Our results and others (*8–11*) are compatible with several possible defects in the main class II antigen-processing and presentation pathway. (i) Class II molecules and processed antigen may initially occupy separate compartments; conjunction of these compartments may be defective in .174. (ii) Processed antigenic peptides may have to be transported from the compartment in which they are produced to a compartment containing class II molecules; their transport may be impaired in .174. (iii) A chaperonin-like protein may facilitate the binding of peptides to class II molecules in the main antigen presentation pathway (*29*) and may be absent in .174 cells. (iv) Antigen-processing proteinases or conditions that affect their activities may be defective in .174. Proteolysis occurs in the class II pathway of .174, but it may not be the proteolysis predominantly used to generate antigenic peptides.

It is not yet possible to decide between these four hypotheses. Any explanation of the defect in .174 should also account for the relative scarcity of long, I_i-derived peptides on DR3 molecules produced in .82(DR3) and the presence of these same peptides on DR1 produced by another human cell line, in which antigen processing is apparently normal (*30*). The long, I_i-derived peptides may be produced in a compartment other than that in which cells generate the highly diverse shorter peptides that are associated with class II molecules in nondefective cells. This interpretation is supported by the absence on .174(DR3)-derived DR3 of the 14– to 15–residue long I_i peptides that were relatively abundant on DR1 of normal cells (*30*).

Peptides derived from homologous segments of the human and mouse invariant chains associate with DR3, DR1 (*30*), I-A^b (*27*), and I-A^d (*24*). However, such peptides do not bind to I-E^b (*27*), and the 17–amino acid peptide that binds to I-A^d binds poorly to I-E^d (*24*). The part of I_i that is bound to the DR3 and I-A molecules is unlikely to occlude the peptide-binding groove (*24*). This suggests that after proteolytic cleavage and dissociation of I_i from class II MHC molecules, the I_i peptides bind to certain class II molecules because those class II molecules have motifs that favor the association. However, the possibility that the I_i-derived peptides are not derived from the peptide-binding groove but are derived from some other binding site on the class II molecule cannot be excluded at present. Analysis of peptides that are bound to DR1, DR2, and DR52b that are produced in existing transfectants of .174 (*8*) should clarify this point.

Most class II–associated peptides derived from exogenous proteins are produced in the endocytic pathway, but some viral peptides presented on class II molecules are derived by the processing of cytosolic proteins (*31, 32*). Both pathways can contribute to the presentation of foreign antigens. Our data suggest that a third source of class II–peptide complexes is from a B cell compartment that contains abundant peptides derived from the invariant chain and perhaps other proteins. Possible contributions of this pathway of peptide loading to the presentation of foreign and autoantigens can now be investigated with class II–expressing derivatives of .174.

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Expression Directed from HIV Long Terminal Repeats in the Central Nervous System of Transgenic Mice

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Infection with the human immunodeficiency virus (HIV) is frequently accompanied by the AIDS (acquired immunodeficiency syndrome) dementia complex. The role of specific HIV genetic elements in the pathogenesis of central nervous system (CNS) disease is not clear. Transgenic mice were constructed that contained the long terminal repeats (LTRs) of two CNS-derived strains and a T cell tropic strain of HIV-1. Only mice generated with CNS-derived LTRs directed expression in the CNS, particularly in neurons. Thus, some strains of HIV-1 have a selective advantage for gene expression in the brain, and neurons can supply the cellular factors necessary for their transcription.

During the course of AIDS an individual is host to many different strains of HIV-1 (*1*). The role of these different strains in the pathogenesis of AIDS and the frequently associated neurologic disease called AIDS dementia complex (ADC) is not clear. AIDS dementia complex is characterized by subacute encephalitis that results in progressive memory loss, cognitive impairment, and motor and behavioral abnormalities (*2*). Examination of the brains of patients with ADC has revealed significant neuronal cell loss (*3*). The cause of neuronal loss remains unclear because HIV-1 replication occurs primarily in the microglia and macrophages (*3*). A number of HIV-1 strains have been isolated from the CNS (*4*) of patients with ADC; however, it is difficult to determine

