of any of the three fragments generated by Xba I; thus the SSV-related *onc* gene in humans is probably interrupted by noncoding sequences.

The FeSV *onc*-specific probe also detected a single locus in human DNA digested with Eco RI (Fig. 2). The size of this fragment (16 kbp) is different from the one detected by SSV. Two and three bands, respectively, were produced by Pst I and Sac I (Fig. 2). The sum of the sizes of these fragments is again greater than that of the *onc* sequences.

The HaMSV probe hybridized to one Hind III fragment and two Kpn I and Bam HI fragments of human DNA (Fig. 2). Although HaMSV contains, in addition to the *onc* gene, sequences homologous to rat 30*S* RNA, the endogenous virus-like 30*S* RNA sequences would be expected to be less well conserved phylogenetically than the *onc* sequences. However, we cannot rule out the possibility that a subset of the bands detected in human DNA is due to hybridization of the portion of HaMSV genome homologous to the 30*S* RNA.

Our studies confirm earlier observations that the cellular *onc* genes are well conserved among vertebrate species. We were able to detect specific fragments in human DNA homologous to sarcoma viruses isolated from cat, rat, and primate, using hybridization conditions of moderate stringency (50 percent formamide; triple-strength standard saline citrate; 60°C). Our results indicate that each cellular *onc* gene is confined to a single genetic locus, distinct from the loci of the others. In at least two cases, the size of the *onc* locus is greater than that of the viral *onc* specific sequences, suggesting the presence of intervening sequences not related to *onc*. This is borne out by direct cloning of the human cellular homologs of the FeSV- and SSV-related *onc* genes (*27*). The presence of introns has been reported in cellular *onc* genes of Abelson-MuLV (mouse) (*28*) and FeSV (cat) (*23*), and they may be a common feature of other *onc* genes. An exception is the Moloney-MSV system, where complete and uninterrupted homology was observed between viral and cellular *onc* sequences (*29*).

There are now about a dozen known viral *onc* genes. All of them may have counterparts in human DNA. The presence of these genes in humans raises the question whether they are expressed constitutively or are turned on specifically in certain differentiating or neoplastic cells. Since these genes have been conveniently parceled into retroviruses, they can be used as probes to answer these questions.

Note added in proof: In surveying a large number of human DNA samples, we have found more than one allelic forms of the *onc* loci of SSV and HaMSV (*30*).

