of the synthetic HSEs were made by cloning the Xho I–Sal I fragments that contain the synthetic HSEs of pHSE1-4 into the Xho I site at the 5′ end of the HSEs of pw1-4. In these combinations, the spacing between two synthetic HSEs is 9 bp, the same as that between the two native copies in the regulatory region of the *hsp*70 gene. The sequence of the synthetic regulatory regions of all the constructs was determined by DNA sequence analysis by the chain-termination method of Sanger *et al.* (*14*). Additional details of plasmid constructions are available on request.

14. F. Sanger, S. Nicklen, A. R. Coulson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463 (1977).
15. An Xho I–Mbo I fragment from 5′ region of *hsp*70 (Xho I at −194 and Mbo I at +260 of 132E3) (*16*) and a Bam HI fragment containing a 3-kb *lacZ* coding sequence from pMC1871 (*17*) were isolated and cloned into Xho I–Bam HI digested pΔZX [a pUC13X (*7*) derivative in which a Nar I–Eco RI fragment containing the entire *lacZ* sequence was removed]. Ligation of the Mbo I and Bam HI sticky ends put the two reading frames in phase. This hybrid gene contains only the first seven amino acids from *hsp*70 fused to the eighth amino acid of *lacZ* and appears to express severalfold more β-galactosidase in transformant flies than the previously published *hsp*70-*lacZ* hybrid gene (*7*). All the constructs used in this study are derivatives of this new fusion gene.
16. S. Artavanis-Tsakonas, P. Schedl, M.-E. Mirault, L. Moran, J. Lis, *Cell* **17**, 9 (1979).
17. M. J. Casadaban, A. Martinez-Arias, S. K. Shapira, J. Chou, *Methods Enzymol.* **100**, 293 (1983).
18. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
19. H. R. B. Pelham, *Cell* **30**, 517 (1982).
20. Program written by and provided by R. Schleif (Brandeis University).
21. R. S. Cohen and M. Meselson, *Cell* **43**, 737 (1985).
22. D. Pauli, A. Spierer, A. Tissières, *EMBO J.* **5**, 755 (1986).
23. R. Mestril, P. Schiller, J. Amin, H. Klapper, A. Jayakumar, R. Voellmy, *ibid.*, p. 1667.
24. R. Mestril, D. Runger, P. Schiller, R. Voellmy, *ibid.* **4**, 2971 (1985).
25. G. Riddihough and H. R. B. Pelham, *ibid.* **5**, 1653 (1986).
26. E. Hoffman and V. Corces, *Mol. Cell. Biol.* **6**, 663 (1986).
27. R. Klemenz and W. J. Gehring, *ibid.*, p. 2011.
28. A. Amy, R. Southgate, A. Tissières, *J. Mol. Biol.* **182**, 469 (1985).
29. (i) To construct plasmid pMTI, we isolated an Msp I–Pst I fragment containing sequences from positions −38 to +89 of *hsp*70 from pXTI (a pUC13X (*7*) derivative containing the Xho I–Pst I fragment from −89 to +89 of *hsp*70 between the Xho I and Pst I sites), and the Msp I sticky ends were filled in using the Klenow fragment of DNA polymerase I. An Xho I–Bam HI adapter sequence, CCTCGAGGGATC, was added to the filled-in Msp I site, creating a Bam HI site at position −39. This fragment was then cloned into Xho I and Pst I digested pUC13X. (ii) To construct pXNI, we first digested pUC13X with restriction enzyme Xba I, and the sticky ends were removed with mung bean nuclease, and then digested with Xho I. The Xho I–Nru I fragment containing sequences from −89 to −50 of *hsp*70 was isolated from pXTI and cloned into the Xba I– and Xho I–digested pUC13X. This construction recreated the 14-bp heat-shock consensus of site I with a Sal I site (in italics) immediately downstream of the consensus, CTcGAAtgTTCgAG*tcgac*. (iii) To construct plasmid pXBSB, we cloned a Bam HI–Pst I fragment from pMTI (−39 to +89 of *hsp*70) into pUC13 to generate plasmid pSB. pSB was then digested with restriction enzymes Bgl I and Sma I, and the vector fragment was purified and ligated to a purified Bgl I–Xho I nonvector fragment from pΔZX (Xho I end was filled in with the Klenow fragment), and excess Bgl II linker, GGGAGATCTCCC. The Bgl II linker was inserted between the Sma I and Xho I sites. The sequence TTTCTAGAAA was then cloned as dimer or monomer into the Sma I site of pXBSB. The Bgl II–Bam HI fragment containing the dimer or monomer was polymerized with T4 DNA ligase to generate the regulatory regions for 70Z2.2 and 70Z4.1, respectively.
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Multipotent Precursors Can Give Rise to All Major Cell Types of the Frog Retina

