ERK MAP Kinase Links Cytokine Signals to Activation of Latent HIV-1 Infection by Stimulating a Cooperative Interaction of AP-1 and NF-κB*

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Human immunodeficiency virus type 1 (HIV-1) can establish latent infection following provirus integration into the host genome. NF-κB plays a critical role in activation of HIV-1 gene expression by cytokines and other stimuli, but the signal transduction pathways that regulate the switch from latent to productive infection have not been defined. Here, we show that ERK1/ERK2 mitogen-activated protein kinase (MAPK) plays a central role in linking signals at the cell surface to activation of HIV-1 gene expression in latently infected cells. MAPK was activated by cytokines and phorbol 12-myristate 13-acetate in latently infected U1 cells. The induction of HIV-1 expression by these stimuli was inhibited by PD98059 and U0126, which are specific inhibitors of MAPK activation. Studies using constitutively active MEK or Raf kinase mutants demonstrated that MAPK activates the HIV-1 long terminal repeat (LTR) through the NF-κB sites. Most HIV-1 inducers activated NF-κB via a MAPK-independent pathway, indicating that activation of NF-κB is not sufficient to explain the activation of HIV-1 gene expression by MAPK. In contrast, all of the stimuli activated AP-1 via a MAPK-dependent pathway. NF-κB and AP-1 components c-Fos and c-Jun were shown to physically associate by yeast two-hybrid assays and electrophoretic mobility shift assays. Coexpression of NF-κB and c-Fos or c-Jun synergistically transactivated the HIV-1 LTR through the NF-κB sites. These studies suggest that MAPK acts by stimulating AP-1 and a subsequent physical and functional interaction of AP-1 with NF-κB, resulting in a complex that synergistically transactivates the HIV-1 LTR. These results define a mechanism for signal-dependent activation of HIV-1 replication in latently infected cells and suggest potential therapeutic strategies for unmasking latent reservoirs of HIV-1.

The human immunodeficiency virus type 1 (HIV-1)1 can establish latent infection following provirus integration into the host genome. NF-κB plays a critical role in activation of HIV-1 gene expression by cytokines and other stimuli, but the signal transduction pathways that regulate the switch from latent to productive infection have not been defined. Here, we show that ERK1/ERK2 mitogen-activated protein kinase (MAPK) plays a central role in linking signals at the cell surface to activation of HIV-1 gene expression in latently infected cells. MAPK was activated by cytokines and phorbol 12-myristate 13-acetate in latently infected U1 cells. The induction of HIV-1 expression by these stimuli was inhibited by PD98059 and U0126, which are specific inhibitors of MAPK activation. Studies using constitutively active MEK or Raf kinase mutants demonstrated that MAPK activates the HIV-1 long terminal repeat (LTR) through the NF-κB sites. Most HIV-1 inducers activated NF-κB via a MAPK-independent pathway, indicating that activation of NF-κB is not sufficient to explain the activation of HIV-1 gene expression by MAPK. In contrast, all of the stimuli activated AP-1 via a MAPK-dependent pathway. NF-κB and AP-1 components c-Fos and c-Jun were shown to physically associate by yeast two-hybrid assays and electrophoretic mobility shift assays. Coexpression of NF-κB and c-Fos or c-Jun synergistically transactivated the HIV-1 LTR through the NF-κB sites. These studies suggest that MAPK acts by stimulating AP-1 and a subsequent physical and functional interaction of AP-1 with NF-κB, resulting in a complex that synergistically transactivates the HIV-1 LTR. These results define a mechanism for signal-dependent activation of HIV-1 replication in latently infected cells and suggest potential therapeutic strategies for unmasking latent reservoirs of HIV-1.

