Retroviral-Mediated Gene Transfer

Applications in Neurobiology

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Abstract

There are now many examples of the successful expression of genes transduced by retroviruses in studies from outside the field of neuroscience. Retroviruses will undoubtedly also prove to be effective tools for neuroscientists interested in expressing cloned neurotransmitter and receptor genes. There are also other less obvious applications of retroviruses, such as their insertional mutagenic effects, which may be useful in studies of the genetic factors and biochemical mechanisms involved in, for example, neurotoxicity. Strong cellular promoters have been identified by retroviral infection and subsequent rescue of the flanking genomic DNA. Retroviruses can be employed again to reintroduce these regulatory sequences back into cells. In this way the complexities of gene expression in the many subpopulations of neurons may be unraveled. Retroviruses can also serve as very useful genetic markers in studies of development and lineage relationships. Retroviruses may be used to efficiently transfer oncogenes into neuronal cells to create new cell lines. This application exploits one of the natural traits of retroviruses—oncogenesis—which led to their original discovery. Finally, there are neurotropic retroviruses that could serve as important vectors for delivering genes into neurons. Studying these retroviruses may lead to an understanding of how they cause neuropathologic changes in the CNS.

Index Entries: Gene transfer; retroviral vectors; insertion mutagens; lineage analysis.

Introduction

Viruses have long been suspected to play a role in the etiology of a number of disease states, particularly those involving disruptions in normal cellular function, such as cancer. Intensive research over many years has shown that in some cases cellular transformation may be caused by a class of RNA tumor virus known as retroviruses. Recently, one member of this class, the human immunodeficiency virus (HIV), has received much attention as the causative agent of the human acquired immunodeficiency syndrome (AIDS). Retroviruses consist of an RNA genome encapsidated in a multisubunit protein coat. Replication is accomplished by an RNA-dependent DNA polymerase, reverse transcriptase (Fig. 1). Retroviruses are endemic to many diverse groups of animals (Teich, 1984), and may be transmitted horizontally, by infection between somatic cells, or vertically, by either congenital transmission or integration into the germline. Mouse mammary tumor virus provides a classic example of germline transmission (Teich et al., 1984).

Retroviruses are characteristically flanked by long terminal repeat (LTR) sequences, resulting in RNA genomes resembling the transposable elements of yeast and flies (Bukhari et al., 1977; Shiba and Saigo, 1983; Flavell, 1984; Boeke et al., 1985). Retroviral integration into the host genome following infection is very efficient and stable, is a random event, and, unlike some DNA viruses, may often occur without causing cell lysis or morphological change (Varmus, 1982). With the advent of molecular biological techniques, retroviruses may be exploited as powerful tools in studies of the genetic regulatory mechanisms of, especially, cellular differentiation, normal cellular function, and development.

Retroviruses have recently emerged as particularly useful tools for the transfer of genes into cells. Only the intact viral genome integrates into the host chromosome, and once achieved, integration is stable. An additional advantage of retroviral infection is the resulting low copy number of virus, often only one, per infected cell (Barklis et al., 1986). Viruses, with their built in mechanisms for infecting cells, are naturally very efficient vectors for gene transfer; in particular, they have the ability to transfer

