(9), including challenges performed with the same doses of the same challenge stocks that were protected against by the SIV/*nef*-deletion vaccine. Either the nature of the neutralizing antibodies being elicited is different with the different approaches or the key protective component is something other than neutralizing antibodies. A possible role of cytotoxic T lymphocytes is certainly worthy of intensive investigation.

Most viral vaccines currently in use in humans are of the live attenuated variety. It is simply difficult to match live virus infection for the strength, breadth, nature, and duration of the immune response that is generated. Our results suggest that live attenuated HIV-1 may also be the most potent, effective vaccine for the prevention of AIDS. Concern for safety is likely to be the key issue for the eventual development of this approach. In addition to *nef*, other genetic elements can be deleted from HIV-1 to help ensure long-term safety. Gibbs *et al.* (*23*) have identified an SIV mutant deleted in three genetic elements that replicates well in cell culture, infects rhesus monkeys, and induces neutralizing antibody responses that are only slightly less than those induced by the SIVmac239/*nef*-deletion mutant. The use of deletion mutations is an important part of this strategy. The use of deletion mutants eliminates the possibility of reversion and helps to ensure the safety of inoculation with the attenuated virus. If other vaccine approaches indeed show little or no efficacy under field conditions, limited safety testing of live, multiply deleted HIV-1 in high-risk human volunteers seems warranted.

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17. Two rhesus monkeys were preselected as negative for antibodies to SIV, type D retrovirus, simian T-lymphotropic virus type I (STLV-I), and foamy retrovirus. We prepared PBMCs from each by banding over Ficoll-Hypaque and stimulated them with phytohemagglutinin (PHA; 1 μg/ml) for 2 to 4 days in complete RPMI 1640 medium with 20% fetal calf serum. Cells were then cultured in complete media that contained interleukin-2 but lacked PHA. Aliquots of each monkey's PBMCs were infected separately with cloned SIVmac239/*nef*-open (*13, 24*) (produced by transfection of cloned DNA into rhesus monkey PBMC cultures) and with uncloned, original, early passage SIVmac251 (*15*). Culture supernatants were pooled at day 8, filtered through a 0.45-μm filter, and stored in aliquots in the vapor phase of liquid nitrogen (−160°C). SIVmac239/*nef*-open stock contained p27 antigen (46 ng/ml) and 2.5×10^4 median tissue culture infectious doses ($TCID_{50}$) per milliliter as determined on CEM×174 cells. SIVmac251 stock contained p27 antigen (96 ng/ml) and 5×10^3 $TCID_{50}$ per milliliter in CEM×174 cells. Virus stocks were negative for type D virus on Raji cells and negative for foamy virus on Vero cells.
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Targeted Degradation of c-Fos, But Not v-Fos, by a Phosphorylation-Dependent Signal on c-Jun

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The proto-oncogene products c-Fos and c-Jun heterodimerize through their leucine zippers to form the AP-1 transcription factor. The transcriptional activity of the heterodimer is regulated by signal-dependent phosphorylation and dephosphorylation events. The stability of c-Fos was found to also be controlled by intracellular signal transduction. In transient expression and in vitro degradation experiments, the stability of c-Fos was decreased when the protein was dimerized with phosphorylated c-Jun. c-Jun protein isolated from phorbol ester–induced cells did not target c-Fos for degradation, which suggests that c-Fos is transiently stabilized after stimulation of cell growth. v-Fos protein, the retroviral counterpart of c-Fos, was not susceptible to degradation targeted by c-Jun.

The AP-1 family consists of dimeric, sequence-specific DNA binding transcription factors that are part of the pathway by which intracellular signals are converted into changes of gene activity. Stimulators of AP-1 include tumor-promoting agents [phorbol 12-myristate 13-acetate (TPA)], growth factors, and oncogene products, all of which have been implicated in the stimulation of cell growth (*1*). Two components of AP-1, the products of the c-*jun* and the c-*fos* proto-oncogenes (c-Jun and c-Fos, respectively), are required for the stimulation of growth of quiescent cells and for continuous cell proliferation (*2*). These proteins also function in the transformation of cells by nonnuclear oncogenes, such as activated alleles of *ras* (*3*). The function of c-Jun and c-Fos as dominant regulators of cell growth is under stringent control. Inducible phosphorylations and dephosphorylations of c-Jun may modulate its DNA binding and transcription activation potential (*4–6*). We have found that the stability of c-Fos is also regulated by phosphorylation of c-Jun.