1 The abbreviations used are: HIV-1, human immunodeficiency virus type 1; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; PMA, phorbol 12-myristate 13-acetate; LTR, long terminal repeat; ERK, extracellular signal-regulated kinase; TNF, tumor necrosis factor; EMSA, electrophoretic mobility shift assay; CAT, chloramphenicol acetyltransferase.
NF-IL6, ATF-2, and Elk-1 (28, 29). Other mitogen-activated protein kinases in mammalian cells are c-Jun N-terminal kinase/stress-activated protein kinase and p38/HOG, which are activated by stress stimuli and inflammatory cytokines (30–32).

Mitogens and cytokines that activate HIV-1 gene expression in latently infected cells are known to activate MAPK (28, 29, 33–35). We therefore investigated the role of MAPK in the activation of latent HIV-1 infection. We performed studies in the U1 human monocytic cell line, an in vitro model for post-integration HIV-1 latency (36–38). U1 cells contain two integrated copies of the HIV-1 genome and under unstimulated conditions express low levels of viral transcripts encoding Tat, Rev, and Nef but little or no full-length viral RNA (20, 21). The pattern of HIV-1 RNA expression is similar to that in other latently infected cell lines (23) and peripheral blood mononuclear cells (22, 24). HIV-1 gene expression can be induced in U1 cells by stimulation with phorbol ester or cytokines, such as TNF-α, IL-1β, and IL-6 (15, 16, 23, 36–38), or by Tat (25, 39). Here, we demonstrate that activation of MAPK is required for induction of HIV-1 gene expression in latently infected U1 cells by cytokines and other stimuli. MAPK acts by stimulating AP-1 and a subsequent physical and functional interaction of AP-1 with NF-κB, resulting in a complex that synergistically transactivates the HIV-1 LTR. These results define a mechanism for signal-dependent activation of HIV-1 replication in latently infected cells.

**EXPERIMENTAL PROCEDURES**

**Plasmids—**pLTR-Luc was made by subcloning the 720-base pair Xhol–HindIII fragment containing the HIV-1 LTR from the pNL4–3 HIV-1 proviral DNA plasmid into pGL3-Luc basic (Promega). pLTRRaf-Luc was made by deleting LTR sequences upstream from the NF-κB sites (a 480-base pair AvaI–Avai fragment) from pLTR-Luc. pLTRmxB-Luc, derived from pLTR-Luc, contains mutations of the two NF-κB binding sites within the LTR (40). pSVLTat expresses the HXB2 Tat protein under the control of the SV40 promoter. pMEKko (pMEK-R4F, also called AN3/S218E/S222D) and pMEKdn (pMKK1-K37M) (41, 42) were a gift of Dr. Natalie Ahn. pRaf-BXB and pRaf-BXB301 (43) were a gift of Dr. Jeff Rupp.

**Cell Culture—**The human monocytic cell lines U1 and U937 were maintained in RPMI medium containing 10% fetal calf serum. HeLa cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum.

**Transfections and Reporter Assays—**U937 cells (10^2 cells) were transfected with 10 μg of pLTR-CAT alone or together with 5 μg of the pSVLTat HIV-1 Tat expression plasmid by the DEAE-dextran method (40). pSVLTat expresses the HXB2 Tat protein under the control of the SV40 promoter. pMEKko (pMEK-R4F, also called AN3/S218E/S222D) and pMEKdn (pMKK1-K37M) (41, 42) were a gift of Dr. Natalie Ahn. pRaf-BXB and pRaf-BXB301 (43) were a gift of Dr. Jeff Rupp.

**Immunoblotting—**Immunoblotting of U1 or HeLa cell lysates was performed with anti-p24 (Intracell), antiphospho-MAPK (New England Biolabs), or anti-ERK1 and anti-ERK2 MAPK (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) as described (44).

