Gene Transfer into the Nervous System

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Index Entries: Gene transfer; retrovirus; herpes; transgenic mice; viral vectors; nervous system.

Potential Uses

Several techniques have been developed that allow the introduction of recombinant DNA molecules into the cells of the nervous system. These methods include DNA uptake (transfection), microinjection of DNA, and infection with viral vectors. Gene transfer can be used for two general purposes: (1) To evaluate the regulation and function of a cloned gene following its modification and introduction into different cell types. Such studies include the definition of the regulatory elements that control levels of gene expression, alternate modes of RNA splicing, post-translational processing of peptides, sorting of proteins to their appropriate cellular locations, and biological activities of proteins. (2) To modify cells of the

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nervous system in culture and in vivo. Such studies involve analysis of cell lineage, alteration of phenotypic properties, and ablation of specific cell populations, as well as creation and correction of hereditary disease states (*see* Table 1). Gene transfer techniques can thus be used as a tool in understanding molecular aspects of the development, function, and survival of cells in the nervous system. This review is intended to spark interest in this new approach and to bring together relevant information from several fields.

Gene transfer technology will be useful to investigators in many different fields of the neurosciences. To the neurochemists perhaps the most immediate applications are in elucidating the processing of neural peptides and the functional domains of proteins. Cloned cDNA or genomic sequences for neural proteins can be introduced into different cell types in culture to study cell type-specific differences in processing and cellular fate. By placing the coding sequences under the control of a strong promoter, a substantial amount of the protein can be made, thus avoiding difficulties in characterizing trace amounts. Furthermore, the specific residues involved in protein processing, intracellular sorting, or biological activity can be determined by mutational changes in discrete residues of the coding sequence.

To the neurophysiologist interested in analysis of the function of neural proteins, gene transfer provides a method to control expression of a protein and assess its modulation of cellular events. Some functions of neural proteins, such as their role in differentiation, may be studied in tissue culture, whereas others will require reintroduction into the nervous system at different times in development or aging to monitor changes in receptor density, cell number, fiber growth, electrical activity, and other relevant properties.

To the molecular biologist, gene transfer provides a means to study the DNA sequences and cellular factors that regulate expression of neural specific genes. Often the experimental approach is to fuse the regulatory elements to be studied to a reporter gene and subsequently assay for expression of the reporter gene. Regulation of gene expression takes place in vivo, in maintaining homeostasis and probably in mediating information retention in response to external and internal signals (Black et al., 1987). During development, coordinate regulation of gene expression serves to produce a differentiated phenotype, e.g., as in catecholamine metabolism and myelin biosynthesis. Regulation depends on many factors including chromatin structure, DNA methylation, and transacting factors, which respond to phosphorylation, hormones, and other signals. It is a complex process that allows sets of genes to be expressed together or differentially, and may involve a combinatorial code of regulatory sequences.

TABLE 1

Why Transfer Genes into Nervous System

Elucidate regulatory elements of genes
Study action and functional domains of neural proteins
Mark cells during development
Kill or transform select cell populations
Therapy for defective genes
Create animal models for neurologic diseases: dominant disease genes and antisense blockade

For the cellular and developmental neurobiologist, issues of cellular fate and interactions in the nervous system can be addressed by gene transfer. A particularly active area of research has been the introduction of genes that encode histological markers into embryonic cells to determine lineage relationships during development and to elucidate neuronal pathways. In addition, genes encoding growth factors, oncogenic proteins, toxic peptides, or other physiologically important proteins, can be introduced into specific areas of the nervous

system to study their effects on cell division, survival, and differentiation. For some studies, gene transfer or gene expression must be restricted to specific cells in the nervous system. The means to do this has not been resolved but possible approaches using tissue-specific promoters and cell targeting are discussed below.

Finally a discussion of gene transfer would not be complete without considering its potential use in understanding and providing therapy for disease states. There are a number of inherited neurologic diseases in which defective genes are known and have been cloned. In some cases the function of these cloned genes is known, in others not. In humans, genes for defective enzymes have been identified for several lysosomal storage diseases (e.g., beta hexosaminidase, Korneluk et al., 1986; Myerowitz et al., 1985; and glucocerebrosidase, Sorge et al., 1985; Tsuji et al., 1987); the Lesch-Nyhan syndrome (HPRT; Stout and Caskey, 1985); amyloid polyneuropathies (prealbumin, Sasaki et al., 1984); Alzheimer amyloid (Tanzi et al., 1987; Goldgaber et al., 1987), Duchenne's muscular dystrophy (uncharacterized muscle protein; Monaco et al., 1986), and retinoblastoma (uncharacterized protein expressed in the retina and other tissues, Lee et al., 1987; Friend et al., 1986); and in mice for the *shiverer* (myelin basic protein, Roach et al., 1987; Molineaux et al., 1986) and *jimpy* (proteolipoprotein, Nave et al., 1986; Hudson et al., 1987) mutations. These diseases fall into two classes: deficiency states, usually of enzymes, which are inherited in a recessive manner; and unbalanced states, at least sometimes involving structural or regulatory proteins, which are inherited in a dominant manner. For deficiency states gene transfer could be used to bring a normal gene into affected tissues for replacement therapy, as well as to create animal models for the disease using antisense mutations. For unbalanced states gene transfer could be used to create the disease state in a model system and attempt to counteract its effects. The possibility of site-specific integration of DNA sequences to cause mutations and correct defects is also under study (Thomas and Capecchi, 1986).

Issues to be Resolved

Delivery into the Nervous System

If a gene is to be delivered to every cell of the entire organism, including the nervous system, the most effective means is through introduction into preimplanation mouse embryos and subsequent propagation of transgenic pedigrees (Fig. 1). For these studies microinjection of DNA or infection with viral vectors is possible. For more limited delivery in post-implantational embryos it is possible to inject viral vectors into the amniotic sac before the neural tube has closed (Calof and Jessell, 1986) or directly into the brain (Sanes et al., 1987). Here transfer will occur into a number of different progenitor cells and hence into all their progeny. Delivery into cells of the nervous system after birth presents complications because of its relative inaccessibility, diversity of cell types, and postmitotic state of mature neurons. Here viral vectors should provide effective and diversified means of delivery.

A virus vector is one in which the gene to be transferred is fused to a subset of viral sequences and encapsulated in a virus particle or virion. The infective properties of this virion are the same as those containing the wild type viral genome. Perhaps the four most important considerations of the virus vector are that: the vector will only be delivered into cells that can be either abortively or productively infected by the wild-type virus; if the virus is capable of persisting in cells for extended periods of time without causing cell death, then the vector may be maintained and expressed in cells for long periods of time; if the viral fusion gene exists as double stranded DNA in the nucleus of a cell, then its promoter(s) may retain appropriate re-

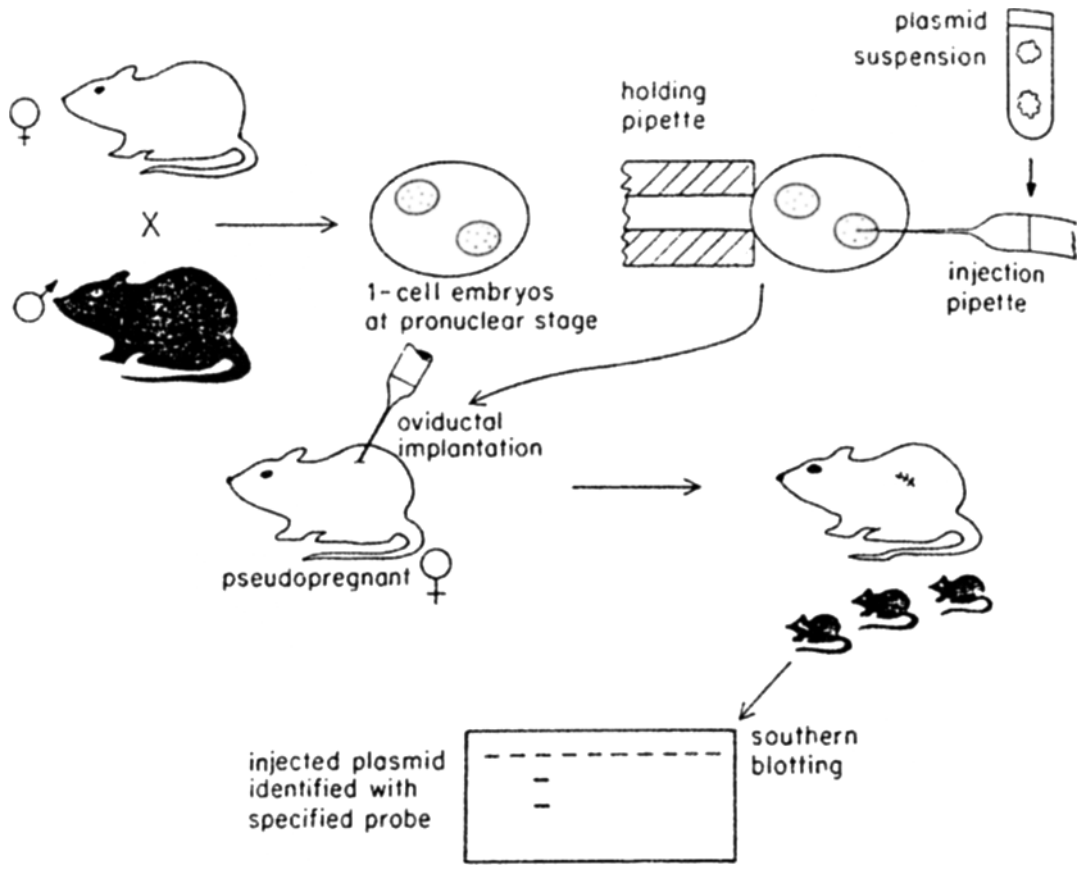

Fig. 1. Procedure for producing transgenic mice. Immature albino females were superovulated and mated to males homozygous for the wild-type allele at the albino locus. One-cell embryos at the pronuclear stage of development (d 1 of gestation) were removed from the oviducts, after which one of the pronuclei was microinjected with recombinant plasmid DNA in solution. Embryos were then reimplanted into the oviducts of the albino females rendered pseudopregnant by mating with albino vasectomized males. The pigmentation markers were employed to eliminated the possibility of the embryos giving rise to live young from oocytes fertilized by spermatozoa from inadequately vasectomized males. In this scheme, such embryos would be of the albino pigmentation phenotype. After normal delivery, pigmented mice were evaluated by Southern blot hybridization for the presence of microinjected DNA. Reprinted with permission from Gordon and Ruddle, 1985.

sponses to transacting regulatory factors; and the size of the gene(s) to be transferred will be limited by the size of the viral genome. The ability of a virus vector to infect cells in tissue culture or in vivo will be determined by properties of the cells, the virus, and genetic elements in the vector.

Several features of viral infections can be used to manipulate delivery of genes. (1) The viral particle itself may attach to and be taken up by essentially all cell types, e.g., retrovirus, herpes simplex, and vaccinia, or may demonstrate cell selective entry through binding to specific cell surface molecules, e.g., rabies virus to the nicotinic acetylcholine receptor (Lentz et al., 1986), reovirus to the beta-adrenergic receptor (Co et al., 1985), and poliovirus to a protein enriched in the synaptosomal fraction (Brown et al., 1987). Once inside the cell, viruses have developed special means to avoid cellular degradation of their nucleic acids. For example, the Semliki Forest virus particle con-

tains a protein that allows it to be expelled from lysosomes by virtue of the low pH encountered there (Mellman et al., 1986). (2) Viruses demonstrate different modes of reproduction. Some DNA viruses replicate as autonomous elements in the cytoplasm (e.g., vaccinia) or nucleus (e.g., papilloma). Vectors derived from these viruses may be especially effective in producing high level, transient expression of transferred genes. Other RNA and DNA viruses integrate into the host cell genome and replicate with it. These are more stably expressed in cell progeny but levels of expression will be affected by the site of integration. (3) Viruses may replicate or be maintained preferentially in certain cells. Many viruses are neurotrophic, e.g., reoviruses, herpes simplex, some retroviruses, and poliovirus. Features that confer neural selectivity are under study. In the future, as sequences encoding various viral determinants are elucidated it may be possible to control cell entry, DNA replication and expression of transferred genes by attaching together different functional cassettes and packaging them in appropriate viral particles.

The best means to introduce the vectors into the nervous system has yet to be determined. Possible means include injection of virus or genetically altered cells (Fig. 2; Gage et al., 1987; Selden et al., 1987), and delivery of cells or virus through the circulation, CSF, or nerve endings. Stereotaxically guided injection should allow accurate delivery of virus or cells to selected areas. One limitation will be the volume that can be injected without seriously disrupting brain functions, about 1–10 μL in an adult brain. If the virus vector cannot be replicated in vivo, then the number of cells receiving the vector will be limited by the number of infectious particles in the inoculum. For example, with a virus stock containing 10^6 plaque forming units (pfu)/mL, an injection of 5 μL would contain about 5×10^3 pfu, only a portion of which would effect gene transfer. Thus, several hundred to a thousand cells might express the transferred gene, if there was no further cell proliferation. This type of delivery would be suitable for experiments in which gene transfer to a small number of neural cells is desired. Injections could be done near cell bodies or nerve terminals. In the latter case retrograde transport mechanisms might be exploited to deliver the genes back to the cell nucleus. In the peripheral nervous system it might be possible to gain entry to neurons through nerve endings on the skin, gut, or blood vessels. Alternately, a helper virus could be included in the injection that would result in production of progeny virus in vivo and thus spread infection. However, such a helper virus might have deleterious effects on the nervous system; and this might confound interpretation of the effects of transferred genes. Alternately, the inoculum could contain cells that had been geneticaly altered in culture. About 10^6 cells could be delivered in a 10 μL volume. Advantages of this approach are that gene transfer could be carried out on dividing cells under optimal conditions and successful gene recipients could be selected. Cell candidates would include embryonic neuroblasts and supporting cells, such as Schwann cells or astrocytes. Once introduced back into the nervous system some of these might continue to proliferate and/or migrate, thus spreading the transferred signal over a wider range of the nervous system. This approach has the advantages and disadvantages of cell transplantation studies. Third, where gene transfer into many cells of the central nervous system is desired, viral vectors could be introduced into the cerebrospinal fluid of brain ventricles or spinal cord, or even into the bloodstream with temporary disruption of the blood-brain barrier (Brady, 1983).