We expressed human c-Jun in HeLa and 293 cells to measure the stability of differentially phosphorylated forms of the c-Fos–c-Jun heterodimer in an in vitro protein degradation assay. Tissue culture cells were transiently transfected with cytomegalovirus (CMV)-based plasmid vectors that encode a c-Jun derivative with an artificial hexahistidine sequence at its NH_2-terminus that allows rapid purification (*7, 8*) while preserving the posttranslational phosphorylation of the protein (*6*). This expression system yields a c-Jun protein (cmv-H_6cJun) that is phosphorylated in the TPA-responsive manner described for the endogenous

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factor (*4*, *6*). ^{35}S-labeled c-Fos was synthesized in a cell-free transcription-translation system that contained a rat *c-fos* cDNA and that used rabbit reticulocyte lysate supplemented with [^{35}S]methionine (*9*). The [^{35}S]c-Fos was mixed with (i) buffer alone (Fig. 1A), (ii) an excess of cmv-H_6cJun that had been digested with potato acid phosphatase (PAP) to remove all accessible phosphate groups (Fig. 1B), or (iii) the same material that had been treated with PAP in the presence of a combination of PAP inhibitors (inactivated PAP; Fig. 1B) (*10*). As a control, [^{35}S]c-Fos was also incubated with nonphosphorylated c-Jun expressed in *Escherichia coli* [ec-cJun (*11*)] that had been treated with PAP or inactivated PAP (Fig. 1B). Both protein immunoblot analysis with a polyclonal antiserum to c-Jun and DNA binding assays (*6*, *12*) confirmed that the different Jun proteins were not proteolyzed and were functional after PAP treatment (*10*).

After incubation at 37°C to facilitate heterodimerization of Fos and Jun, a mixture that contained fresh rabbit reticulocyte lysate was added. Rabbit reticulocyte lysates are well described in in vitro systems that reproduce specific degradation of cellular proteins (*13*). The different samples were incubated under suitable conditions (37°C for 2 hours), and the concentration of [^{35}S]c-Fos was monitored by SDS–polyacrylamide gel electrophoresis (SDS-PAGE). Only a small amount of [^{35}S]c-Fos was lost after incubation of the protein in the presence of the degradation mixture regardless of its phosphorylation state (Fig. 1A). Similarly, incubation of [^{35}S]c-Fos with nonphosphorylated forms of c-Jun (either the PAP-treated cmv-H_6cJun or bacterially expressed ec-cJun) did not influence the stability of [^{35}S]c-Fos (Fig. 1B). When cmv-H_6cJun treated with inactivated phosphatase (and thus still carrying the phosphate groups acquired in the expressing cells (*6*) was present in the reaction, the amount of [^{35}S]c-Fos recovered was diminished (Fig. 1B). The disappearance of c-Fos therefore requires a specific proteolytic event that is dependent on the presence of phosphorylated c-Jun protein. Fos degradation apparently happens very quickly with no detectable intermediates in a manner typical of specific proteolytic reactions. Phosphorylation of c-Fos that might occur during the in vitro translation reaction (*14*) does not seem to affect its stability because the same results were obtained regardless of whether [^{35}S]c-Fos was treated with PAP or inactivated PAP (*10*) (Fig. 1B).

To test if the degradation of c-Fos in the presence of c-Jun requires physical interaction of those proteins, we repeated the experiment with a mutant of cmv-H_6cJun (MUT 22-23) (*15*) in which Leu^3 and Leu^4 within the dimerization domain were replaced by Pro and Ala, respectively, so that it does not have the ability to homodimerize or heterodimerize (*16*). This form of c-Jun did not promote Fos degradation in vitro (Fig. 2), which demonstrates that an intact leucine zipper, and hence complex formation between cmv-H_6cJun and c-Fos, are required to elicit the degradation effect.