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A prospective lineage analysis was performed to determine the variety of cell types that could be formed by individual precursor cells of the developing frog retina. Fluorescent dextran was iontophoretically injected into single cells of the embryonic optic vesicle. After further development of the embryo, labeled descendants were observed in all three layers of the larval retina. Furthermore, different clones were composed of various combinations of all major cell types, including the glial Müller cells. Hence, single optic vesicle cells have the potential to form any type of retinal cell, suggesting that the interactions that specify the differentiation pathway of retinal cells must occur late in development.

ONE OF THE CENTRAL QUESTIONS of developmental biology is how different cell types are created in the correct numbers and positions. One approach to this question is to examine the lineages of the cells that make up the embryo. The cell lineage reveals what cell types share common ancestors and when different lineages diverge. In some embryos, such as that of the nematode *Caenorhabditis elegans*, the complete cell lineage was elucidated by direct observation of the embryo with Nomarski optics (*1*). These data were required for subsequent analyses of both the cell interactions and the molecular mechanisms that might subserve them (*2*). Such an approach has not been possible in the vertebrate nervous system, because the large numbers and undifferentiated state of the precursor cells make it impossible to directly identify and follow single cells and their descendants. Thus, it remains possible that the wide variety of neural cell types arises either from a collection of prespecified precursors, each restricted to only one cell type, or from multipotent precursors, each able to give rise to the full range of cell types.

We have examined cell lineages in the neural retina of the frog by injecting single cells of the optic vesicle with lysinated rhodamine dextran (LRD). Fluorescent dextrans (*3*) serve well as lineage markers because their size and charge prevent their escape from the injected cell or from its descendants through either cell junctions or cell membranes. Microinjection of fluorescent dextrans into blastomeres, which are relatively large, making it easy to inject dye into them, has permitted studies of early embryonic lineages in a variety of invertebrates (*4*) and vertebrates (*5*). We have refined the techniques for iontophoretically injecting fluorescent dextrans into small and fragile cells, such as retinal precursor cells. The neural retina has been extensively studied (*6*) and offers several advantages for cell lineage studies. (i) It has relatively few cell types: the photoreceptors in the outer nuclear layer (ONL); the horizontal, bipolar, amacrine, and glial Müller cells in the inner nuclear layer (INL); and the ganglion cells in the ganglion layer (GL). The laminar organization to some extent facilitates the identification of cell types. (ii) The precursor cells in the lateral portion of the optic vesicle are accessible for microinjection. (iii) In the frog, the cells quickly develop into a functional retina. Neuron birthdates begin soon after the optic vesicle stage, at stage 29 (*7*), and the lamination begins around stage 39 (3 days after fertilization).

At the end of each microinjection (*8*), direct visual observation of a single fluorescently labeled cell confirmed a successful dye fill. In some cases this visual confirmation was ambiguous because of suboptimal viewing conditions (distortion by the overlying tissue or by the meniscus of the solution). To independently verify that the technique consistently yields single dye-filled cells, six animals with several injections each were fixed immediately (within 5 minutes) after injection. Histological sections (Fig. 1A) revealed that 16 of 17 injections resulted in single labeled cells. In the remaining case,

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one labeled cell was in the optic vesicle, while another was in the overlying epidermis, through which the electrode must pass. The clones arising from these two precursors would have been easily distinguished because of their different locations and derivatives. Hence, clones arising after intracellular injection of fluorescent dextran are probably descendants of a single marked cell.

In 27 animals, single optic vesicle cells were injected iontophoretically with LRD at stages 22 to 24, and the animals were raised to free-swimming tadpoles (stages 46 to 52). The labeled founder cell proliferated, forming a clone of cells, and the descendants differentiated, often spreading over all three layers of the retina (Fig. 1, B and C). In most of the clones, the labeled cells were distributed in a radial column, with little spread circumferentially (laterally), similar to the narrow columns of clonally related cells that have been described in the rat retina (*9, 10*). In the narrowest clones, the cells of the INL were completely unmixed with the neighboring, unlabeled cells. In the widest clones, the spread was only about six cell diameters. Since each layer in the retina is composed of characteristic cell types, the laminar position of labeled cells offers information about their cell types. In all but seven of the 61 clones examined, the labeled cells were distributed over two or three of the retinal layers (Table 1). This predominance of multilaminar clones indicates that most of the injected cells are multipotent.