Expression in the Nervous System

The expression of transferred genes can be constitutive in all cells, limited to specific cell

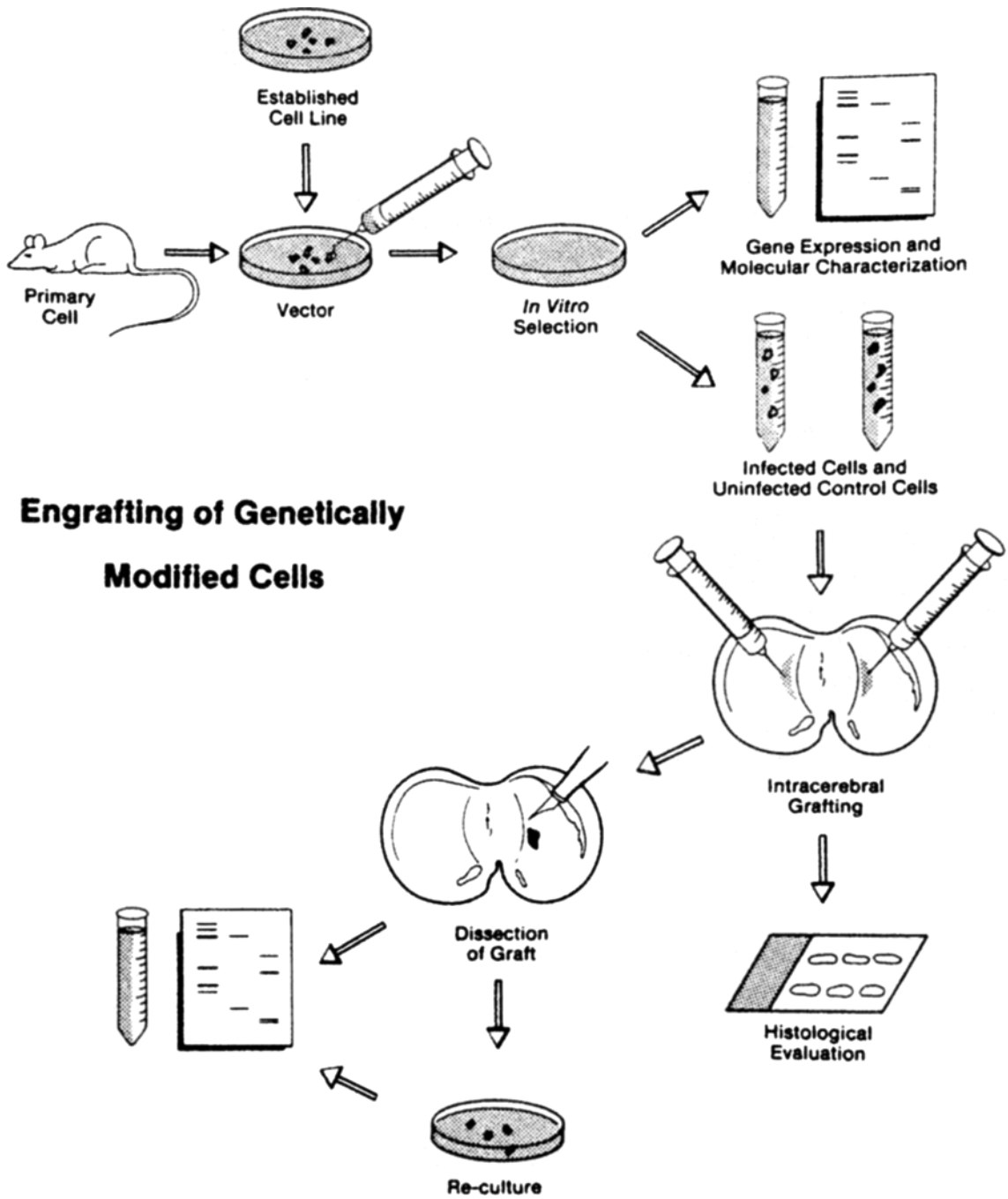

Fig. 2. Grafting of genetically modified cells to the CNS. Established cell lines or primary cell cultures are genetically altered by transfection or infection with a viral vector. Cells expressing the exogenous gene are enriched or cloned in culture on the basis of growth selection or expression of a marker protein. Expression of the exogenous genes in recipient cells is characterized in vitro. The "transgenic" cells are injected intracerebrally into rats and uninfected control cells are injected contralaterally. Sometime later, e.g., 1 wk to 3 mo, rats are sacrificed and the brains are examined histologically to evaluate cell survival, and grafted regions are dissected out to evaluate expression of the exogenous gene. In addition, portions of the graft are recultured and continued presence and expression of the transgenes is determined. Reprinted with permission from Gage et al., in press.

types, and/or responsive to selective signals depending on regulatory elements attached to it. What little is known about the transcriptional regulatory elements of genes expressed in the cells of the CNS is consistent with the behavior of transcriptional regulatory elements in other systems. A brief review of basic concepts is provided here. Eucaryotic genes are transcribed by one of three RNA polymerases that have been defined by their differential sensitivities to the drug alpha-amantin. RNA polymerase I transcribes primarily ribosomal RNA genes; RNA polymerase II transcribes single copy genes encoding the proteins familiar to neurochemists; and RNA polymerase III transcribes primarily tRNA and 5S ribosomal genes. This section will be concerned with the regulation of genes transcribed by RNA polymerase II.

There are two broad categories of genetic elements that regulate the initiation of transcription, termed promoters and enhancers. Promoters determine the site of initiation of transcription by RNA polymerase II. Promoters contain a transcriptional start site (cap site), a TATAA box, and often a CAAT sequence. Their structure has been reviewed recently (Buchner and Trifonov, 1986). Enhancers are elements that regulate the rate of transcription through their action on a promoter. Enhancers must be present on the same molecule of DNA as the promoter with which they interact, but they can function at a distance of up to several kb away from it and in an orientation independent manner (reviewed by Khoury and Grass, 1983; Serfling et al., 1985). The recently postulated "silencer" element has the same properties as an enhancer except it acts to reduce rather than increase the rate of transcription (Larsen et al., 1986). Either the promoter, the enhancer, or both may contain elements that determine cell type specific expression, temporal specific expression during development, and responses to extra- and intracellular stimuli.

It is not yet clear whether genes selectively active in neural cells contain common regulatory elements. One candidate element has been identifier (ID) sequences. These are moderately repetitive elements 72 bp in size that reside within the introns or flanking sequences of some genes. A subset of them appear to be transcribed by RNA polymerase III as well as RNA polymerase II. Transcripts of ID sequences can be found in the cytoplasm of neural cells (for review, *see* Chikaraishi, 1986). In initial studies, two such transcripts were found to be expressed shortly after birth in the brain and not in other tissues. This led to the hypothesis that these repeat elements and their RNA transcripts might have a role in regulating brain-specific expression of genes (Sutcliffe et al., 1984). Since these initial observations, data have emerged that both support and weaken this hypothesis. On the positive side, the cytoplasmic presence of some, but not all, transcripts from such elements is brain-specific. Further, when ID sequences are linked to reporter sequences under the control of constitutively expressed viral enhancer and promoter sequences, they increase transcription of these genes in cells that contain cytoplasmic ID transcripts, and not in cells that do not contain them (McKinnon et al, 1986). On the negative side these ID sequences occur in the introns of nonneural as well as neural genes; among species the numbers of these sequences in the genome varies dramatically and they are not highly conserved; and neural specific transcripts are expressed in cultured lines of nonneural cells. Further work is needed to establish whether ID elements will prove useful in regulating expression of genes transferred into the nervous system.

Neural regulatory elements can be used to effect selective expression of genes in the nervous system. First, short-term temporal regulation could be obtained with defined elements that mediate responses to second messages. Genetic elements mediating responses to cyclic AMP and glucocorticoids have been defined in 5' flanking components of some neural genes. Second, long term cell-type specific regulation

can be obtained using cell-type specific promoters from either viruses or cellular genes. For example, the human papovavirus JC, which causes progressive multifocal leukoencephalopathy, contains a 98 base pair repeat sequence that increases expression of JC genes in glial cells (Kenny et al., 1984). Similarly, sequences within the long terminal repeats (LTRs) of retroviruses can serve to enhance their expression in the CNS (DesGroseillers et al., 1985). Other "neurotrophic" viruses may also utilize such regulatory elements. Neural genes contain elements that limit and regulate their expression. Such regulatory elements usually lie within 1 kb 5' to the promoter sequence, but may occur further away in 5' sequences as well as in introns and 3' flanking sequences. As more neural genes are cloned and characterized, more genetic regulatory elements specific to neural cells will be available, e.g., some regulatory elements of the genes for glial fibrillary acidic protein (Lewis et al, 1987) and S100 (Kuwano et al., 1984) should be selective for astrocytes; myelin basic protein (Roach et al., 1987) for oligodendrocytes; neurofilaments for neurons, some neuropeptides (e.g., neuropeptide Y., Minth et al., 1984; Larhammer et al., 1987), tyrosine hydroxylase (Lewis et al., 1987), or the beta-adrenergic receptor (Dixon et al., 1986) for select neuronal populations (Table 2).

Progress to Date

DNA Mediated Gene Transfer

A number of methods have been developed to introduce (transfect) either genomic or cloned DNA sequences into cells. These include coprecipitation with calcium phosphate (McBride and Peterson, 1980; Klobutcher and Ruddle, 1981), treatment with DEAE dextran (Camper et al., 1985), microinjection, and electroporation (Potter et al., 1984). For transfection of cells in culture calcium phosphate precipitation and electroporation have proven to be the most effective. The efficiency of the calcium phosphate technique can be increased with glycerol (Parker and Stark, 1979) and chloroquine (Luthman and Magnusson, 1986).

DNA transfection can be used either in a transient assay system for expression of genes within several days, or for isolation of transformants that have stably incorporated the transferred DNA into their genome. The transient method takes advantage of the fact that a relatively large portion of cells (1–50%) will take up and express the foreign DNA for a short period of time. With the long-term approach only a few cells (10^{-4}–10^{-6}) will undergo stable transformation. However, if a selectable marker is included it is possible to clone and propagate these transformants, most of which will also retain the transferred nonselectable gene of interest. Selectable markers include hypoxanthine-guanine phosphoribosyltransferase (HPRT; Graf et al., 1979); adenosine phosphoribosyltransferase (APRT; Wigler et al., 1979a), dihydrofolate reductase (DHFR; Wigler et al., 1979b; Subramani et al., 1981) and HSV-1 thymidine kinase (TK; Wigler et al., 1977), all of which require introduction into mutant cells lacking the corresponding enzyme. Dominant selectable markers, in contrast, can be used with most wild-type cells; these include the bacterial enzyme xanthine-guanine phosphoribosyltransferase (XGPRT; Mulligan and Berg, 1981) and those conferring resistance to neomycin (neo; Colbere-Grapin et al., 1981; Southern and Berg, 1982) and hygromycin (Bernard et al., 1985). Use of a methotrexate-resistant allele of the DHFR gene can be used to amplify it as well as adjoining DNA sequences by progressively increasing methotrexate levels in the medium (Choo et al., 1986).

Given the low efficiency of DNA-mediated gene transfer and difficulties of microinjection into somatic cells, it seems unlikely that these methods will prove useful for gene transfer in vivo. There is a report (Dubensky et al., 1984),

TABLE 2

Some Cloned Neural Proteins (References indicated in parentheses)[a]

Actin *(1)*	Nerve growth factor receptor *(15)*
Beta-adrenergic receptor *(2)*	Neurofilaments *(11)*
Calcitonin gene-related peptide *(1)*	Neuroleukin *(12)*
Calmodulin*(1)*	Neuron-specific enolase *(13)*
Cholecystokinin *(1)*	Neuropeptide Y *(14)*
Cholinesterase *(3)*	Nicotinic acetylcholine receptor *(1)*
Corticortropin-releasing factor *(1)*	alpha-subunit
Dynorphin *(1)*	beta-subunit
Enkephalin *(1)*	gamma-subunit
Epidermal growth factor *(1)*	delta-subunit
Epidermal growth factor receptor *(1)*	Potassium A-channel *(22)*
GAP-43 *(4)*	Phenylethanolamine-*N*-methyltransferase *(1)*
Glial fibrillary acidic protein *(20)*	Prolactin *(1)*
GABA receptor *(5)*	Pro-opiomelanocortin *(1)*
Glutamic acid decarboxylase *(6)*	Proteolipoprotein *(16)*
Glycine receptor *(7)*	S100 *(9)*
Growth hormone-releasing factor *(1)*	Sodium channel *(23)*
Monoamine oxidase A *(8)*	Somatostatin *(1)*
Muscarinic acetylcholine receptor *(18)*	Substances P and K *(1)*
Myelin associated glycoprotein (MAG) *(21)*	Tubulin *(1)*
Myelin basic protein *(1)*	alpha-subunit
N-CAM *(10)*	beta-subunit
Nerve growth factor *(1)*	Tyrosine hydroxylase *(1)*
beta-subunit	Vasoactive intestinal peptide *(17)*
gamma-subunit	Vasopressin, arginine *(1)*

[a]To save space, references published previously can be found in (1) (Rosenberg et al., 1985). Other representative original references are: (2) Strader et al., 1987; Dixon et al., 1986; (3) Prody et al., 1987; (4) Basi et al., 1987; (5) Schofield et al., 1987; (6) Kaufman et al., 1986; (7) Grenningloh et al., 1987; (8) Hsu et al., in press (9) Kuwano et al., 1984; (10) Hemperley et al., 1986 (11) Robinson et al., 1986; Lewis and Cowan, 1985; Julien et al., 1987; (12) Gurney et al., 1986; (13) Forss-Petter et al., 1986; (14) Minth et al., 1984; Larhammar et al., 1987; (15) Chao et al., 1986; Radeke et al., 1987; (16) Milner et al., 1985; (17) Tsukadas et al., 1985; (18) Kubo et al., 1986; (19) Kuwano et al., 1984; (20) Lewis et al., 1984; and (21) Bloom et al., 1985; (22) Papazian et al., 1987; and (23) Noda et al., 1984.

however, of successful calcium-phosphate-mediated gene transfer into mouse spleen and liver using polyoma virus DNA and a polyoma-plasmid DNA construct. Both resulted in active viral infections.

DNA-mediated gene transfer has been used to clone genes, study processing and release of peptide hormones, and analyze genetic regulatory elements. For cloning purposes, genomic DNA or a cDNA library, prepared from RNA isolated from cells that express the mRNA, is introduced into a continuous cell line, which does not normally express the gene product of interest. Cells that express the gene are isolated either by genetic selection or by screening for the desired gene product using a wide range of methods including biochemical and electrophysiological assays, ligand binding, and antibody recognition. The cells containing the gene are propagated and the gene is isolated and identified. For example, the human nerve growth factor receptor gene was isolated by transforming mouse fibroblast L cells with human genomic DNA and screening for ex-

TABLE 3

Homology in 5' Flanking Regions of Genes Containing the Somatostatin Palindrome

Genes	Sequence	Ref(s).
Somatostatin	CTGGGGGCGCCTCCTTGGCTGACGTCAGAGAGAGAG (–32)	(13,21,22)
PEPCK*[a]	TGATCCAAAGGCCGGCCCCTTACTTCAGAGGCGAGC (–74)	(23)
VIP*[a]	TCCCATGGCCGTCATACTGTGACGTCTTTCAGAGCA (–60)	(24)
Parathyroid hormone	GGGAGTGACGTCATCT (65)	(25)
Proenkephalin	GGGCCTGCGTCAGC (–87)	(26)
Gamma-chorionic gonadotropin	AAAATTGACGTCATGG (–113)	(27)
C-fos*[a]	CCGCCCAGTGACGTAGGA (–57)	(28)
Cytomegalovirus enhancer	CCACCCCATTGACGTCAATGGAGTT (–124)	(29)
BLV LTR	ACCAGACAGAGACGTCAGCTGCCAGA (–144)	(30)
HTLV-11 LTR	CCACGGCCCTGACGTCCCTCCCCCCC (–162)	(31)
Intracisternal A particle	CCICICCCGTGACGTCATCTGGGG (–86)	(32)

[a] Asterisks indicate genes that are known to be transcriptionally regulated by cAMP. Boldface sequences indicate homology with the palindrome. The positon of the 3'-most nucleotide is listed in parentheses at the end of each sequence. PEPCK, phosphoenolpyruvate carboxykinase; BLV, bovine leukemia virus; LTR, long terminal repeat; HTLV-11, human T-cell leukemia virus type II.

pression of the receptor with a monoclonal antibody (Chao et al., 1986; Radeke et al., 1987). Following isolation and cloning of DNA from transformed cells, human genomic elements were identified by repetitive sequences unique to the transfected DNA (Gusella et al., 1980).