Phosphorylation of c-Jun can impair the DNA binding activity of the molecule (*4*, *6*). Accordingly, we reasoned that the differential stability of c-Fos detected above might not be a direct consequence of the phosphorylation of c-Jun but rather might be caused by the inability of a c-Jun–phosphate–c-Fos heterodimer to bind to DNA that might be present in the reticu-

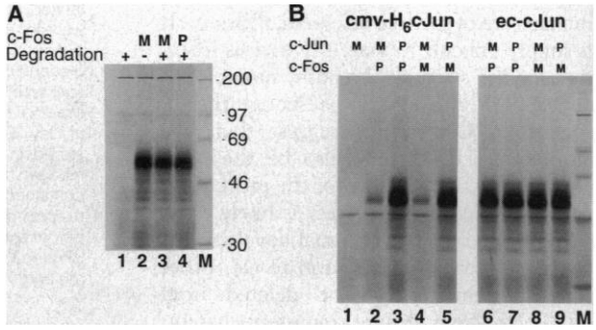

Fig. 1. (**A**) Degradation of c-Fos in vitro after treatment with active (PAP-treated, P) or inactive (mock-treated, M) PAP. A synthetic RNA that encoded rat c-Fos protein was translated in rabbit reticulocyte lysate in the presence of [^{35}S]methionine (*9*). A portion of the in vitro translation reaction (2 μl out of 50 μl) was treated (c-Fos pretreatment) with PAP in the presence (M, lanes 2 and 3) or in the absence (P, lane 4) of a cocktail of PAP inhibitors (*10*). PAP-treated samples were subsequently quenched with PAP inhibitors, and aliquots of each reaction (5 μl, containing 1 μl of in vitro–translated [^{35}S]c-Fos) were mixed with dialysis buffer [5 μl (*8*)] and left at 37°C for 30 min. Samples were brought to 30 μl (final volume) with a degradation mixture that contained (final concentrations) 50 mM tris-HCl (pH 7.5), 3 mM DTT, and 50% the concentration of PAP-protease inhibitors employed in the initial PAP treatment (*10*) and with (+) or without (−) freshly thawed rabbit reticulocyte lysate (7 μl). Reaction mixtures were assayed for degradation at 37°C and terminated after 2 hours by the addition of fourfold-concentrated Laemmli sample buffer (10 μl) and heating for 5 min at 95°C. The samples were then analyzed by SDS-PAGE, and radioactively labeled c-Fos was visualized by autoradiography of the fixed and dried gel. Lane 1 contains [^{35}S]methionine-supplemented rabbit reticulocyte lysate that was treated with inactivated PAP and processed exactly as described for the [^{35}S]c-Fos–containing lanes. Lane M, molecular size markers are shown at right in kilodaltons. (**B**) Degradation of heterodimeric c-Fos in vitro. The cmv-H_6cJun protein (lanes 2 through 5) purified from transiently transfected HeLa TK$^-$ cells (*7*, *8*) or the ec-cJun protein (lanes 6 through 9) isolated from *E. coli* cells (*11*) (8 μl, ~50 ng of c-Jun in each preparation) was treated with PAP (P, lanes 3, 5, 7, and 9) or inactivated PAP (M, lanes 2, 4, 6, and 8) as described (*10*). At the end of the incubation, PAP inhibitors were added to reactions that lacked them, and aliquots (5 μl) of each reaction (~25 ng of c-Jun protein) were mixed with [^{35}S]c-Fos (5 μl that contained 1 μl of in vitro–translated material) that had been treated with PAP (P, lanes 2, 3, 6, and 7) or inactivated PAP (M, lanes 4, 5, 8, and 9) as described in (A). Reaction mixtures were then incubated at 37°C for 30 min to enhance heterodimerization, brought to 30 μl (final volume) with a degradation mixture that contained freshly thawed rabbit reticulocyte lysate (7 μl), incubated at 37°C for 2 hours, and analyzed as in (A). Lane 1 contains [^{35}S]methionine-supplemented rabbit reticulocyte lysate that had been treated with inactivated PAP, mixed with similarly reacted (M) cmv-H_6cJun, and processed as described for the [^{35}S]c-Fos–containing lanes. Lane M is as in (A).

