmids (Fig. 4, D and E). These results are consistent with the hypothesis that the effect of Vpr as a coactivator depends also on the presence of an intact TFIIB-binding domain.

We also studied the interactions of Vpr with GR and components of the transcription complex in dexamethasone- and mock-treated pCMV-FLAG-VPR–transfected cells by coimmunoprecipitations of cell extracts. As shown in Fig. 5, FLAG-Vpr was coimmunoprecipitated by anti-TFIID (TBP), anti-TFIIB, or anti-GR antibodies in dexamethasone-treated cells, suggesting that Vpr binds to components of the transcription machinery and to the GR, as part of the glucocorticoid-activated transcription initiation complex (23). FLAG-VprL64A was coimmunoprecipitated by anti-TFIIB antibody, suggesting that the TFIIB-binding site of this mutant remains functional, whereas FLAG-Vpr(36–96) was not precipitated by either GR, TFIID, or TFIIB antibodies. Coprecipitation of FLAG-Vpr was not efficient by TFIIB antibodies in the absence of dexamethasone, whereas it increased in its presence. FLAG-VprL64A was similarly precipitated by TFIIB antibodies, but this did not increase in the presence of dexamethasone. These results suggest that Vpr binds directly to TFIIB through the $NH_2$-terminal part of the molecule and to the GR through the LXXLL coactivator domain. Binding to both factors leads to enhanced incorporation of Vpr into a large transcription complex also including other transcription factors such as TFIID. The binding of the VprL64A mutant to TFIIB but not to the GR may explain its transdominant negative phenotype as competition of the mutant with wild-type Vpr for the TFIIB-binding site. If Vpr becomes part of a bigger transcription complex in the presence of dexamethasone, then it may be coprecipitated with antibodies for other proteins known to be in the GR transcription complex, such as the coregulators p300. In coimmunoprecipitation experiments using lysate of FLAG-Vpr–transfected A204 cells, Vpr and p300 were either weakly or strongly coprecipitated by anti-FLAG antibody in the absence or presence of dexamethasone, respectively (data not shown). This may reflect the presence of Vpr and p300 in the same complex. Alternatively, it may indicate additional contacts of Vpr with p300. It was recently suggested that Vpr transactivation on the HIV promoter is mediated through p300 (45). To study any potential interactions of Vpr and p300, we compared the effects of transfected Vpr and p300/CBP coregulators on the MMTV promoter (Fig. 6 A). We also studied the interaction of Vpr with another GR coactivator, steroid receptor coactivator (SRC)-1, by cotransfecting pCDNA3-VPR with an SRC-1 expression vector. As shown in Fig. 6, A and B, Vpr potentiated dexamethasone activity >20-fold. Vpr and p300 or SRC-1a synergistically enhanced ligand-activated GR activity on the MMTV promoter. No enhancement was observed in the absence of glucocorticoid. Therefore, Vpr appears to synergize with other coactivators in the activation of the MMTV promoter.

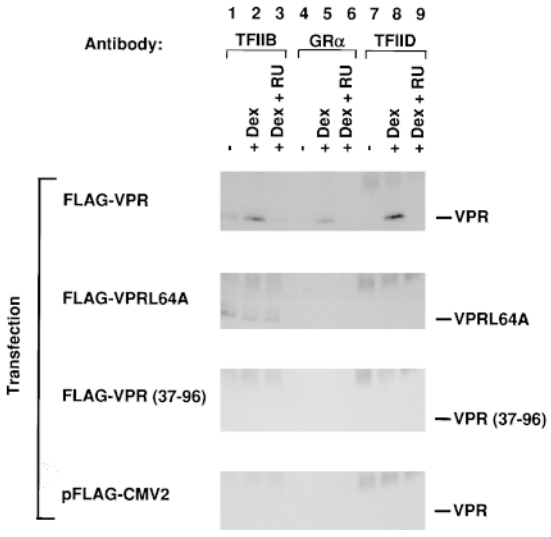


**Figure 5.** Vpr can be precipitated with anti-TFIID (TBP), anti-TFIIB, or anti-hGR antibodies in dexamethasone-treated A204 cells. A204 cells were transfected with pCMV-FLAG-VPR, pCMV-FLAG-VPRL64A, pCMV-FLAG-VPR(36–96), or pFLAG-CMV-2 (control). Cells were treated with dexamethasone (Dex, $10^{-6}$ M) and RU 486 (RU, $10^{-5}$ M) as indicated at the top, lysed in lysis buffer, and precipitated by using anti–human TFIID (TBP) antibody, anti–human TFIIB antibody, or anti-GRα antibody. FLAG-Vpr, FLAG-VprL64A, and FLAG-Vpr(36–96) were detected after blotting by using anti-FLAG (M2) antibody. Lane 1 (marker), cell extracts from FLAG-Vpr–, FLAG-VprL64A–, and FLAG-Vpr(36–96)–expressing cells before immunoprecipitation. In the pFLAG-CMV2 (negative control) panel, FLAG-Vpr–containing extract was included in lane 1 as marker.

