References and Notes

1. C. D. Little, M. M. Nau, D. N. Carney, A. F. Gazder, J. D. Minna, *Nature (London)* **306**, 194 (1983).
2. R. Dalla-Favera, F. Wong-Staal, R. C. Gallo, *ibid.* **299**, 61 (1982).
3. S. Collins and M. Groudine, *ibid.* **298**, 679 (1982).
4. K. Alitalo, M. Schwab, C. C. Lin, H. E. Varmus, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1707 (1983).
5. S. J. Collins and M. T. Groudine, *ibid.*, p. 4813.
6. G. T. Merlino *et al.*, *Science* **224**, 417 (1984).
7. M. Schwab, K. Alitalo, H. E. Varmus, J. M. Bishop, D. George, *Nature (London)* **303**, 497 (1983).
8. P.-G. Pelicci, L. Lanfrancone, M. D. Brathwaite, S. R. Wolman, R. Dalla-Favera, *Science* **224**, 1117 (1984).
9. F. W. Alt, N. E. Kohl, J. Murphy, C. E. Gee, *UCLA Symp. Mol. Cell. Biol. N.S.*, in press.
10. N. E. Kohl *et al.*, *Cell* **35**, 359 (1983).
11. M. Schwab *et al.*, *Nature (London)* **305**, 245 (1983).
12. G. M. Brodeur, R. C. Seeger, M. Schwab, H. E. Varmus, J. M. Bishop, *Science* **224**, 1121 (1984).
13. W.-H. Lee, A. L. Murphree, W. F. Benedict, *Nature (London)* **309**, 458 (1984).
14. N. Kohl *et al.*, unpublished data.
15. N. Kohl, C. Gee, F. Alt, in preparation.
16. T. Maniatis, E. F. Fritsch, J. Sambrook, in *Molecular Cloning, A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982), p. 202.
17. G. D. Yancopoulos *et al.*, *Nature (London)*, **311**, 727 (1984).

18. We thank S. Latt, G. Bruns, and G. Yancopoulos for advice and assistance and T. K. Blackwell and M. Goldfarb for critical reading of the manuscript. We thank L. Kedes for pHF1. Supported by NIH grant 2-P01 CA 23767-06, ACS grant NP-393, an award from the Searle Scholars program (F.A.), and an Irma T. Hirschl Career Scientist Award (F.A.).

* Present address: Department of Pathology, Dana-Farber Cancer Institute, Harvard University Medical School, Boston, Mass. 02115.

26 July 1984; accepted 4 October 1984

JC Virus Enhancer-Promoter Active in Human Brain Cells

Abstract. *A human papovavirus, JCV, is the etiologic agent of the fatal demyelinating disease, progressive multifocal leukoencephalopathy. The JCV 98-base-pair tandem repeats, located to the late side of the viral replication origin, were shown to be a transcriptional regulatory element with enhancer-like activity in human fetal glial cells. These tandem repeats share significant homology with the 82-nucleotide rat brain–specific identifier RNA sequence.*

The JC virus (JCV), a human papovavirus, is the etiologic agent of progressive multifocal leukoencephalopathy and has been isolated from brain lesions of patients with this fatal demyelinating disease (*1*). It has also been isolated from the urine of pregnant women (*2*) and of immunocompromised patients (*3*). In contrast to the closely related viruses SV40 and BKV, JCV has an extremely restricted host range. Although human uroepithelial cells, amnion cells, and endothelial cells are able to support JCV reproduction to a limited degree, JCV grows well only in human fetal glial cells (*4–7*).

