(R,K)-X-S or (R,K)-(R,K)-X-X-S for adenosine 3', 5'-monophosphate (cAMP)–dependent phosphorylation sites [B. E. Kemp, D. J. Graves, E. Benjamini, E. G. Krebs, *J. Biol. Chem.* **252**, 4888 (1977)] and (S,T)-X-(R,K) for protein kinase C (PKC)–dependent phosphorylation [J. R. Woodget, K. L. Gould, T. Hunter, *Eur. J. Biochem.* **161**, 177 (1986); A. Kishimoto *et al.*, *J. Biol. Chem.* **260**, 12492 (1985)].
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37. We dedicate this paper to the memory of Tom Elkins, our colleague and friend whose pioneering studies provided the groundwork for this analysis. We thank C. Petersen, and R. Kreber for technical assistance; F. Blattner, L. Marr, and members of the Blattner lab for help with sequencing; E. Knust and J. Campos-Ortega for providing stocks and clones; J. Tamkun, T. Schwarz, L. Jan, Y. N. Jan, B. Hamilton, and E. Meyerowitz for providing libraries; and our colleagues for advice and comments on the manuscript. Supported by NIH grant NS15390, the Markey Charitable Trust, and a Klingenstein Fellowship to B.G. N.S.A. received support from NIH grant T32 GM07131 and the Muscular Dystrophy Association. G.A.R. received support from the American Heart Association of Wisconsin, NIH grant T32 GM07131, and an NIH postdoctoral fellowship. This is paper number 3194 from the Laboratory of Genetics, University of Wisconsin–Madison.

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Progressive Encephalopathy and Myopathy in Transgenic Mice Expressing Human Foamy Virus Genes

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Transgenic mice carrying the *bel* region of human foamy retrovirus (HFV) under transcriptional control of its own long terminal repeat expressed the transgene in their central nervous systems and in smooth and striated muscle tissues. The animals developed a progressive degenerative disease of the central nervous system and of the striated muscle. Because expression of the transgene was closely correlated with the appearance of structural damage and inflammatory reactions were scanty, the disease is likely to be caused directly by the HFV proteins. These unexpected findings call for a reevaluation of the pathogenic potential of HFV in humans.

HFV IS A RETROVIRUS ORIGINALLY isolated 20 years ago from individuals with various diseases (*1, 2*). Although the prevalence of HFV was reported to be high in certain geographical areas (*3*), it has not been possible to identify HFV as the causative agent of any human disease (*4*). As with the human T cell lymphoma virus (HTLV) and human immunodeficiency virus (HIV), the HFV genome has, in addition to structural retroviral genes, a region containing three open reading frames, *bel-1* to *bel-3*. Bel-1 has some homology to HIV-2 Tat protein and, similarly to HIV Tat and HTLV-I Tax, functions as a transcriptional transactivator (*5–8*).

Transgenic mice (*9*) have proved useful in the dissection of the pathogenic potential of the regulatory genes of the human retroviruses HTLV-I and HIV (*10*). To analyze the biological activity of HFV regulatory proteins we generated transgenic mice with two constructs: (i) $pHFV_{Af}$, which contains the complete HFV genome in a noninfectious form because of a frameshift mutation in the integrase domain that created a premature stop codon in *pol*, and (ii) pΔgpe, in which 2.3 kb of *gag-pol* and 1.6 kb of *env* were deleted (*7*) (Fig. 1A). In both constructs, expression is controlled by the HFV long terminal repeat (LTR). Eight and nine founder animals (*11*) were derived with $pHFV_{Af}$ and pΔgpe, respectively. Five $pHFV_{Af}$ and six pΔgpe mice developed a neurological syndrome consisting of ataxia, spastic tetraparesis, and blindness. The symptoms in the $pHFV_{Af}$ mice appeared as early as 5 to 6 weeks after birth, progressed rapidly, and led to death within 4 to 6 weeks. The pΔgpe transgenic mice displayed a later onset of the disease (1 to 4 months of age) with a slower progression, which allowed their breeding. The remaining six founder animals, which were asymptomatic, did not express the transgene (*12*). Northern (RNA) hybridization analysis of the expression of the transgene revealed RNAs of sizes corresponding to a total transcript and to *bel* subgenomic mRNA only in the forebrain and in the cerebellum of all animals with disease symptoms (Fig. 1B).