The processing and release of peptide hormones has been studied in a number of different cells using gene transfer techniques. The first experiments demonstrated that some peptide hormones could be synthesized and secreted in an active form by cells that do not normally produce them. Examples include expression of rat growth hormone by mouse fibroblast 3T3 cells (Doehmer et al., 1982), fish somatostatin by monkey kidney COS cells (Warren and Shields, 1984), human growth hormone by rat pheochromocytoma PC12 cells (Schweitzer and Kelly, 1985), human enkephalin by mouse pituitary line AtT-20 (Comb et al., 1985), and a hybrid protein containing sequences for a constitutively secreted viral protein and human growth hormone by AtT-20 cells (Moore and Kelly, 1986). Some of the interesting findings elucidated by such studies include: (1) Alternate splicing of the same RNA transcript can occur in different cell types (Schweitzer and Kelly, 1986). (2) Peptide leader sequences can determine whether a peptide is secreted through constitutive or regulated routes (Kelly, 1985). For example, the secretion of human growth hormone follows primarily a regulated route in AtT-20 cells, which have both regulated and constitutive pathways. However, when sequences encoding the *N*-terminal of the protein are removed and replaced with *N*-terminal sequences from a protein that follows the constitutive route, the hybrid protein takes the constitutive route (Moore and Kelly, 1986). (3) Specific features of a protein determine its mode of post-translational processing. Some precursor proteins, for example proenkephalin, preprosomatostatin, and preproNGF, from which the biologically active peptide can be released by proteolytic cleavage at basic amino acid residues, can be successfully processed by many different cell types. In contrast, other precursor proteins,

such as proinsulin and proforms of lysosomal enzymes, require more complex processing events, including glycosylation, and can only be produced in a limited number of cell types (Moore et al., 1983). (4) Discrete protein domains involved in certain cellular events. Transfection of beta-adrenergic receptor genes specifically altered by oligonucleotide-directed mutagenesis has been used to show that the same region of the protein is involved in coupling to a G protein and agonist-mediated sequestration (Strader et al., 1987).

Genetic regulatory elements of neural genes have also been studied using gene transfer techniques. For example, sequences 5' from the start site of transcription have been fused to reporter genes, such as bacterial chloramphenicol acetyltransferase (CAT) and neo, and expression monitored in a transient assay system 24–48 h later. Studies of neural genes have examined elements needed for tissue-specific expression and for cyclic-AMP mediated inducibility. Tissue-specific expression has been evaluated in genes for parathyroid hormone (PTH; Igarashi et al., 1986) and tyrosine hydroxylase (TH; Harrington et al., 1987), which bear regulatory elements within 700 bp of the promoter. Elements from the PTH gene confer expression of a fused neo gene onto rat pituitary, but not onto human carcinoma cells. Fusion of the TH promoter to the CAT gene results in CAT activity in rat pheochromocytoma and pituitary lines, but not in two neural lines or two fibroblast lines. Since TH is normally expressed in chromaffin cells and some neurons, and not in pituicytes or fibroblasts it appears that continuous cell lines can be somewhat deregulated for gene expression. Interestingly, tissue-specific expression of thy-1, a surface glycoprotein on neurons, T-lymphocytes, and fibroblasts, appears to lie in sequences, possibly introns, downstream from the promoter (Ingraham and Evans, 1986). Expression of transfected thy-1 sequences is 50-fold lower in L-cells, which although "fibroblastic," do not normally express this surface marker, as compared to lymphoid or neuronal lines (Evans et al., 1984).

A large number of genes demonstrate cyclic AMP inducibility presumably mediated by interaction of the DNA with cyclic AMP binding proteins or proteins phosphorylated in a cyclic AMP dependent manner. A DNA element responsible for cAMP inducibility has been localized by deletion analysis to an approximately 35 bp region that resides 5' to the promoter for the TH (Lewis et al., 1987), somatostatin (Montminy et al., 1986), proenkephalin (Comb et al., 1985), and vasoactive intestinal peptide (Tsukada et al., 1987) genes. These regions contain a common core consensus sequence shared with other cylic AMP inducible genes (Table 3). Some neural genes inducible by glucocorticoids, e.g., tyrosine hydroxylase (Lewis et al., 1987), pro-opiomelanocortin (Birnberg et al., 1983), and monoamine oxidase A (Edelstein and Breakefield, 1986), will probably also contain enhancer elements that respond to glucocorticoids (Yamamoto, 1985).

Transgenic Mice

Mouse strains with new genes can be created by microinjection of cloned DNA sequences into the pronuclei of fertilized eggs (Fig. 1; for review, *see* Palmiter and Brinster, 1985). These eggs are reintroduced into pseudopregnant females and allowed to develop. Resulting transgenic mice will usually contain the introduced gene in all the cells of the body including the germ line. By breeding these transgenic mice, pedigrees are produced that can be homozygous or heterozygous for the new gene. The new genes are usually integrated at a single, random site in the genome as multiple copies in a tandem array. This is a moderately efficient process, with about 25% of surviving injected embryos successfully incorporating and expressing the new gene.

Transgenic mice have been used to perform several different kinds of experiments, including insertional mutagenesis of endogenous genes; determination of DNA sequences important for cell specific gene expression; alteration of cellular phenotypes, such as correction of gene defects and insertion of cell markers; exploration of the effects of oncogenes; and selective destruction of specific cells. Examples will be given of some general information gained from transgenic mice, with special focus on the few studies involving genes expressed in the cells of the nervous system.

Since introduced genes integrate into the genome at random sites about 10–20% of the time integration occurs in an endogenous gene, thus disrupting its function. This insertional mutagenesis must be kept in mind in analyzing the phenotype of transgenic mice. The phenomenon can be used to generate new mutant mouse strains, with the advantage that the defective gene can be isolated later and characterized by virtue of its proximity to inserted sequences. Insertional mutagenesis may prove useful in generating new neurologic mutants in previously unidentified genes.

To create a mutant animal lacking expression of a particular gene, it is possible to produce antisense sequences for the RNA encoded by this gene. This involves insertion of gene sequences inverted relative to their promoter. If these sequences are placed under the control of a strong promoter and the antisense RNA will outnumber the endogenous mRNA, hybridize to it and block its processing and/or translation. This approach has been undertaken for the HPRT gene in order to create an animal model of the Lesch-Nyhan syndrome (Stout and Caskey, in press).

Transgenic mice can also be used as an assay system to study gene expression by exchanging, deleting, or altering regulatory sequences in and around genes. For example, cell-type specific expression has been obtained for the elastase gene in pancreatic cells and the regulatory elements have been localized to a 213 bp sequence immediately 5' to the promoter (Ornitz et al., 1985). In contrast, some regulatory elements for beta-globin genes appear to lie within introns or 3' flanking sequences (Townes et al., 1985). Tissue-specific regulatory sequences can also be analyzed visually by placing the *E. coli lacZ* gene under the control of a eukaryotic promoter. Tissues are then stained histochemically for beta-galactosidase. Thus, the regulatory elements for the gamma 2-crystallin gene confer expression of this enzyme exclusively onto central nuclear fiber cells of the lens (Goring et al., 1987).

As yet there has been little analysis of regulatory elements for neural genes using transgenic mice. Transgenic animals containing exogenous genes for thy 1.1. (Kollias et al., 1987) and myelin basic protein (Readhead et al., 1987) do show developmentally regulated expression in the nervous system. Introduction of the calcitonin/calcitonin gene-related peptide (CGRP) gene under the control of the metallothionein (MT) promoter has demonstrated that the RNA splicing pathway that generates CGRP is neuron-specific (Leff et al., 1987). Three studies have identified DNA sequences serendipitously capable of neuronal specific regulation. First, when a human cDNA for HPRT was introduced under the control of the MT promoter, exogenous gene expression was high in brain and low in liver (Stout et al., 1985), in contrast to expression of the endogenous MT gene, which is normally high in liver and low in brain. Two possible explanations have been considered: that features of the construct itself act as neural-specific enhancers of transcription, or that factors in neural cells act to stabilize the HPRT mRNA. Second, transgenic mice were constructed that contained the MT promoter linked to the human growth hormone gene (Swanson et al., 1985). A high level of expression of growth hormone, normally restricted to pituitary cells, was observed both in hepatocytes and select sub-

populations of neurons in several brain regions, including the hypothalamus, neocortex, and hippocampus. The same novel pattern of expression was observed in several transgenic mouse pedigrees containing this construct, indicating that it was independent of the site of chromosomal insertion. One possible explanation is that sequences in the fusion gene interact with a transacting regulatory factor shared by these neurons. Third, transgenic mice were constructed harboring a fusion gene consisting of the SV40 early region, including the small t and large T antigens, but lacking the enhancer element, and the human growth hormone gene under control of the MT promoter (Messing et al., 1985). These mice suffered from a demyelinating peripheral neuropathy, in addition to developing hepatocellular carcinomas and islet cell adenomas. The demyelination apparently resulted from expression of the transferred fusion gene in Schwann cells or adjacent neurons. Introduction of a similar fusion gene that retains the SV40 enhancer caused tumors in other cell types and no demyelination (*see below*).

Other uses of transgenic mice are the genetic engineering of new mice strains and correction of genetic defects. The classic example of the first is increasing the size of mice through introduction into the germline of a growth hormone gene under the control of an MT promoter resulting in elevated levels of growth hormone in animals (Palmiter et al., 1983). Transgenic mice have also been used as a model system for gene therapy through the correction of recessive mutations. Examples include the correction of selective antigen immune deficiency through transfer of the E alpha immune response gene (LeMeur et al., 1985); beta-thalassemia with the beta-globin gene (Constantini et al., 1986); and demyelination in the *shiverer* mouse with the myelin basic protein gene (Roach et al., 1987). In each case, the normal regulatory elements of the genes were used to confer tissue specific expression. The level of expression varied depending on the site of chromosomal integration and the amount of 5' and 3' flanking sequences used in the constructs.

Correction of the *shiverer* defect warrants further discussion, since it is a CNS disease. This is an autosomal recessive condition caused by deletion of a portion of the myelin basic protein gene. Affected animals make no myelin basic protein, show deficient myelination in the CNS, and suffer from tremors, convulsions, and an early death. The myelin basic protein gene, including 4 kb of 5' flanking sequence, introns, and 1 kb of 3' flanking sequence (total 37 kb), was introduced into embryos heterozygous for the *shiverer* mutation and the resulting animals were analyzed and bred (Roach et al., 1987). Resulting animals homozygous for both the *shiverer* mutation, and the new myelin basic protein gene had 25% normal levels of the mRNA for myelin basic protein. The new mRNA was expressed in appropriate cells in the proper developmental sequence. Myelination occurs in these animals to some extent, and they have only mild tremors, no convulsions, and a near normal lifespan. The subnormal levels of expression of the transferred gene may reflect its site of integration, and/or missing enhancer elements. There also appears to be a gene dosage effect; animals with one copy of the transferred gene have a lower level of expression than those with two copies (Popko et al., 1987).

Another use of transgenic mice is to target a particular cell type for tumor formation. This is accomplished by transferring an oncogene under the control of a tissue specific promoter. For example, fusion genes containing the *c-myc* gene and enhancer elements from the mouse mammary tumor virus lead to formation of mammary tumors in transgenic mice (Stewart et al., 1984). Transgenic mice containing the SV40 early region, which includes the enhancer and large T antigen coding sequences, frequently develop tumors of the choroid plexus in the central nervous system (Palmiter et al.,

1985). Transfer of this early SV40 region can also lead to altered cell migration and other tumor types in the CNS (Kelley and Herrup, 1987). In addition, HTLV-1 sequences cause, in transgenic mice, neurofibromas that are presumed to arise from benign tumors of Schwann cells (Hinrichs et al., 1987).

There are a number of oncogenes that have been found to be associated with tumor formation in the nervous system (for review, *see* Breakefield and Stern, 1986). These oncogenes may be useful in obtaining transgenic mice with particular types of CNS tumors and deriving transformed lines from specific cells of the CNS. Such oncogenes include the recessive *rb* gene on chromosome 13q, which can lead to retinoblastoma formation when the normal allele is lost through somatic mutation (Friend et al., 1986); the *N-myc* gene which is amplified in most malignant neuroblastomas (Schwab et al., 1984); the V-*erb* gene (related to the EGF receptor gene), which is altered and/or amplified in some glioblastomas (Libermann et al., 1985); the *neu* gene (related to the PDGF receptor), which is found in mutagen-induced neuroglioblastomas in rats (Schechter et al., 1984) and its human counterpart erbB-2/HER2, which is amplified in some mammary tumors (Kraus et al., 1987: Slamon et al., 1987); the *N-ras* gene, which is altered in some neuroblastomas (Taparowsky et al., 1983); and *gli*, which is expressed in some glioblastomas (Kinzler et al., 1987).

Selective cell ablation has been achieved in transgenic mice using a cell-specific promoter. Ligation of the diptheria toxin A chain gene to an elastase I gene enhancer/promoter resulted in mice either lacking a pancreas or having only a rudiment of it (Palmiter et al., 1987). Transgenic mice provide an opportunity to study the tissue-specific and developmental regulation as well as the critical coding domains in oncogenes and their normal counterparts.

Retroviral Vectors

One of the most promising areas of gene transfer into neural cells involves use of retrovirus vectors (for review, *see* Gilboa et al., 1986; Weiss et al., 1985; Mulligan, 1982). Retrovirus vectors derived from modification of Moloney murine leukemia virus (MoMuLV) can deliver genes into cells in culture and in vivo without themselves being able to replicate (Mann et al., 1983). The first part of this section will provide an overview of retroviral mediated gene transfer, the second part will address transfer into neural cells.

The organization of retroviral sequences and the events during viral replication have been well characterized. The viral RNA sequences are converted to double stranded DNA within the cell through the action of reverse transcriptase. Following circularization via the long terminal repeats (LTRs, Fig. 3), the DNA enters the cell nucleus and integrates in the host genome at apparently random sites. Integrated sequences are transcribed to yield mRNAs using the promoter and enhancer in the 5' LTR and the polyadenylation site in the 3' LTR. The translational products of the unspliced mRNA are *gag*, a structural component of the virus particle, and *pol*, the reverse transcriptase. Excison of an intron generates a shorter mRNA, which is translated to yield *env*, the virus coat protein. *Env* is embedded in the lipid bilayer of the virus envelope and determines the host range of the virus particle.