Recently, transcriptional regulatory elements called "enhancers" have been identified within the genomes of a number of viruses (*8–11*). These elements increase the level of transcription of many genes in a manner that is relatively independent of position and orientation. A second control element, the "TATA" box (T, thymine; A, adenine), also influences the level of transcription and positions the cap site of the messenger RNA (mRNA) at a distance 25 to 30 base pairs (bp) downstream. A characteristic property of enhancers is their relative host cell specificity. Some cellular enhancer elements such as those associated with the immunoglobulin heavy chain and light chain genes (*12–15*) and the insulin and chymotrypsin genes (*16*) appear to function only in specific tissues. The strong T-cell tropism of several retroviruses, including the type II human T-cell leukemia virus, is derived from information encoded within the long terminal repeats that contain their enhancer element (*17–19*). In this study, we have identified an element in the JCV genome which shows greatest enhancer-like activity in human fetal glial cells. This preferential enhancer activity is likely to be a major determinant in the restricted host range of JCV.

The region of the origin of DNA replication has recently been sequenced in the JCV genome (*20*) (Fig. 1). It contains a tandem duplication of 98 bp located in a position analogous to that of the SV40 enhancer (a 72-bp repeat) and the BKV enhancer (a 68-bp triplication). In terms of nucleotide sequence homology, SV40, BKV, and JCV are very similar except for the regions of tandem repeats. Both 98-bp repeats in JCV contain a TATA box; in contrast, the SV40 and BKV enhancer elements are located upstream of single TATA boxes. We determined

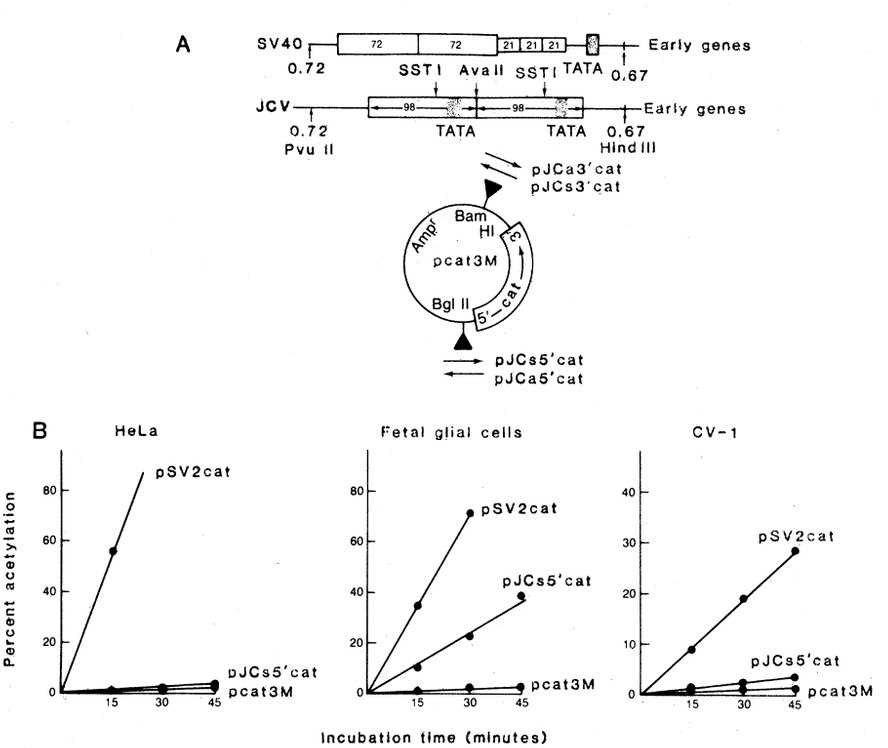

Fig. 1. (A) Construction of the JCV-cat plasmids. The 286-bp JCV fragment from 0.67 to 0.72 map units was excised with Pvu II and Hind III and ligated into the pcat3M plasmid. The pcat3M plasmid contains the bacterial CAT gene without promoter elements (*19*). The JCV fragment was placed in the Bgl II site, 5′ of the CAT gene, in both the sense (pJCs3′cat) and antisense (pJCa5′cat) orientations (relative to the JCV early transcription unit), and likewise in both the sense (pJCs3′cat) and antisense (pJCa3′cat) orientations in the Bam HI site 3′ of the CAT gene. (B) Activation of the CAT gene by JCV 98-bp repeats. Equivalent amounts (20 μg) of the CAT plasmids containing either the JCV 98-bp repeats (pJCs5′cat), the SV40 72-bp repeats and TATA box (pSV_2cat), or no promoter element (pcat3M) were transfected into the different cell types by calcium phosphate precipitation (*22*). The cells were harvested 72 hours after transfection and analyzed for CAT activity (*24*). The percent acetylation of ^{14}C-labeled chloramphenicol was determined by thin-layer chromatography and subsequent radioactive scintillation counting.