The restriction of some clones to only one layer may result from either a lineage restriction or limited cell mixing in the radial direction. To gain some insight into these possibilities, the sizes of the different clones were compared to their laminar distribution. In general, larger clones were distributed over more layers (see Table 1).

The identification of the labeled cell types by their laminar position, especially in the INL in which there are four major cell types, is incomplete. For some cells, a more complete identification, based on cell morphology, was made possible by the Golgi-like images (*11*) produced when the LRD fills a

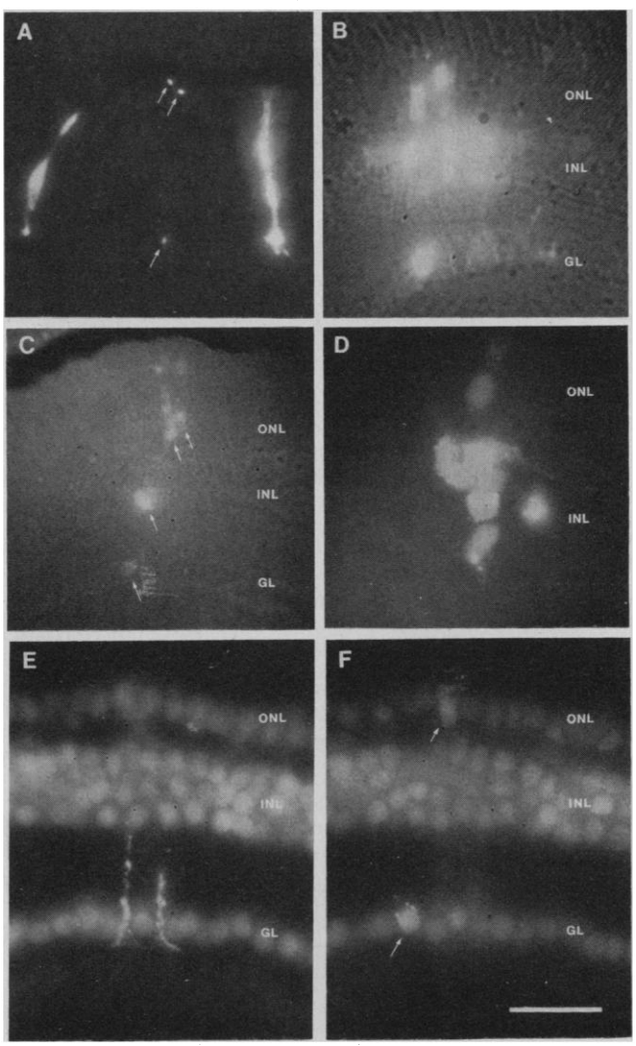

Fig. 1. Cells labeled with LRD. (**A**) Single precursor cells in the optic vesicle of a stage 23 *Xenopus* labeled by iontophoretic microinjection of LRD. Portions of three individual dye injections can be seen: two complete individual cells (left and right), and the end-feet of a third cell (arrows), whose cell body was in the adjacent section. (**B**) A large clone with 22 labeled cells located in all three layers of the retina. Since each cell type of the retina is characteristically located in one of the three layers, this clone is composed of multiple cell types. Because of the high magnification and the thickness of the section, not all of the labeled cells are in the same plane of focus. (**C**) A smaller clone composed of multiple cell types. Of the eight cells in this clone, two rods, an amacrine, and a ganglion cell are shown (arrows). (**D**) A high magnification view of a clone of nine labeled cells spread over two layers. Three of the cells were identifiable: a cone at the top, with wide outer segments; a horizontal cell below the cone, with a process running to the right in the outer plexiform layer; and an amacrine cell at the inner edge of the INL, with a process (out of the plane of focus) running to the right in the inner plexiform layer. (**E** and **F**) A clone composed of both glial and neuronal cells. The glial Müller cells (E) are recognized by the characteristic radial processes in the inner plexiform layer and their end-feet at the vitreal surface. In a different plane of focus (F), labeled cone and ganglion cells (arrows) are visible. The high autofluorescence in this section allows the three retinal layers to be visualized. The vitreal surface of the retina is toward the bottom of all figures. Scale bar is 70 μm in (A), 30 μm in (B), 50 μm in (C), (E), and (F), and 20 μm in (D).