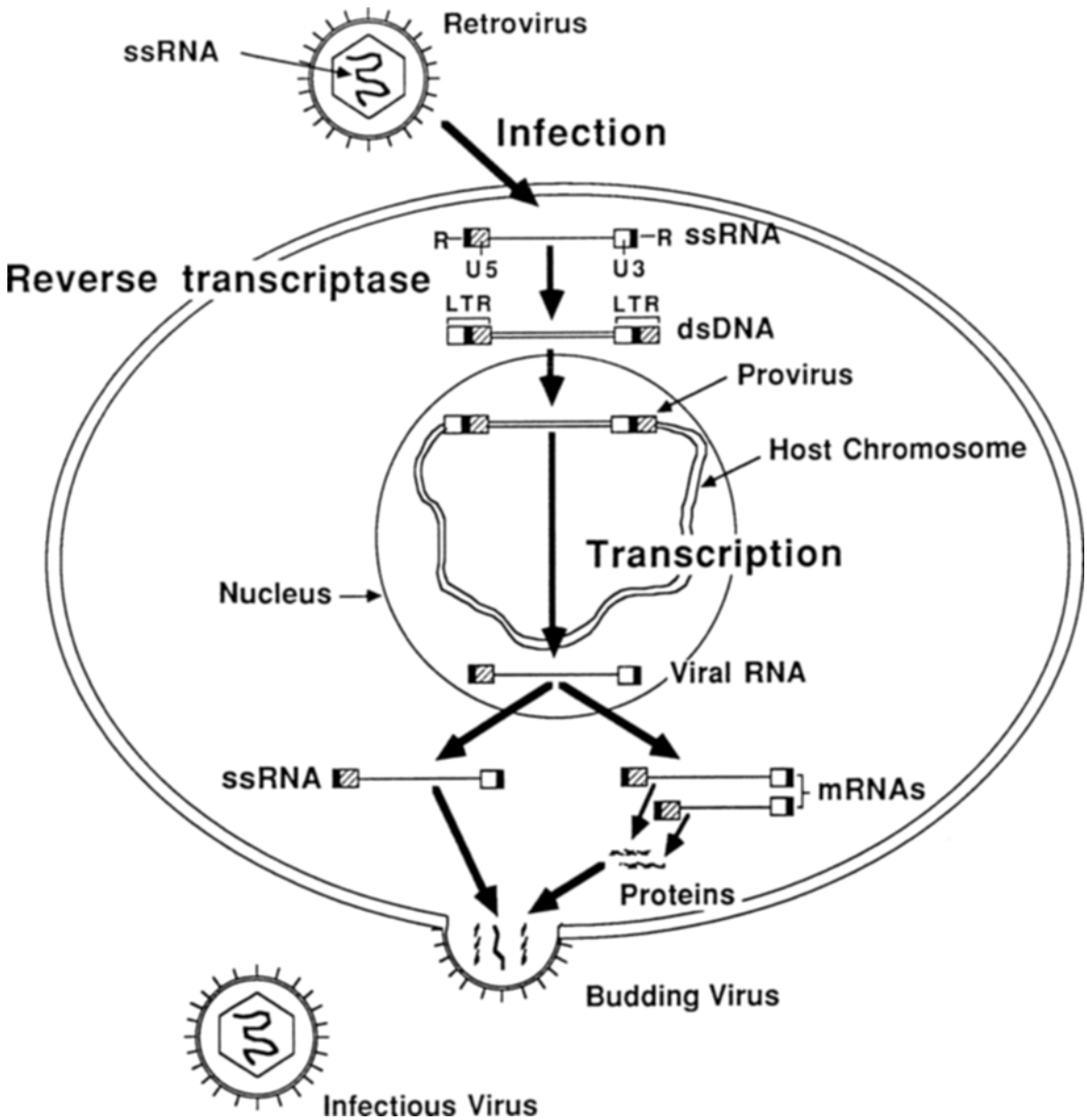

Fig. 1. Retrovirus life cycle. A replication competent retrovirus is shown infecting a host cell. The retrovirus consists of a single stranded RNA (ssRNA) genome packaged in an infectious virion. After infection, a double stranded DNA molecule is generated from the viral RNA by the viral reverse transcriptase. The DNA integrates stably into the host DNA, resulting in a provirus. Expression of viral genes can now proceed from the viral LTR, resulting in new viral RNA genomes and viral messenger RNAs (mRNAs). Proteins generated from the mRNAs result in packaging of the ssRNA viral genomes into infectious virus particles.

multiple genes within the same virus into eukaryotic cells.

Recombinant retroviruses, carrying foreign genetic material, can be constructed for this purpose as well. However, the introduction of foreign genes disrupts the function of retroviral genes required for viral reproduction. Consequently, the simultaneous presence of a wild-type virus is required in order to complement the impaired function of the replication-defective recombinant. Only then is it possible for the cell to produce intact virus particles containing the recombinant viral genome. This poses a problem because wild-type retroviruses, once integrated, may often continue to propagate, a situation that is undesirable for the ideal experimental system.

Recently, Mulligan and coworkers have devised a helper-free producer system, using cotransfection of recombinant retroviruses with a packaging defective Moloney mouse leukemia virus (Mo-MuLV), allowing for the production of recombinants that are infectious once only, and eliminating the necessity for the introduction of any wild-type virus into experimental cells. This helper-free producer system is now a basic tool for retroviral gene transfer (Mann et al., 1983).

There have already been many studies in a variety of fields in which recombinant retroviruses have been used. One obvious application of recombinant retroviral technology is the transfer of a cloned gene fragment for the purpose of studying its function in different eukaryotic cells. However, the construction of a recombinant virus carrying a functional cloned gene is still largely a trial-and-error process, since many molecular mechanisms involved are not as yet fully understood. Nevertheless, there are many other ways retroviruses can be used to study biological, including neurological, phenomena. Since retroviruses integrate randomly into the host genome, causing random disruption of the normal DNA, identification of loss of normal function could lead to discovery of the gene regulating that function. Therefore, they can be used as insertion mutagens. Normally silent genes (like the extensively studied oncogenes) may become expressed should the virus, with its promoter and enhancer sequences ("cis-acting" factors), integrate directly upstream of the gene. Genes carried by the retrovirus may become expressed by transcription from some endogenous host promoter and/or enhancer in the flanking genomic DNA, thus allowing direct and rapid cloning of such regulatory cellular genes. Since retroviruses integrate in a stable way, they become powerful tools for the study of developmental neurobiology through lineage analysis. Finally, genetic rearrangements that may occur during differentiation could also be studied using viral gene markers.

Development of Helper-Free Producers

Several methods are presently available for the in vitro transfer of genetic material into cells. One is infection by viral vectors. The use of a virus allows for limited target selection (i.e., cells of a particular species), but the other methods are currently totally nonselective, and include DNA transfection with calcium phosphate (Wigler et al., 1977; Graham and Van der Eb, 1973), DEAE dextran (Wigler, et al., 1977; McCutchen and Pagano, 1968), or in phage particles (Ishiura et al., 1982), direct nuclear microinjection (Anderson et al., 1980), fusion with liposome packages (Franley et al., 1980), protoplasts (Sandri-Goldin, et al., 1981), or erythrocyte ghosts (Wiberg et al., 1983), and electroporation with high voltage fields (Neumann et al., 1982). These latter methods are useful only for transfer of genetic material into preselected cell populations. The transferred genetic material is initially epichromosomal, and even many viruses are often only transiently expressed. Only rarely is stable integration achieved. In addition, the structural integ-

rity of any transfected DNA molecule cannot be guaranteed (whereas retroviruses usually integrate at their LTRs, thereby improving the chances of introducing intact genes into cellular genomes). Therefore, studying gene expression by these means is relatively inefficient.

Early attempts to use retroviruses in gene transfer experiments focused on the selection, following integration, of a gene carried by the retrovirus. For example, a recombinant virus was constructed in which the envelope gene was replaced by the thymidine kinase (TK) gene of the *Herpes simplex* virus. The recombinant virus was used to transfect 3T3 cells, previously infected with Mo-MuLV (to provide the helper function), which were deficient in TK. The cells were then selected on aminopterin, which kills cells lacking TK. Surviving cells had been successfully transfected with the recombinant virus (Shimotohno and Temin, 1981; Wei et al., 1981; Tabin et al., 1982). This sort of system is useful under a very limited set of circumstances, such as when one is using specific cell lines. A further disadvantage is the fact that the wild-type helper Mo-MuLV is always present in the experimental cells.