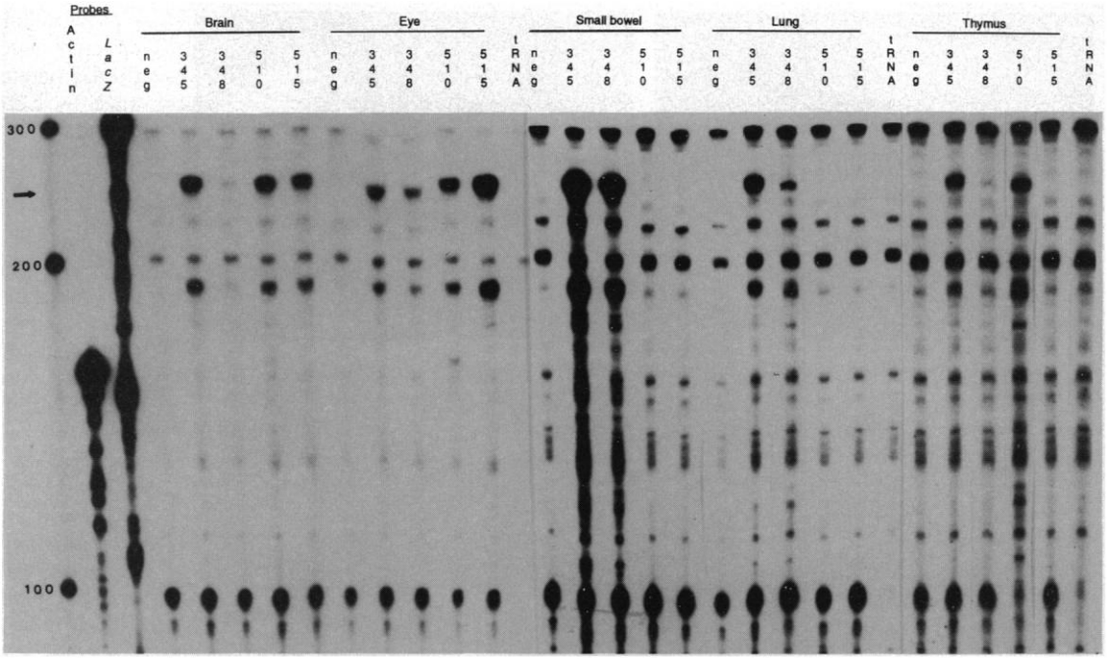

Fig. 1. RNase protection assay was used to quantitate β-gal mRNA in tissues of the transgenic mice. RNA was isolated from 6- to 12-week-old G2 transgenic offspring (*24*). A 250-bp fragment from the 3′ end of the *lacZ* gene (bp 2780 to 3029) was cloned in the pGEM-7Z(+) vector. The plasmid was linearized at the Bam HI site, and a ^{32}P-labeled 295-bp antisense RNA was synthesized with T7 polymerase. This RNA probe (5 × 10^5 cpm) was hybridized with 30 μg of RNA extracted from each tissue. The RNA hybrid was digested for 1 hour at 30°C with RNase A (62 μg/ml) and RNase T_1 (50 units). This resulted in a 250-bp–protected β-gal RNA hybrid (*25*). Numbers at the left side indicate the base pairs of end-labeled double-stranded oligonucleotides. Human β-actin cDNA (*26*) was used to generate an antisense β-actin RNA probe (2.5 × 10^3 cpm per lane); digestion produced protected species of 100 bp. The RNase protection products were analyzed on a 6% acrylamide–urea gel and exposed to x-ray film for 8 to 11 days with intensifying screens. Neg, tissue from negative control littermates.

the role of these viral strains in ADC because no animal model of neuronal loss exists. In order to compare the expression of different strains of HIV-1 in the CNS, we constructed transgenic mice with the LTRs of two CNS-derived strains of HIV-1. We chose the LTR because transcription of retroviruses is regulated by sequences in the U3 region of the LTR and the LTR has been implicated in tissue-specific gene expression and disease (*5*). Other transgenic mice that contained the HIV-$1_{IIIB/LAV}$ LTR or the entire HIV-$1_{IIIB/LAV}$ provirus did not have any detectable gene expression or replication in the CNS (*6*).