FLOSSIE WONG-STAAL
RICCARDO DALLA-FAVERA
GENOVEFFA FRANCHINI
EDWARD P. GELMANN
ROBERT C. GALLO

Laboratory of Tumor Cell Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

References and Notes

1. P. H. Duesberg, *Cold Spring Harbor Symp. Quant. Biol.* **14**, 13 (1980).
2. B. G. Neel, W. S. Hayward, H. L. Robinson, J. Fang, S. M. Astrin, *Cell* **23**, 323 (1981).
3. W. S. Hayward, B. G. Neel, S. M. Astrin, *Nature (London)* **290**, 475 (1981).
4. M. Shibuya, T. Hanafusa, H. Hanafusa, J. R. Stephenson, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 6536 (1980).
5. W. J. M. Van de Ven, F. W. Reynolds, J. R. Stephenson, *Virology* **101**, 185 (1980).
6. M. Barbacid, K. Beemon, S. G. Devare, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5158 (1980).
7. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
8. O. N. Witte, A. Dasgupta, D. Baltimore, *Nature (London)* **283**, 826 (1980).
9. P. Mellon, A. Dawson, K. Bister, G. S. Martin, P. H. Duesberg, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5874 (1978).
10. M. J. Hayman, B. Royer-Pokora, T. Graf, *Virology* **92**, 31 (1979).
11. R. A. Feldman, T. Hanafusa, H. Hanafusa, *Cell* **22**, 757 (1980).
12. M. Barbacid, A. V. Lauver, S. G. Devare, *J. Virol.* **33**, 196 (1980).
13. S. K. Ruscetti, L. P. Turek, C. J. Sherr, *ibid.* **35**, 259 (1980).
14. C. J. Sherr, L. A. Fedele, L. Donner, L. P. Turek, *ibid.* **32**, 860 (1979).
15. Y. H. Chien, M. Lai, T. Y. Shih, I. M. Verma, E. M. Scolnick, P. Roy-Burman, N. Davidson, *ibid.* **31**, 752 (1979).
16. R. W. Ellis, D. Defeo, J. M. Maryak, H. A. Young, T. Y. Shih, E. H. Chang, D. R. Lowry, E. M. Scolnick, *ibid.* **36**, 408 (1980).
17. T. Y. Shih, M. O. Weeks, H. A. Young, E. M. Scolnick, *Virology* **96**, 64 (1979).
18. E. P. Gelmann, F. Wong-Staal, R. Kramer, R. C. Gallo, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
19. D. J. Donoghue, P. A. Sharp, R. A. Weinberg, *J. Virol.* **32**, 1015 (1979).
20. C. J. Sherr, L. A. Fedele, M. Oskarsson, J. Maizel, G. F. Vande Woude, *ibid.* **34**, 200 (1980).
21. F. Wong-Staal, E. P. Gelmann, R. Dalla-Favera, S. Szala, S. Josephs, R. C. Gallo, unpublished data.
22. ———, V. Manzari, unpublished data.
23. G. Franchini, J. Even, C. J. Sherr, F. Wong-Staal, *Nature (London)* **290**, 154 (1981).
24. G. L. Hager *et al.*, *J. Virol.* **31**, 795 (1979).
25. P. N. Rigby, M. Dieckmann, C. Rhodes, P. Berg, *J. Mol. Biol.* **113**, 236 (1977).
26. A. E. Frankel, J. H. Gilbert, K. J. Porzig, E. M. Scolnick, S. A. Aaronson, *J. Virol.* **30**, 821 (1979).
27. R. Dalla-Favera, E. P. Gelmann, R. C. Gallo, F. Wong-Staal, *Nature (London)*, in press; G. Franchini, F. Wong-Staal, R. Dalla-Favera, R. C. Gallo, unpublished data.
28. S. P. Goff, E. Gilboa, O. N. Witte, D. Baltimore, *Cell* **22**, 777 (1980).
29. M. Oskarsson, W. L. McClements, D. G. Blair, J. V. Maizel, G. F. Vande Woude, *Science* **207**, 1222 (1980).
30. F. Wong-Staal, R. Dalla-Favera, G. Franchini, E. P. Gelmann, R. C. Gallo, E. Westin, S. Arya, unpublished results.
31. We thank M. Martin for the gift of cloned HaMSV plasmid DNA and C. Sherr for FeSV recombinant phage from which subclones of the *onc* region were constructed.

2 February 1981; revised 14 April 1981

Perivascular Meningeal Projections from Cat Trigeminal Ganglia: Possible Pathway for Vascular Headaches in Man

Abstract. *Peroxidase-containing cell bodies were found in the ipsilateral trigeminal ganglia after horseradish peroxidase was applied to the proximal segment of the middle cerebral artery in seven cats. Cell bodies containing the enzyme marker were located among clusters of cells that project via the first division. The existence of sensory pathways surrounding large cerebral arteries provides an important neuroanatomical explanation for the hemicranial distribution of headaches associated with certain strokes and migraine.*

The proximal segments of large cerebral arteries are among the few structures in the cranium which, when stimulated, give rise to the sensation of pain (*1*). Afferent nerve fibers that convey this information have not been identified. However, the areas to which pain is referred (such as the forehead) suggest the possibility of a relation between the trigeminal nerve, which receives afferent fibers from the cranium, and head pain of vascular origin. Thus perivascular trigeminal projections, transmitting sensory information from large cerebral arteries, may explain the unilateral head pains associated with certain vascular syndromes such as strokes, classical migraine, and cluster headaches (*2*).