Subsequently, it was realized that a specific viral sequence is required for encapsidation of the viral genome into infects virions (Watanabe and Temin, 1982). This led to the construction of the currently widely used helper-free producer system mentioned above. This requires the use of a "cis-packaging" mutant virus, called pMOVψ- (Fig. 2). It was created by the deletion from Mo-MuLV of 350 nucleotides between the putative 5' donor splice site for the envelope message and the AUG start codon of the *gag* gene. This mutant virus, when transfected into NIH/3T3 cells, produces all the proteins necessary for the manufacture of infectious viral particles. However, since it lacks the packaging sequence, the required protein binding to this sequence cannot occur, and thus the virus is theoretically unable to package its own genome into infectious particles (*see below*). But, any recombinant virus carrying the packaging sequence and cotransfected into the same cell with pMOVψ- will become packaged, even though lacking other essential viral gene products, as these are provided by the defective helper virus. The recombinant virus is only infectious one time, as after successful infection of a new host cell, genes essential for further viral replication are lacking (having been replaced by marker, or other, genes of interest) (Mann et al., 1983). This system works very well in practice, in spite of the fact that it has recently been realized that pMOVψ- is packaged at a very low frequency (Dzierzak and Mulligan, personal communication). This method eliminates the presence of any wild-type virus in the experimental system.

pMOVψ – is simply a mutant Mo-MuLV, and therefore its host range is restricted to rodents, as, of course, is that of any recombinant virus produced as described above. Another packaging deficient helper mutant, called pMAVψ⁻, has been constructed by replacing the Mo-MuLV *env* gene with an *env* fragment from an amphotropic virus, 4070A. This allows for construction of recombinant virions possessing a much broader host range, including human cells (Cone and Mulligan, 1984).

The pMOVψ- mutant has been incorporated into the genome of a stable producer cell line called ψ2, and pMAVψ- resides in a line called ψ AM. When a recombinant is needed in quantity, initially it is constructed as plasmid DNA. This is then transfected into the appropriate producer cell line. Initially, the transfected DNA is in the nucleus, but is extrachromosomal. Transcription can occur at this stage, producing genomic viral RNA. This is only a transient phase, but recombinant virus may be harvested from the cell supernatant during this time. Usually the recombinant either carries or is cotransfected with a selectable marker. Selection for stable expression of this marker allows identification of clones with integrated virus. Since the recombinant is initially transfected rather than infected, its integrity may be vari-

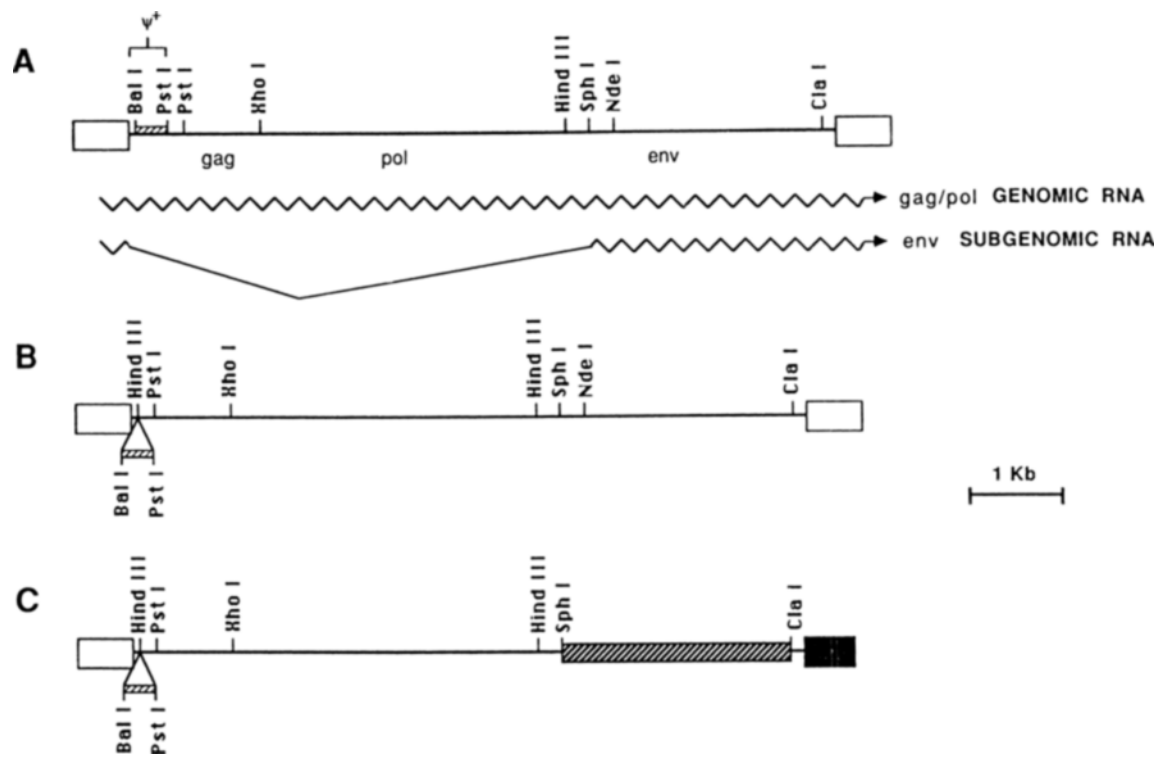

Fig. 2. Structure of wild-type Mo-MuLV, pMOVψ^-, and pMAVψ^-. A. The wild-type Mo-MuLV consists of three genes: *gag*, encoding for the group associated antigen found in the viral core, *pol*, coding for the reverse transcriptase, and *env*, coding for the viral specific envelope proteins. Some sites of restriction enzyme activity are shown. Transcription proceeds from the viral LTR and results in two mRNA strands, the complete or genomic strand, and a spliced or sub-genomic mRNA containing only the *env* message. B. pMOVψ^-, with the packaging sequence between the Bal 1 and Pst 1 restriction sites deleted. C. pMAVψ^-, containing the packaging deletion as well as a foreign *env* gene from 4070A, an amphotropic virus, which broadens the host range for recombinants produced with this mutant helper virus.

able, therefore affecting viral function in the producer line. However, once a stable producer cell line has been established, supernatant containing shed virus can be harvested and used to infect target cells. A potential target may be another packaging defective producer line. At least one other such line has been created using the Mo-MuLV (Miller et al., 1985). In addition, the rous sarcoma virus (RSV) with a packaging sequence deletion has been used as a helper virus, giving recombinants an avian host range (Majors, J., Grey, G., and Sanes, J. R., in preparation). This was used in studies of ontogenesis of the chick brain (*see below*).