In all animals expressing either construct, the pathological findings were restricted to the central nervous system (CNS) and to the striated muscle. The disease phenotype was transmitted to the offspring with a penetrance of 100%. Animals with the clinical symptoms described above showed variable degrees of selective nerve cell degeneration in the forebrain, with tissue atrophy and reactive gliosis. The areas affected most frequently were the CA3 layer of the hippocampus and the telencephalic cortex (Fig. 2). Some lesions exhibited a more abnormal pattern, with incomplete cortical and subcortical necrosis and occasionally with macrophages and mild lymphocytic infiltrates in the meninges and in the perivascular spaces (*13*). In situ hybridization analysis revealed a complex pattern of expression in the CNS, with groups of neurons, oligodendrocytes, and astrocytes containing high amounts of transcript in the brain and spinal cord. The distribution of positive cells was bilaterally symmetrical. In animals 4 weeks old, most neurons in the CA3 sector of the hippocampus and a subpopulation of cortical neurons were strongly positive for HFV RNA, even in animals in which onset of symptoms had not yet occurred (Fig. 2A). In sick animals older than 6 weeks, most CA3 neurons had degenerated (Fig. 2B), and the highest levels of expression were seen in cortical neurons.

Two $pHFV_{Af}$ and four pΔgpe mice showed focal degeneration of striated mus-

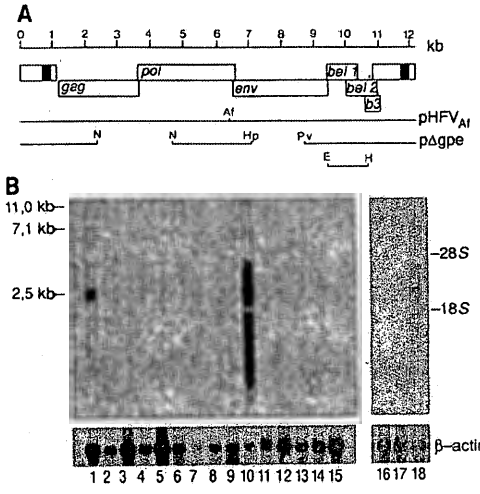

Fig. 1. (**A**) Genomic organization of HFV and constructs used for microinjection. Both constructs have been shown to transactivate HFV transcription in cultured cells (*8*). Af, AfI II; E, Eco RI; H, Hind III; Hp, Hpa II; N, Nco I; Pv, Pvu II. (**B**) Expression of HFV-RNA in various tissues of transgenic mice. Mice 75, 253, 267, and 881 harbor the pΔgpe transgene, and mouse 362 is transgenic for $pHFV_{Af}$. Lanes 1 to 9 were from mouse 75: lane 1, brain; lane 2, liver; lane 3, lung; lane 4, heart; lane 5, thymus; lane 6, kidney; lane 7, muscle; lane 8, lymph nodes; lane 9, spleen. Lane 10, mouse 362 brain; lane 11, mouse 253 testis; lane 12, mouse 253 brain; lane 13, mouse 267 brain; lane 14, nontransgenic littermate brain; lane 15, nontransgenic littermate brain; lane 16, mouse 881 forebrain; lane 17, mouse 881 parietal and midbrain; lane 18, mouse 881 cerebellum and brain stem.

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cles. These changes ranged from degeneration of single myotubes to large areas of myogenic atrophy with extensive necroses (Fig. 2D). In situ hybridization of cross sections of the affected striated muscles demonstrated that expression preceded degeneration and was confined to the myotubes.

Of the organs that did not show pathological changes, transgene expression could be detected by in situ hybridization in a small fraction of retinal interneurons and in a few smooth muscle cells in almost all organs analyzed (*13*). Higher numbers of positive cells were detected in the smooth muscle layer of the digestive tract.