We have initiated neuroanatomical studies to determine uptake and transport of horseradish peroxidase (HRP) (*3*) by neurons surrounding the middle cerebral artery to cell bodies in the trigeminal ganglia. Our results indicate that, in the cat, sensory afferents surround the proximal segment of the middle cerebral artery and project ipsilaterally from cell bodies in the first division of the trigeminal ganglia. Should such first-order afferent fibers exist in man (*1*), this would provide an explanation for the distribution of hemicranial vascular headaches.

Using microsurgical techniques and a dissecting microscope (magnification, ×25), we applied HRP to the right middle cerebral artery in seven cats (2 to 4 kg) (*4*). To restrict the diffusion of HRP away from the artery, HRP (3 mg) was

0036-8075/81/0710-0228S00.50/0

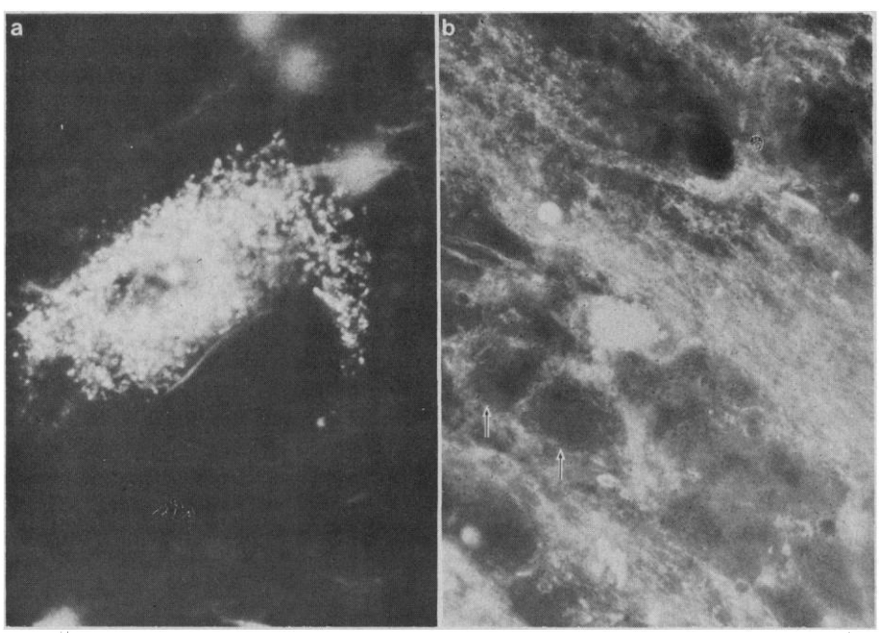
mixed with 3 g of the viscous polymer polyvinyl alcohol (16 percent, weight to volume) in distilled water and applied to the surface of the blood vessel with a spatula. Thus the local spread of HRP was limited to an area < 10 mm on either side of the vessel (*5*). After 72 hours the animals were perfused transaortically with 2.5 percent glutaraldehyde in 0.1*M* phosphate buffer (*p*H 7.4). Both trigeminal ganglia were removed under the dissecting microscope. In three animals, both superior cervical ganglia were removed and examined for HRP reaction product. The tissues were embedded in gelatin albumin (*6*), serially sectioned (30 μm) in a freezing microtome, and processed with the chromogen tetramethylbenzidine (*7*). Uncounterstained sections were examined under dark-field illumination for the HRP reaction product.

Peroxidase activity was identified in the cells of the ipsilateral trigeminal ganglia in all seven animals (Fig. 1). The number of HRP-positive cells varied between 1 and 20 per animal (approximately 0.02 percent of trigeminal neurons) for a total of 63 cells. Their diameter ranged from 20 to 60 μm. No peroxidase-containing cells were observed in the contralateral ganglia of the trigeminal nerve. No endogenous peroxidase activity was detected in the bodies of trigeminal neurons, nor did HRP appear in ganglion cells after the tracing enzyme was placed on the meninges at a distance from large cerebral blood vessels (*8*). Horseradish peroxidase was also identified in cell bodies of the ipsilateral superior cervical ganglia.