Insertion Mutations

Just as in spontaneously or chemically mutated cells, those infected with viruses sometimes undergo disruptive changes affecting normal funtion. Although some of these effects may be owing to the introduction of virally encoded protein products into the cell, viruses occasionally insert into critical areas of the host genome, interrupting actual coding sequences or essential regulatory stretches of the DNA. Clearly, the controlled introduction of easily identifiable genome-disrupting viruses into cells of interest would provide a distinct advantage over classic mutagenesis techniques in the investigation of cell function and genetic regulation.

Viral Integration

Viral infection of cells is a very specific event determined by the *env* gene in the viral genome and by receptors on the cell membrane. Integration of the viral DNA into the host genome is dependent on the host's enzyme availability, and typically occurs in the presence of active DNA synthesis, most often during the S phase of mitotic cell division. Another factor that could be important during the S phase is the greater accessability of chromosomal DNA following breakdown of the nuclear membrane. Integration appears to be a random event, and is nonselective for particular host DNA sequences. Studies of proviral positions of the avian sarcoma virus (ASV) in transformed rat cells showed random integration at many different sites. Transformation was owing to the *src* oncogene carried by ASV (Hughes et al., 1978). In this and other studies (Shimotohno and Temin, 1980; Majors and Varmus, 1981), examination of the flanking sequences of the virus-cell junctions of several different proviruses revealed no common features of the host DNA integration site sequences. In addition, integration has been studied in detail in the cell-free bacteriophage lambda system. In this in vitro system retrovirus integrated into naked target DNA. These results indicate that supercoiling, chromatin structure, transcription, and replication of the lambda DNA were not required for integration of viral DNA (Brown et al., 1987).

Although viral integration occurs with no apparent host DNA sequence specificity, and intergration does not require that active transcription or replication be underway, there does appear to be some preferential integration into regions directly upstream of active sites of transcription (Harbers et al., 1984; Hawley et al., 1982; Schubach and Groudine, 1984; Wolf and Rotter, 1984; Ymer et al., 1985). Active transcription is usually seen on chromosomes in regions of open chromatin structure (Weintraub, 1985). These regions have been shown to be hypersensitive to DNase digestion, probably as a result of increased accessibility to the enzyme (Elgin, 1981). Likewise, preferential viral integration in the 5' region of actively transcribed genes may result from the increased accessibility to the virus of host DNA with open chromatin (van der Putten et al., 1982; Breindl et al., 1982).

Proviral Location in Selected Phenotypes

Studies of genotypes in cells transformed with retroviruses have shown that a variety of endogenous oncogenes, including Ha-*ras*, c-

erb-B, *int* 1 and *int* 2, c-*myc*, c-*mos*, and c-*myb*, can become activated by the presence of provirus. A vast body of work on tumors has thus far shown that

1. Certain viruses induce certain tumors; for example, Mo-MuLV and MCF proviruses induce lymphomas;
2. In experimental models, a particular oncogenic virus may integrate into 4 to 5 common sites within the genome of a given resultant tumor type;
3. Some of the common integration sites are not near the oncogene, and may even be on a separate chromosome; for example, in mouse lymphomas, c-*myc* is located on chromosome 15, and one selected integration site is on chromosome 17;
4. Integration may at times induce rearrangements within the transforming oncogene involved; and
5. Tumor formation may require simultaneous integration at more than one site.

An important point to note is that particular transformed phenotypes have been found to contain common viral integration sites (Teich et al., 1985).

Viral integration may influence genes other than oncogenes. Spontaneous insertion mutagenesis has been shown to be associated with the dilute and yellow lethal alleles (*see below*). In addition, intentional insertion mutagenesis (Goff, 1987) has identified a number of normal genes (Table 1).

The effectiveness of viruses as insertion mutagens has been more critically assessed in vitro in two studies. In the first a cell line transformed with a single copy of Rous sarcoma virus (RSV) was infected with Mo-MuLV. Reversion of the *src* transformed phenotype occurred, caused by inactivation of the single RSV *src* gene (Varmus et al., 1981). In the second study, inactivation of the cellular hypoxanthine-guanosine phosphoribosyl transferase (HPRT) gene on the X chromosome, which confers resistance to 8-azaguanine and 6-thioguanine, was observed following infection with Mo-MuLV (King et al., 1985).

Expression of Transduced Genes in Somatic Cells

In beginning to understand a complex biological system, the molecular biologist first turns to a prokaryotic system in order to isolate and clone a single discrete fragment of the eukaryotic genome. Eukaryotic genomes consist of about three billion nucleotides, encoding for between 50,000 and 100,000 genes (Ayala and Kreiger, 1984). Many of these genes are expressed in the nervous system and other tissues, but only a few uniquely expressed genes play critical roles in specific neurological functions. It is these that neurobiologists would be interested in cloning.

While the possession of a cloned gene of interest is very useful indeed, this alone is not sufficient for the elucidation of the mechanisms which regulate expression in eukaryotic systems. For example, specific DNA sequences are involved in recognizing and binding nucleoproteins and receptors (called "trans-acting factors"), thereby performing crucial roles in genetic regulation. Other mechanisms involving post-transcriptional and translational steps may also be important in the ultimate control of gene function. It is therefore essential to return cloned DNA fragments into eukaryotic cells where their function can be tested. The successful accomplishment of this task may well provide answers to questions about why specific gene products are differentially expressed in specific subsets of mature neurons.