In addition to the lesions described above, the $pHFV_{Af}$ transgenic mice showed spotty symmetric areas of grayish color in the white matter because of severe damage of the myelinated tracts in the anterior commissure, the corpus callosum, the optic nerves, the brain stem, the cerebellar white matter, and, to a lesser extent, in the spinal cord. These lesions consisted of fluid-filled vacuoles in the white matter (Fig. 3). Immunocytochemistry for glial fibrillary acidic protein (GFAP) revealed a striking reactive gliosis in the affected areas, whereas oligodendrocytes showed only occasional swellings. Transgene-expressing cells were invariably clustered in the immediate proximity of the lesions. Some of these cells had the typical size and structure of oligodendrocytes, but a small fraction was identified as being composed of astrocytes by combined in situ hybridization for HFV RNA and immunohistochemistry for GFAP (*13*). The extent and time of onset of these lesions varied only slightly between individual animals and between families derived from distinct founders.

The observation that only a small fraction of cells in specific lineages expressed high levels of transgene RNA, whereas other cells of the same lineages were negative, is unusual for transgenes controlled by promoters with restricted tissue specificity, such as retroviral LTRs. Mosaicism and positional effects of the transgene integration could be excluded because these observations were confirmed in founder animals from 11 independent integration events and in their offspring. Also, we found no evidence for

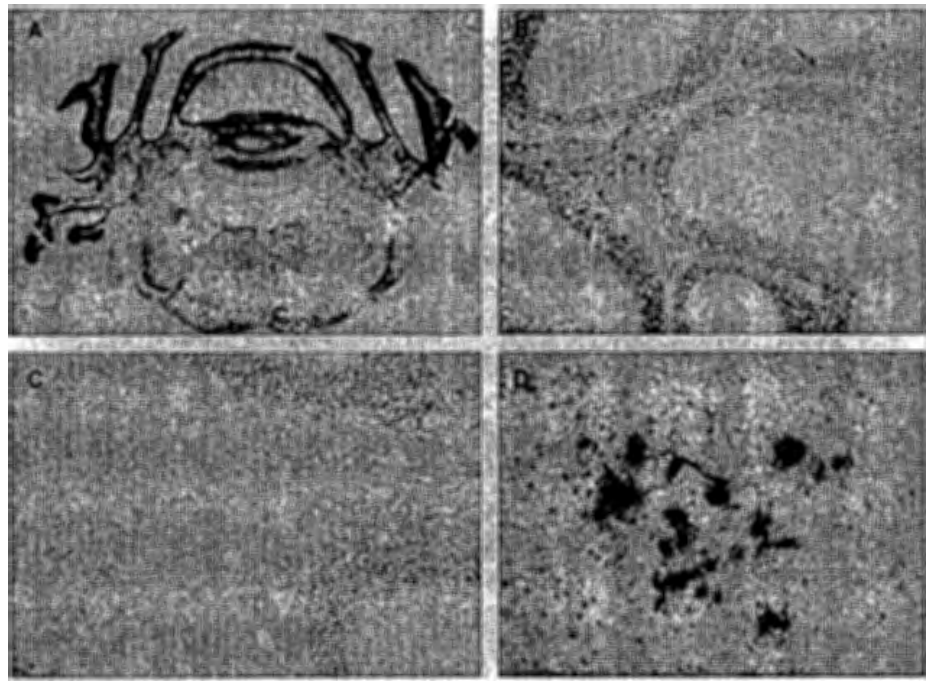

Fig. 3. White matter lesions. (**A**) Cross section through the cerebellum of a 97-day-old $pHFV_A$ transgenic animal. There is widespread vacuolar degeneration in the white matter of the cerebellum (Luxol fast blue–Nissl, 12,5×). (**B**) HFV expression in the cerebellum of the same animal (in situ hybridization, 50×). Most cells expressing HFV RNA are located in the white matter and are closely associated with the microcystic changes. Some positive cells are present in the granular layer. (**C**) Immunohistochemical demonstration of myelin basic protein (MBP) (100×). In the immediate vicinity of the vacuoles, MBP immunoreactivity is essentially preserved and virtually no macrophages with myelin degradation products are present, indicating that structural loss and degradation of myelin has not yet taken place. (**D**) HFV expression in the anterior commissure. Large amounts of transgenic RNA are present in the cells of the anterior commissure but not in the neurons of the surrounding basal ganglia (in situ hybridization, 200×).