To identify the division within the trigeminal ganglia in which neurons supplying perivascular afferents reside, HRP was applied to the proximal cut end of the supraorbital nerve—a branch of the ophthalmic division—in three cats (*9*). After 48 hours, the animals were perfused with phosphate buffer (0.1*M*, *p*H 7.4) containing 2.5 percent glutaraldehyde and processed for the blue reaction product. In each of these studies, numerous cell bodies in the ipsilateral ganglia contained the transported HRP. The regional distribution of cell bodies that project via the supraorbital nerve appeared similar to the distribution of cell bodies that project to the perivascular plexus of the middle cerebral artery (Fig. 2). This suggests that either the same neuron sends axons to both terminal fields or that each of these pathways modulates the activity of the other, either at the ganglia or distally. Modulation may also occur more proximally via axoaxonic synapses in the descending tract of the trigeminal nerve (*10*). A similar association may exist between vascular afferents and other branches of the ophthalmic division and between extracranial arteries and the trigeminal nerve.

A number of clinical observations have been recorded in the human and other species to support the existence of a perivascular pathway for the transmission of cerebrovascular pain. When pain accompanies the intracarotid injection of histamine or an occlusion of the middle cerebral artery or occurs during internal carotid angiography, it is most often localized to one side of the forehead or behind the eye on that side (*11*). It also appears that an intact trigeminal pathway is required for headaches to be induced by the infusion of histamine (*12*); interruption of this ganglia or tract prevents the development of these headaches and reportedly is the single most effective surgical procedure for relieving migraine headaches (*13*). The unilateral nature of the pathways from the first division of the trigeminal nerve agrees with the results of morphological (*14*) and clinical studies (*15*), but differs somewhat from the results of other studies showing a transmedian distribution of afferents with the maxillary and mandibular divisions in other mammals (*16*). Our studies do not rule out the possibility that perivascular fibers arise contralaterally, but if they do they are too few to be visualized by current histochemical methods for tracing HRP.

The limited number of HRP-positive cells which project from endings around the middle cerebral artery would in no way diminish the functional significance of this small population of neurons. The



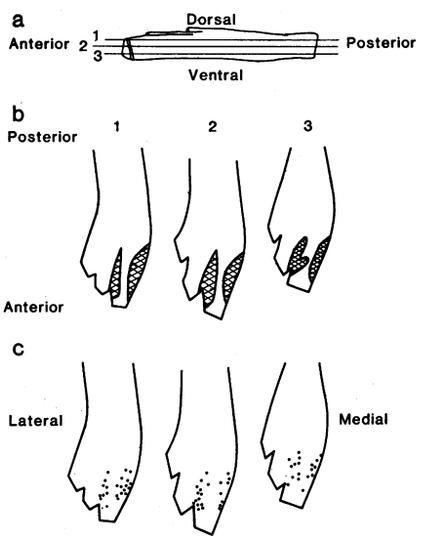


Fig. 1 (left). (a and b) Dark-field photomicrographs of ipsilateral trigeminal ganglia neurons that are filled with typical granular reaction product. Note the peroxidase granules extending into cell processes. The arrows in (b) point to unlabeled cell bodies. Original magnification: (a) ×1000; (b) ×400. Fig. 2 (right). Schematic diagrams of representative horizontal sections through cat trigeminal ganglia. (a) Orientation angles for the sections in (b) and (c). The large nerve trunk emerging most medially from the ganglia is the ophthalmic nerve, or the first division of the trigeminal nerve. (b) Localization and distribution of HRP-containing cells after enzyme marker was applied to the supraorbital nerve. The crosshatched areas represent the region occupied by cells containing HRP from ten adjoining sections. (c) Points depicting the location of HRP-containing neurons after the marker enzyme was applied to the proximal segment of the right middle cerebral artery in seven cats. Each diagram represents a composite of ten adjacent sections.