**Electrophoretic Mobility Shift Assays—**Nuclear extracts were prepared from U1 cells as described (14). Five μg of DNA–protein complexes was used for EMSAs. NF-κB binding reactions were performed as described (46) in a total volume of 20 μl containing 20 μM Hepes, pH 7.9, 50 mM KC1, 1 μM dithiothreitol, 2.5 mM MgCl2, 4% Ficol, 1 μg of poly(dI–dC), 2 μg of bovine serum albumin, and 20,000 cpm of 32P-labeled NF-κB oligonucleotide probe (5’-AGTGGAGGGACTTCCCAGG-3’) (Promega) or HIV-1 LTR κB probe (nucleotides –108 to –78, 5’-CAAGG-GACCTTCCCGCTGGAGCATTCCCAGG-3’) (12, 13). AP-1 binding reactions were carried out in a reaction mixture containing 20 μM Hepes, pH 7.9, 100 mM KC1, 5 mM dithiothreitol, 1 mM MgCl2, 0.3 mM phenylmethylsulfonyl fluoride, 0.6 mM EDTA, 4% Ficol, 1 μg of poly(dI–dC), and 15,000–20,000 cpm of 32P-labeled AP-1 oligonucleotide (5’-CGCT-TGATGGATCGCACCGGAAA-3’) (Promega) as described (47). Antibodies to p65 (1 μl), c-Fos and c-Jun (1 or 2 μl) (anti-NF-κB antibodies), c-Fos (4–10G), and anti-c-Jun (N), respectively, from Santa Cruz Biotechnology or normal rabbit serum (1 μl) was preincubated on ice with nuclear extracts prepared from U1 cells stimulated with TNF-α for 40 min before the addition of the 32P-labeled oligonucleotides and performing EMSAs using a probe containing the HIV-1 LTR κB sites. After 20 min at room temperature, samples were loaded onto a nondenaturing polyacrylamide gel and run in 0.5–x TBE buffer. After electrophoresis, gels were dried and exposed to x-ray film.

**RESULTS**

**MAPK Activation Is Required for Cytokine-induced HIV-1 Gene Expression in Latently Infected Cells—**To examine a potential role of MAPK in mediating activation of latent HIV-1, we first studied whether MAPK is activated by stimuli that activate HIV-1 gene expression in latently infected cells by using the MEK inhibitor PD98059, which specifically inhibits activation of MAPK (48, 49). The high degree of specificity of PD98059 is indicated by its failure to inhibit >20 other serine/threonine kinases, including other MEK homologs (i.e. c-Jun N-terminal kinase/stress-activated protein kinase and p38/HOG kinase kinases) (48). MAPK phosphorylation induced by phorbol 12-myristate 13-acetate (PMA) or cytokines was demonstrated by immunoblotting with antiphospho-MAPK, which specifically detects MAPK only when activated by phosphorylation (Fig. 1A). Equivalent amounts of MAPK were present in all samples (Fig. 1A), indicating that these stimuli induced activation rather than increased expression of MAPK. PD98059 abolished MAPK phosphorylation induced by these stimuli (Fig. 1A). The induction of HIV-1 expression by PMA or TNF-α was suppressed by PD98059 in a dose-dependent manner with an IC50 of approximately 10 μM, and 90–98% inhibition at 50 μM (Fig. 1, B and C). This suppression was not due to cytotoxic effects of the drug as determined by trypan blue staining and counting the number of viable cells (Fig. 1B).

Additional results were obtained using the latently infected T cell line ACH-2 and promyelocytic cell line OM10.1 (23) (data not shown). PD98059 also inhibited the induction of HIV-1 expression by IL-1β and IL-6 alone in combination by 95–98%, while induction by TNF-α/IL-6 was inhibited by 70% (Fig. 1C). Induction of the HIV-1 protein synthesis by PMA or cytokines was also inhibited by PD98059 (Fig. 1D), with the same dose dependence as that observed for induction of viral RNA as measured by reverse transcriptase assays (data not shown). The level of MAPK activation induced by the different stimuli correlated directly with the level of activation of HIV-1 expression (Fig. 1, A, C, and D). Conversely, the inhibition of MAPK activation by PD98059 correlated with its inhibitory effects on induction of HIV-1 expression (Fig. 1, B and C). U0126, another MEK inhibitor, which specifically inhibits activation of MAPK (50), also inhibited the induction of HIV-1 expression by PMA, TNF-α, and TNF-α/IL-6 (Fig. 1E). Similar to PD98059 (Fig. 1C), these results suggest that activation of MAPK plays a critical role in the induction of HIV-1 expression by PMA and TNF-α.
expression in U1 cells.