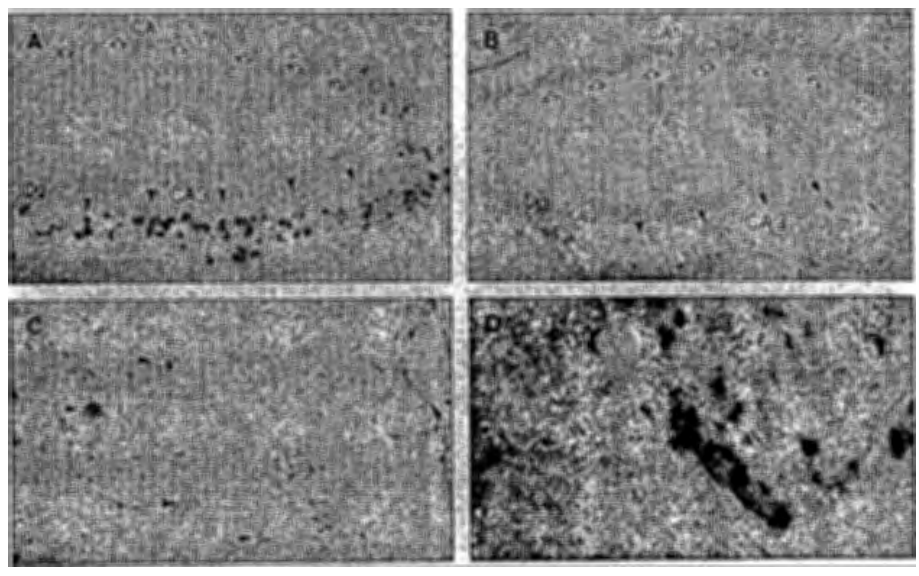

Fig. 2. Neuronal and muscle pathology in HFV transgenic mice (*18*). (**A**) In situ hybridization with an HFV probe of the hippocampus of a 4-week-old animal before onset of symptoms. Most neurons in the CA3 layer (arrowheads) expressed high levels of HFV RNA, whereas the CA1 area (open arrows) and the dentate gyrus (Dg) were essentially negative (hematoxylin-eosin, 100×). (**B**) Selective nerve cell loss in the CA3 layer of the hippocampus in a 6-week-old animal. The lesioned segment is sharply demarcated and corresponds precisely to the area of HFV expression in the younger animal (Luxol fast blue–Nissl stain, 100×). (**C**) Frontal cortex from a 7-week-old transgenic animal (GFAP immunohistochemistry, 200×) showing loss of neurons. Several GFAP-positive reactive astrocytes (arrowheads) and activated microglial cells (arrows) are present. (**D**) Paravertebral striated muscles of a transgenic animal. There is severe myogenic atrophy with size variation of the muscle fibers, increased number of internal nuclei, loss of cross-striation, hyaline degeneration of the sarcoplasm, and hypertrophy of adjacent myotubes. A majority of viable muscle fibers express HFV RNA.

reversion of the constructs to an infectious form or for horizontal spread of HFV (*13*). It is unlikely that expression of the HFV constructs depends on the cell cycle because neurons are irreversibly postmitotic in adult animals. These observations indicate that additional, unknown cellular factors may be required for high levels of HFV LTR–directed transcription.