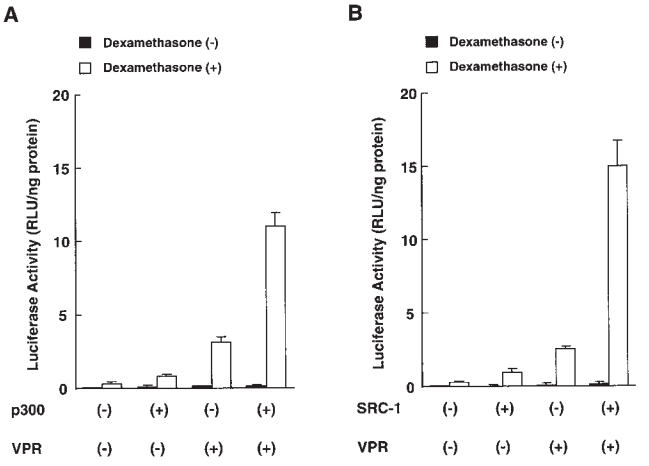


**Figure 6.** Vpr enhances the GR coactivation by p300 or SRC-1. A204 cells were transfected with 1.5 μg/well of pMMTV-luc, 0.6 μg/well of pCDNA3-VPR, 2.0 μg/well of CMVβ-p300-CHA, or 2.0 μg/well of pCR-SRC-1a as indicated. pCDNA3 (instead of pCDNA3-VPR and pCMVβ-p300-CHA) or pCR3.1 (instead of pCR-SRC-1a) was cotransfected as needed to maintain the same amount of DNA. Black and white bars, mean ± SEM values obtained in the absence or presence of $10^{-7}$ M dexamethasone, respectively.

## Discussion

Vpr stimulates HIV replication in lymphocytes and is important for the efficient viral replication in macrophages due to both transcriptional activation and its role in nuclear targeting of the preintegration complex (5, 12–15). The present work suggests that, in addition, Vpr may render cells more sensitive to glucocorticoids. Though it is not immediately clear what advantage such an activity may give to the virus, this new effect may be of importance for AIDS pathogenesis.

We found that in several lymphoid (CEM, Jurkat), rhabdomyosarcoma (A204, HS729), and kidney (CV1) cell lines Vpr dramatically increases the effect of glucocorticoids on the MMTV promoter (Fig. 1). This effect of Vpr is mediated by binding to the GR through a helical part of the molecule containing the LQQLL sequence; disruption of the motif is detrimental for both GR binding and the transcriptional effect of Vpr (Figs. 3 and 4). This region has been shown to interact with transcription factor Sp1 (46). Mutations in this region also decrease the nuclear localization of Vpr (47). Similar motifs, LXXLL, were shown to be involved in binding of several coactivator molecules to nuclear receptors (41). These findings suggest that Vpr is a virally encoded coactivator of the GR. Vpr also works as a coactivator for other nuclear receptors, such as the progesterone and estrogen receptors, as would be expected from the presence of a functional LXXLL motif. A previous report has associated Vpr to the activated GR complex, presumably through interaction with a cytosolic protein, named Rip-1 (16). Our results suggest that the observed association to the activated GR complex is through a direct interaction to GR.

Classical nuclear receptor coactivators, like SRC-1 and CBP/p300, were shown to interact with the receptors in the presence of the appropriate ligand as well as with general transcription factors, components of the RNA polymerase II complex (29, 48). They also possess histone acetyltransferase activity that may overcome the inhibitory effect of chromatin on gene expression, leading to efficient transcription (28, 30).

Coprecipitation experiments (Fig. 5) showed that in the presence of glucocorticoid Vpr became associated not only with the GR, but also with TFIIB and TFIID, consistent with its incorporation into a stable transcription initiation complex, reminiscent of other nuclear receptor coactivators. Notably, mutant VprL64A, which is unable to enhance the glucocorticoid response, was excluded from such a complex, but was still able to bind to TFIIB. This observation is consistent with the reported ability of the $NH_2$-terminal part of Vpr to interact with TFIIB (44) and may explain the dominant-negative phenotype of the L64A mutation that disrupts GR binding but retains the ability to bind, and thus block, a general transcription factor.