kB Sites Are Required for MAPK Activation of the HIV-1 LTR—To determine whether MAPK mediates induction of HIV-1 expression through activation of the HIV-1 LTR, we transfected U937 cells, the uninfected parental cell line of U1, with an HIV-1 LTR chloramphenicol acetyltransferase (CAT) reporter plasmid (Fig. 2A). Transfected cells were then stimulated with PMA or cytokines in the presence or absence of PD98059 and assayed for CAT activity. The induction of HIV-1 LTR expression by the different stimuli was inhibited by PD98059 (Fig. 2B, left). However, PD98059 had little or no effect on activation of the HIV-1 LTR induced by coexpression of an HIV-1 Tat expression plasmid (Fig. 2B, middle and right), indicating that MAPK does not affect Tat transactivation of the HIV-1 LTR.

We next examined whether activation of MAPK is sufficient
to induce expression of the HIV-1 LTR. HeLa cells were transfected with an HIV-1 LTR luciferase reporter plasmid (Fig. 2A) together with MEK or Raf kinase expressor plasmids. Expression of constitutively active mutant MEK (41) or Raf (43, 45), which induce constitutive MAPK activation in the absence of extracellular stimulation (41–43), increased luciferase expres-
sion by 8–33-fold, whereas dominant negative mutant MEK or Raf had no significant effect (Fig. 2C). Thus, activation of MAPK through the Raf/MEK pathway can activate the HIV-1 LTR. The HIV-1 LTR contains NF-AT sites at −255 to −217, NF-κB sites at −104 to −81, and Sp1 sites at −78 to −47 (Fig. 2A). Deletion of sequences upstream from the κB sites between positions −641 and −158 did not affect activation of the HIV-1 LTR by coexpression of constitutively active mutant MEK or Raf (Fig. 2C). In contrast, when the NF-κB binding sites were mutated, HIV-1 LTR expression was not significantly induced by coexpression of these mutant kinases (Fig. 2C). The HIV-1 LTR lacking NF-κB sites was not activated by TNF-α, TNF-α/IL-6, or PMA but could still be activated by sodium butyrate (18) alone or together with TNF-α (Fig. 2D). Thus, this mutant HIV-1 LTR can still be activated by NF-κB-independent mechanisms (15–19). MAPK activation by the constitutively active mutant MEK and Raf plasmids was confirmed by transfection of HeLa (Fig. 2E) and 293T cells (44) and immunoblotting cell lysates with an antiphosphorylated MAPK antibody. Together, these results suggest that MAPK activation of the HIV-1 LTR occurs through the κB sites.

MAPK Regulation of NF-κB and AP-1 Activity—We then examined whether activation of NF-κB is involved in the mechanism of HIV-1 LTR activation by MAPK. NF-κB binding activity was analyzed by electrophoresis mobility shift assays (EMSA). PMA, TNF-α, TNF-α/IL-6, and IL-1/β/IL-6 markedly activated NF-κB binding activity, whereas activation by IL-1β and IL-6 was minimal (Fig. 3A). PD98059 inhibited the induction of NF-κB binding activity by PMA, but not TNF-α, TNF-α/IL-6, and IL-1β/IL-6 (Fig. 3A). Thus, activation of NF-κB occurred by MAPK-dependent and MAPK-independent routes depending on the stimulus. NF-κB binding activity did not correlate with HIV-1 gene expression based on the discrepancy between PD98059 inhibition of NF-κB binding and its effects on HIV-1 gene expression. Therefore, NF-κB by itself cannot account for the induction of HIV-1 gene expression by these stimuli. AP-1, which consists of homo- or heterodimers of members of the Jun and Fos families, is regulated by MAPK (51, 52) and has been shown to physically associate with NF-κB in vitro (53). The preceding experiments demonstrate that MAPK-dependent activation of the HIV-1 LTR requires the κB sites but not two upstream sites with weak homology to AP-1 sites (54).