Designing Vectors

The recombinant retrovirus contains only a few essential features; the packaging sequence required for encapsidation of the genomic viral RNA, and two flanking long terminal repeat sequences, which contain the promoter and enhancer elements. A great advantage of retrovirus recombinants as cloning vectors is the fact that multiple foreign genes may be introduced

Table 1

Normal Genes Identified Using Insertion Mutations Caused by Retroviruses[a]

Cell of origin	Mutagen	Affected gene	Mutant phenotype	Ref.[b]
Inherited mutations				
mouse germ line	ectropic MLV	coat color	"dilute" (d)	*1*
mouse germ line	ectropic MLV	agouti coat color	lethal	*2*
mouse germ line	Mo-MVL (mov-13)	α1 (I) collagen gene	embryonic arrest	*3*
Oncogenic mutations				
avian B cell	RAV-1, RAV-2 CSV, MAV, RPV	c-*myc*	lymphoma	*4*
chicken erythroblast	RAV-1	c-*erb-B*	erythroblastosis	*5*
mouse mammary cell	MMTV	*int*-1, *int*-2	mammary carcinoma	*6*
tissue culture cell		*src*	reversion of transformation	*7*
mouse T cell	MLV	MLVI-1,MLVI-2,c-*myc*	lymphoma	*8*
Somatic, inactivating mutations				
RSV-transformed rat-1	Mo-MLV	RSV provirus	morphological revertant	*9*
mouse hybridoma lines	IAP	κ light-chain Ig	decreased Ig production	*10*
mouse teratocarcinoma lines	MLV	HPRT	resistance to 8-azaguanine	*11*

[a] Adapted from Varmus and Swanstrom, 1985.

[b] *1.* Jenkins et al., 1981; Copeland et al., 1983a; *2.* Copeland et al., 1983a,b; *3.* Jaenisch et al., 1983; Schneike et al., 1983b; *4.* Hayward et al., 1981; Noori-Daloii et al., 1981; Payne et al., 1982; *5.* Fung et al., 1983; *6.* Nusse and Varmus, 1982; Peters et al., 1983; Nusse et al., 1984; Dickson et al., 1984; *7.* Varmus et al., 1981; *8.* Tsichlis et al., 1983a, b; Corcoran et al., 1984; *9.* Varmus et al., 1981; *10.* Hawley et al., 1982; Kuff et al., 1983; *11.* King et al., 1985.

simultaneously into a host cell. A very useful inclusion is an easily selectable marker, such as the *neo* gene, derived from the transposon Tn5, which confers resistance to G418 in mammalian cells and to kanamycin in *E. coli.* Other useful markers are *eco*GPT, from *E. coli*, which encodes for xanthine-guanine phosphoribosyl transferase; a mutant dihydrofolate reductase (DHFR), which confers resistance to methotrexate; and *his*, which encodes for an enzyme that converts L-histidinol to histidine (Hartman and Mulligan, personal communication).

The prototypic vector, pZIPNEOSV(X), is derived from the Mo-MuLV virus, and contains a unique BamHl cloning site, and the *neo* gene (Fig. 3). Any gene of interest may be cloned into the BamHl restriction site, and transcription proceeds from the viral 5' LTR. A spliced transcript containing *neo* also arises from the 5' LTR. Splicing occurs between the 5' donor and 3' acceptor sites. This splicing event permits the transcripts for both genes to proceed from the same promoter in the 5' LTR. These splice sites are normally involved in the formation of subgenomic messenger RNA encoding for the Mo-MuLV envelope protein. pZIPNEOSV(X) also contains two additional DNA fragments, from the SV40 virus and the pBR plasmid, encoding for their respective origins of replication. These are constructed into the vector to facilitate the rescue of the proviral sequence once it has integrated into the host genome (Cepko et al., 1984).

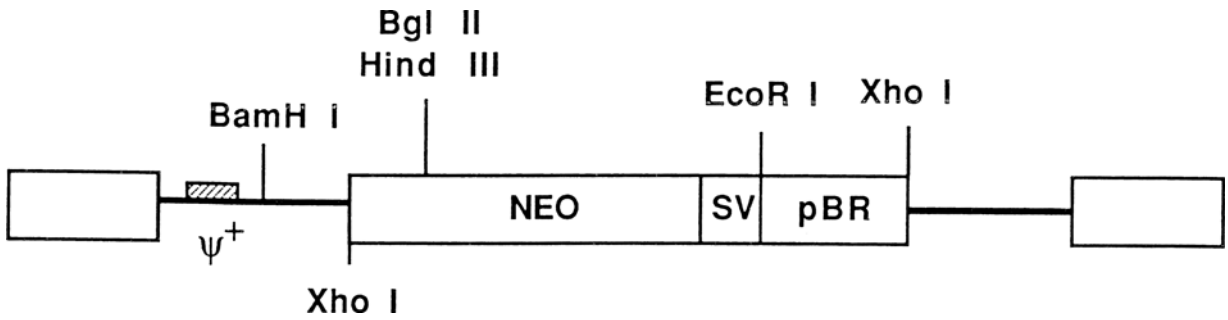

Fig. 3. pZIPNEOSV(X), a prototypic shuttle vector. This recombinant virus contains the packaging sequence, the *neo* gene, and origins of replication from the SV40 virus and the pBR plasmid. The origins of replication are included to facilitate subsequent rescue of the provirus from infected cells. Some restriction sites are shown.

A disadvantage of this vector is the fact that the gene introduced into the BamHl cloning site may contain cryptic splice sites, thereby interfering with the formation of both the genomic and subgenomic messages.

In order to avoid undesired splicing, the splice sites may be removed altogether and unique promoters may be introduced into the vector instead. One such vector is called DO (direct orientation), in which *neo* is transcribed from an internal SV40 promoter (Korman et al., 1987). There are several powerful promoters that can be incorporated into recombinants. Some, such as the promoter of the metallothionine gene from human or mouse cells, are inducible, in this case by the addition of heavy metals or steroids.

Initial studies, which demonstrated that such vector systems can be used to infect cells, were done using only selectable markers. However, more recently studies have involved the use of a variety of genes with more interesting functions, including those encoding for growth hormone (Miller et al., 1984), parathyroid hormone (Hellerman et al., 1984), β-globin (Soriano et al., 1986; Dzierzak et al., 1988), phenylalanine hydroxylase (Ledley et al., 1986), adenosine deaminase (Friedman, 1985; Kantoff et al., 1986), and the T-cell CD4 protein—the HIV I (human immunodeficiency virus I) receptor (Sleckman et al., 1987) (Table 2).