Table 1. Laminar distribution of labeled cells of single clones. Each entry represents one clone (all descendants of a single injected cell), and the value indicates the number of cells in that clone. The columns give the number of layers over which each clone is spread. Sixty-one clones from 27 animals are presented. In those animals with more than one clone, each cluster of LRD-labeled cells was distinctly separate from all of the other clones in the same retina. Although all injections were performed at essentially the same stage, some clones are quite small, yet others are relatively large. The smaller clones are not located closer to the central retina, where the cells withdraw from the cell cycle earlier than in the periphery (*7, 16*). The wide variety of clone sizes suggests that the processes controlling withdrawal from the mitotic cycle and differentiation might be somewhat stochastic. The mean size of the three-layer clones is significantly larger than the means of the one- and the two-layer clones ($P < 0.001$, protected t test), and the one- and two-layer clones are significantly different from one another ($P < 0.05$). This relation between clone size and laminar distribution suggests that precursors may have had the potential to form many cell types but by chance gave rise to only a few cells, and hence only a few cell types were actually formed.

Number of cells		
One layer	Two layers	Three layers
1	2	4
1	2	8
1	4	8
3	5	10
3	5	10
4	6	11
9	6	12
	6	15
	6	16
	8	16
	8	17
	8	17
	8	18
	9	20
	9	21
	9	21
	9	22
	9	22
	11	27
	12	28
	12	29
	12	30
	18	31
	18	34
	25	34
	31	34
	42	36
3.1 ± 1.08*	11.1 ± 1.73*	20.4 ± 1.78*

*Mean ± SEM.

cell's processes (*12*). All major types of neuronal and glial cells could be identified within clones, which also include other cell types (Fig. 1, D, E, and F). Any combination of different cell types could be derived from a single labeled precursor (Table 2). Even small clones located in one or two layers appeared to consist of multiple cell types. The same range of cell types was seen in clones of all sizes. Together these observations show that optic vesicle cells are multipotent for all major types of retinal cells.

The present fluorescent dextran study confirms and extends the results of experiments in which retroviral vector was used to study lineages in the postnatal rat retina (*9, 10*). On the basis of the argument that a clustered group of labeled cells represented the descendants from a single infected precursor, the retroviral study suggested that single precursor cells can give rise to any three of the four retinal cell types that arise postnatally. Turner and Cepko (*10*) further suggested that the data from their smallest clones support the proposal that cell type might be specified as late as after the last cell division. Because our LRD injections were performed at the optic vesicle stage, the majority of our clones were relatively large compared to the retrovirus-labeled clones, which were infected at a later stage of retinal development. The larger size of our clones prevents a direct determination of the exact time of specification of the differentiated phenotype; however, the diverse cell types observed in even the smallest LRD-labeled clones supports the suggestion of Turner and Cepko (*10*). Because the LRD labeling was accomplished at an earlier stage, all major cell types were represented in the clones reported here, including cone, horizontal, and ganglion cells. Hence, the present study extends the retrovirus study in two ways, by following the descendants of confirmed single precursors and by showing that retinal precursors are multipotent for all types of retinal cells (*13*). The two approaches support the same conclusion: cell type specification occurs late in vertebrate retinal development. The similar results obtained from frog and rat suggest that the developmental mechanisms that specify cell types in the retina may be universal to all vertebrates.

The wide variety of cells produced by any one precursor brings to the forefront the question of how the relative numbers of different cell types are regulated. There is evidence that specification of cell type may occur by short-range, cell-cell interactions. In one study, the tyrosine hydroxylase–immunoreactive neurons of the frog retina were ablated with 6-hydroxydopamine (*14*). The ciliary margin, which produces new cells throughout the animal's life, increased its production of tyrosine hydroxylase–immunoreactive cells. The specificity of the overproduction suggests that differentiated postmitotic cells provide feedback information to the ciliary margin, and thereby influence the differentiation of new cells. Preliminary evidence from fluorescent dextran injections indicate that the ciliary margin is composed of multipotent proliferative cells similar to those in the optic vesicle (*15*). Therefore, it appears that preexisting neurons can influence the phenotypes of the cells produced by these multipotent precursor cells. In the developing eye vesicle, a similar feedback mechanism may be operating to ensure that the proper types of cells are formed in the correct proportions. The fluorescent dextran approach to retinal lineage has provided the cellular background required for studying the molecular events that regulate the commitment and differentiation of specific cell types.