These results suggest that Vpr functions by bridging the ligand-bound nuclear receptor and general transcription factors, resulting in the stabilization of the transcription preinitiation complex. It is also highly probable that Vpr cooperates with and enhances the activity of other coactivators, the same way coactivators cooperate with each other. We found synergistic effects of Vpr with both p300 and SRC-1 on the MMTV promoter (Fig. 6). The simplest interpretation of these results is that each coactivator, including Vpr, contributes to the efficiency and stability of the transcription initiation complex (Fig. 7).

The relation of nuclear receptor coactivator activity of Vpr described in this work to previously described transcriptional effects of this HIV protein is not clear. The stimulatory effect of Vpr on several promoters, including HIV-1 LTR, correlates with Vpr's ability to block cell cycle. It was proposed that G2 arrest in T cells was sufficient to enhance LTR transcription (5). The ability of Vpr mutants to arrest dividing cells correlated also with transcription stimulation in nondividing macrophages, suggesting that cell cycle arrest is not a prerequisite for transcriptional activation by Vpr (15). In either case, it appears that the Vpr effect on nuclear receptors is distinct from HIV-1 LTR activation and cell cycle arrest. Our mutational analysis showed that there is no correlation between the ability of Vpr to stimulate glucocorticoid response and its cell cycle–blocking activity. VprL64A, a mutant unable to activate GR, was proficient in the cell cycle arrest, whereas VprR80A, known to be inactive in the arrest (49), was fully able to activate glucocorticoid response (33).

The contribution of the glucocorticoid coactivator effect of Vpr on the replication of HIV-1 remains to be defined. The reported effects of glucocorticoids on HIV-1 expression have been controversial and both mildly stimulatory and inhibitory (5, 16, 50–53). However, there is a role for the Vpr coactivator activity in the pathophysiology of HIV-1 infection. Patients with AIDS have clinical manifes-

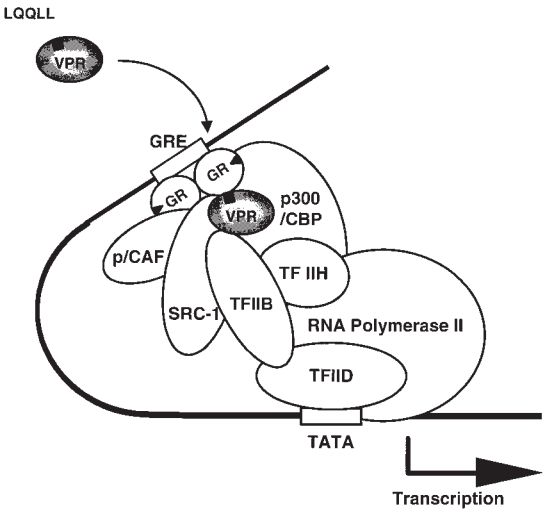


**Figure 7.** A model of the involvement of Vpr in glucocorticoid-dependent activation of transcription. Vpr enhances the glucocorticoid response by direct association with components of the glucocorticoid-induced transcription initiation complex, including the GR itself and TFIIB. Interactions with p300/CBP and SRC-1 are also possible. This function of Vpr is similar to that of other known mammalian coactivators.

tations compatible with glucocorticoid hypersensitivity, reflected in severe immune suppression and profound myopathy and muscle wasting, all recognized effects of chronically elevated levels of glucocorticoids. Thus, the glucocorticoid coactivator actions of Vpr may contribute, along with actions of other viral proteins, to the development of HIV-1–associated pathologies. Furthermore, Vpr may mimic glucocorticoid effects on apoptosis and on immune system suppression through induction of IκB transcription (54). The role of HIV-1 on muscle wasting cannot be explained by direct effects of the virus. However, Vpr can be detected outside of infected cells and in the plasma of HIV-1–infected patients (17, 18). Like Tat, Vpr appears also to affect uninfected cells in a paracrine or endocrine fashion (17, 18, 55, 56). If Vpr contributes to increased tissue sensitivity to glucocorticoids, our data suggest a role for steroid hormone receptor antagonists, such as RU 486, or Vpr antagonists in the treatment of HIV-1 disease, even in the absence of hypercortisolism.

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