Retroviral vectors contain retroviral LTR and packaging (psi) sequences, as well as plasmid sequences for replication in bacteria, and may include the SV40 early promoter and enhancer for potential replication in eukaryotic cells. Much of the rest of the viral genome is removed and replaced with other promoters and genes (*see below*). Vectors are packaged as RNA in virus particles following transfection of DNA constructs into packaging cell lines (Fig

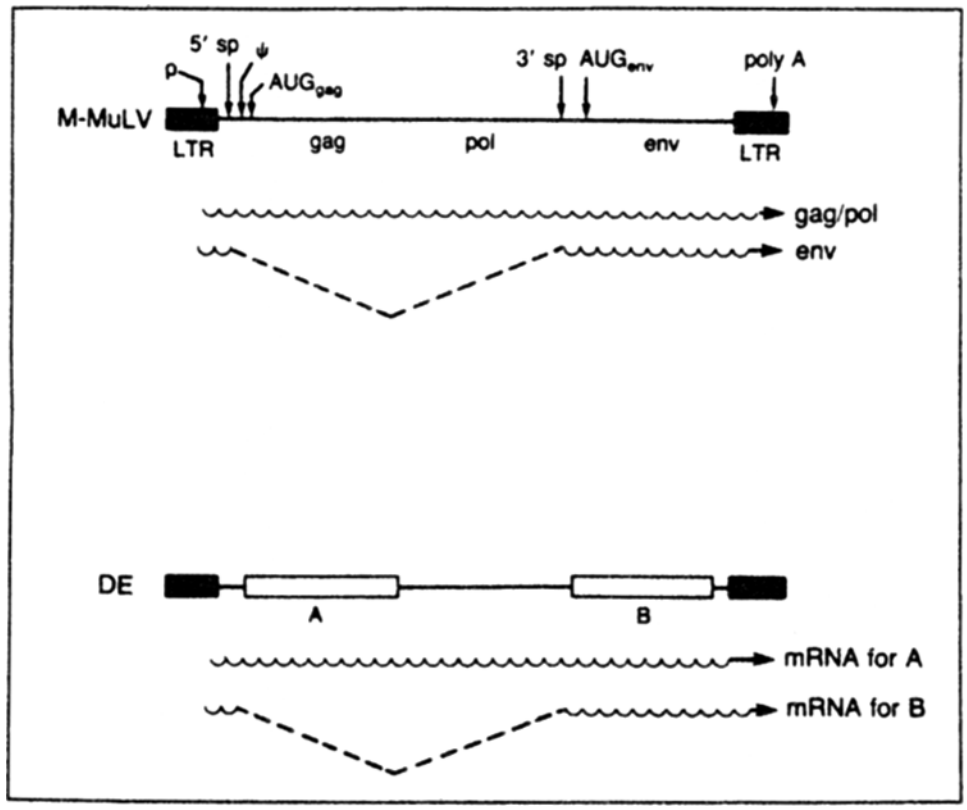

Fig. 3. Retroviral sequences from wild-type M-MuLV and a vector derived from it. (A) M-MuLV (MoMuLV) generates two types of transcripts (wavy lines): unspliced (gap/pol) and spliced (env) RNA, both under regulation of the LTR (black boxes). p = promoter; sp = RNA splice sites; psi (ψ) = RNA packaging signal; AUG = start sites of protein translation; poly A = poly A$^+$ addition site. (B) In a vector (DE) the *gap/pol* and *env* genes are replaced with exogenous genes A and B. Then, the normal splicing mechanisms are used to generate mRNAs for Genes A and B, with expression of both regulated by the 5' LTR. Reprinted with permission from Gilboa et al., 1986.

4). These include psi two (Mann et al., 1983), which produces viral particles that can infect rodent cells, and psi am (Cone and Mulligan, 1984) and PA 12 (Miller et al., 1985), which produce particles that can infect a broad range of species.

The advantages of retroviral vectors are that: (1) Gene transfer is highly efficient, with up to 100% of the cells in culture being infected. In contrast, using the calcium-phosphate-DNA uptake technique only 0.01–0.001% of the cells are transfected; (2) Vectors are available that contain selectable genes and restriction sites for insertion of other genes; (3) The host cell range is very large; and (4) Depending on the types of genes inserted, and the host range, these vectors present little or no biological hazard (*see also* Miller et al., 1983). The disadvantages to date (some of which may be surmountable), are that: (1) Viral sequences only integrate into mitotic cells (i.e., not mature neurons); (2) The titers of virus stocks of some of the vectors are low; (3) Levels of expression of incorporated genes can vary depending on the nature of the construct and the site of insertion into the genome; and (4) There has been some difficulty

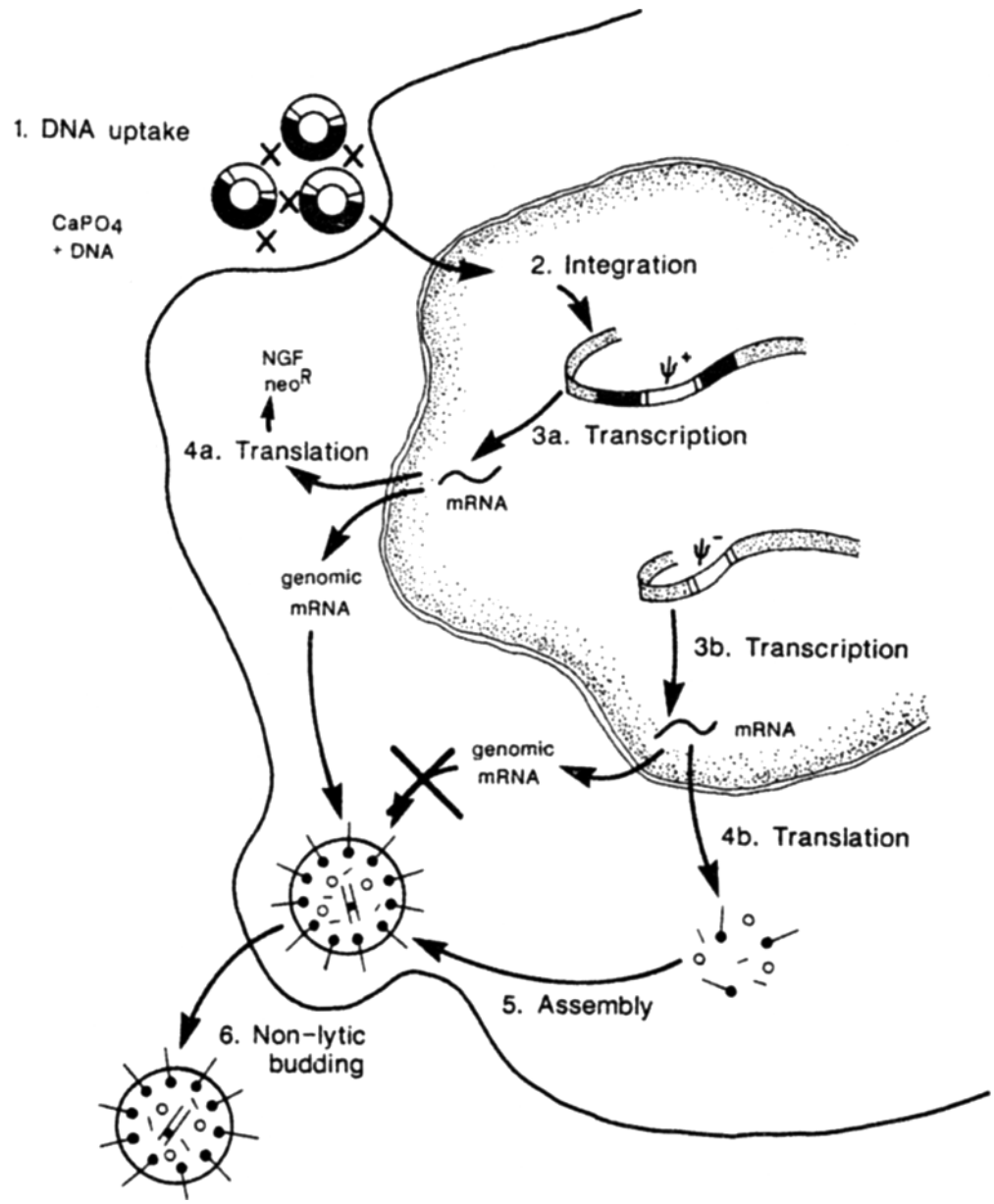

Fig. 4. Packaging of replication-defective retroviral vectors. The packaging cell line contains wild type retroviral sequences mutated at the psi (ψ) packaging signal. These sequences code for viral proteins that make up the viral particles but cannot themselves be packaged. DNA plasmid constructs corresponding to retroviral vectors containing two genes of interest, here genes for beta-NGF (NGF) and neomycin resistance (neo), are transfected into the packaging cell line. Successful tranfectants are cloned under neomycin selection and express an RNA transcript encoding NGF and neo that can be packaged because of an intact psi signal. Replication-defective particles are harvested from the medium and used to infect other cells. Modified from Cepko and Turner, unpublished figure.

in obtaining expression following gene transfer into preimplantation embryos, perhaps because of methylation of viral LTR sequences. This latter limitation may be overcome by using promoters and enhancers normally associated with the gene of interest instead of the retroviral LTRs (*see below*).

Retroviral vectors have been used successfully in three types of experiments: (1) Incorporation of exogenous genes into cultured cells; (2) Introduction of genes into preimplantation and midgestation embryos; and (3) Transfer of genes into postnatal and adult cells. The virally encoded mRNA can be distinguished from endogenous mRNA on the basis of size and heterologous sequences such as virus sequences, and the gene products can be distinguished biochemically or immunologically. The level of expression of the protein depends on many factors, including the rates of transcription and translation of the mRNA and the stability of the message and protein, as well as whether or not the protein has toxic effects on the recipient cell.

Most retrovirus vectors currently in use have two genes inserted in them—one is a dominant selectable marker and the other is the gene of interest. The selectable marker is used to isolate transfectants of the packaging line and other cells in culture, and to titer the replication defective virus particles. This gene is placed under the control of a strong promoter such as a retrovirus LTR, SV40 early, or HSV-1 thymidine kinase promoter. Some of the selectable markers that have been used include HPRT (Miller et al., 1983), methotrexate-resistant dihydrofolate reductase (Miller et al., 1985), and neomycin resistance (Ledley et al., 1986; Hock and Miller, 1986). Expression of the transferred genes of interest has been observed following infection of continuous cell lines or primary cultures. Genes associated with differentiated phenotypes that have been transferred using retroviruses include phenylalanine hydroxylase into mouse 3T3 cells and hepatoma cells (Ledley et al., 1986), adenosine deaminase into murine lymphoid cell lines and human lymphoctye lines (Williams et al., 1986), preproparathyroid hormone into rat pituitary line GH4 (Hellerman et al., 1984), the *src* oncogene into PC12 cells (Alema et al., 1985), and nerve growth factor into mouse pituitary line AtT20 (*see below*). "Replacement therapy" for adenosine deaminase deficiency has also been achieved in cultured skin fibroblasts (Palmer et al., 1987) and lymphocytes (Kantoff et al., 1986) from patients; for HPRT in fibroblasts (Miller et al., 1983) and lymphoblasts (Willis et al., 1924) from Lesch-Nyhan patients; and for glucocerebrosidase in Gaucher fibroblasts (Sorge et al., 1987).

Retroviral gene transfer into pre-implantation embryos has provided useful information. First, cell lineages have been elucidated by introducing unique DNA sequences, such as bacterial genes, and analyzing the cellular distribution of these sequences in genomic DNA later in development. Using this approach the time of separation of somatic and germ line lineages was elucidated (Soriano and Jaenisch, 1986). Second, since retroviruses integrate at many sites in the genome, they can cause insertional mutagenesis. One such recessive embryonic lethal mutation was determined to lie in the alpha 1(1) collagen gene (Schnieke et al., 1983). This approach has also been used to create a mouse strain lacking HPRT activity as a model for the Lesch-Nyhan syndrome. In this case, embryonal carcinoma cells were infected with replication defective retroviruses and selected for HPRT deficiency prior to use in creating chimeric mice (Kuehn et al., 1987). Surprisingly, HPRT-deficient male progeny showed no apparent neurologic dysfunction. Third, retrovirus vectors have been used to study tissue specific expression of developmentally regulated genes. Here too, the site of integration of the vector into the genome is important, since sequences adjacent to the vector influence the expression of genes within it. Jaenisch

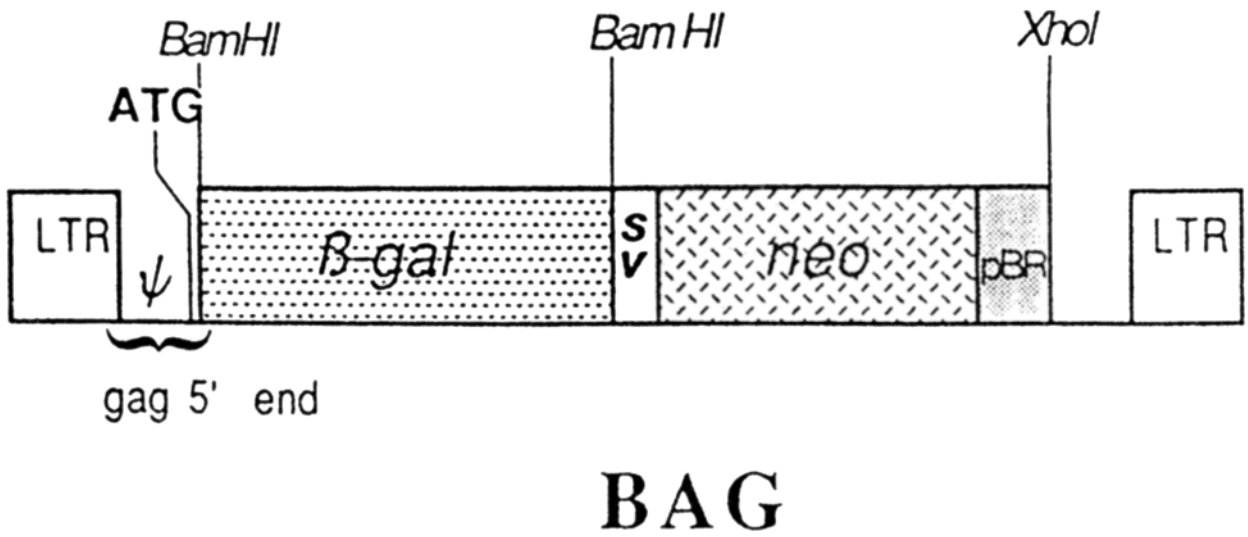

Fig. 5. Retroviral vector "BAG" encoding *lacZ* and *neo* genes. The bacterial gene for beta-galactosidase was inserted in the unique Bam HI site of the pLJ vector downstream from the ATG translational start site of the retroviral gene *gag*. Neo sequences are under an SV40 early promoter, which also contains an origin of replication allowing replication in cos cells. The pBR origin of replication is also present for replication in bacteria. RNA splice sites are absent. Reprinted with permission from Price et al., 1987.

and coworkers (Soriano et al., 1986) infected 4–16 cell embryos with a retrovirus vector containing the entire human beta globin gene plus almost 1 kb each of 5' and 3' flanking sequences. Relatively selective, tissue-specific expression of beta globin in blood, bone marrow, and spleen of mice was observed. Retroviral infection of early embryos is technically simpler than microinjection of DNA into fertilized eggs and should facilitate studies of transgenic mice.

In model systems for gene therapy, retrovirus vectors have also been used to infect bone marrow cells of mice. A small proportion of these cells, about 0.01%, are hematopoietic stem cells that can give rise to granulocytes, lymphocytes, erythrocytes, macrophages, and platelets throughout the life of the individual. Retrovirus infection of bone marrow cells in tissue culture is very efficient and expression of transferred genes continues in cells reintroduced into mice. Successful long-term gene transfer has been reported using HPRT (Miller et al., 1984) and neo-resistance genes (Miller et al., 1984; Keller et al., 1985; Dick et al., 1985). For successful gene therapy of postnatal animals with retrovirus vectors, at the present state of the art, the disease phenotype must be ameliorated by gene transfer into a small population of dividing cells, ideally ones that constitute a stem cell population, and the cells must be able to tolerate a wide range of gene expression.