previously reported observation that only five HRP-positive cells were visualized after the marker enzyme was injected into a pain-sensitive structure, tooth pulp (*17*), supports this notion. Nevertheless, limited numbers of HRP-positive cells found in our studies may reflect differences between species (cat versus human), the relatively small area to which HRP was applied, the wide receptive fields from which trigeminal neurons project (*16*), or perhaps the uptake properties of these nerve terminals. The possibility that other cranial nerves convey sensory information from meningeal blood vessels also deserves consideration. By "painting" HRP mixed with a polymer directly onto the surface of the middle cerebral artery, uptake of the marker by perivascular nerve endings was assured. In this instance, uptake presumably occurred within myelinated and poorly myelinated neurons surrounding large cerebral arteries (*18*).

MARC MAYBERG
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 02139, and *Department of Neurosurgery, Massachusetts General Hospital, Boston 02114*

ROBERT S. LANGER
Department of Nutrition and Food Science, Massachusetts Institute of Technology, and *Department of Surgery, Children's Hospital Medical Center, Boston, Massachusetts 02115*

NICHOLAS T. ZERVAS
Department of Neurosurgery, Massachusetts General Hospital

MICHAEL A. MOSKOWITZ*
Department of Nutrition and Food Science, Massachusetts Institute of Technology, and *Section of Neurology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115*

References and Notes

1. B. S. Ray and H. G. Wolff, *Arch. Surg. (Chicago)* **41**, 813 (1940); F. L. McNaughton, *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* **18**, 178 (1938); H. G. Wolff, *Headache and Other Head Pain* (Oxford Univ. Press, New York, 1963).
2. M. A. Moskowitz, J. F. Reinhard, J. Romero, E. Melamed, D. Pettibone, *Lancet* **1979-II**, 883 (1979).
3. J. H. LaVail and M. M. LaVail, *Science* **176**, 1416 (1972).
4. The cats were anesthetized with subcutaneous ketamine hydrochloride (20 mg/kg) and intraperitoneal sodium pentobarbital (5 mg/kg). Under the operating microscope, a right subtemporal and subfrontal approach enabled the middle cerebral artery to be exposed at its origin from the internal carotid artery. The floor of the middle cranial fossa was covered with a sheet of polyethylene to prevent any contact between the coated vessel and the underlying trigeminal ganglia. After HRP was applied to the vessel embedded in the polymer, this mixture was sealed from adjacent structures with polyethylene film and petrolatum. The wound was closed and the animals were allowed to recover.
5. M. A. Moskowitz, M. Mayberg, R. S. Langer, *Brain Res.* **212**, 460 (1981).
6. C. W. Bowers and R. E. Zigmond, *J. Comp. Neurol.* **185**, 381 (1979).
7. M. M. Mesulam, *J. Histochem. Cytochem.* **26**, 106 (1978).
8. In one cat, HRP-containing polymer was applied to a 1-cm^2 area of parietal cortex (between the inferior and superior divisions of the middle cerebral artery) in an area devoid of large cerebral arteries. The animal was allowed to recover and was perfused with glutaraldehyde after 72 hours.
9. The right supraorbital nerve was exposed at its emergence from the supraorbital foramen and dissected free for 1 cm. The nerve was transected at this point and the proximal end was placed in the polyethylene tube containing a 50 percent solution of horseradish peroxidase (Sigma type VI) for 2 hours. After 48 hours, the ganglia were removed after perfusion and sectioned serially.
10. S. Gobel, *J. Neurocytol.* **3**, 219 (1974).
11. C. M. Fisher, in *Pathogenesis and Treatment of Cerebrovascular Disease*, W. Fields, Ed. (Thomas, Springfield, Va., 1960), pp. 123–164.
12. G. W. Pickering and W. Hess, *Clin. Sci.* **1**, 77 (1933); G. A. Schumacher, B. S. Ray, H. G. Wolff, *Arch. Neurol. Psychiatry* **44**, 701 (1940).
13. J. C. White and W. H. Sweet, *Pain: Its Mechanisms and Neurological Control* (Thomas, Springfield, Va., 1955).
14. B. Arvidson, *Acta Neuropathol.* **38**, 49 (1977); C. W. Morgan, E. Nadelhaft, W. C. de Groat, *Neurosurgery* **2**, 252 (1978).
15. H. Cushing, *Bull. Johns Hopkins Hosp.* **15**, 213 (1904).
16. K. V. Anderson and G. S. Pearl, *Exp. Neurol.* **47**, 357 (1975); G. S. Pearl, K. V. Anderson, H. S. Rosing, *ibid.* **54**, 432 (1977).
17. L. Furstman, S. Saporta, L. Kruger, *Brain Res.* **84**, 320 (1975).
18. G. C. Huber, *J. Comp. Neurol.* **9**, 1 (1899); S. Clarke, *ibid.* **60**, 21 (1929).
19. Supported by NIH grants NS 15201, HL 22573, and GM 26698. M.A.M. is an established investigator of the American Heart Association.
* Send requests for reprints to M.A.M. Present address: Departments of Neurology and Neurosurgery, Massachusetts General Hospital, Boston 02114.