These observations raise the possibility that MAPK-dependent activation of HIV-1 gene expression might be mediated by a cooperative physical interaction of AP-1 and NF-κB (53). We therefore performed EMSAs to examine whether activation of MAPK by the different stimuli induced activation of AP-1. Stimulation of U1 cells by PMA or cytokines induced AP-1 binding activity (Fig. 3B). PD98059 inhibited the induction of AP-1 binding activity by PMA, TNF-α, and IL-6 and to a lesser extent by IL-1β, TNF-α/IL-6, and IL-1β/IL-6 (Fig. 3B). We then analyzed the functional activation of AP-1 by transient expression assays using the AP-1-dependent reporter plasmid 5XTR-TKCAT (55) in U937 cells and demonstrated that PD98059 abolished induction of AP-1 transcriptional activity by all stimuli examined (Fig. 3C). These results are consistent with previous studies, which have shown that AP-1 binding activity does not always mirror its transcriptional activity (51, 52). EMSAs performed using probes to detect activation of NF-AT or PU.1 showed no significant activation of these transcription factors by PMA or cytokine stimulation (data not shown), suggesting that they are unlikely to contribute to activation of the HIV-1 LTR by these stimuli. These results suggest that stimuli that induce HIV-1 expression in U1 cells increase AP-1 activity via the MAPK pathway.

Physical Interaction of NF-κB and AP-1 in Yeast Two-Hybrid Assays and HIV-1-infected Cells—We next examined whether AP-1 physically interacts with NF-κB. First, we demonstrated a physical association of NF-κB and AP-1 by a yeast two-hybrid system in which the interaction of NF-κB with c-Fos or c-Jun allowed growth of yeast in absence of histidine and activated LacZ reporter gene expression (Fig. 4A). The expected interaction of c-Jun with either c-Jun or c-Fos was also demonstrated by the yeast two-hybrid system. We then examined whether a physical interaction of NF-κB and AP-1 occurs in HIV-1-infected cells by EMSAs. Nuclear extracts prepared from U1 cells stimulated with TNF-α were preincubated with antibodies to NF-κB, c-Fos, or c-Jun, and the resultant complexes were an-
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NF-κB and AP-1 Synergistically Transactivate the HIV-1 LTR—To determine whether AP-1 and NF-κB can cooperatively activate the HIV-1 LTR, activation of the HIV-1 LTR was assessed after cotransfection of expression plasmids for c-Fos, c-Jun, and the NF-κB p65 subunit. Expression of c-Fos or c-Jun alone had no significant effect on LTR activity, whereas coexpression of c-Fos and c-Jun stimulated expression of pLTR-Luc by 3–6-fold but had no significant effect on pLTRs-Luc activity (Fig. 5). Expression of the NF-κB p65 subunit alone stimulated luciferase expression of pLTR-Luc and pLTRs-Luc by 3.5–7-fold (Fig. 5). Synergistic activation with up to 27-fold stimulation of HIV-1 LTR expression was observed when c-Fos or c-Jun was coexpressed with the NF-κB p65 subunit. In contrast, when the κB sites were mutated, no significant activation of HIV-1 LTR expression was observed with any of the cotransfected plasmids alone or in combination. Together with the results of the preceding experiments, these results suggest that a physical association between NF-κB and AP-1 results in synergistic transcriptional activation of the HIV-1 LTR via the κB binding sites.