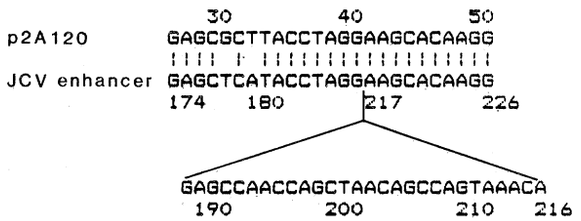

Fig. 2. The JCV enhancer region (*20*) was compared to the 82-nucleotide sequence (p2A120) found in precursor RNA specific to rat brain tissue (*25, 26*). The homologous JCV nucleotide sequences from nucleotide 174 to 188 and from 217 to 226, have been aligned with the rat brain RNA sequence. The nonhomologous JCV nucleotides 189 to 216 are shown as an insert below the homologous sequences. The proximal 98-bp JCV repeat extends from nucleotides 129 to 226. The TATA box is located from 132 to 146.

that the nucleotide sequence in the region of the replication origin of a MAD-1 strain of JCV was identical to that reported by Frisque (*20*) except for an extra 4-bp insertion between the two 98-bp repeats (*21*). The 286-bp fragment from 0.67 to 0.72 map units containing the two 98-bp repeats, but not the T-antigen translational initiation site, was isolated by restriction enzyme cleavage with Hind III and Pvu II and inserted into the plasmid pcat3M. The recipient plasmid contains the bacterial gene coding for the enzyme chloramphenicol acetyltransferase (CAT) and an SV40 polyadenylation signal, but not the SV40 TATA box, 21-bp repeats, or an enhancer element. The JCV fragment was placed in the sense and antisense orientations (relative to its position in the JCV genome) at both the 5′ and 3′ ends of the CAT gene (Fig. 1).

DNA from the four JCV-cat plasmids, the pSV2cat plasmid (which contains the SV40 enhancer and TATA box linked to the CAT gene), and the RSVcat plasmid (which contains the RSV enhancer and TATA box linked to the CAT gene), were transfected into four different cell types using the calcium precipitation method (*22*). Cells were harvested after 72 hours and protein extracts were prepared and analyzed for CAT activity (*23*). This assay provides a sensitive and quantitative measurement of gene activity induced by the added enhancer-promoter elements in the absence of a background signal.

The JCV fragment enhances CAT gene expression to a much greater degree in human fetal glial cells than in CV-1 cells; it has almost no activity in HeLa cells (Fig. 1). The pSV2cat and RSVcat plasmids, on the other hand, induced significant CAT activity in all cell types (*21*). In human embryonic kidney (HEK) cells, the CAT activity of all plasmids tested was less than that observed in the other three cell lines (*21*). However, the relative activity of the different plasmids in HEK cells was very similar to that in CV-1 cells.

The effects of orientation and position of the JCV fragment on CAT gene expression are shown in Tables 1 and 2. The JCV fragment increased CAT activity in either orientation when placed 5′ to the CAT gene in both fetal glial and CV-1 cells. However, in fetal glial cells, the sense orientation was twice as active as the antisense orientation, whereas in CV-1 cells, the sense orientation was less than one-half as active as the antisense orientation. Although enhancers function with different efficiencies in various positions and orientations (*10, 11*), the specific determinants of these effects remain obscure. When the JCV fragment was placed 3′ to the CAT gene, activity was greatly diminished; nevertheless, it remained consistently greater than that of the pcat3M plasmid with no associated enhancer element. The decrease in 3′ activity probably results in part from the fact that the JCV repeat contains a TATA box which can initiate transcription in its vicinity, and that these transcripts are inefficient translational templates.