Studies have begun using retrovirus vectors to introduce genes into neural cells in culture and in vivo in order to study cell lineages and the processing and function of neural proteins. A histological marker gene is defined as one that encodes a protein that labels a cell and its descendants in a stable, distinctive, and innocuous manner. Two such genes used to date are the bacterial *lacZ* gene encoding the enzyme, beta-galactosidase, and the human gene encoding the cell surface glycoprotein, T8. The *lacZ* gene has been coupled to both the SV40 early promoter (Sanes et al., 1987) and the retroviral LTR (Price et al., 1987; Fig. 5). Either construct readily confers expression of beta-ga-

lactosidase onto a number of different continuous cell lines in culture, including neuroblastoma, retinoblastoma, pheochromocytoma, glioma, and fibroblast lines. This enzyme is readily detected by histochemical staining using the substrate X-gal, which is hydrolyzed to form an insoluble blue precipitate. Although mammalian cells contain lysosomal (acidic) beta-galactosidase, this endogenous enzyme is not active at the neutral pH used for staining. Cepko and coworkers (Price et al., 1987) also infected primary cultures of E15 rat cerebral cortex at low multiplicities of infection and observed isolated clusters of stained cells of several morphologies, suggestive of astrocytes, fibroblasts, and neurons. Presumably different cell types represent, at least in some cases, the descendants of a pluripotent precursor cell. If neurons express this transferred gene they must have integrated it as neuroblasts prior to their last division and terminal differentiation.

Retroviral constructs containing the *lacZ* gene have also been used to label cells in vivo. Sanes et al. (1986) injected midgestation (E9-11) mouse embryos through the uterine wall with such a vector and analyzed expression of beta-galactosidase 2–6 d later. About 60% of injected embryos survived and 25% of these showed X-gal staining in cell clusters in the subdermal fascia, skull, meninges, choroid plexus, and cerebral cortex. This work suggests that a number of different cell types in vivo can express detectable levels of this bacterial enzyme. Retroviral vectors containing *lacZ* have also been injected into the retina of newborn rats (Price et al., 1987; Turner and Cepko, 1987). Staining a few days to months later revealed clusters of intensely labeled photoreceptor cells, as well as some bipolar cells, Mueller glia, and pigment epithelia. All these cells are thought to be generated from a zone that is mitotic at the time of injection.

Preliminary results using another reporter gene, *T8*, also appear successful in labeling neural cells (Calof and Jessell, 1986). This cell surface glycoprotein can be detected immunocytochemically using a monoclonal antibody, and is normally expressed only by T lymphocytes. Successful labeling of neurons and other cell types was observed in dissociated cultures of mouse newborn cerebellum and E13 dorsal root ganglia. In addition, injection of virus into the amniotic sac of mouse embryos before closure of the neural tube resulted in labeling of cells in the skin and nervous system. The full potential for analysis of cell lineage using retrovirus-introduced marker genes into early embryos has yet to be evaluated. However, in conjunction with other embryologic techniques, including ^{3}H-thymidine labeling, transplantation, and immunocytochemistry, this promises to be a powerful approach.

Work has also begun on inserting genes encoding neural proteins into cells in culture. Nerve growth factor (beta-NGF) has long been known to be necessary for survival and differentiation of sensory and sympathetic neurons (Greene and Shooter, 1980; Thoenen and Edgar, 1985) and has recently been implicated in peripheral nerve regeneration (Taniuchi et al., 1986)., as well as in survival and phenotypic expression in CNS neurons; (Gnahn et al., 1983; Williams et al., 1986b). A retrovirus vector has been constructed that contains sequences encoding the shorter form of the pro-beta-NGF messenger RNA (Edwards et al., 1986) under control of the LTR, as well as neo-resistance sequences under the SV40 early promoter (Wolf et al., submitted). This vector is able to confer expression and release of biologically active beta-NGF onto mouse pituitary AtT20 cells in culture. Secretion appears to occur through both constitutive and regulated pathways (Fig. 6; Kelly, 1985). It is likely that in the near future a large number of other biologically active proteins under the control of different promoters will be incorporated into retroviral vectors in order to study their effects on development, homeostasis, and aging in the nervous system.

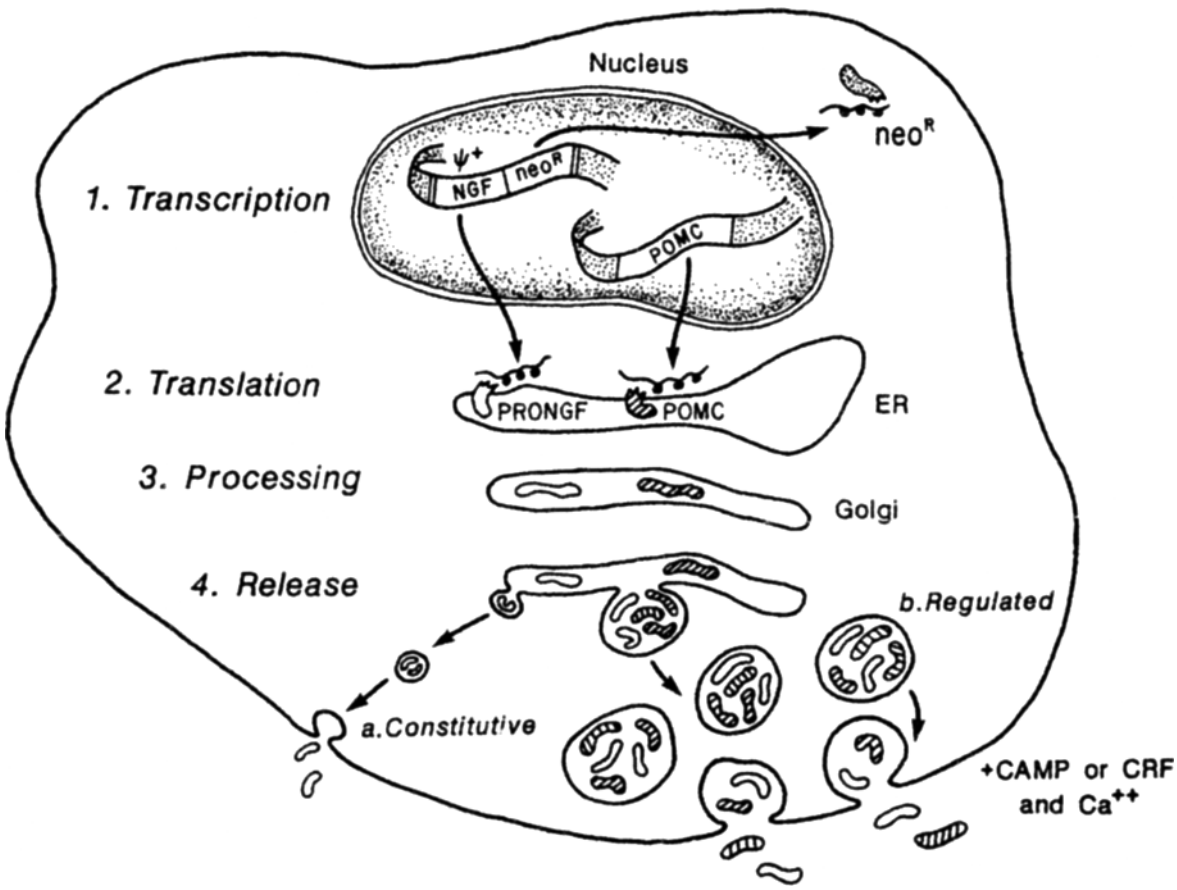

Fig. 6. AtT-20 cells infected with a retroviral vector containing sequences for NGF and *neo*. AtT-20 cells were infected with a replication defective retroviral vector encoding the precursor for beta-NGF and neomycin resistance. Cells were cloned under neo selection. These cells normally produce and process pro-opiomelanocortin to beta-endorphin and ACTH, which are released predominantly through the regulated pathway (Kelly, 1985). Release is stimulated by corticotropin releasing factor (CRF) and cyclic AMP (cAMP) and is calcium dependent. These cells can process the beta-NGF precursor to biologically active beta-NGF and release it through both the constitutive and regulated routes (Wolf et al., manuscript submitted).

Other Viral Vectors

Many virus vectors have already been developed for gene transfer and it is likely that more will be developed in the future (Table 4). In choosing the best vector system for a particular experiment, there are four criteria that must be considered: First, the vector can be delivered only into cells that are either abortively or productively infected by the virus. Second, if the virus is capable of persisting in a cell for an extended period of time without causing cell death, then the possibility exists that the vector can be stably maintained and expressed in the cell. Third, it is possible, in theory, to obtain proper function of cellular promoters such as neuronal cell type specific promoters only if at some stage during the life cycle of the virus its genome exists as double stranded DNA in the nucleus of a cell. Fourth, the size of the virus genome determines the upper limit on the size of the vector and genes that can be inserted in

TABLE 4

Possible Viral Vectors for Gene Transfer into the Nervous System

Virus	Virion nucleic acid	Approx. size, kb	Site of replication	Infects neurons	Integration in genome
Retrovirus (MoMuLV)	RNA	10	Nucleus	–	+
Herpes simplex	DNA	150	Nucleus	+	–Lytic ? +Latent
Papovavirus (JC, SV40 + polyoma)	DNA	5	Nucleus	–	–Lytic +Transform.
Adenovirus	DNA	35	Nucleus	–	–Lytic +Transform.
EBV	DNA	160	Nucleus	–	–Lytic –Transform.
Papilloma	DNA	8	Nucleus	–	–Transform.
Vaccinia	DNA	250	Cytoplasm	–	–Lytic
Poliovirus	RNA	10	Cytoplasm	+	–Lytic

it. Animal viruses have been sorted into different classes dependent on the strategy they use to replicate their nucleic acid. Some of these classes are more useful and easily adaptable to virus vectors than others. A more theoretical discussion of the different strategies animal viruses have adopted to replicate their nucleic acid has been presented elsewhere (Baltimore, 1971).

Perhaps the virus that holds the greatest potential for gene transfer into neurons is Herpes Simplex Virus (HSV-1). Here we discuss the properties of HSV-1 in relation to the criteria outlined above for evaluating a virus vector system: First, a virus vector can only transfer genes into cells normally infected by the virus. The host range of HSV-1 is broad; HSV-1 infects most mammals and birds including chickens, rats, mice, monkeys, and humans (for a general review of HSV-1, *see* Spear and Roizman, 1981). HSV-1 can lytically infect a wide variety of cells including neurons, fibroblasts, and macrophages. Neurons can harbor HSV-1 in the latent state, and HSV-1 is not usually maintained for more than several days in non-neuronal cells. Among the immortal neuronal cell lines that can be infected by HSV-1 include the mouse neuroblastoma cell lines NS20Y and N1E-115, and rat pheochromocytoma cell line PC12. HSV-1 virions have been detected in neurons in vivo by electron microscopy (Cook et al., 1974). Furthermore, two lines of evidence suggest that HSV-1 can infect most if not all kinds of neurons in the central nervous system. Following innoculation of HSV-1 in the periphery a burst of virus production ascends the neuroaxis, initially in the sensory or motor neurons innervating the site of innoculation, then in the spinal cord, brainstem, cerebellum, and cerebral cortex (Koprowski, 1978). Attempts to mimic HSV-1 latency in tissue culture with different preparations of neurons have required high temperature, DNA synthesis inhibitors, and antisera directed against HSV-1 virions to prevent a lytic infection from spreading to all the neurons (Wigdahl et al., 1984).

Second, if the virus genome exists as double stranded nucleic acid in the nucleus of the cell then it may be possible to obtain proper regu-

lated expression of a cellular promoter. The genome of HSV-1 is double stranded DNA which is replicated and transcribed in the nucleus of a cell. Two other properties of HSV-1 argue well for the possibility of obtaining proper regulated expression of a neuronal specific promoter. First, HSV-1 genes are transcribed by the cellular RNA polymerase II. Second, HSV-1 gene expression is itself complex. HSV-1 contains approximately 100 genes. Five immediate early genes encode the major regulatory proteins of the virus. The immediate early genes induce expression of the early genes that are responsible for DNA replication. The late genes are induced after DNA replication and encode the structural components and enzymes required for assembly of virus particles. When the late genes are induced, transcription of the immediate early genes is reduced. Thus, during the lytic cycle of HSV-1 expression of the three classes of genes follows in a complex sequential cascade. In contrast, in the latent state, expression is generally restricted to the immediate early genes. Thus, in both the lytic and latent states extensive gene regulation occurs and closely mimicks the regulation of cellular genes. This is an encouraging foundation for attempts to obtain proper regulated expression of cellular promoters in HSV-1 vectors.

Third, if a virus is capable of persisting in cells for long periods of time without causing cell death then it may be possible for a vector to be maintained stably in a cell. Post-mitotic neurons harbor HSV-1 in the latent state (for review, *see* Stevens, 1985). Once HSV-1 attains the latent state it can be retained for the life of the neuron. As mentioned above, latent HSV-1 is capable of expressing genes; expression of genes encoded by HSV-1 has been detected by immunohistochemistry in latently infected neurons. Furthermore, HSV-1 is transported both anterogradely and retrogradely in neurons. This is an attractive property of HSV-1, since it should allow HSV-1 vectors to reach cells of interest some distance away from the injection site. In summary, the latent state of HSV-1 should allow stable maintenance of an HSV-1 vector in a neuron and regulated gene expression of transcription units in the vector.

Fourth, the size of the vector is limited by the size of the virus genome. The 150 kb genome of HSV-1 is considerably larger than retroviruses, adenoviruses, or SV-40 and suggests that HSV-1 vectors could be designed to accommodate large molecules of DNA.

There has been some initial work on HSV-1 vectors (Spaete and Frenkel, 1982, 1985; Kwong and Frenkel, 1984; Stow and McMonagle, 1982; Shih et al., 1984). These vectors require an HSV-1 packaging site, an HSV-1 origin of DNA replication, and pBR sequences for growth in *E. coli*. Virus stock containing a vector is obtained by transfecting fibroblasts with a mixture of vector DNA and helper HSV-1 DNA. The vectors are maintained because of their growth advantage over the helper virus (Frenkel et al., 1980). HSV-1 contains three origins of replication (ori), one every 50 kb; the vectors contain 1 ori every 5–15 kb. In virions, vectors are composed of head to tail repeats 5–15 kb in size, up to 150 kb, the size of HSV-1. An HSV-1 vector has been constructed that contains the *lacZ* gene under the control of an HSV-1 immediate early promoter. Virus stocks of this vector efficiently express the lacZ gene in cultured fibroblasts and neurons, as well as in a number of continuous cell lines (Geller, Gusella, and Breakefield, in preparation).

Other viral vectors that might be considered for delivery of genes into the nervous system are discussed briefly here. Early virus vectors were based on the papova virus SV40 and polyoma, which replicate in the nucleus (Mulligan et al., 1979; Hamer and Leder, 1979). The genomes of these viruses are small, about 5 kb, double-stranded circular DNA. Whereas their own promoters give a high level of expression, expression with cellular promoters has generally been lower. Most papova viruses do not productively infect or persist in the cells of the

nervous system. However, JC virus infects human glial cells in vivo and can be grown on primary cultures of these cells. This virus appears to contain regulatory enhancer elements that are induced by transacting factors in glial cells (Kenney et al., 1984). The recent immortalization of a human glial cell line permissive for JC virus may allow further development of JC virus vectors for gene transfer (Mandl et al., 1987).

Adenovirus vectors are also well developed. The genome of adenovirus is a large, 35 kb, double-stranded linear DNA molecule that is replicated in the nucleus of the cell. It has been possible to obtain proper function of cellular promoters and stable transfection of cells with adenovirus vectors. For example, human fetal globin genes introduced by these vectors are properly regulated in human K562 cells and can be induced to express human fetal globins (Karlsson et al., 1986). Adenovirus host range is restricted to humans, however, and it does not productively infect or persist for extended periods of time in the cells of the nervous system.

Epstein-Barr virus (EBV) vectors have proven to be of great use for transfecting lymphocytes (Yates et al., 1985). EBV is a member of the herpes family of viruses but differs from HSV-1 in several important ways. The host range of EBV is limited to lymphocytes where it can persist in the nucleus of a cell without causing cell death. It exists in the nucleus as an extrachromosomal circular molecule of double stranded DNA at a copy number of several tens to hundreds per cell. EBV vectors are of great use to immunologists, but probably not to neuroscientists.