Table 2. Combinations of recognized cells in clones derived from single optic vesicle cells. For each clone, the total number of cells in the clone and the number of layers involved are presented. An "x" in a column indicates that at least one of that cell type was present in the clone. These 15 clones illustrate that many combinations of different cell types were observed after labeling single optic vesicle cells. There is no evidence that any of our 61 clones were restricted to producing specific cell types. Abbreviations: Ph, photoreceptor; Hor, horizontal cell; Bi, bipolar cell; Am, amacrine cell; Mü, Müller cell; and Gl, ganglion cell.

Clone name	Cells/layers	Cell type					
		Ph	Hor	Bi	Am	Mü	Gl
506F-D	8/3	x	x		x		x
506F-C	11/3	x	x	x		x	x
928A	22/3	x		x		x	x
928C	9/2	x	x		x		
601Z	12/3	x	x	x			x
612E	12/2	x			x	x	
612F-C	2/2	x				x	
612F-D	4/3	x		x			x
612V	8/2	x	x			x	
720J	29/3	x		x	x	x	x
720K-N	12/2	x			x	x	
720K-T	9/2	x		x	x		
403H	16/3	x	x	x	x		x
922F	3/1	x					
1006E	1/1						x

REFERENCES AND NOTES

1. J. E. Sulston and H. R. Horvitz, *Dev. Biol.* **56**, 110 (1977); J. Kimble and D. Hirsh, *ibid.* **70**, 396 (1979); J. E. Sulston, E. Schierenberg, J. G. White, J. N. Thomas, *ibid.* **100**, 64 (1983); J. E. Sulston and J. G. White, *ibid.* **78**, 577 (1980); C. Kenyon, *Philos. Trans. R. Soc. London Ser. B* **312**, 21 (1985).
2. W. A. Harris and M. McKeown, *Trends Neurosci.* **9**, 383 (1986).
3. R. L. Gimlich and J. Braun, *Dev. Biol.* **109**, 509 (1985).
4. D. A. Weisblat and M. Shankland, *Philos. Trans. R. Soc. London Ser. B* **312**, 39 (1985); R. A. Cameron *et al.*, *Genes Dev.* **1**, 75 (1987).
5. H. Balakier and R. A. Pedersen, *Dev. Biol.* **90**, 352 (1982); C. B. Kimmel and R. M. Warga, *Science* **231**, 365 (1986); P. Sheard and M. Jacobson, *ibid.* **236**, 851 (1987); R. Wetts, N. A. O'Rourke, S. E. Fraser, in *The Making of the Nervous System*, J. G. Parnavelas, C. D. Stern, R. V. Sterling, Eds. (Oxford Univ. Press, Oxford, 1988), pp. 52–69.
6. R. Adler and D. Farber, Eds., *The Retina: A Model for Cell Biology Studies* (Academic Press, Orlando, FL, 1986), parts 1 and 2; J. E. Dowling, *The Retina: An Approachable Part of the Brain* (Harvard Univ. Press, Cambridge, MA, 1987).
7. D. H. Beach and M. Jacobson, *J. Comp. Neurol.* **183**, 603 (1979); M. Jacobson, *Dev. Biol.* **17**, 219 (1968).
8. Frog embryos (*Xenopus laevis*) at stages 22 to 24, obtained by artificial fertilization [N. A. O'Rourke and S. E. Fraser, *Dev. Biol.* **114**, 265 (1986)], were placed in an agar dish filled with modified frog Ringer solution, supplemented with 5 m*M* Ca^{2+}, 10 m*M* Hepes (*p*H 7.4), and methylsulfonate anesthetic. With some embryos, part of the epidermis covering the eyebud was removed with a sharpened watchmaker's forceps to facilitate access. Microelectrodes (thin-walled aluminosilicate, 10 to 40 megohms) were filled at the tip with LRD (100 mg/ml, Molecular Probes) and backfilled with 1.2*M* LiCl. The fluorescent dextran was ejected with positive current pulses (200 msec, 2 to 5 nA) at 2 Hz for 10 to 30 seconds. Embryos were fixed in 4% paraformaldehyde in 0.1*M* phosphate buffer (*p*H 7.4) at 4°C for 6 to 8 hours, dehydrated in 95% ethanol, embedded in methacrylate plastic (Historesin, LKB), and sectioned at 20 or 30 μm. A light-intensifying camera (SIT) was used to amplify the signal from the fluorescent label, and an image-processing system (Imaging Technologies) was used to average several video frames to eliminate camera noise.
9. J. Price, D. Turner, C. Cepko, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 156 (1987).
10. D. L. Turner and C. L. Cepko, *Nature (London)* **328**, 131 (1987).
11. S. Ramon y Cajal, *The Structure of the Retina*, S. A. Thorpe and M. Glickstein, Translators. (Thomas, Springfield, IL, 1972); J. E. Dowling, in *Frog Neurobiology*, R. Llinas and W. Precht, Eds. (Springer-Verlag, New York, 1976), pp. 278–296; N. Matsumoto and K.-I. Naka, *Brain Res.* **42**, 59 (1972).
12. Many cells had incompletely filled processes or overlapped with neighboring cells; such cells were classed as unidentifiable.
13. A possible limitation of injected tracers such as LRD is dilution by cell division. This is minimized in amphibians, because they develop from eggs to free-swimming tadpoles without a significant increase in total volume. Because our interpretations are based only on clearly labeled cells, dilution of the dye to undetectability in some cells, had it occurred, would not alter our conclusions concerning the multipotency of optic vesicle cells.