We constructed transgenic mice by using the LTRs from HIV-1_{JR-CSF} and HIV-1_{JR-FL}, derived from the cell-free cerebrospinal fluid (CSF) and frontal lobe (FL) of a patient, JR, with ADC (*7*). Comparison of the sequences in the U3 region of the LTRs of HIV-$1_{IIIB/LAV}$, HIV-1_{JR-CSF}, and HIV-1_{JR-FL} showed that two common sites of HIV-1 transcriptional regulation were completely conserved (nuclear factor NF-κB and SP-1). However, there were multiple (>27) base differences upstream of these sites in the U3 region of both HIV-1_{JR-CSF} and HIV-1_{JR-FL} as compared with the U3 region of HIV-$1_{IIIB/LAV}$ (*8*). The U3 regions of the LTRs of HIV-1_{JR-CSF} and HIV-1_{JR-FL} differed from each other by 16 bp, all of which were upstream of the NF-κB and SP-1 sites. We report here that the LTRs of both CNS-derived strains of HIV-1 directed the expression of the bacterial reporter gene β-galactosidase (β-gal) in the CNS of transgenic mice, but in different neuroanatomical locations.

Six transgenic mice were generated, two containing the LTR of HIV-1_{JR-CSF} and four the LTR of HIV-1_{JR-FL} [Table 1 and (*9*)]. Both of the HIV-1_{JR-CSF} (345 and 348) and two of the four HIV-1_{JR-FL} (510 and 515) transgenic mouse lines expressed β-gal protein and mRNA in multiple tissues. Southern (DNA) blot analysis was used to estimate the number of copies of the transgene in each line (Table 1) and to demonstrate that the transgenes were integrated at single sites in a head-to-tail array (*10*). No clinical signs related to the presence of the transgenes have been detected in animals during 8 months of observation. Six- to 12-week-old second-generation (G2) heterozygote offspring of the four transgenic founder mice were analyzed for expression of the β-gal mRNA by ribonuclease (RNase) protection assay (Fig. 1). Significant amounts of β-gal mRNA were demonstrated in the brain and eyes, with less in the thymus (Fig. 1). Only the two HIV-1_{JR-CSF} lines expressed β-gal mRNA in the small bowel and lung (Fig. 1). Mice from one HIV-1_{JR-FL} line (510) expressed comparable amounts of mRNA in the heart, brain, and eyes and in the liver and thymus. Quantitative comparison was done by laser densitometry and normalization to actin mRNA for each RNA sample. None of the four transgenic lines expressed β-gal in skin, spleen, or mesenteric lymph nodes.

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Table 1. HIV-1 LTR transgenic founder lines. β-gal DNA, β-gal expression (mRNA and enzymatic activity).

Strain	Line	Sex	PCR	Southern	Copy number	β-Gal expression
HIV-1_{JR-CSF}	345	F	+	+	5 to 10	+
	348	M	+	+	≤50	+
HIV-1_{JR-FL}	510	F	+	+	10	+
	513	M	+	+	100	−
	514	M	+	+	2 to 3	−
	515	M	+	+	2 to 3	+