Because expression of HFV was predominantly seen in anatomical structures that will eventually degenerate, it is likely that the gene products of HFV are directly responsible for the observed lesions. However, we are unable to identify conclusively which proteins of HFV are responsible for neurotoxicity. Nerve cell degeneration was present in animals carrying the pΔgpe DNA, which essentially contains only the *bel* reading frames. A possible candidate for neurotoxicity is the *bel-1* gene product, which is functionally comparable to the retroviral transactivating factors, HIV *tat* and HTLV-I *tax* (*5–7*). However, transgenic mice carrying LTR *tat* and LTR *tax* develop Kaposi sarcomas and peripheral neurogenic and mesenchymal tumors, respectively (*10*), but no degenerative encephalopathy. These differences may relate to the tissue-specific expression pattern caused by the HFV cis regulatory elements or to different cellular and molecular targets of the *bel-1* protein.

The pathologies observed in HFV mice are reminiscent of human retroviral disease of the CNS: neuronal loss and white matter damage are often present in HIV-associated encephalopathy (*14*) and vacuolar myelopathy (*15*) and in HTLV-I–induced disease of the CNS (*16*). The mechanisms of neurotoxicity operating in these conditions may be related to those leading to neurological damage in HFV transgenic mice. In fact, one of the original isolates of HFV was obtained from the brain of a patient with a neurodegenerative disease with features reminiscent of the lesions in the HFV mice (*2*).

Although a specific pathology has not been unequivocally associated with HFV infection, and foamy viruses generally have been regarded as benign (*4*), detection of HFV in human diseases might be problematic if virulence is restricted to immunocompromised hosts or to abortive forms of infection (*17*). The findings described here prompt a critical reappraisal of the potential role of HFV as a human pathogen. It will be crucial to search for evidence of HFV infection, or superinfection, in neurodegenerative and myopathic disorders of unknown etiology and neurological syndromes in immunodeficient patients.

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11. Transgenic mice were derived by pronuclear microinjection of (C57Bl/6 × C3H)F_2 fertilized eggs as described [B. Hogan *et al.*, in *Manipulating the Mouse Embryo: A Laboratory Manual*, B. Hogan *et al.*, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1986)]. Eight $pHFV_{\Delta f}$ and nine pΔgpe transgenic animals were obtained from 87 and 93 newborns, respectively. The number of integrated copies of the constructs varied from 3 to 30 per haploid genome (*12*). Total RNA (15 μg per lane) was separated on 1.5% formaldehyde-agarose gels, transferred to a nylon membrane, and hybridized to an Eco RI–Hind III fragment encoding nucleotides 9475 to 10697 of HFV (Fig. 1A). Blots were rehybridized with a β-actin probe for quantification of the amount of RNA.
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Identification of Profilin as a Novel Pollen Allergen; IgE Autoreactivity in Sensitized Individuals

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A complementary DNA encoding a pollen allergen from white birch (*Betula verrucosa*) that was isolated from a pollen complementary DNA library with serum immunoglobulin E from a birch pollen–allergic individual revealed significant sequence homology to profilins. The recombinant protein showed high affinity to poly-L-proline. Immunoglobulin E antibodies from allergic individuals bound to natural and recombinant birch profilin and also to human profilin. In addition, birch and human profilin induced histamine release from blood basophils of profilin-allergic individuals, but not of individuals sensitized to other plant allergens. The structural similarity of conserved proteins might therefore be responsible for maintaining immunoglobulin E antibody titers in type I allergy.

ALLERGIES OF THE IMMEDIATE TYPE are a major health problem in industrialized countries, where up to 15% of the population suffers from type I allergic symptoms (rhinitis, conjunctivitis, and bronchial asthma). An understanding of the complex pathogenetic mechanisms leading to allergy requires information about the structure and function of allergenic proteins. Comparison of the sequences of cloned allergens with those of known proteins has already contributed to the characterization of some allergenic proteins. The major birch (*Betula verrucosa*) pollen allergen Bet v I (*1*) and the white-faced hornet venom allergen antigen 5 Dol m V (*2*) were shown to be highly homologous to pathogenesis-related plant proteins. The major house dust mite allergen Der p I is homologous to proteases-

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