The only papilloma virus developed as a gene transfer vector to date is bovine papilloma virus type I (BPV) (DiMaio, 1984). It has a limited host range and will not infect cells in culture, but can be used for transformation of primary mouse and hamster fibroblasts in culture by DNA-mediated gene transfer. These vectors exist as several tens to hundreds of copies per cell of autonomously replicating circular DNA molecules in the cell nucleus. Shuttle vectors have been constructed that can replicate in bacteria, and that contain selectable markers. Genes incorporated into these vectors express at reproducible levels and respond to transacting, cellular regulatory factors. Although very useful in the study of regulatory sequences that are active in fibroblasts, this vector does not appear to offer any advantages for the study of neural genes or for gene transfer into neural cells.

Retroviruses have been extensively discussed in the previous section. Perhaps the primary drawback of retrovirus vectors for the neuroscientist is that retrovirus vectors require mitotic cells for integration, and neurons are post-mitotic. Some retroviruses, however, appear to be able to infect at least some post-mitotic cells in the nervous system. Human immunodeficiency virus (HIV) primarily infects capillary endothelial cells and macrophages in the CNS; in severe cases low level infection has also been seen in astrocytes and neurons (Wiley et al., 1986). Some retroviruses are neurotropic in that infection tends to localize in the CNS, these include type C retrovirus (cas-Br-E; Sharpe et al., 1987) and Visna (Narayan and Cork, 1985). Whether it will prove possible (and safe) to incorporate the unique host range of these viruses into vectors and to extend the host range to post-mitotic neurons remains to be seen.

Vaccina virus vectors (for review, *see* Mackett and Smith, 1986) have proven useful for expressing large amounts of a particular gene product. Vaccinia or pox viruses are large double stranded DNA viruses, approximately 200 kb in size. They replicate in the cytoplasm and cause a lytic infection in a wide variety of cell types. Vectors which place a gene (up to 25 kb) under the control of a vaccinia late promoter can be used to produce large amounts of a polypeptide of interest. Production of biologically

active met-enkephalin by AtT-20 cells (Thomas et al., 1986) and beta-NGF by a number of cell types in culture (Edwards and Rutter, in preparation) has been possible using this vector. However, the limitations of the vectors are first, they cause cell death rather than persisting for long periods of time; second, they replicate in the cytoplasm; third, only vaccinia promoters are effective; and fourth, they do not infect cells in the nervous system.

There are a number of "slow" viruses, e.g., scrapie, that persist for an extended period of time in the cells of the nervous system (reviewed by Meulen and Hall, 1978; Gajdusek, 1985). Vectors derived from these viruses have the potential for stable long term transfection of neurons. Such vectors have not yet been developed either because other properties of these viruses present difficulties for vector design or because not enough is known about the molecular biology of the virus to design them.

Finally, there are a number of viruses whose molecular biology is well understood and which can infect at least some cells in the nervous system in addition to nonneuronal cells. These viruses also have limitations as a neurotrophic vectors. Poliovirus can infect motor neurons. However, poliovirus is an RNA virus that replicates in the cytoplasm and can only produce a lytic infection. Theilers murine leukemia virus infects glia and some CNS neurons, principally those in the spinal cord and brainstem, such as motor neurons, but again causes a lytic infection (Chamarro et al., 1986). There are several segmented RNA viruses that can infect neurons under some circumstances. These include lymphocytic choriomeningitis virus and reovirus. Developing a virus vector system based on a segmented RNA virus may be a difficult undertaking, however.

Conclusion

Gene transfer into the cells of the nervous system has the potential to provide interesting information for many fields of the neurosciences, including neurochemistry, neurophysiology, developmental neurobiology, molecular neurobiology and neuropathology, and perhaps may even to contribute to the ongoing debate between psychologists and neurobiologists about the nature of learning and memory. Although the field is in its infancy, however, there are already several different approaches for delivering DNA into the cells of the nervous system. These include transfection of cells with DNA, microinjection of fertilized eggs to construct transgenic animals, and infection with viral vectors. Each of these systems has its advantages and drawbacks, and improvements in the technology are needed. Many different genes of interest to neurobiologists have been cloned, including those for enzymes that synthesize or degrade neurotransmitters, neuropeptides, neurotransmitter receptors, ion channels, and proteins that mediate second messenger effects. Functional characterization of these genes using gene transfer technology has already yielded information. At present far more experiments have been done in tissue culture systems than in vivo. In five years time it should be possible to write a comprehensive review, detailing the technological improvements to allow routine gene transfer in vivo, the cloning of many more genes of interest to neurobiologists, and functional studies of these genes on a molecular level that demonstrate unifying and diversifying principles of neuronal function.

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References

Alema S., Casalbore P., Agostini E., and Tato F. (1985) Differentiation of PC12 phaeochromocytoma cells induced by v-src oncogene. *Nature* **316,** 557–559.

Baltimore D. (1971) Expression of animal virus genomes. *Bacteriol. Rev.* **35,** 235–241.

Basi G. S., Jacobson R. D., Virag I., Schilling J., and Skene J. H. K. (1987) Primary structure and transcriptional regulation of GAP-43, a protein associated with nerve growth. *Cell* **49,** 785–791.

Birnberg N. C., Lissitzky J.-C., Hinman M., and Herbert E. (1983) Glucocorticoids regulate proopiomelanocortin gene expression in vivo at the levels of transcription and secretion. *Proc. Natl. Acad. Sci. USA* **80,** 6982–6986.

Bernard H. U., Krammer G., and Rowekamp W. G. (1985) Construction of a fusion gene that confers resistance against hygromycin B to mammalian cells in culture. *Exp. Cell Res.* **158,** 237–243.

Black I. B., Adler J. E., Dreyfus C. F., Friedman W. F., LaGamma E. F., and Roach A. H. (1987) Biochemistry of information storage in the nervous system. *Science* **236,** 1263–1268.

Bloom F. E., Battenberg E. L. F., Milner R. J., and Sutcliffe J. G. (1985) Immuncytochemical mapping of IB236: A brain specific neuronal polypeptide deduced from the sequence of a cloned mRNA. *J. Neurosci.* **5,** 1781–1802.

Brady R. O. (1983) Genetic errors and enzyme replacement strategies, *Genetics of Neurological and Psychiatric Disorders* (Kety, S. S., Rowland L. P., Sidman R. L., Matthysee S. W., eds), pp. 181–193. Raven Press, New York.

Breakefield X. O. and Stern D. F. (1986) Oncogenes in neural tumors. *TINS* **9,** 150–155.

Brown Jr. R. H., Johnson D., Ogonowski M., and Weiner H. L. (1987) Type 1 human poliovirus binds to human synaptosomes. *Ann. Neurol.* **21,** 64–70.

Bucher P. and Trifonov E. N. (1986) Compilation and analysis of eucaryotic Pol II promoter sequences. *Nuc. Acids. Res.* **14,** 10,009–10,026.

Calof A. L. and Jessell T. M. (1986) Use of retroviral vector to transfer and express a foreign gene in murine neurons and neural ectoderm. *Soc. Neurosci. Abst.* **12,** 183.

Chamorro M., Aubert C., and Brahic M. (1986) Demyelinating lesions due to Theilers virus are associated with ongoing central nervous system infection. *J. Virol.* **57,** 992–997.

Chikaraishi D. M. (1986) The ID, brain identifier, model of neuronal gene expression: a reevaluation. *TINS* **9,** 543–546.

Chao M., Bothwell M., Ross A., Koprowski H., Lanahan A., Buck C., and Sehgal A. (1986) Gene transfer and molecular cloning of the human NGF receptor. *Science* **232,** 518–521.

Choo K. H., Filby R. G., Jennings I. G., Peterson G., and Fowler K. (1986) Vectors for expression and amplification of cDNA in mammaliam cells: Expression of rat phenylalanine hydroxylase. *DNA* **5,** 529–537.

Co M. S., Gaulton G. N., Tominaga A., Homcy C. J., Fields B. N., and Greene M. I. (1985) Structural similarities between the mammalian beta-adrenergic and reovirus type 3 receptors. *Proc. Natl. Acad. Sci. USA* **82,** 5315–5318.

Colbere-Grapin F., Horodniceanu F., Kourilsky P., and Garapin A. C. (1981) A new dominant hybrid selective marker for higher eukaryotic cell. *J. Molec. Biol.* **150,** 1–14.

Comb M., Liston D., Martin M., Rosen H., and Herbert E. (1985) Expression of the human proenkephalin gene in mouse pituitary cells: Accurate and efficient mRNA production and proteolytic processing. *EMBO J.* **4,** 3115–3122.

Cone R. D. and Mulligan R. C. (1984) High efficiency gene transfer into mammalian cells: generation of helper-free recombination retrovirus with broad mammalian host range. *Proc. Natl. Acad. Sci. USA* **81,** 6349–6353.

Cook M. L., Bastone V. B., and Stevens J. G. (1974) Evidence that neurons harbor latent herpes simplex virus. *Infection and Immunity* **9,** 946–951.

Costantini F., Chada K., and Magram J. (1986) Correction of murine beta-thalassemia by gene transfer into germ line. *Science* **233,** 1192–1194.

DesGroseillers L., Rassart R., Robitaille Y., and Jolicoeur P. (1985) Retrovirus-induced spongiform encephalopathy: The 3'-end long terminal repeat-containing viral sequences influence the incidence of the disease and the specificity of the neurological syndrome. *Proc. Natl. Acad. Sci. USA* **82,** 8818–8822.

Dick J. E., Magli M. C., Huszar D., Phillips R. A., and Bernstein A. (1985) Introduction of a selectable gene into primitive stem cells capable of long-term reconstitution of the hemopoietic System of W/W^v mice. *Cell* **42**, 71–79.

DiMaio D. (1984) Eukaryotic cloning vectors based on bovine papilloma viruses. *Bio. Essays* **1**, 23–26.

Dixon R. A. F., Kobilka B. K., Strader D. J., Benovic J. L., Dohlman H. G., Frielle T., Bolanowski M. A., Bennett C. D., Rands E., Diehl R. E., Mumford R. A., Slater E. E., Sigal I. S., Caron M. G., Lefkowitz R. J., and Strader C. D. (1986) Cloning of the gene and cDNA for mammalian beta-adrenergic receptor and homology with rhodopsin. *Nature* **321**, 75–79.

Doehmer J., Barinaga M., Vale W., Rosenfield M., Verma I., and Evans R. (1982) Introduction of rat growth hormone gene into mouse fibroblasts via a retroviral DNA vector: Expression and regulation. *Proc. Natl. Acad. Sci. USA* **79**, 2268–2272.

Dubensky T. W., Campbell B. A., and Villarreal L. P. (1984) Direct transfection of viral and plasmid DNA into the liver or spleen of mice. *Proc. Natl. Acad. Sci. USA* **81**, 7529–7533.

Edelstein S. B. and Breakefield X. O. (1986) Monoamine oxidases A and B are differentially regulated by glucocorticoids and "aging" in human skin fibroblasts. *Cell. and Molec. Neurobiol.* **6**, 121–150.

Edwards R. H., Selby M. J., and Rutter W. J. (1986) Differential RNA splicing predicts two distinct nerve growth factor precursors. *Nature* **319**, 784–787.

Evans G. A., Ingraham H. A., Lewis K., Cunningham K., Seki T., Moriuchi T., Chang H. C., Silver J., and Hyman R. (1984) Expression of the Thy-1 glycoprotein gene by DNA-mediated gene transfer. *Proc. Natl. Acad. Sci. USA* **81**, 5532–5536.

Forss-Petter S., Danielson P., and Sutcliffe J. G. (1986) Neuron-specific enolase: Complete structure of rat mRNA, multiple transcriptional start sites and evidence suggesting post-transcriptional control. *J. Neurosci. Res.* **16**, 141–156.

Frenkel N., Locker H., and Vlazny D. A. (1980) Studies of defective herpes simplex viruses. *Annals NY Acad. Sci.* **354**, 347–370.

Friend S. H., Bernards R., Rogelj S., Weinberg R. A., Rapaport J. M., Albert D. M., and Dryja T. P. (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* **323**, 643–646.

Gage F. H., Wolf F. J. A., Rosenberg M. B., Xu L., Yee J.-K., Shults C., and Friedmann T. Grafting genetically modified cells to the brain. *Neuroscience*, in press.

Gajdusek D. C. (1985) Unconventional viruses causing subacute spongiformencephalopathies, *Virology* (Fields B. N., Knipe D., Chanock R., Melnick J., Roizman B., and Shope R., eds), pp. 1519–1557. Raven Press, New York.

Gibbs R. A. and Caskey C. T. (1987) Identification and localization of mutations at the Lesch-Nyhan locus by ribonuclease A cleavage. *Science* **236**, 303–305.

Gilboa E., Eglitis M. A., Kantoff P. W., and Anderson, W. F. (1986) Transfer and expression of cloned genes using retroviral vectors. *Biotech.* **4**, 504–512.

Gnahn H., Hefti F., Heumann R., Schwab M. E., and Thoenen H. (1983) NGF-mediated increase of choline acetyltransferase (ChAT) in the neonatal forebrain: evidence for a physiological role of NGF in the brain? *Brain Res.* **285**, 45–52.

Goldgaber D., Lerman M. I., McBride O. W., Saffiotti U., and Gajdusek D. C. (1986) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* **235**, 877–880.

Gordon J. W. and Ruddle F. H. (1985) DNA-mediated genetic transformation of mouse embryos and bone marrow—a review. *Gene.* **33**, 121–136.

Goring D. R., Rossant J., Clapoff S., Breitman L., and Tsui L.-C. (1987) *In situ* detection of beta-galactosidase in lenses of transgenic mice with a γ-crystallin/*lacZ* gene. *Science* **235**, 456–458.

Graf Jr., L. H., Urlaub G., and Chasin L. (1979) Transformation of the gene for hypoxanthine phosphoribosyltransferase. *Somat. Cell Genet.* **6**, 1031–1044.

Greene L. A. and Shooter E. M. (1980) The nerve growth factor: biochemistry, synthesis and mechanism of action. *Annu. Rev. Neurosci.* **3**, 353–402.

Grenningloh G., Rienitz A., Schmitt B., Methfessel C., Zensen M., Beyreuther K., Gundelfinger E. D., and Betz H. (1987) The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature* **328**, 215–220.

Gurney M. E., Heinrich S. P., Lee M. R., and Yin H. S. (1986) Molecular cloning and expression of neuroleukin, a neurotrophic factor for spinal and

sensory neurons. *Science* **234,** 566–574.

Gusella J., Keys C., Varsanyi-Breiner A., Kao F.-T., Jones C., Puck T., and Housman D. (1980) Isolation and localization of DNA segments from specific human chromosomes. *Proc. Natl. Acad. Sci. USA* **77,** 2829–2833.

Hamer D. H. and Leder P. (1979) Expression of the chromosomal mouse beta maj-globin gene cloned in SV40. *Nature* **281,** 35–40.

Hamer D. H. and Leder P. (1979) SV40 recombinants carrying a functional RNA splice junction and polyadenylation site from the chromosomal mouse beta-globin gene. *Cell* **17,** 737–747.

Harrington C. A., Lewis E. J., Krzemien D., and Chikaraishi D. M. (1987) Identification and cell-type specificity of the tyrosine hydroxylase gene promoter. *Nucleic Acids Res.* **15,** 2363–2384.