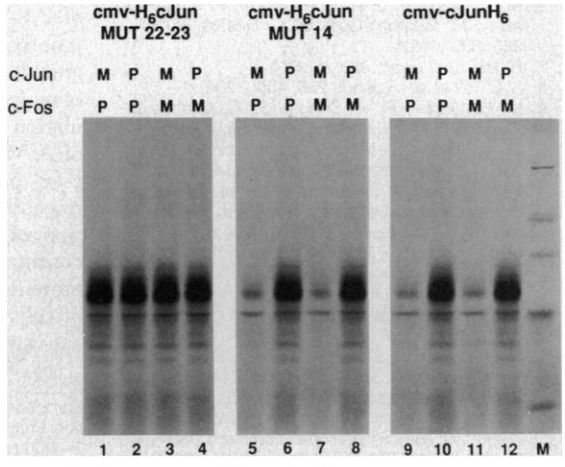

Fig. 2. Degradation of c-Fos in the presence of wild-type and mutant c-Jun. In vitro–translated [^{35}S]c-Fos was treated with PAP (P, lanes 1, 2, 5, 6, 9, and 10) or inactivated PAP (M, lanes 3, 4, 7, 8, 11, and 12), mixed with cmv-H_6cJun MUT 22-23 (lanes 1 through 4), cmv-H_6cJun MUT 14 (lanes 5 through 8), or cmv-cJunH_6 (lanes 9 through 12) proteins purified from parallel transient transfections of HeLa TK$^-$ cells (*8*) that had been treated with PAP (P, lanes 2, 4, 6, 8, 10, and 12) or inactivated PAP (M, lanes 1, 3, 5, 7, 9, and 11), and assayed for degradation as described (Fig. 1). The conditions for PAP treatment and the amounts of protein mixed in each reaction were identical to those used in Fig. 1. Lane M is as in Fig. 1.

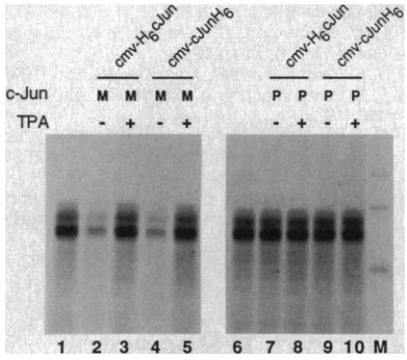

Fig. 3. Degradation of c-Fos in the presence of c-Jun purified from cells exposed to TPA. In vitro–translated [^{35}S]c-Fos was treated with inactivated PAP and mixed with either cmv-H_6cJun (lanes 2 and 3 and 7 and 8) or cmv-cJunH_6 (lanes 4 and 5 and 9 and 10) proteins purified from TPA-stimulated (+) or control (−) transiently transfected 293 cells (*8, 18*) that had been treated with PAP (P, lanes 7 through 10) or inactivated PAP (M, lanes 2 through 5). Reaction mixtures were then assayed for degradation as described (Fig. 1). Lanes 1 and 6 contain [^{35}S]c-Fos mixed with dialysis buffer that had been treated with inactivated PAP or with PAP, respectively, and processed exactly as the material in all other lanes. Lane M is as in Fig. 1.

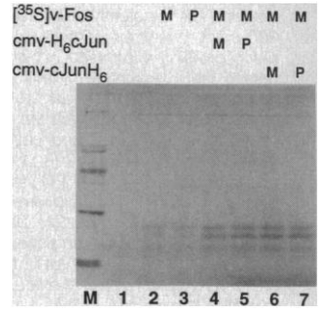

Fig. 4. Insensitivity of heterodimeric v-Fos to degradation. In vitro–translated [^{35}S]v-Fos (*9*) was treated with inactivated PAP (M, lanes 4 through 7), mixed with either cmv-H_6cJun (lanes 4 and 5) or cmv-cJunH_6 (lanes 6 and 7) proteins purified from transiently transfected 293 cells (*8*) that had been treated with PAP (P, lanes 5 and 7) or inactivated PAP (M, lanes 4 and 6), and assayed for degradation as described (Fig. 1). Lanes 2 and 3 contain [^{35}S]v-Fos treated with PAP (P, lane 3) or inactivated PAP (M, lane 2), mixed with dialysis buffer, and processed exactly as the material in lanes 4 through 7. Lane 1 contains [^{35}S]methionine-supplemented rabbit reticulocyte lysate that had been treated with inactivated PAP and processed exactly as described for the [^{35}S]v-Fos–containing lanes. Lane M is as in Fig. 1.