DISCUSSION

These studies demonstrate that MAPK plays a central role in signal-dependent activation of HIV-1 gene expression in latently infected cells. The results suggest a model in which MAPK acts by stimulating AP-1 and a subsequent physical and functional interaction of AP-1 with NF-κB, resulting in a complex that synergistically transactivates the HIV-1 LTR at the κB sites (Fig. 6). c-Fos and c-Jun were shown to physically associate with NF-κB by the yeast two-hybrid system and in HIV-1-infected cells by EMSA and supershift assays. Furthermore, expression of c-Fos or c-Jun was shown to activate the HIV-1 LTR in synergy with NF-κB, suggesting that a physical interaction represents the molecular basis of the functional synergy. Consistent with the proposed model, a physical interaction of the Rel homology domain of NF-κB with the bZIP regions of c-Fos or c-Jun was previously demonstrated in vitro, and functional cooperation of NF-κB and AP-1 was demonstrated with a reporter plasmid containing only the HIV-1 LTR κB sites linked to a TATA box (53). The κB sites but not potential AP-1 sites upstream in the LTR (54), downstream of the transcription start site (at +95 and +160) (56, 57), or in the pol gene (58) were required for HIV-1 activation by MAPK. Consistent with these findings, other studies have shown that Ras and Raf can increase HIV-1 LTR activity via the κB sites (43, 45). Furthermore, the upstream AP-1-like sites appear to be nonfunctional (59).

Our studies suggest that MAPK-dependent activation of AP-1 is involved in the mechanism of induction of HIV-1 gene expression upon cellular stimulation with mitogens and cytokines. AP-1 was activated through a MAPK-dependent pathway by all stimuli examined. The activation of AP-1 by MAPK involves induction of c-Fos synthesis, and possibly phosphorylation and activation of c-Jun (28, 29, 51, 52). c-Fos transcription is controlled in part by a serum response element, which binds a ternary complex between serum response factor and TCF/Elk-1, which is phosphorylated and activated by MAPK (51). In contrast to AP-1, the finding that PD98059 inhibited NF-κB activation by PMA but not TNF-α or IL-1β/IL-6 suggests that NF-κB activation in stimulated U1 cells occurred either by MAPK-dependent or MAPK-independent pathways depending on the stimulus. The activation of NF-κB involves phosphorylation and subsequent degradation of inhibitory proteins called IxBs, allowing NF-κB to translocate to the nucleus (60, 61). PMA, TNF-α, IL-1β, and IL-6 activate distinct signal transduction pathways that ultimately converge on the NF-κB-inducing kinase/IxB kinase pathway (60, 61). PMA may activate NF-κB through MAPK-dependent activation of pp90<sup>rsk</sup>, leading to phosphorylation of IxB (61) (Fig. 6). However, most stimuli induced NF-κB activation via a MAPK-independent pathway, consistent with previous studies (61, 62). Thus, activation of NF-κB is not sufficient to explain the activation of HIV-1 gene expression by MAPK. This conclusion is further supported by our finding that activation of NF-κB by IL-1β and IL-6 was minimal and is consistent with a previous study that showed that IL-1β alone and in combination with IL-6 can activate HIV-1 gene expression by an NF-κB-independent mechanism (16).