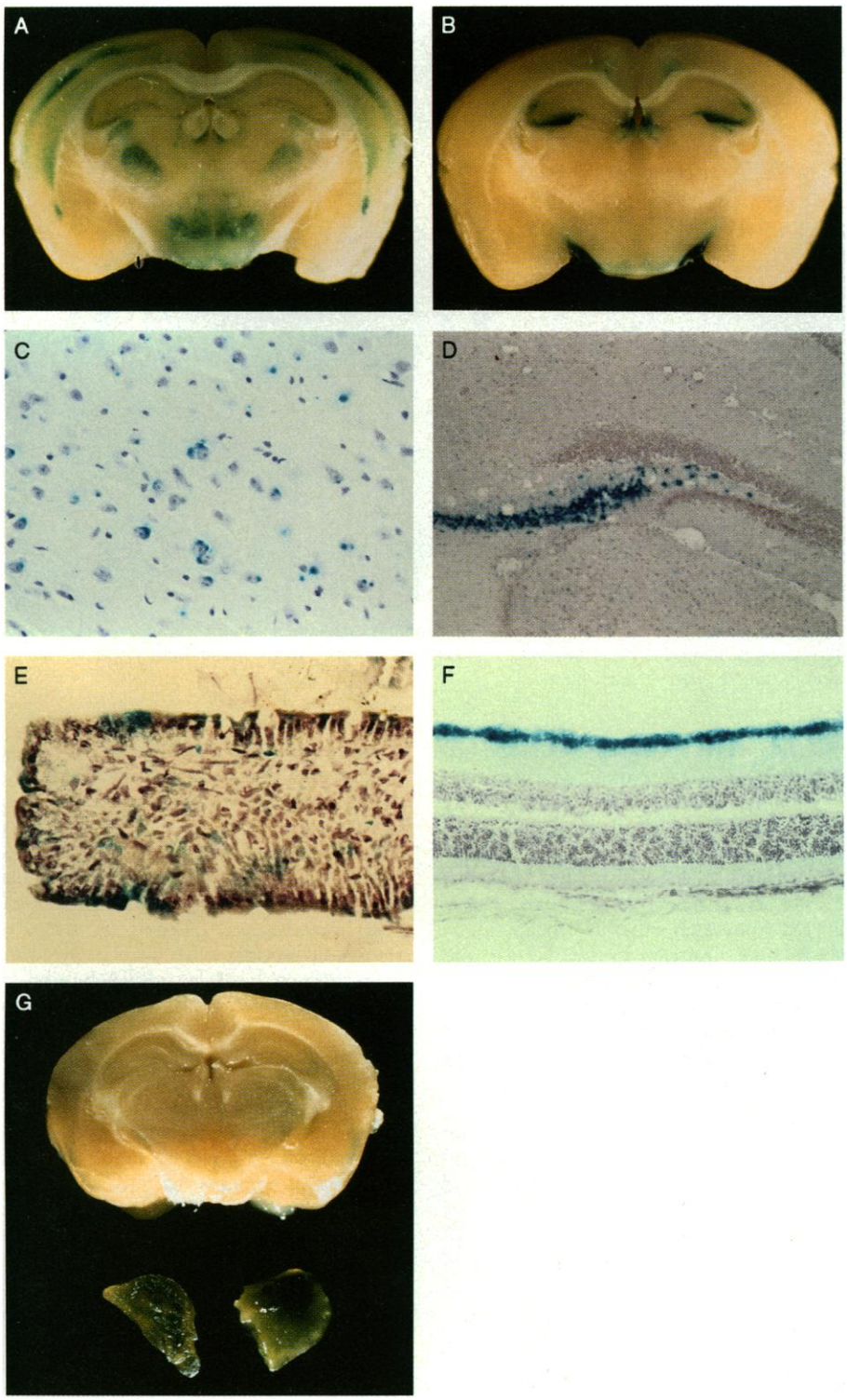

Fig. 2. Histological detection of β-gal in the CNS and intestine of HIV-LTR transgenic mice. Adult G2 transgenic mice HIV-$1_{JR\text{-}CSF}$ (**A**) and HIV-$1_{JR\text{-}FL}$ (**B**) were anesthetized and perfused with 4% paraformaldehyde, and coronal sections of the brain were stained for β-gal activity overnight (original magnification, ×2) (*11*). Tissues from negative control littermates contained no blue stain. (C to G) Detection of β-gal protein by histochemical staining of 10-μm frozen cryostat sections. (**C**) Neurons of the dorsolateral geniculate nucleus of HIV-$1_{JR\text{-}CSF}$ LTR transgenic mouse counterstained with hematoxylin (×40). (**D**) Hippocampal neurons of CA3 in HIV-$1_{JR\text{-}FL}$ transgenic mouse (×20). (**E**) Columnar epithelium in the ileum of HIV-$1_{JR\text{-}CSF}$ transgenic mouse (×40). (**F**) Retinal ganglion cells positive in all transgenic lines show discrete neuronal localization in HIV-$1_{JR\text{-}FL}$ β-gal and hematoxylin stains (×20). Adult G2 transgenic mice HIV-$1_{IIIB/LAV}$ brain contained no β-gal activity (coronal section, ×1.5). In contrast, (**G**) the thymus of HIV-$1_{IIIB/LAV}$ [seen in cross-sectional (left) and surface views] contained significant β-gal activity.