locyte lysate. Free leucine zipper protein dimers have a less rigid structure than DNA-bound dimers and therefore may be more susceptible to proteolytic attack (*17*). To investigate this possibility, we analyzed in a degradation assay a mutant of cmv-H_6cJun (MUT 14) (*15*) that is incompetent for DNA binding even when present in a heterodimer with wild-type Fos (*16*) (Fig. 2). This mutant behaved indistinguishably from wild-type cmv-H_6cJun (Fig. 1), which rules out indirect effects on c-Fos stability mediated by its DNA binding state in our assays. We also examined cmv-cJunH_6, a variant of cmv-H_6cJun that carries the hexahistidine tag at the COOH-terminus instead of at the NH_2-terminus (Fig. 2) (*15*). Both proteins behaved identically (Fig. 1), which shows that an artifactual activity of cmv-H_6cJun that was a result of the presence of an altered NH_2-terminus cannot explain the observed effects.

Inducible changes in the phosphorylation state of Jun seem to be important in signal transduction (*4–6*). We therefore tested whether c-Fos degradation might also be regulated by extracellular signals by treating cells that had been transfected with the vectors encoding cmv-H_6cJun or cmv-cJunH_6 with the tumor-promoting agent TPA (*18*). TPA activates protein kinase C and thus mimics the effect of some extracellular inducers of c-Jun activity. The cmv-H_6cJun and cmv-cJunH_6 proteins were purified from TPA-treated and control cells (*8*), and the material obtained was quantitated by protein immunoblot and DNA binding assays (*12*) and then analyzed in an in vitro degradation assay with [^{35}S]c-Fos (Fig. 3). The c-Jun proteins from TPA-treated cells caused much less degradation of c-Fos than c-Jun isolated from untreated cells. Co-immunoprecipitation experiments confirmed that all c-Jun preparations used in this analysis heterodimerize similarly with c-Fos (*12*). Because in vitro dephosphorylation of cmv-H_6cJun elicits the same loss of trans-degradation activity as TPA treatment (Fig. 3), we propose the involvement of a signal-induced dephosphorylation event. This effect could serve to transiently stabilize c-Fos–c-Jun dimers in stimulated cells. Such a stabilization would, in conjunction with the elevated c-*fos* and c-*jun* mRNA synthesis after cell induction (*19*), lead to an increase of AP-1 concentration. After withdrawal of the stimulus, the relevant phosphate groups on c-Jun might be restored, which would cause a rapid decrease of AP-1 activity as a result of protein degradation.

To examine if v-Fos is subject to the same trans-degradation as its cellular progenitor c-Fos, we investigated whether v-Fos could be destabilized by phosphorylated c-Jun. A strongly transforming version of v-*fos*, the retrovirally derived gene encoding the E300 protein (*20*), was used to generate in vitro–translated radiolabeled protein (^{35}S-labeled v-Fos) for the degradation assay (*9*). The [^{35}S]v-Fos protein was not destabilized by cmv-H_6cJun or cmv-cJunH_6, regardless of their phosphorylation state (Fig. 4); rather, dimerization with c-Jun seemed to slightly stabilize v-Fos treated with PAP or inactivated PAP. Co-immunoprecipitation analyses confirmed that all the c-Jun preparations retained the ability to heterodimerize with v-Fos (*12*). Moreover, the stability of v-Fos was not affected when c-Jun purified from TPA-stimulated cells or PAP-treated [^{35}S]v-Fos was used in the assay (*12*). This insensitivity to destabilization might explain part of the transforming potential of v-Fos.

Trans-targeted protein degradation requires a degradation signal on a subunit of a multimeric protein complex that is different from the subunit attacked by the protease. Such phenomena have been described with artificial substrates (*21*) and for p53, which is degraded after forming a complex with the human papilloma virus E6 protein (*22*). Our findings differ in that the target is a transcription factor that participates in positive growth control pathways and in that the degradation signal on c-Jun is regulated by reversible protein phosphorylation. An advantage of the proposed c-Fos degradation mechanism is that it would target only the biologically active form of c-Fos, namely the one present in a DNA binding–competent complex with c-Jun, whereas monomeric c-Fos or c-Fos that is complexed with other AP-1 family members would be unaffected. Our findings are specific to Fos and Jun inasmuch as the c-Myc and Max proteins, which dimerize like Jun and Fos to form a functional transcription factor, do not influence each other's stability (*23*).