Self-Inactivating Vectors

It would be useful to use retroviral vectors for the study of regulatory genes. However, this is complicated by the fact that the Mo-MuLV LTR carries very powerful promoter and enhancer elements that may interfere with any promoters cloned into the recombinant. To overcome this problem, self-inactivating vectors were designed (Yu et al., 1986) (Fig. 4). In these constructs, the promoter and enhancer in the 3' U3 region are deleted from the viral LTR. During the process of viral replication, the U3 regions of both progeny LTRs are generated only from the U3 of the original virus' 3'LTR (Varmus, 1982). Therefore, all new viruses generated from the U3 defective virus (possible because transcription proceeds from the original virus' intact 5'LTR) will completely lack viral promoter/enhancer sequences. The same authors reported that when 3T3 cells were infected with self-inactivating vectors containing *neo*, no G418 resistance was found, demonstrating the efficacy of viral inactivation.

A similar construct was used to study expression of a cloned human genomic β-globin gene, which carries its own native promoter, in transgenic mice. Expression of the human gene was observed in all the hematopoietic tissues of the mice. However, the quantitative level of expression was directly dependent on proviral posi-

Table 2

Examples of Retroviral-Mediated Gene Transfer into Cells

Transduced gene	Target cell	Parent vector	Producer	Ref.[a]
neo	3T3TK$^-$	MSV	C1-1	*1*
Growth hormone	3T3, rat 208F	MSV		*2*
Growth hormone	3T3	MSV		*3*
HPRT	hematopoietic	MSV	Mo-MuLV	*4*
HPRT	human cells	MSV	Amphotropic	*5*
HPRT	3T3HGPT$^-$	MSV	Amphotropic	*6*
Col El replicon	mouse cell line MOP	SB302 SB401	ψ2	*7*
Parathyroid hormone	mouse GH4	MSVgpt	ψ2	*8*
Adenosine deaminase	mouse cells	ZIPNEO	ψ2	*9*
Human HLA-DR	3T3	Dolmtgpt	ψ2	*10*
Phenylalanine hydroxylase	hepatoma	SVNEO, ZIPNEO	ψ2	*11*
Growth hormone	Primary epithelial	DOL	ψ2, ψAM	*12*
neo, ElA	3T3, CV-1	ZIPNEO	ψ2	*13*
CD4	mouse T-helper hybridoma	lMNST4	Amphotropic DAMP	*14*
β-galactosidase	*in vivo* retina	DOL	ψ2	*15*
DHFR	*in vivo* hematopoietic	ZIPNEO	ψ2	*16*
neo, fos	3T3	SIN	ψ2	*17*
β-globin	whole mouse	ZIPNEOenh$^-$	ψ2	*18*
*eco*gpt	whole mouse	MSVgpt	ψ2	*19*

[a]*1.* Hwang and Gilboa, 1984; *2.* Miller et al., 1984b; *3.* Doehmer et al., 1982; *4.* Miller et al., 1984a; *5.* Miller et al., 1983; *6.* Miller et al., 1985; *7.* Berger and Bernstein, 1985; *8.* Hellerman et al., 1984; *9.* Friedman., 1985; *10.* Korman et al., 1987; *11.* Ledley et al., 1986; *12.* Morgan et al., 1987; *13.* Cepko et al., 1984; *14.* Steckman et al., 1987; *15.* Price et al., 1987; Turner and Cepko., 1987; *16.* Williams et al., 1984; *17.* Yu et al., 1986; *18.* Soriano et al., 1986, Dzierzak et al., 1988; *19.* Stuhlman et al., 1984.

tion in the host genome, demonstrating the important contribution of host genomic regulatory factors (Soriano et al., 1986).

Level of Expression of Transduced Genes

Most studies to date have been designed to show qualitative expression of transduced genes. A few have also successfully quantitated expression of the gene of interest. Human skin cells infected with a recombinant containing the growth hormone gene produced 11.4 ng/mg tissue/d, as compared with human pituitary cells that secrete as much as 1μg/mg tissue/d (Morgan et al., 1987). A mouse T-cell line infected with the human adenosine deaminase (ADA) gene produced human ADA activity at 25–50% of the endogenous mouse ADA activity level. A mouse T-cell hybridoma with a transduced CD4 gene produced 6–10 times more interleukin 2 when exposed to human class II HLA-DR antigens than did the uninfected par-