NF-κB has been shown to physically and functionally interact with a number of transcription factors, including AP-1 (53), CCAAT/enhancer-binding protein, serum response factor, members of the ATF/CREB family, steroid receptors, TFIIB, and coactivator proteins (63, 64). The κB sites in the HIV-1
LTR can be synergistically activated through the \( \kappa B \) sites by NF-\( \kappa B \) and NF-AT in T cell lines (65). However, we were unable to demonstrate activation of NF-AT in stimulated U1 cells, suggesting that NF-AT does not mediate activation of latent HIV-1 infection in this model. Previous studies suggest that Ets proteins (66) and possibly other signal-dependent transcription factors (43) may also activate the HIV-1 LTR through the \( \kappa B \) motif. The \( \kappa B \) sites in the HIV-1 LTR may therefore represent a special class of \( \kappa B \) motifs capable of giving rise to synergistic transactivation (65). Together, these findings support a model in which a multiprotein complex containing NF-\( \kappa B \) and one or more signal-dependent transcription factors can synergistically activate HIV-1 gene expression through the \( \kappa B \) sites. In Vivo it is likely that rate-limiting concentrations of various regulatory factors acting on the HIV-1 LTR are not conducive to efficient gene expression in resting T cells. Thus, activation of the HIV-1 LTR through the \( \kappa B \) motif may not be sufficient for maximal gene expression and exit from latency in peripheral blood lymphocytes. It will be important to elucidate the mechanisms of synergistic activation in the context of chromatin structure (67) and other cellular factors such as transcriptional coactivators (63, 64, 68) in future studies.

It has been proposed that a deficiency of Tat may be responsible for the latent state of HIV-1 in U1 cells (25, 39). However, the finding that a virus containing a functional tat gene does not replicate in U1 cells (69) suggests that the intracellular environment rather than a deficiency of Tat per se may be the key factor in determining latency. Previous studies suggest that Tat can activate both NF-\( \kappa B \) and AP-1 (71–74). Therefore, activation of these transcription factors by MAPK may initiate a reinforcing mechanism in which an initial increase in Tat synthesis may act to further increase HIV-1 gene expression through activation of NF-\( \kappa B \) and AP-1, in addition to stimulating elongation of initiated transcripts (8, 9, 11).

Signal transduction pathways distinct from the ERK1/ERK2 MAPK cascade may also participate in the activation of HIV-1 gene expression by certain stimuli. This possibility is supported by our finding that PD98059 inhibited but did not abolish the activation of HIV-1 gene expression by TNF-\( \alpha \)/IL-6. Furthermore, studies using the p38/HOG MAPK inhibitor SB203580 suggest that the p38/HOG MAPK pathway may also be involved in activation of the HIV-1 LTR in response to certain cytokines and stress (75) and activation of HIV-1 from latency (76). In addition to regulating HIV-1 gene expression, ERK1/ERK2 MAPK may be incorporated into HIV-1 virions (77, 78) and may also regulate other steps of the virus life cycle. For example, activation of ERK1/ERK2 MAPK in producer cells has been shown to enhance the infectivity of HIV-1 virions by phosphorylating Vif (44) as well as other mechanisms (78, 79). Conversely, inhibition of MAPK by PD98059 reduces virion infectivity (79). Thus, MAPK activation in producer cells may contribute to the activation of HIV-1 replication at the level of transcription as well as during subsequent steps in the virus life cycle.

Understanding the signal transduction pathways that activate HIV-1 replication in response to mitogens and other extracellular stimuli may provide new insights into pathogenic mechanisms involved in HIV-1 disease and may contribute to the development of new therapeutic strategies. HIV-1 replication in infected cells in vivo most likely results in death of the

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**Fig. 5.** Synergistic activation of the HIV-1 LTR by a physical interaction of NF-\( \kappa B \) and AP-1. HeLa cells were transfected with pLTR-Luc, pLTRs-Luc, or pLTRm\( \kappa B \)-Luc and pRSVRel-p65, pRSVcFos, or pRSVcJun as indicated. To maintain the same amount of transfected DNA, the total amount of DNA was adjusted using the vector control plasmid pSG5. Luciferase activity was determined at 48 h after transfection.

**Fig. 6.** Model for induction of HIV-1 gene expression in latently infected U1 cells by ERK MAPK through regulation of a cooperative interaction between AP-1 and NF-\( \kappa B \).
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cell. The finding that stimulation of MAPK activates HIV-1 expression in latently infected cells therefore raises the possibility that this strategy in combination with antiviral drugs or other therapies may facilitate eradication of virus by unmasking latent reservoirs of HIV-1.

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