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7. The vector used for the expression of cmv-H_6cJun, pKH6, was derived from pCMV-Jun (*24*). The first nine codons of the human c-Jun open reading frame in pCMV-Jun were replaced with an oligonucleotide encoding the NH_2-terminal peptide Met-Asp-Pro-His-His-His-His-His-His-Asp-Pro. The HeLa thymidine kinase–negative (TK$^-$) or 293 cell lines were propagated as described (*6*), and ~8 × 10^5 cells were seeded on 10-cm tissue culture plates 24 hours before transfection by the calcium phosphate co-precipitation method (*25*). CsCl-purified wild-type or mutant DNA (3 μg) was transfected with 22 μg of pUC18 as carrier DNA, and approximately 24 hours after transfection, the cells were washed three times with ice-cold phosphate-buffered saline and scraped into lysis buffer (*8*).
8. Wild-type and mutant cmv-H_6cJun–cmv-cJunH_6 proteins were purified from untreated or TPA-

treated (*18*) transiently transfected HeLa TK$^-$ or 293 cells. Every experiment was repeated with c-Jun proteins expressed in both cell lines. The results were essentially identical for both cell types, except the effect of TPA treatment was clearly detectable only with the material derived from 293 cells. Transfected cells were lysed by the addition of lysis buffer [6 M guanidinium-HCl and 0.1 M Na_2HPO_4-NaH_2PO_4 (pH 8.0), with 1 ml per 10-cm dish], and the overexpressed protein was purified by nickel-chelate affinity chromatography as described (*6*) and brought into dialysis buffer [30 mM Pipes KOH (pH 6.0), 10% glycerol, 0.1% lauryldimethylamine oxide, 10 mM KCl, 2 mM $MgCl_2$, 0.2 mM EDTA, 1 mM dithiothreitol (DTT), 2 mM phenylmethylsulfonyl fluoride (PMSF), E-64 (4 μg/ml), aprotinin (5 μg/ml), pepstatin (2 μg/ml), and leupeptin (2 μg/ml)]. The yield of both wild-type and mutant cmv-cJun per plate of HeLa TK$^-$ or 293 cells was ~1 μg per dish.

9. As a source of c-Fos and v-Fos E300 proteins, a combined in vitro transcription-translation system was used. A plasmid that contained a rat cDNA encoding the wild-type c-Fos (*26*) or pTZE300 encoding v-Fos (*20*) was transcribed (after linearization with Hind III) essentially as suggested by the supplier with either SP6 (c-Fos) or T7 (v-Fos) RNA polymerases (Promega). The resulting mRNAs (from 6 μg of template DNA) were used in a translation reaction (50 μl) that contained nuclease-treated rabbit reticulocyte lysate (40 μl; Promega) in the presence of 6 μl of L-[^{35}S]methionine (>1000 Ci/mmol; Amersham).
10. PAP as a suspension in $(NH_4)_2SO_4$ solution (Grade I, Boehringer Mannheim) was prepared as described (*6*); 0.05 U (in 0.5 μl) of enzyme were used in a reaction (10 μl) that contained either wild-type or mutant c-Jun protein (8 μl, ~50 ng) in dialysis buffer (*8*) or in vitro–translated c-Fos or v-Fos [2 μl (*9*)] and a final concentration of 25 mM Pipes KOH (pH 6.0), 2 mM PMSF, E-64 (4 μg/ml), aprotinin (5 μg/ml), pepstatin (2 μg/ml), and leupeptin (2 μg/ml). Digestions were performed at 25°C for 1 hour in the presence or absence of a mixture of competitive and noncompetitive phosphatase inhibitors [100 mM NaF, 15 mM Na_2MoO_4, 20 mM (Na_2)-*p*-nitrophenyl phosphate, and 10 mM (Na_2)-bis-glycerophosphate].
11. The pHJmet5 vector was used to express (from a promoter recognized by T7 RNA polymerase) the complete human c-Jun protein in *E. coli* strain BL21 starting at Met^5, which is the probable initiation codon utilized in vivo (*16*). The ec-cJun protein was also brought into dialysis buffer (*8*) before PAP treatment.
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15. We created the cmv-H_6cJun MUT 22-23 construct from pKH6 by substituting the codons for Leu^4 and Leu^3 of the dimerization domain with codons specifying Ala and Pro, respectively, by oligonucleotide-directed in vitro mutagenesis. Similarly, we generated the cmv-H_6cJun MUT 14 construct from pKH6 by substituting the codons for Lys and Cys of the amino acid sequence Ala-Ser-Lys-Cys-Arg-Lys within the basic DNA binding domain with codons specifying Ile and Asp, respectively. Both mutant clones were sequenced with the dideoxy nucleotide chain termination method to confirm the presence of the mutations. We derived the vector used for the expression of cmv-cJunH_6 from pHJ40 (*16*) by replacing (using the polymerase chain reaction technique) the COOH-terminal two codons (Thr and Phe) of the human c-Jun sequence with an oligonucleotide stretch that encoded the peptide Glu-Phe-His-His-His-His-His-His. The resulting construct was sequenced to verify the COOH-terminal addition.
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18. For TPA treatment of the cell cultures, TPA (as a stock solution (100 μg/ml) in dimethyl sulfoxide) or solvent alone (control cells) was added to the medium 24 hours after transfection to a final concentration of 100 ng/ml, and cells were incubated for an additional 1 hour before harvesting.
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Retinoids Selective for Retinoid X Receptor Response Pathways