In an attempt to separate the enhancer-like activity from the other viral promoter elements, we isolated subfragments of the JCV control region, inserted them in the sense orientation 5′ to the CAT gene of pcat3M, and transfected these new constructs into fetal glial cells. The 128-bp fragment from Hind III to Ava II, which contains the entire proximal 98-bp repeat plus an additional 30-bp downstream, had only 7.3 to 9.3 percent of the CAT activity produced by both repeats together (plasmid pJCs5′cat). The 158-bp fragment from Ava II to Pvu II, which contains the entire distal 98-bp repeat plus an additional 60-bp sequence upstream, produced 7.3 to 19.3 percent of the CAT activity of both repeats together. A smaller 50-bp subfragment from Ava II to Sst I, which contains the distal TATA box, had 6.0 to 8.6 percent of the CAT activity of both repeats together. Thus, both JCV tandem repeats are necessary to give full enhancer-like activity. Since we have not yet effectively separated the JCV enhancer sequences from other viral promoter elements, we will refer to the JCV tandem repeat region as the JCV enhancer-promoter or transcriptional regulatory region.

The JCV enhancer-promoter shows a much stronger tissue-type specificity than the enhancers of the two closely related papovaviruses, SV40 and BKV. The SV40 enhancer has significant activity in all cell types tested (*24*). Likewise, the BKV enhancer functions almost equally well in CV-1 cells, HeLa cells, and mouse L cells (*10*). The JCV enhancer, on the other hand, is more than 20 times as strong in fetal glial cells as in HeLa cells, with intermediate activity in CV-1 cells. This enhancer-promoter activity correlates well with the ability of JCV to grow in fetal glial cells and uroepithelial cells but not in HeLa cells.

An unexpected finding was that the

Table 1. The effect of orientation and position of the 286-bp JCV fragment upon CAT activity in fetal glial cells. The JCV fragment was inserted at the 5′ or 3′ ends of the CAT gene in either the sense (s) or antisense (a) orientation. Protein extracts were prepared and analyzed for CAT activity as described (*23*) with an incubation time of 45 minutes. CAT activity is normalized to give a pcat3M CAT activity of 1.0. Each experiment was repeated three times. Although transfection efficiency varied among experiments, the ratio of CAT activity between the different plasmids remained similar.

Plasmid	Mean CAT activity	Range
pcat3M	1.0	
JCa3′cat	2.3	2.0 to 2.5
JCs3′cat	2.1	1.5 to 2.8
JCa5′cat	15.4	12.9 to 19.1
JCs5′cat	29.2	19.4 to 38.0

Table 2. The CAT activity of the JCV fragment ligated into the Bgl II site of pcat3M plasmid in either the sense orientation (JCs5′cat) or antisense orientation (JCa5′cat). In the CAT activity assays (*23*), an incubation time of 30 minutes (HeLa, fetal glial, or CV-1 cells) or 1 hour (HEK cells) was used. The preference of fetal glial cells for the sense orientation was observed in seven separate experiments (ratio of activity in JCs5′cat to JCa5′cat varied from 1.5 to 2.8). The preference of CV-1 cells for the antisense orientation was also reproducible in seven separate experiments (the ratio of activity in JCs5′cat to JCa5′cat in CV-1 cells ranged from 0.2 to 0.6).

Plasmid	Percent acetylation			
	HeLa	Fetal glial	CV-1	HEK
pcat3M	0.4	0.8	0.5	0.1
JCs5′cat	1.1	23.0	2.8	0.4
JCa5′cat	0.9	11.1	6.8	0.6

286-bp JCV fragment functioned better in the sense orientation in fetal glial cells, but in the antisense orientation in CV-1 cells. This is in contrast to the SV40 and BKV enhancers, which are consistently more active in the sense orientation, regardless of cell type. This difference in orientation preference among cell types may relate to the JCV enhancer activity or to a difference in the activity of the two TATA boxes. It may be significant that the only cell type in which JCV grows well is also the cell type in which the sense orientation of the JCV enhancer is more active. This preference for the sense orientation of the JCV enhancer region in fetal glial cells could relate to the orientation of transcription of T antigen, which in turn is necessary for DNA replication.