Hellerman J. G., Cone R. C., Potts Jr., J. T., Rich A., Mulligan R. C., and Kronenberg H. M. (1984) Secretion of human parathyroid hormone from rat pituitary cells infected with a recombinant retrovirus encoding preproparathyroid hormone. *Proc. Natl. Acad. Sci. USA* **81,** 5340–5344.

Hemperly J. J., Murray B. A., Edelman G. M., and Cunningham B. A (1986) Sequence of a cDNA clone ending the polysialic acid-rich and cytoplasmic domains of the neural cell adhesion molecule N-CAM. *Proc. Natl. Acad. Sci. USA* **83,** 3037–3041.

Hinrichs S. H., Nerenberg M., Reynolds R. K., Khoury G., and Jay G. (1987) A transgenic mouse model for human neurofibromatosis. *Science* **237,** 1340–1343

Hock R. A. and Miller A. D. (1986) Retrovirus-mediated transfer and expression of dmp resistance genes in human haematopoietic progenitor cells. *Nature* **320,** 275–277.

Hooper M., Hardy K., Handyside A., Hunter S., and Monk M. (1987) HPRT-deficient (Lesch-Nyhan) mouse embryos derived from germline colonization by cultured cells. *Nature* **326,** 292–298.

Hsu Y.-P. P., Powell J., Chen S., Weyler W., Ozelius L., Bruns G., Utterback M., Mallet J., Gusella J. F., and Breakefield X. O. Molecular genetic studies of MAO genes in Sixth International Catecholamine Symposium,Raven Press, NY, in press.

Hudson L. D., Berndt J. A., Puckett C., Kozak C. A., and Lazzarini R. A. (1987) Aberrant splicing of proteolipid protein mRNA in the dysmyelinating jimpy mutant mouse. *Proc. Natl. Acad. Sci. USA* **84,** 1454–1458.

Igarashi T., Okazaki T., Potter H., Gaz R., and Kronenberg H. M. (1986) Cell-specific expression of the human parathyroid hormone gene in rat pituitary cells. *Mol. Cell. Biol.* **6,** 1830–1833.

Ingraham H. A. and Evans G. A. (1986) Characterization of two atypical promoters and alternate mRNA processing in the mouse Thy-1.2 glycoprotein gene. *Mol. Cell. Biol.* **6,** 2923–2931.

Itoh N., Obata K., Yanaihara N., and Okamoto H. (1983) Human preprovasoactive intestinal polypeptide contains a novel PHI-27 like peptide, PHM-27. *Nature* **304,** 547–549.

Julien J. P., Grosveld F., Yazdanbaksh K., Flavell D., and Mushynski W. (1987) The structure of a human neurofilament gene (NF-L): a unique exon-intron organization in the intermediate filament gene family. *Biochim. Biophys. Acta.* **909,** 10–20.

Kantoff P. W., Kohn D. B., Mitsuya H., Armentano D., Sieberg M., Zweibel J. A., Eglitis M. A., McLachlin J. R., Wiginton D. A., Hutton J. J., Horowitz S. D., Gilboa E., Blaese R. M., and Anderson W. F. (1986) Correction of adenosine deaminase deficiency in human T and B cells using retroviral-mediated gene transfer. *Proc. Natl. Acad. Sci. USA* **83,** 6563–6567.

Karlsson S., Van Doren K., Schweiger S. G., Nienhuis A. W., and Gluzman Y. (1986) Stable gene transfer and tissue-specific expression of a human globin gene using adenoviral vectors *EMBO J.* **5,** 2377–2385.

Karns L. R., Ng S. C., Freeman J. A., and Fishman M. C. (1987) Cloning of complementary DNA for GAP-43, a neuronal growth related protein. *Science* **236,** 597–600.

Kaufman D. L., McGinnis J. F., Krieger N. R., Tobin A. J. (1986) Brain glutamate decarboxylase cloned in lambda gt-11: fusion protein produces gamma -aminobutyric acid. *Science* **232,** 1138–1140.

Keller G., Paige C., Gilboa E., and Wagner E. F. (1985) Expression of a foreign gene in myeloid and lymphoid cells derived from multipotent haematopoietic precursors. *Nature* **318,** 149–154.

Kelley K. A. and Herrup K. (1987) Unexpected CNS tumor cytologyin wild type and mutant SV-40 T-antigen transgenic mice. *Soc. Neurosci. Abst.* 154.6.

Kelly R. B. (1985) Pathways of protein secretion in eukaryotes. *Science* **230,** 25–31.

Kenney S., Natarajan V., Strike D., Khoury G., and Salzman N. (1984) JC virus enhancer-promoter active in human brain cells. *Science* **266**, 1337–1339.

Khoury G. and Gruss P. (1983) Enhancer elements. *Cell* **33**, 313–314.

Kinzler K. W., Bigner S. H., Bigner D. D., Trent J. M., Law M. L., O'Brien S. J., Wong A. J., and Vogelstein B. (1987) Identification of an amplified highly expessed gene in a human glioma. *Science* 236, 70–73.

Klobutcher L. A. and Ruddle F. H. (1981) Chromosome-mediated gene transfer. *Ann. Rev. Biochem.* **50**, 533–554.

Kollias G., Spanopoulou E., Grosveld F., Ritter M., Beech J., and Morris R. (1987) Differential regulation of a Thy-1 gene in transgenic mice. *Proc. Natl. Acad. Sci. USA* **84**, 1492–1496.

Koprowski H. (1978) Possible role of herpes virus in chronic CNS disease, in *Persistent Viruses* (Stevens F. G., eds.), pp. 691–699, Academic, NY.

Korneluk R. G., Mahuran D. J., Neote K., O'Dowd B. F., Tropak M., Willard H. F., Anderson M. J., Lowden J. A., and Gravel R. A. (1986) Isolation of cDNA clones coding for the alpha-subunit of human beta-hexosaminidase. *J. Biol. Chem.* **261**, 8407–8413.

Kraus M. H., Popescu N. C., Amsbaugh C., and King C. R. (1987) Overexpression of the EGF receptor-related proto-oncogene *erb*-2 in human mammary tumor cell lines by different molecular mechanisms. *EMBO J.* **6**, 605–610.

Kubo T., Fukuda K., Mikami A., Maeda A., Takahashi H., Mishina M., Haga T., Haga K., Ichiyama A., Kangawa K., Kojima M., Matsuo H., Hirose T., and Numa S. (1986) Cloning, sequencing and expression of complementary DNA encoding the muscarinic acetylcholine receptor. *Nature* **323**, 411–416.

Kuehn M. R., Bradley A., Robertson E. J., and Evans M. J. (1987) A potential animal model for the human Lesch-Nyhan syndrome through introduction of HPRT mutations into mice. *Nature* **326**, 295–298.

Kuwano R., Usui H., Maeda T., Fukui T., Yamanari N., Ohtsuka E., Ikehara M., and Takahashi Y. (1984) Molecular cloning and the complete nucleotide sequence of cDNA to mRNA for S-100 protein of rat brain. *Nuc. Acids Res.* **12**, 7455–7465.

Kwong A. D. and Frenkel N. (1984) Herpes simplex virus amplicon: Effect of size on replication of constructed defective genomes containing eucaryotic DNA sequences. *J. Virol.* **51**, 595–603.

Larhammar D., Ericsson A., and Persson H. (1987) Structure and expression of the rat neuropeptide Y gene. *Proc. Natl. Acad. Sci. USA* **84**, 2068–2072.

Larsen P. R., Harney J. W., and Moore D. D. (1986) Repression mediates cell-type specific expression of the rat growth hormone gene. *Proc. Natl. Acad. Sci. USA* **83**, 8283–8287.

Ledley F., Grennet H., McGinnis-Shelnutt M., and Woo S. (1986) Retroviral-mediated gene transfer of human phenylalanine hydroxylase into NIH 3T3 and heptoma cells. *Proc. Natl. Acad. Sci. USA* **83**, 409–413.

Lee W.-H., Bookstein R., Hong F., Young L.-J., Shew J.-Y., and Lee E. Y.-H.P. (1987) Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* **235**, 1394–1399.

Leff S. E., Evans R. M., and Rosenfeld M. G. (1987) Splice commitment dictates neurospecific alternative RNA processing in calcitonin/CGRP gene expression. *Cell* **48**, 517–524.

LeMeur M., Gerlinging P., Benoist C., and Mathis D. (1985) Correcting an immune-response deficiency by creating E alpha gene transgenic mice. *Nature* **316**, 38–42.

Lentz T. Z., Benson R. J., Klimowicz D., Wilson P. T., and Hawrot E. (1986) Binding of rabies virus to purified *Torpedo* acetylcholine receptor. *Brain Res.* **387**, 211–219.

Lewis S. A., Balcarek J. M., Krek V., Shelanski M., and Cowan N. J. (1984) Sequence of a cDNA clone encoding mouse glial fibrillary acidic protein: structural conservation of intermediate filaments. *Proc. Natl. Acad. Sci. USA* **81**, 2743–2746.

Lewis S. A. and Cowan N. J. (1985) Genetics, evolution, and expression of the 68,000-mol-wt neurofilament protein: isolation of a cloned cDNA probe. *J. Cell. Biol.* **100**, 843–850.

Lewis E. J., Harrington C. A., and Chikaraishi D. M. (1987) Transcriptional regulation of the tyrosine hydroxylase gene by glucocorticoid and cyclic AMP. *Proc. Natl. Acad. Sci. USA* **84**, 3550–3554.

Libermann T. A., Nusbaum H. R., Razon N., Kris R., Lax I., Soreq H., Whittle N., Waterfield M. D., Ullrich A., and Schessinger J. (1985) Amplification, enhanced expression and possible rearran-

gement of EGF receptor gene in primary human brain tumors of glial origin. *Nature* **313,** 144–147.

Luthman H. and Magnusson G. (1983) High efficiency polyoma DNA transfection of chloroquine treated cells. *Nuc. Acids Res.* **11,** 1295–1308.

Mackett M. and Smith, G. L. (1986) Vaccinia virus expression vectors. *J. Gen. Virol.* **67,** 2067–2082.

Mandl C., Walker D. L., and Frisque R. J. (1987) Derivation and characterization of POJ cells, transformed human fetal glial cells that retain their permissivity for JC virus. *J. Virol.* **61,** 755–763.

Mann R., Mulligan R. C., and Baltimore D. (1983) Construction of retrovirus packaging mutant and its use to produce helper-free defective retrovirus. *Cell* **33,** 153–159.

McBride O. W. and Peterson J. L. (1980) Chromosome-mediated gene transfer in mammalian cells. *Ann. Rev. Genet.* **14,** 321–345.

McKinnon R. D., Shinnick T. M., and Sutcliffe J. G. (1986) The neuronal identifier element is a *cis*-acting positive regulator of gene expression. *Proc. Natl. Acad. Sci. USA* **83,** 3751–3755.

Mellman I., Fuchs R., and Helenius A. (1986) Acidification of the endocytic and exocytic pathways. *Annu. Rev. Biochem.* **55,** 663–700.

Messing A., Chen H. Y., Palmiter R. D., and Brinster R. L. (1985) Peripheral neuropathies, hepatocellular carcinomas, and islet cell adenomas in transgenic mice. *Nature* **316,** 461–463.

Meulen V. T. and Hall W. W. (1978) Slow virus infections of the nervous system: Virological, immunological, and pathogenetic considerations. *J. Gen. Virol.* **41,** 1–25.

Miller A. D., Eckner R. J., Jolly D. J., Friedmann T., and Verma I. M. (1984) Expression of a retrovirus encoding human HPRT in mice. *Science* **225,** 630–631.

Miller A. D., Jolly D. J. Friedmann T., and Verma I. (1983) A transmissable retrovirus expressing human hypoxanthine phosphoribosyl transferase (HPRT): gene transfer into cells obtained from humans deficient in HPRT. *Proc. Natl. Acad. Sci. USA* **80,** 4709–4713.

Miller A. D., Law M.-F., and Verma, I. M. (1985) Generation of helper-free amphotropic retroviruses that transduce a dominant-acting, methotrexate-resistant dihydrofolate reductase gene. *Mol. Cell. Biol.* **5,** 431–437.

Milner R. J., Lai C., Nave K.-A., Lenoir D., Ogata J., and Sutcliffe J. G. (1985) Nucleotide sequences of two mRNAs for rat brain myelin proteolipid protein. *Cell* **42,** 931–939.

Minth C. M., Bloom S. R., Polak J. M., and Dixon J. E. (1984) Cloning, characterization and DNA sequence of a human cDNA encoding neuropeptide tyrosine. *Proc. Natl. Acad. Sci.* **81,** 45774581.

Molineaux S. M., Engh H., de Ferra F., Hudson L., and Lazzarini R. A. (1986) Recombination within the myelin basic protein gene created the dysmyelinating shiverer mouse mutation. *Proc. Natl. Acad. Sci. USA* **83,** 7542–7546.

Monaco A. P., Neve R. L., Colletti-Feener C., Bertelson C. J., Kurnit D. M., and Kunkel L. M. (1986) Isolation of candidate cDNAs for portions of the Duchenne muscular dystrophy gene. *Nature* **323,** 646–650.

Montminy M. R., Sevarino K. A., Wagner J. A., and Mandell G. (1986) Identification of a cyclic-AMP-responsive element within the rat somatostatin gene. *Proc. Natl. Acad. Sci. USA* **83,** 6682–6686.

Moore H. and Kelly R. (1986) Re-routing of a secretory protein by fusion with human growth hormone sequences. *Nature* **321,** 443–462.

Moore H. P., Walker M. D., Lee F., and Kelly R. B (1983) Expressing a human proinsulin cDNA in a mouse ACTH-secreting cell. Intracellular storage, proteolytic processing, and secretion on stimulation. *Cell* **35,** 531–538.

Mulligan R. C., Howard B. H., and Berg P. (1979) Synthesis of rabbit beta-globin in cultured monkey kidney cells following transfection with a SV40 beta-globin recombinant genome. *Nature* **277,** 108–144.

Mulligan R. C. and Berg P. (1981) Selection for animal cells that express the *Escherichia coli* gene coding for xanthine-guanine phosphoribosyltransferase. *Proc. Natl. Acad. Sci. USA* **78,** 2072–2076.

Mulligan R. C. (1982) Development of new mammalian transducing vectors, *Eukaryotic Viral Vectors* (Gluzman, Y., ed.), pp. 133–137. Cold Spring Harbor Lab, NY.

Myerowitz R., Piekarz R., Neufeld E. F., Shows T. B., and Suzuki K. (1985) Human beta-hexosaminidase alpha-chain: coding sequence and homology with the beta-chain. *Proc. Natl. Acad. Sci. USA* **82,** 7830–7834.

Narayan O. and Coile L. C. (1985) Lentiviral diseases of sheep and goats: chronic pneumonia, leukoencephalomyelitis, and arteritis. *Rev. Infect. Dis.* **7,** 89–98.

Nave K.-A., Lai C., Bloom F. E., and Milner R. J. (1986) Jimpy mutant mouse: a 74-base deletion in the mRNA for myelin proteolipid protein and evidence for a primary defect in RNA splicing. *Proc. Natl. Acad. Sci. USA* **83,** 9264–9268.

Ornitz D. M., Palmiter R. D., Hammer R. E., Brinster R. L., Swift G. H., and MacDonald R. J. (1985) Specific expression of an elastase-human growth hormone fusion gene. *Nature* **313,** 600–602.

Palmer T. D., Hock R. A., Osborne W. R. A., and Miller A. D. (1987) Efficient retrovirus-mediated transfer and expression of a human adenosine deaminase gene in diploid skin fibroblasts from an adenosine deaminase-deficient human. *Proc. Natl. Acad. Sci. USA* **84,** 1055–1059.