The cellular distribution of HIV LTR–directed gene expression was analyzed in the G2 heterozygote offspring of all founder mice by histochemical staining for β-gal activity. Six- to 8-week-old males and females were anesthetized and perfused with 4% paraformaldehyde, and tissues were stained for β-gal activity with the substrate 5-bromo-4-chloro-3-indoyl-β-D-gal (X-gal) (*11*). The anatomical patterns of β-gal expression in the CNS and nonneural tissues of the two HIV-$1_{JR\text{-}CSF}$ lines were similar; small differences (noted in Table 1) were attributed to the intensity of X-gal staining. In tissues from the two HIV-$1_{JR\text{-}FL}$ lines, the pattern of β-gal protein expression and intensity were very similar. The amounts of mRNA determined by RNase protection assay correlated with the β-gal protein expression. Moreover, these patterns were the same in either sex throughout several generations tested.

Within the CNS the X-gal stain was bilaterally symmetrical (Fig. 2, A and B) and was located primarily in cortical and subcortical neuronal regions involved in both motor and cognitive function and in the retina of the eye. The greatest gene expression observed in the CNS of HIV-$1_{JR\text{-}CSF}$ mice was in layers 2, 3, and 6 of the cerebral cortex; in the dorsolateral geniculate and posterior nuclei of the thalamus (Fig. 2A); and in the pontine nuclei. To a lesser extent, the HIV-$1_{JR\text{-}CSF}$ LTR directed expression of β-gal in the hypothalamus and in all layers of the hippocampus. In contrast, the HIV-$1_{JR\text{-}FL}$ LTR–directed expression of β-gal strongly in the CA3 and CA4 regions of the hippocampus (Fig. 2D), the septal nuclei, the habenular nuclei (Fig. 2B), the optic tract, and in the pontine nuclei. No β-gal expression was seen in the spinal cord of any animal. Table 2 summarizes the CNS regional β-gal expression in the transgenic mice.

The discrete cellular localization of X-gal staining in the brain and in the retina of the eye suggested that these LTRs directed expression of β-gal in neurons. To more precisely identify the cell type that expressed β-gal, we stained 10- to 20-μm frozen and paraffin-embedded sections with X-gal and various markers specific for CNS cells. X-gal staining was observed in cells that were morphologically compatible with neurons in the dorsolateral geniculate nucleus of HIV-$1_{JR\text{-}CSF}$ mice (Fig. 2C). In this location cells were double-stained only infrequently by X-gal and histochemical markers for astrocytes and microglial cells (glial fibrillary acidic protein and microglial lectin RCA-1, respectively, and S-100β in both) (*12*). No expression was detected in endothelial cells or choroid plexus in any of these transgenic animals. In the HIV-$1_{JR\text{-}FL}$ mice the X-gal staining of the hippocampus

Table 2. Distribution of HIV-1 LTR–directed gene expression in the CNS (relative density of β-gal activity). In HIV-1_{JR-CSF} the data refer to both 345 and 348; however, when intensity is less than (++), 348 is low to undetectable.

Tissue	HIV-1_{JR-CSF}	HIV-1_{JR-FL}
Telencephalon		
Cerebral cortex		
Cingulum	−	+
Frontal parietal somatosensory layers 3 to 4 + 6	+++	++
Frontal parietal motor layers 3 to 4 + 6	+++	+
Striate	++	+
Retrosplenial	+++	−
Basal ganglia	+	−
Rhinencephalon-hippocampus		
Olfactory	+	+
Septum	+	+++
Hippocampus		
CA1 + CA2	+	−
CA3 + CA4	+	+++
Diencephalon		
Thalamus		
Ventroposterior	++	−
Reticular	+	+
Geniculate, DLG	+++	−
Habenula	+	+++
Hypothalamus		
Supraoptic		
Medial preoptic	++	++
Anterior area	+	−
Paraventricular	++	−
Tuberomammillary nucleus	+	−
Mammillary		
Submammillothalamic nucleus	++	+
Premammillary nucleus dorsal	++	+
Retina, ganglion cell layer	++	+++
Optic tract	−	+++
Mesencephalon		
Superior colliculus, zonal layer	+	++
Pontine nucleus	+++	+++
Cerebellum, Purkinje cell	++	+
Spinal cord	−	−

occurred in the CA3 neurons (Fig. 2D). All the transgenic mice had β-gal staining in the retinal ganglion cell layer of the eye, although expression in HIV-1_{JR-FL} animals was higher (Fig. 2F). Both LTRs directed expression in a subset of Purkinje cells in the cerebellum. These specific patterns of expression do not correspond with any known neurotransmitter patterns. We conclude that the LTRs from CNS-derived strains of HIV-1 directed gene expression in the CNS neurons of transgenic mice. The LTRs from both CNS-derived strains of HIV-1 directed expression in CNS neurons and may therefore represent neuroadapted strains of HIV-1.