The mechanism by which the JCV enhancer-promoter stimulates brain-specific transcription is currently unknown. In the case of viruses which have evolved from cellular sequences, it seems reasonable that the proteins which interact with viral regulatory sequences perform an analogous function for cellular genes. An 82-nucleotide element, the identifier sequence has been found within the introns of precursor RNA molecules isolated from rat brain that was not detected in other rat tissues (*25, 26*). We have compared the nucleotide sequence of the JCV 98-bp repeat with this 82-nucleotide rat brain specific sequence and found marked regions of homology [significance level of 10^{-8}; (*27*)] (Fig. 2). This suggests the presence of a brain-specific transcription factor that recognizes both the JCV transcriptional regulatory region and the 82-nucleotide rat brain sequence. Whether these identifier sequences serve a regulatory role in the transcription of brain-specific genes remains to be determined.

SHANNON KENNEY
VENKATACHALA NATARAJAN
DAVID STRIKE
GEORGE KHOURY
NORMAN P. SALZMAN

Laboratory of Biology of Viruses, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

References and Notes

1. B. L. Padgett, D. L. Walker, G. M. Zurhein, A. E. Hodach, S. M. Chow, *J. Infect. Dis.* **133**, 686 (1976).
2. D. V. Coleman *et al.*, *ibid.* **142**, 1 (1980).
3. T. F. Hogan, E. C. Borden, J. A. McBain, B. L. Padgett, D. L. Walker, *Ann. Intern. Med.* **92**, 373 (1980).
4. B. L. Padgett, C. M. Rogers, D. L. Walker, *Infect. Immun.* **15**, 656 (1977).
5. A. M. Beckmann, K. V. Shah, B. L. Padgett, *ibid.* **38**, 774 (1982).
6. K. K. Takemoto, P. M. Howley, T. Miyamura, *J. Virol.* **30**, 384 (1979).
7. G. C. Fareed, K. K. Takemoto, M. A. Gimbrone, in *Microbiology*, D. Schlessinger, Ed. (American Society for Microbiology, Washington, D.C., 1978), p. 427.
8. C. Benoist and P. Chambon, *Nature (London)* **290**, 304 (1981).
9. P. Gruss, R. Dhar, G. Khoury, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 943 (1981).
10. N. Rosenthal, M. Kress, P. Gruss, G. Khoury, *Science* **222**, 749 (1983).
11. L. Laimons, P. Gruss, R. Pozzatti, G. Khoury, *J. Virol.* **49**, 183 (1984).
12. S. D. Gillies, S. L. Morrison, V. T. Oi, S. Tonegawa, *Cell* **33**, 717 (1983).
13. J. Banerji, L. Olson, W. Schaffner, *ibid.*, p. 729.
14. C. Queen and D. Galhmore, *ibid.*, p. 741.
15. D. Picard and W. Schaffner, *Nature (London)* **307**, 80 (1984).
16. M. Walker, T. Edlund, A. Boulet, W. Rutter, *ibid.* **306**, 557 (1983).
17. I. S. Chen, J. McLaughlin, D. W. Golde, *ibid.* **309**, 276 (1984).
18. J. Lenz, *et al.*, *ibid.* **308**, 467 (1984).
19. L. DesGroseillers, E. Rassart, P. Jolicoeur, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4203 (1983).
20. R. Frisque, *J. Virol.* **46**, 170, (1983).
21. Unpublished data.
22. F. Graham and A. Van der Erb, *Virology* **52**, 456 (1973).
23. C. Gorman, L. Moffat, B. Howard, *Mol. Cell. Biol.* **2**, 1044, (1982).
24. C. Gorman, G. Merlino, M. Willingham, I. Pastan, B. Howard, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6777 (1982).
25. R. Milner, F. Bloom, C. Lai, R. Lerner, J. Sutcliffe, *ibid.* **81**, 713 (1984).
26. J. Sutcliffe, R. Milner, J. Gottesfeld, R. Lerner, *Nature (London)* **308**, 237 (1984).
27. W. B. Goad and M. I. Kanehisa, *Nucleic Acids Res.* **10**, 247 (1982).
28. We thank Peter Howley for his gift of cloned JCV; K. Takemoto for his assistance in growing fetal brain cells; Charles Buckler and Jacob Maizel for assistance in computer analysis of the JCV and rat brain sequences; and Joslyn Buller and Jeanne Carolan for preparation of the manuscript.