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Retinoids have a broad spectrum of biological activities and are useful therapeutic agents. Their physiological activities are mediated by two types of receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). RARs, as well as several related receptors, require heterodimerization with RXRs for effective DNA binding and function. However, in the presence of 9-*cis*-retinoic acid, a ligand for both RARs and RXRs, RXRs can also form homodimers. A series of retinoids is reported that selectively activates RXR homodimers but does not affect RAR-RXR heterodimers and thus demonstrates that both retinoid response pathways can be independently activated.

Retinoic acid (RA) and its natural and synthetic analogs (retinoids) affect a wide array of biological processes. Retinoids are used in the treatment of many skin diseases (*1*) and are promising drugs for several cancers (*2*). Consistent with their broad biological activity pattern, retinoids have a variety of side effects, including teratogenicity, that limit their therapeutic potential. Specific retinoids with restricted biological activities may have fewer side effects. We have designed and synthesized several retinoids that selectively activate RXRs by inducing the formation of RXR homodimers. Although it was previously thought that both RARs and RXRs function as homodimers, evidence now indicates that RARs require heterodimerization with RXRs for effective DNA binding and function (*3–9*). RXRs also bind several other ligand-regulated receptors, including thyroid hormone receptors and vitamin D_3 receptor (*3–9*). In addition, in the presence of 9-*cis*-retinoic acid (9-*cis*-RA), RXRs form homodimers that have response element specificities that are distinct from those of RAR-RXR heterodimers (*10*), indicating that the two RA response pathways activate distinct sets of genes. Because it binds both RARs and RXRs with high affinity (*11*), 9-*cis*-RA is a very potent activator of RAR-RXR heterodimers (*10*). Thus, 9-*cis*-RA should elicit very broad biological responses. Here, we define a class of retinoids that activates only the RXR homodimer.

We used the TREpal-tk reporter gene (*12*), which is activated by both RAR-RXR heterodimers and RXR homodimers (*10*), in a transient transfection assay (*13*) to evaluate compounds for the induction of RXR activity. When the RXR expression vector was cotransfected with the TREpal-tk reporter gene into African green monkey kidney (CV-1) cells, all-*trans* RA did not efficiently activate the reporter, whereas 9-*cis*-RA did (*10*). Preliminary evaluation of a series of retinoids indicated that several showed activity with cotransfected RXR. The pharmacophoric elements of these retinoid structures were then combined and further modified to produce a subset of retinoids (Fig. 1A) whose activation profiles for RXR were similar to that of 9-*cis*-RA (Fig. 2A). Although none of these compounds revealed activity at 10^{-8} M, the two most potent retinoids, SR11217 and SR11237, showed activities similar to 9-*cis*-RA at 10^{-7} M. The induction profiles were similar to that of a reporter gene carrying CRBPII [the RA response element

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