In multiple previous studies with mice of varying genetic backgrounds, HIV-$1_{IIIB/LAV}$ transgenic mice did not exhibit gene expression or replication of the virus in the CNS (6). We constructed a single HIV-$1_{IIIB/LAV}$ LTR transgenic mouse line in parallel with those described above; β-gal was not expressed in the CNS, in contrast to extensive expression in the thymus (Fig. 2G).

Examination of nonneural tissues from transgenic mice (345, 348, 510, and 515) indicated β-gal activity in the medulla of the thymus; less staining was observed in the spleens of all four lines. No X-gal staining was detected in liver, heart, lung, kidney, adrenal glands, muscles, or freshly isolated peripheral blood leukocytes of animals from any transgenic line. In the HIV-1_{JR-FL} LTR mice, no β-gal expression was detected in the intestine, consistent with the lack of mRNA in that organ. However, the small intestine of the HIV-1_{JR-CSF} LTR animals was positive for β-gal protein in the columnar epithelium that lines the lumen of the intestinal tract (Fig. 2E). Specific cells in the intestine and CNS respond to a family of peptides that act as hormonal regulators in the intestine and as neurotransmitters in the CNS (*13*). Thus, expression from the HIV-1_{JR-CSF} LTR in neurons as well as small intestine may represent a common pathway of transcriptional regulation.

The results in this report are unexpected because HIV-1 replication in the CNS has not been detected in neurons. However, the pathogenesis of the neuronal cell loss seen in ADC is still uncertain. In autopsy studies of the brains of individuals with ADC, macrophages and microglia are the cells most commonly infected with HIV-1 (*14*). Infrequently in autopsy and biopsy studies, other CNS cells, including endothelial cells (*15, 16*), astrocytes and oligodendroglia (*15–18*), and neurons (*15, 17, 19*) are found to be infected. In tissues other than those of the CNS, HIV-1 primarily infects T lymphocytes and macrophages that express the surface molecule CD4 (*20*). However, CD4 may not be necessary for HIV-1 infection of neural-derived primary (*21*) or cultured (*22*) cells, where the virus may use other molecules for entry into cells (*23*). The loss of neurons in ADC has not been readily explained by HIV-1 infection of macrophages and microglial cells; other explanations, including cytotoxicity mediated by cytokines, viral proteins, and the direct infection of neurons, have been suggested. Our data suggest that differences in the nucleotide sequence of the LTRs of different HIV strains can alter the tissue-specific pattern of gene expression directed from their LTRs, as has been observed for other retroviruses. If these CNS-derived strains are able to gain entry into the cell, neurons can produce cellular transcription factors that interact with and direct expression from the LTRs.

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5′ end in the R region (bp +41 to +59) with an Xho I site at the 3′ end. The amplified fragment was gel purified and cloned into the lacZ-containing vector at the Kpn I and Xho I sites upstream of the *lacZ* gene. HIV-1_{JR-CSF} LTR was digested with Hind III to remove it from the vector (mp18-1-1-JRCSF), and the 0.95-kb fragment (including the 0.4-kb 5′ flanking sequences) was cloned into the plasmid pSAFYre upstream of the *lacZ* gene. The HIV-1_{JR-CSF} LTR β-gal construct was digested with Cla I and Bam HI, and the HIV-1_{JR-FL} LTR β-gal construct was digested with Kpn I and Bam HI and LTR-*lacZ* fragments purified by electroelution and CsCl centrifugation [as in B. Hogan, F. Constantine, E. Lacy, in *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986)]. DNA was quantitated by fluorometry and diluted in microinjection buffer [10 mM tris (pH 7.4) and 0.1 mM EDTA] to a final concentration of 2 μg/ml. We made transgeni mice by standard microinjection techniques, using CD-1 donor females and (C57BL/6 × DBA/2) G1 donor males. A total of 45 HIV-1_{JRCSF} β-gal and 15 HIV-1_{JR-FL} β-gal potential transgenic founders were screened by Southern blot analysis of which two and four respectively, were transgenic.
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Activation of Transcription by IFN-γ: Tyrosine Phosphorylation of a 91-kD DNA Binding Protein