14. T. A. Reh, *Soc. Neurosci. Abstr.* **12**, 768 (1986); _____ and T. Tully, *Dev. Biol.* **114**, 463 (1986); T. A. Reh, *J. Neurosci.* **7**, 3317 (1987).
15. R. Wetts and S. E. Fraser, unpublished data; Fig. 1, E and F, show a ciliary margin clone composed of multiple cell types.
16. K. Straznicky and R. M. Gaze, *J. Embryol. Exp. Morphol.* **26**, 67 (1971).
17. Supported by NSF, the McKnight Foundation, and the Monsanto Corporation.

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A Gene for Dihydrofolate Reductase in a Herpesvirus

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The enzyme dihydrofolate reductase (DHFR) is found ubiquitously in both prokaryotes and eukaryotes. It is essential for de novo synthesis of purines and of deoxythymidine monophosphate for DNA synthesis. Among viruses, however, only the T-even and T5 bacteriophage have been found to encode their own DHFR. In this study a gene for DHFR was found in a specific subgroup of the gamma or lymphotropic class of herpesviruses. DNA sequences for DHFR were found in herpesvirus saimiri and herpesvirus ateles but not in Epstein-Barr virus, Marek's disease virus, herpes simplex virus, varicella-zoster virus, herpesvirus tamarinus, or human cytomegalovirus. The predicted sequence of herpesvirus saimiri DHFR is 186 amino acids in length, the same length as human, murine, and bovine DHFR. The human and herpesvirus saimiri DHFRs share 83 percent positional identity in amino acid sequence. The herpesvirus saimiri DHFR gene is devoid of intron sequences, suggesting that it was acquired by some process involving reverse transcription. This is to our knowledge the first example of a mammalian virus with a gene for DHFR.

THREE DISTINCT BUT RELATED groups of herpesviruses have generally been recognized. A human herpesvirus serves as the prototype for each of these groups (*1*). Herpes simplex virus is the prototype of the alpha group, human cytomegalovirus (CMV) is the prototype of the beta group, and Epstein-Barr virus (EBV) is the prototype of the gamma or lymphotropic herpesviruses. Herpesvirus saimiri (HVS) and herpesvirus ateles (HVA) are T-lymphotropic viruses of New World primates closely related to each other in sequence (*2*). Although genetic relatedness between these New World primate viruses and EBV was not obvious from hybridization studies, evidence suggests HVS is more closely related to EBV than to alpha or beta herpesviruses (*3*). HVS and HVA naturally infect squirrel monkeys (*Saimiri sciureus*) and spider monkeys (*Ateles* sp.), respectively.