1 August 1980; revised 18 December 1980

Freeze-Fracture Cytochemistry: Replicas of Critical Point–Dried Cells and Tissues After Fracture-Label

Abstract. *Applications of the new fracture-labeling techniques for the observation of cytochemical labels on platinum-carbon replicas are described. Frozen cells, embedded in a cross-linked protein matrix, and frozen tissues are fractured with a scalpel under liquid nitrogen, thawed, labeled, dehydrated by the critical point drying method, and replicated. This method allows direct, high-resolution, two-dimensional chemical and immunological characterization of the cellular membranes in situ, as well as detection of sites within cross-fractured cytoplasm and extracellular matrix.*

Face views of biological membranes are revealed in platinum-carbon replicas of freeze-fractured cells and tissues. The fracture process appears to split regions of the membrane having a bilayer organization (*1*). The bilayer continuum of biological membranes is interrupted by integral membrane proteins that may span the membrane. In freeze-fracture replicas, these proteins, and possibly their tightly associated lipids, interrupt the smooth fracture plane of the bilayer and appear as membrane particles. Characterization of the chemistry and topology of components represented by the membrane particles is derived from studies of freeze-etched surface-labeled membranes (*2*) and of recombinants of membrane lipids and integral proteins (*3*). The generalization of the correspondence between integral membrane proteins and membrane particles is therefore inferential and largely based on the qualitative homogeneity of the freeze-fracture morphology of all biological membranes.

The low temperature (below −100°C) and high vacuum (below 2×10^{-6} mmHg) at which the fracture faces are produced and replicated have limited the development of techniques for cytochemical characterization of the identity and distribution of fracture face components. We have developed methods that permit the cytochemical labeling of freeze-fractured cells and tissues and observation of the labels by thin-section electron microscopy (*4*). These thin-section fracture-labeling methods involve grinding (that is, multiple fracture) of frozen cells and tissues that are immersed in liquid nitrogen. The fractured specimens are thawed, labeled, and processed for thin sectioning. Initial application of this method to the labeling of freeze-fractured erythrocytes (*4*) showed that anionic and lectin binding sites can be labeled on the fracture faces. In particular, concanavalin A binding sites, associated in human erythrocytes to the band 3 component—a membrane-traversing protein and the principal component of the membrane particles—are partitioned during fracture in a manner similar to that of the particles: about 75 percent of the label is seen on the inner, protoplasmic face (*5*), and the rest is associated with the exoplasmic face of the membrane. This signifies that surface residues and chemical groups associated with integral membrane-traversing proteins may be dragged during fracture from their sites at the outer surface across the frozen outer half of the membrane. In addition, the process of fracture appears to cause exposure of additional chemical groups which in the in-

0036-8075/81/0710-0230$00.50/0