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Interferon-γ (IFN-γ) induces the transcription of the gene encoding a guanylate binding protein by activating a latent cytoplasmic factor, GAF (gamma-activated factor). GAF is translocated to the nucleus and binds a DNA element, the gamma-activated site. Through cross-linking and the use of specific antibodies GAF was found to be a 91-kilodalton DNA binding protein that was previously identified as one of four proteins in interferon-stimulated gene factor–3 (ISGF-3), a transcription complex activated by IFN-α. The IFN-γ–dependent activation of the 91-kilodalton DNA binding protein required cytoplasmic phosphorylation of the protein on tyrosine. The 113-kilodalton ISGF-3 protein that is phosphorylated in response to IFN-α was not phosphorylated nor translocated to the nucleus in response to IFN-γ. Thus the two different ligands result in tyrosine phosphorylation of different combinations of latent cytoplasmic transcription factors that then act at different DNA binding sites.

How different polypeptide ligands elicit transcriptional induction of different sets of genes is not known. However, the pathway through which IFN-α stimulates gene transcription has recently been clarified. Receptor-specific ligand binding at the cell surface results in the intracellular phosphorylation of latent cytoplasmic proteins of 113, 91, and 84 kD (*1–5*); these proteins then translocate to the nucleus to join in a multiprotein complex termed ISGF-3 (*1*) that binds to the IFN-α–responsive DNA element, the interferon-stimulated response element (ISRE). The protein that contacts DNA in the complex is a 48-kD molecule (*2, 6*) that is itself not phosphorylated in response to IFN-α. One kinase that may associate with proteins in the plasma membrane and participate in the phosphorylation of the 113-, 91- and 84-kD proteins is Tyk-2; mutant cells resistant to IFN-α can be restored to responsiveness by transfection of a cDNA encoding Tyk-2 (*7–9*).

IFN-γ causes transcriptional activation of a gene encoding a guanylate binding protein (GBP) (*10, 11*). The DNA site required for activation of transcription by IFN-γ, the GAS (γ-activated site), has been defined (*12*) and binds a protein, the γ-activated factor (GAF), that is activated in the cytoplasm (*13*). IFN-γ stimulates the phosphorylation on tyrosine of a protein of approximately 91 kD (*5*). In this report, we describe the protein composition of GAF and changes in its state of phosphorylation. Although the 91-kD protein in ISGF-3 does not contact DNA, the same 91-kD protein bound specifically to DNA at the GAS element in cells treated with IFN-γ. IFN-γ–dependent cytoplasmic tyrosine phosphorylation of the 91-kD protein was required for this binding. Phosphorylation of the 91-kD protein occurs on the same peptide after treatment of cells with IFN-γ or IFN-α, but the specificity of signaling is maintained because the 113-kD protein of the ISGF-3 protein complex is not phosphorylated in response to IFN-γ as it is in response to IFN-α.

We used an electrophoretic mobility shift assay (*14*) to identify, in extracts of human fibroblasts, a factor that formed an electrophoretically stable DNA-protein complex and that had the same properties as GAF, which was originally identified by an exonuclease III protection assay (*12, 13*; Fig. 1). A complex was formed with the GAS oligonucleotide in extracts from cells treated with IFN-γ but not that from cells treated with IFN-α (Fig. 1A) and was specifically competed by the GAS oligonucleotide but not by the ISRE (*1*). The IFN-γ–dependent activation of this DNA binding factor occurred without new protein synthesis (Fig. 1B), was maximal between 15 and 30 min after treatment of the cells with IFN-γ, and disappeared within 2 to 3 hours (Fig. 1C). This pattern correlates with the time course of IFN-γ induction of the GBP gene in fibroblasts (*10*). The Ly6E gene of mice, which encodes a surface protein, is also activated by IFN-γ through a DNA element very similar to the GAS of GBP (*15*). Just upstream of the GAS in the GBP gene there is an ISRE sequence (*12*). However, no ISRE exists in the Ly6E gene and activation of that gene by IFN-γ clearly operates through the GAS-like element that binds GAF. These results support the conclusion that the GAS site has a general

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