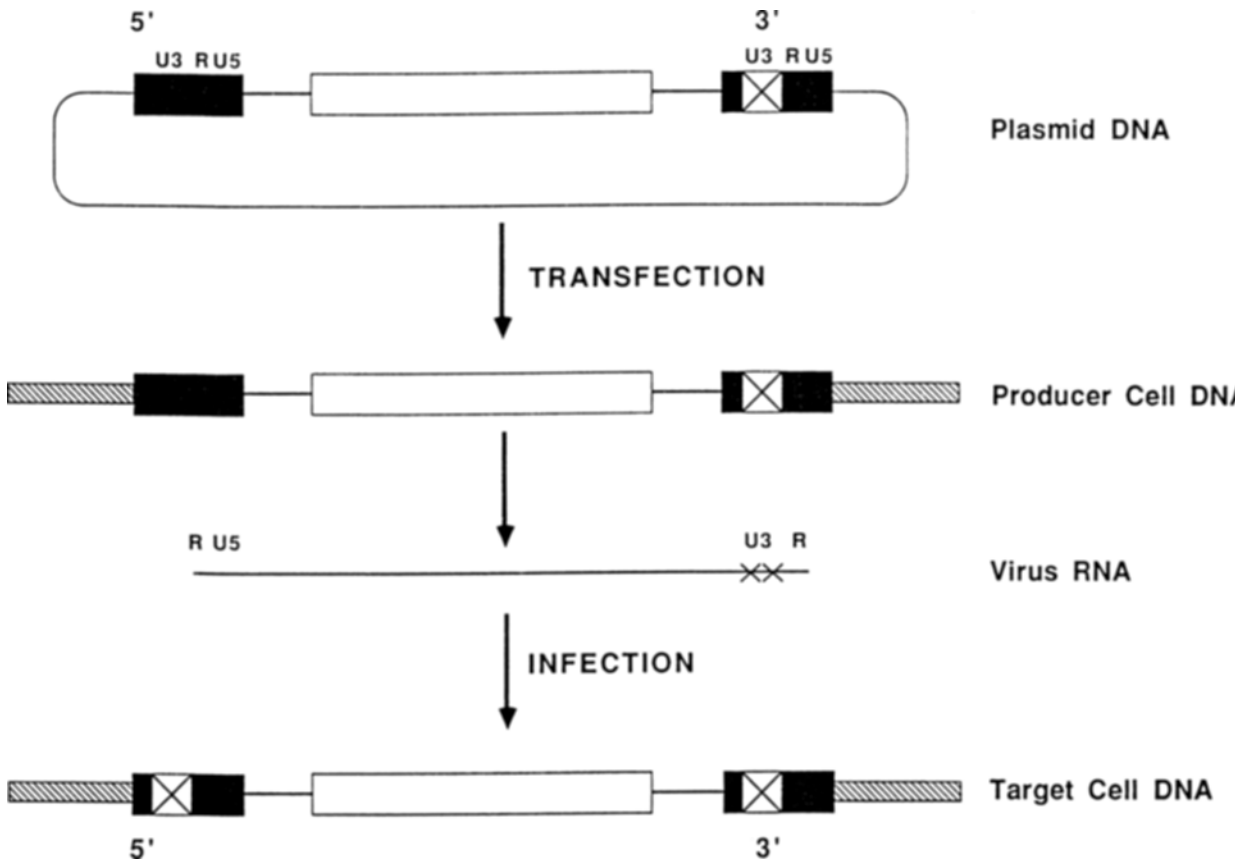

Fig. 4. Self-inactivating vectors. Because of details of viral replication, progeny virus particles contain U3 regions copied only from the 3' U3 region of the parent virus. Replication of the parent is possible because transcription proceeds from its intact 5' LTR. When the parental 3' U3 is defective, all progeny will have only defective LTRs, making further replication in the target cell impossible.

ent hybridoma cells. This demonstrated that the CD4 protein was functional, recognizing the HLA-DR antigens and enhancing the mouse hybridoma responsiveness to the human antigen stimulus.

Very recently, Mulligan and coworkers have obtained quantitatively appropriate levels of human β-globin in the mouse in vivo. Mouse bone marrow stem cells were infected with a retroviral vector containing the human β-globin gene. These cells were then used to salvage lethally irradiated mice. Stable and quantitatively appropriate expression of human β-globin was observed in only those tissues that normally express β-globin. Of particular interest is the fact that the retroviral vector used had a deletion of the enhancer in the 3'LTR. Furthermore, the human DNA fragment, containing the β-globin gene along with its native promoter, was placed into the retrovirus in reversed orientation to that of the normal retroviral genes, which are expressed from the viral LTR. Expression of the human β-globin gene was therefore regulated by its own native promoter (Dzierzak et al., 1988).

Construction of shuttle vectors with the recombinant genes placed in reversed orientation may present some unique problems. The most obvious is the concurrent production of sense and antisense RNA from the native promoter of the transduced gene and the viral promoter, respectively. This would tend to reduce the efficiency of virus production in the helper-free producer cell lines. Also, selectable genes expressed from self-inactivating viral promoters or enhancers that are initially expressed in the producer cell line may not be expressed following viral infection of the target cells. In spite of

these potential problems, it is clear that reverse orientation vectors can be very successfully employed, as is evident from this study. And, in fact, in this study producer cells made equal titers of enhancer minus vectors and those containing viral enhancers.

Therefore, genes transferred by recombinant retroviral infection have been shown to function in a quantifiable, physiological sense. Although viral expression is highly variable, this may well be owing to as yet unclear regulatory mechanisms, involving interactions between the retroviral promoter, trans-acting factors peculiar to the host cell type, and cis-acting factors operational according to the site of viral integration into the cellular genome. As these mechanisms become more clearly understood, the possibility of gene therapy for human diseases becomes increasingly feasible.

Regulation of Expression

Although expression of virally transduced genes is relatively efficient in somatic cells, many viruses are not expressed in preimplantation embryos and undifferentiated cell lines. It was noticed early on that the Mo-MuLV was not expressed when incorporated into the genome of embryonal carcinoma (EC) cells, suggesting that certain host cell factors must be involved in the regulation of expression of viral promoter sequences (Linney et al., 1984). It was further hypothesized that expression is somehow influenced by the stage of cellular differentiation or development. In addition DNA methylation may play a role in the repression of viral expression (Jähner et al., 1982).

In order to unravel the genetic mechanisms underlying the inhibition of expression in embryonal carcinoma cells, Jaenish and coworkers infected these cells with a recombinant virus carrying *neo*. Rare mutants were obtained which expressed *neo* (Fig. 5). It was found that restriction of expression could occur at many levels, but the main restriction appeared to be poor transcription from the viral promoter in the LTR. In one of the rare mutants expressing *neo*, a mutation was found in the viral tRNA primer binding site, which permitted proviral expression. Another mechanism found was transcription from a cellular promoter in the flanking sequence upstream from the provirus. Several mutants were found to have transcription from the same cellular promoter, suggesting that it was this selected integration site that allows transcription to occur. In order to demonstrate this conclusively, they rescued these proviruses along with their flanking sequences. These were attached to a bacterial marker gene, chloramphenicol acetyltransferase (CAT). When these were transfected into EC cells, CAT was expressed, whereas the viruses alone attached to the CAT gene (without their flanking sequences included) did not express CAT after being transfected into EC cells. Thus, an effective embryonic cellular promoter was identified in EC cells using retrovirus as a unique tool (Barklis et al., 1986).

The same strategy clearly could be used to identify a variety of tissue specific promoters. Since many tissues express the Mo-MuLV LTR very well, an obvious improvement would be to remove the viral promoter/enhancer sequences, as was done in the self-inactivating construct described above. In this case, any viral expression would necessarily arise from cellular promoters. Promoters rescued in this manner could conceivably be used for studies of differential gene expression.