26 April 1984; accepted 4 October 1984

Purified Human Granulocyte-Macrophage Colony-Stimulating Factor: Direct Action on Neutrophils

Abstract. *Neutrophil migration inhibition factor from T lymphocytes (NIF-T) is a lymphokine that acts to localize granulocytes. Medium conditioned by the Mo human T-lymphoblast cell line was used to purify NIF-T, a glycoprotein with a molecular weight of 22,000. The NIF-T was found to potently stimulate the growth of granulocyte and macrophage colonies from human bone marrow and colony formation by the KG-1 myeloid leukemia cell line. Thus a human lymphokine (NIF-T) that modulates the activities of mature neutrophilic granulocytes is also a colony-stimulating factor acting on precursors to induce growth and differentiation of new effector cells.*

Neutrophil migration inhibition factor from T lymphocytes (NIF-T) is a lymphokine produced by mitogen- and antigen-stimulated lymphocytes, the Mo T-lymphoblast cell line, and mature T cells transformed by human T-cell leukemia virus (HTLV) (*1, 2*). NIF-T is assayed by measuring its ability to inhibit migration of human peripheral blood neutrophils under agarose, and it is a potent activator of human neutrophils (*1, 3*). The Mo cells and lectin-stimulated lymphocytes also produce colony-stimulating factors (CSF) that act on myeloid precursor cells to give rise to colonies of differentiated granulocytes and mononuclear phagocytes in semisolid culture (*4*). To precisely define the biochemical and biological characteristics of lymphokines that act on early precursor cells (CSF) and mature neutrophils (NIF-T), highly purified preparations of these proteins are required. We recently developed a purification scheme that yields homogeneous erythroid-potentiating activity, a lymphokine regulating early erythropoiesis (*5*); the same approach was used to prepare highly purified NIF-T and CSF. Our results show that NIF-T and CSF activities reside in a single glycoprotein with apparent molecular weight of 22,000, demonstrating that a single human lymphokine acts on progenitor cells in the bone marrow and mature effector cells in the periphery. The identity of these molecules and activities has important implications for human physiology and the pathophysiology of inflammatory and immune disorders.

Ten liters of serum-free Mo-conditioned medium (the cells were grown in Iscove's modified Dulbecco's medium) were concentrated 30-fold with an Amicon hollow-fiber apparatus having an H1P10-8 filter. The concentrated protein was precipitated by ammonium sulfate at 80 percent saturation, dissolved in phosphate-buffered saline (PBS), dialyzed extensively, and clarified by centrifugation. The protein concentrate was applied to a lentil lectin Sepharose 4B column (2.6 by 6 cm; Pharmacia) equilibrated and washed with PBS. Glycoproteins were eluted with 0.5*M* α-methyl-D-mannoside in PBS and concentrated with a Centricon-10 microconcentrator (Amicon). The eluted glycoproteins were fractionated by size with an Ultrogel AcA 44 column (1.6 by 80 cm; LKB) equilibrated and eluted with PBS. Fractions of 2.5 ml were collected and assayed for NIF-T and CSF activities.

Colony-stimulating activity was found over a broad range in the Ultrogel fractions, most likely because of the presence of multiple CSF's in Mo-conditioned medium. However, a well-defined peak of NIF-T activity was found in a