Herpesviruses are known to encode enzymes for nucleotide and DNA synthesis—a luxury afforded perhaps by the large size of their genomes. Herpesvirus-encoded enzymes involved in nucleotide metabolism and DNA synthesis that have been identified to date include thymidine or deoxypyrimidine kinase (*4*), ribonucleotide reductase (*5*), DNA polymerase (*6*), deoxyuridine triphosphatase (*7*), exonuclease (*8*), uracil-DNA glycosylase (*9*) and thymidylate synthase (TS; E.C. 2.1.1.45) (*10*). The TS gene has been found in HVS, HVA, and in varicella-zoster virus but not in other herpesviruses (*10–12*).

Dihydrofolate reductase (DHFR; E.C. 1.5.1.3) catalyzes the reduction of dihydrofolate to tetrahydrofolate, an essential step for de novo glycine and purine synthesis and for the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate (dTMP). The central role of DHFR in DNA precursor synthesis and its sensitivity to inhibition by drugs such as methotrexate have made DHFR the target of anticancer chemotherapy. For these and other reasons, DHFR has been extensively studied and much is known about its synthesis, regulation, and escape from drug inhibition (*13*). Among viruses, however, only the T-even and T5 bacteriophage have been found to encode their own DHFR (*14*). In this report, we describe a naturally occurring DHFR gene in a mammalian virus.

The genome of HVS contains a 110-kilobase-pair (kbp) sequence of DNA called L-DNA (36% G+C). This is flanked at each end by 1444-bp repeat units of repetitive DNA called H-DNA (71% G+C) (*15*). Our nucleotide sequencing near the left H-L DNA border of HVS strain 11 revealed an open reading frame of 246 amino acids that was 187 amino acids (L-DNA nucleotides 4532–3972) in length from the first methionine (Fig. 1). The amino acid sequence predicted by this open reading frame was highly related to sequences for cellular DHFR obtained by others (*16–19*) (Fig. 2). Cellular DHFR of human, murine, and bovine origin is initially synthesized as a 187–amino acid product, with the initiating methionine then being cleaved to yield valine as the first amino acid of mature enzyme

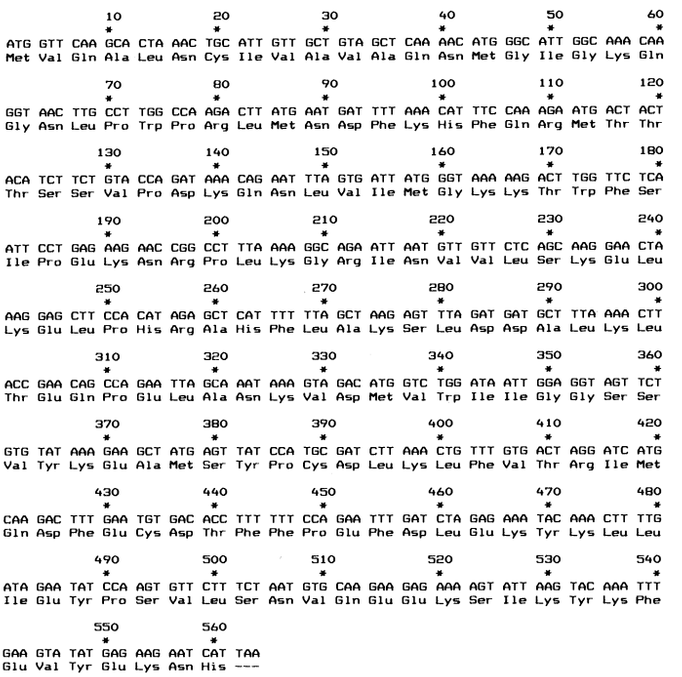

Fig. 1. Nucleotide sequence of herpesvirus saimiri strain 11 DNA encoding a predicted dihydrofolate reductase. Nucleotides 1 and 564 of the sequence shown are nucleotides 4532 and 3969, respectively, of L-DNA from the left H-L border. DNA of this region of HVS was cloned previously (*32, 34*). Several subclones were then generated into PGEM vectors (Promega). In addition, a series of nested deletion clones were obtained by using Exonuclease III and S1 nuclease (*36*). Exonuclease III was used to specifically digest DNA unidirectionally from a 5′ protruding end. Portions were removed at various time intervals, trimmed with S1 nuclease, and religated to yield clones with the desired deletions. Dideoxy sequencing reactions were resolved on buffer gradient, 6% acrylamide (1 m long) sequencing gels (American Bionetics).

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