Palmiter R. D., Behringer R. R., Quiate C. J., Maxwell I. H.. and Brinster R. L. (1987) Cell lineage ablation in transgenic mice by cell-specific expression of a toxin gene. *Cell* **50,** 435–443.

Palmiter R. D., Norstedt G., Gelinas R. E., Hammer R. E., and Brinster R. L. (1983) Metallothionein-human GH fusion genes stimulate growth of mice. *Science* **222,** 809–814.

Palmiter R. D. and Brinster R. (1985) Transgenic Mice. *Cell* **41,** 343–345.

Palmiter R. D., Chen H. Y., Messing A., and Brinster R. L. (1985) SV40 enhancer and large-T antigen are instrumental indevelopment of choroid plexus tumors in transgenic mice. *Nature* **316,** 457–460.

Papazian D. M., Schwarz T. L., Tempel B. L., Jan Y. N., and Jan L. Y. (1987) Cloning of genomic and complementary DNA from *shaker,*a putative potassium channel gene from *Drosophila. Science* **237,** 749–753.

Parker B. A. and Stark G. R. (1979) Regulation of simian virus 40 transcription: Sensitive analysis of the RNA present early in infections by virus or virus DNA. *J. Virol.* **31,** 360–369.

Popko B., Puckett C., Lai E., Shine H. D., Readhead C., Takahashi N., Hunt S. W., Sidman R. L., and Hood L. (1987) Myelin deficient mice: Expression of myeline basic protein and generation of mice with varying levels of myelin. *Cell* **48,** 713–721.

Potter H., Weir L., and Leder P. (1984) Enchancer-dependent expression of human k immunoglobulin genes introduced into mouse pre-beta lymphocytes by electroporation. *Proc. Natl. Acad. Sci. USA* **81,** 7161–7165.

Price J., Turner D., and Cepko C. (1987) Lineage analysis in the vertebrate nervous system by retrovirus-mediated gene transfer. *Proc. Natl. Acad. Sci. USA,* **34,** 156–160.

Prody C. A., Zevin-Sonkin D., Gnatt A., Goldberg O., and Soreq H. (1987) Isolation and characterization of full-length cDNA clones coding for cholinesterase from fetal human tissues. *Proc. Natl. Acad. Sci. USA* **84,** 3555–3559.

Radeke M. J., Misko T. P., Hsu C., Herzenberg L. A., and Shooter E. M. (1987) Gene transfer and molecular cloning of the rat nerve growth factor receptor. *Nature* **325,** 593–597.

Readhead C., Popko B., Takahashi N., Shine H. D., Saavedra R. A., Sidman R. L., and Hood L. (1987) Expression of a myelin basic protein gene in transgenic shiverer mice: correction of the dysmyelinating phenotype. *Cell* **48,** 703–712.

Roach A., Takahashi N., Pravtcheva D., Ruddle F., and Hood L. (1987) Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice. *Cell* **42,** 149–155.

Robinson P. A., Wion D., and Anderton B. H. (1986) Isolation of a cDNA for the rat heavy neurofilament polypeptide (NF-H) *Febs Lett.* **209,** 203–205.

Rosenberg M., Hansen C., and Breakefield X. O. (1985) Molecular genetic approaches to neurologic and psychiatric diseases. *Progress in Neurobiology* **24,** 95–140.

Sanes J. R., Rubenstein J. L. R., and Nicholas J.-F. (1987) Use of a recombinant retrovirus to study postimplantation cell lineage in mouse embryos. *EMBO J.* **5,** 3133–3134.

Sasaki H., Sasaki Y., Matsuo H., Goto I., Kuroiwa Y., Sahashi I., Takahashi A., Shinoda T., Isobe T., and Takagi Y. (1984) Diagnosis of familial amyloidotic polyneuropathy by recombinant DNA techniques. *Biochem. Biophys. Res. Commun.* **125,** 636–642.

Schechter A. L., Stern D. F., Vaidyanathan L., Decker S. J., Drebin J. A., Greene M. I., and Weinberg R. A. (1984) The neu oncogene: an erb-B-related gene encoding a 185,000-M_r tumour antigen. *Nature* **312,** 513–516.

Schnieke A., Harbers K., and Jaenisch R. (1983) Embryonic lethal mutation in mice induced by retrovirus insertion into the alpha 1 (I) collagen gene. *Nature* **304,** 315–320.

Schwab M., Ellison J., Busch M., Rosenau W., Varmus H. E., and Bishop J. M. (1984) Enchanced expression of the human gene N-myc consequent to amplification of DNA may contribute to malignant progression of neuroblastoma. *Proc. Natl. Acad. Sci. USA* **81,** 4940–4944.

Schweitzer E. and Kelly R. (1985) Selective packaging of human growth hormone into synaptic vesicles in rat neuronal (PC12) cell line. *J. Cell Biol.* **101,** 667–676.

Selden R. F., Skoskiewicz M. J., Howie K. B., Russell P. S., and Goodman H. M. (1987) Implantation of genetically engineered fibroblasts into mice: implications for gene therapy. *Science* **236,** 714–718.

Serfling E., Jasin M., and Schaffner W. (1985) Enhancers and eukaryotic gene transcription. *Trends Genet.* **1,** 224–230.

Sharpe A. H., Jaenisch R., and Cuprecht R. M. (1987) Retrovirus and mouse embryos: A rapid model for neurovirulence and transplancental antiviral therapy. *Science* **236,** 1671–1674.

Shih M.-F., Arsenakis M., Tiollais P., and Roizman B. (1984) Expression of hepatitis B virus S gene by herpes simplex virus type I vectors carrying alpha- and beta-regulated gene chimeras. *Proc. Natl. Acad. Sci. USA* **81,** 5867–5870.

Schofield P. R., Darlison M. G., Fujita N., Burt D. R., Stephenson F. A., Rodriquez H., Rhee L. M., Ramachandran J., Reale V., Glencorse T. A., Seeburg P. H., and Barnard E. A. (1987) Sequence and functional expression of the $GABA_A$ receptor shows a ligand-gated receptor super-family. *Nature* **328,** 221–227.

Slamon D. J., Clark G. M., Wong S. G., Levin W. J., Ullrich A., and McGuire W. L. (1987) Human breast cancer: correlation of relapse and survival with amplification of the HER-2/*neu* oncogene. *Science* **235,** 177–182.

Sorge J., Gelbart T., West C., Westwood B., and Beutler E. (1985) Heterogeneity in type I Gaucher disease demonstrated by restriction mapping of the gene. *Proc. Natl. Acad. Sci. USA* **82,** 5442–5445.

Sorge J., Kuhl W., West C., and Beutler E. (1987) Complete correction of the enzymatic defect of type I Gaucher disease fibroblasts by retroviral-mediated gene transfer. *Proc. Natl. Acad. Sci. USA* **84,** 906–909.

Soriano P., Cone R. D., Mulligan R. C., and Jaenisch R. (1986) Tissue-specific and ectopic expression of genes introduced into transgenic mice by retroviruses. *Science* **234,** 1409–1413.

Soriano P. and Jaenisch R. (1986) Retroviruses as probes for mammalian development: allocation of cells to the somatic and germ cell lineages. *Cell* **46,** 19–29.

Southern P. J. and Berg P. (1982) Transformation of mammalian cells to antibiotic resistance with bacterial gene under control of the SV40 early region promoter. *J. Molec. and App. Gen.* **1,** 327–341.

Spear P. G. and Roizman B. (1981) Herpes simplex virus, *DNA Tumor Viruses* (Tooze, J., ed.) pp. 615–746. Cold Spring Harbor Laboratory, NY.

Spaete R. R. and Frenkel N. (1982) The herpes simplex virus amplicon: A new eucaryotic defective-virus cloning amplifying vector. *Cell* **30,** 85–304.

Spaete R. R., and Frenkel N. (1985) The herpes simplex virus amplicon: Analysis of *cis*-acting replication functions. *Proc. Natl. Acad. Sci. USA* **82,** 694–698.

Stevens J. G. (1975) Latent herpes simplex virus and the nervous system. *Curr. Topics in Microbiol. and Immunol.* **70,** 31–50.

Stewart T. A., Pattengale P. K., and Leder P. (1984) Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* **38,** 627–637.

Stewart C. L., Schuetze S., Vanek M., and Wagner E. F. (1987) Expression of retroviral vectors in transgenic mice obtained by embryo infection. *EMBO J.* **6,** 383–388.

Stout J. T., Chen H. Y., Brennand J., Caskey C. T., and Brinster R. (1985) Expression of human HPRT in the central nervous system of transgenic mice. *Nature* **317,** 250–252.

Stout J. T. and Caskey C. T. Anti-sense RNA inhibition of endogenous genes. *Methods Enzymol.*, in press.

Stow N. D. and McGonagle E. C. (1982) Propagation of foreign DNA sequence linked to a herpes simplex virus origin of replication, *Eucaryotic Viral Vectors* (Gluzman Y., ed.), pp. 199–204. Cold Spring Harbor Laboratory, NY.

Strader C. D., Sigal I. S., Blake A. D., Cheung A. H., Register R. B., Rands E., Zemcik B. A., Candelore M. R., and Dixon R. A. F. (1987) The carboxyl terminus of the hamster beta-adrenergic receptor expressed in mouse L cells is not required for receptor sequestration.*Cell* **49,** 855–863.

Subramani S., Mulligan R., and Berg P. (1981) Expression of the mouse dihydrofolate reductase complementary deoxyribonucleic acid in simian virus 40 vectors. *Mol. and Cell. Biol.* **1**, 854–864.

Sutcliffe J. G., Milner R. J., Gottesfeld J. M., and Lerner R. A. (1984) Identifier sequences are transcribed specifically in brain. *Nature* **308**, 237–241.

Swanson L. W., Simmons D. M., Arriza J., Hammer R., Brinster R., Rosenfeld M. G., and Evans R. M. (1985) Novel developmental specificity in the nervous system of transgenic animals expressing growth hormone fusion genes. *Nature* **317**, 363–366.

Taniuchi M., Clark H. B., and Johnson E. M. Jr. (1986) Induction of nerve growth factor receptor in Schwann cells after axotomy. *Proc. Natl. Acad. Sci. USA* **83**, 4094–4098.

Tanzi R. E., Gusella J. F., Watkins P. C., Bruns G. A. P., St. George-Hyslop P., Van Keuren M. L., Patterson D., Pagan S., Kurnit D. M., and Neve R. L. (1987) Amyloid protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* **235**, 880–884.

Taparowsky E., Shimizu K., Goldfarb M., and Wigler M. (1983) Structure and activation of the human N-ras gene. *Cell* **34**, 581–586.

Thoenen H. and Edgar D. (1985) Neurotrophic factors. *Science* **229**, 238–242.

Thomas G., Herbert E., and Hruby D. E. (1986) Expression and cell type-specific processing of human preproenkephalin with a vaccinia recombinant. *Science* **232**, 1641–1643.

Thomas K. R. and Capecchi M. R. (1986) Introduction of homologous DNA sequences into mammalian cells induces mutations in the cognate gene. *Nature* **324**, 34.

Townes T. M., Lingrel J. B., Chen H. Y., Brinster R. L., and Palmiter, R. D. (1985) Erythroid-specific expression of human beta-globin genes in transgenic mice. *EMBO J.* **4**, 1715–1723.

Tsuji S., Choudary P. V., Martin B. M., Stubblefield B. K., Mayor J. A., Barranger J. A., and Ginns E. I., (1987) A mutation in the human glucocerebrosidase gene in neuronopathic Gaucher's disease. *N. Engl. J. Med.* **316**, 570–575.

Tsukada T., Fink J. S., Mandel G., and Goodman R. H. (1987) Identification of a region in the human vasoactive intestinal polypeptide gene responsible for regulation by cyclic AMP. *J. Biol. Chem.* **262**, 8743–8747.

Tsukada T., Horovitch S. J., Montminy M. R., Mandel G., and Goodman R. H. (1985) Structure of the human vasoactive intestinal polypeptide gene. *DNA* **4**, 293–300

Turner D. L and Cepko C. (1987) Cell lineage in the rat retina: a common progenitor for neurons and glia persists late in development. *Nature*, in press.

Warren T. and Sheilds, D. (1984) Expression of preprosomatostatin in heterologous cells: Biosynthesis, postranslational processing, and secretion of mature somatostatin. *Cell* **39**, 547–555.

Waterman M., Murdoch G. H., Evans R. M., and Rosenfeld M. G. (1985) Cylic AMP regulation of eukaryotic gene transcription by two discrete molecular mechanisms. *Science* **229**, 267–269.

Weiss R., Tsnich N., Varmus H., and Coffin J. (1985) *RNA tumor viruses*. Cold Spring Harbor Labs, NY

Wigdahl B., Smith C. A., Iraglia A. M., and Rapp F. (1984) Herpes simplex virus latency in isolated human neurons. *Proc. Natl. Acad. Sci. USA* **81**, 6217–6221.

Wigler M., Silverstein S., Lee L. S., Pellicer A., Cheng Y. C., and Axel R. (1977) Transfer of purified herpes virus thymidine kinase gene to cultures mouse cells *Cell* **11**, 223–232.

Wigler M., Pellicer A., Silverstein S., Axel R., Urlaub G., and Chasin L. (1979a) DNA-mediated transfer of the adenine phosphoribosyltransferase locus into mammalian cells. *Proc. Natl. Acad. Sci. USA* **76**, 13731–13736.

Wigler M., Sweet R., Sim G. K., Wold B., Pellicer A., Lacy E., Maniatis T., Silverstein S., and Axel R. (1979b) Transformation of mammalian cells with genes from procaryotes and eucaryotes. *Cell* **16**, 777–785.

Wiley C. A., Shrier R. D., Nelson J. A., Lampert P. W., and Oldstone B. A. (1986) Cellular localization of human immunodeficiency virus infection within the brains of acquired immune deficiency syndrome patients. *Proc. Natl. Acad. Sci. USA* **83**, 7089–7093.

Williams D. A., Lemishchka I. R., Nathan D. G., and Mulligan R. C. (1984) Introduction of new genetic material into pluripotent haematopoietic stem cells of the mouse. *Nature* **310**, 476–480.

Williams D., Orkin S., and Mulligan R. (1986a) Retrovirus-mediated transfer of human adenosine deaminase gene sequences into cells in culture and into murine hematopoietic cells in vivo. *Proc. Natl. Acad. Sci. USA* **83,** 2566–2570.

Williams L. R., Varon S., Peterson G. M., Wictorin K., Fischer W., Bjorklund A., and Gage F. H. (1986b) Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. *Proc. Natl. Acad. Sci. USA* **83,** 9231–9235.

Willis R. C., Jolly D. J., Miller A. D., Plent M. M., Esty A. C., Anderson P. J., Chang H.-C., Jones O. W., Seegmiller J. E., and Friedmann T. (1984) Partial phenotypic correction of human Lesch-Nyhan (hypoxanthine-guanine phosphoribosyl-transferase-deficient) lymphoblasts with a transmissable retroviral vector. *J. Biol. Chem.* **259,** 7842–7849.

Wolf D., Richter-Landsberg C., Short M. P., Cepko C., and Breakefield X. O. Retrovirus-mediated gene transfer of nerve growth factor into mouse pituitary line AtT-20. Manuscript submitted.

Yamamoto K. R. (1985) Steroid receptor regulated transcription of specific genes and gene networks. *Ann. Rev. Genet.* **19,** 209–252.

Yates J. L., Warren N., and Sugden B. (1985) Stable replication of plasmids derived from Epstein-Barr virus in various mammalian cells. *Nature* **313,** 812–815.