Another group has used a recombinant retrovirus consisting of the Mo-MuLV backbone lacking the enhancer in the LTR, and carrying instead an enhancer from polyoma virus F101, which allows this virus to express itself in embryonal carcinoma cells, unlike the Mo-MuLV. Resultant transgenic mice generated by infection of preimplantation embryos showed expression of certain viral sequences in a limited number of tissues (van der Putten et al., 1985).

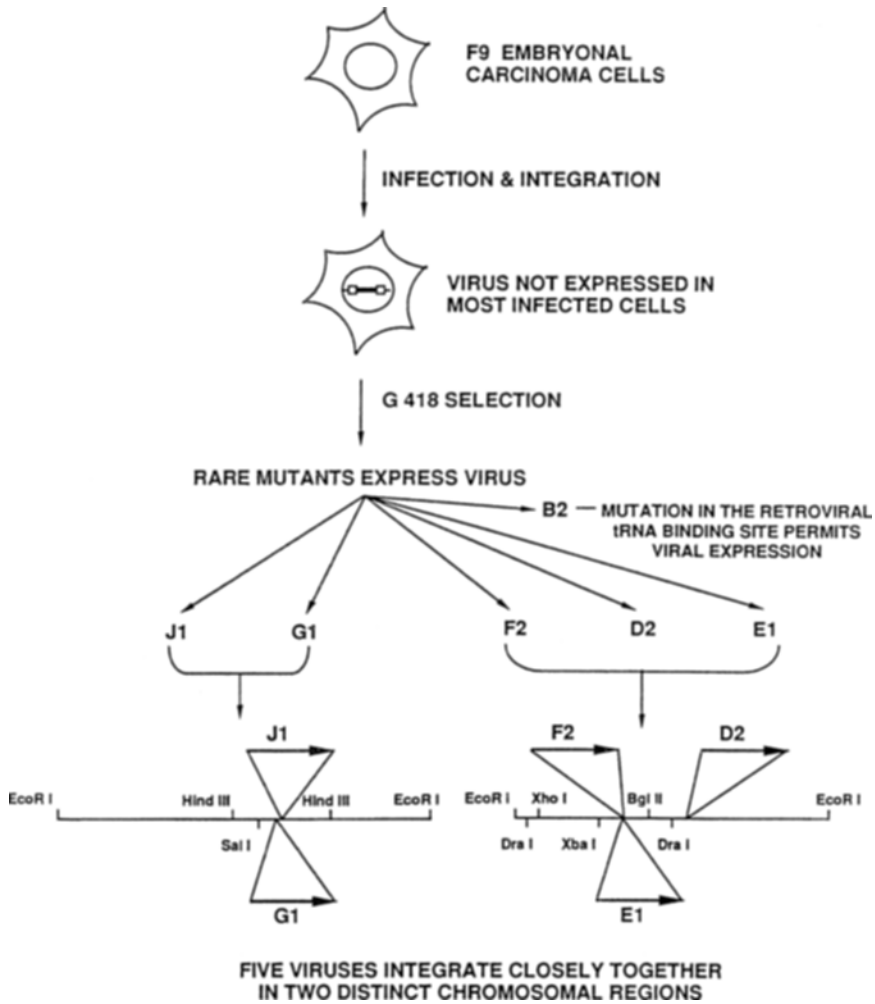

Fig. 5. Insertion mutation of embryonal carcinoma cells allows rare mutants to express the virus. Two chromosomal regions held five of the expressing proviruses, suggesting that these regions of the host DNA are important in allowing expression to occur. Three other expressing proviruses were found in three different, unrelated chromosomal regions. One of these, B2, was found to have a mutation within the viral genome that permitted expression (Barklis et al., 1986).

Insertion Mutagenesis in Whole Animals

Recombinant retroviruses have been used to introduce foreign genetic material into the embryonic tissues of mice at a variety of developmental stages. Retroviral insertion causes mutations with differing consequences depending on the gestational age of the embryo and the particular virus used for infection. Viral integration into genomic DNA is likely augmented by active DNA synthesis, which opens the chromatin structure, permitting access for integration (Breindl et al., 1984; Rohdewohld et al., 1987).

Advantages of retroviral infection compared to the technique first employed—microinjection of naked DNA—include the relative ease of *in utero* embryonic infection vs in vitro injection followed by implantation into foster mothers;

the intactness of the transferred genetic material is ensured, as viral integration occurs at the LTRs; and the resulting copy number per cell infected can be very low (often only one) as compared with microinjection techniques. In both methods, the viral DNA integrates randomly into the host genome, and can be transmitted through the germ line, although this does not always occur. Initially the mutant "transgenic" mice are heterozygous for the new genetic material, since only one allele of the diploid animal will be affected. Therefore, in these individuals, only dominant, nonlethal mutations will be identified. Intercrosses between these individuals must be done in order to identify recessive mutations in the offspring (possible only if germline transmission is present). These recessive mutations may, of course, be lethal. Transgenic animals have proven to be extremely useful for the identification of a number of normal genes (Table 1). Studies so far have been limited to the mouse.

In studies utilizing Mo-MuLV, mice arising from infected preimplantation stage embryos display integration in the germ line as well as in a variety of other tissue types. Intact Mo-MuLV proviral integration at this stage of development does not result in disruption of normal gene function, and animals develop normally (Jaenisch et al., 1975; Jaenisch, 1976). Mechanisms such as methylation and interruption of the formation of open chromatin structures interfere with the expression of the integrated provirus. However, post-implantation embryos microinjected *in utero* with the Mo-MuLV retrovirus do express integrated viral DNA in somatic cells (Jaenisch, 1980), and in one instance also carried the provirus in the germline. The strain resulting from this mutated embryo was ultimately found to have its α1 (I) collagen gene disrupted, causing death of homozygous embryos between d 13 and 14 of gestation (Harbers et al., 1984; Jaenisch et al., 1985).

Very recently, the first animal models for the human neuropsychiatric disorder, the Lesch-Nyhan syndrome, were described. One has been generated by an elegant use of retroviral insertional mutagenesis (Kuehn et al., 1987) (Fig. 6). Mouse embryonic stem cells grown in culture were infected with the *mos*$^{-1}$*neo* retroviral vector (produced in the ψ 2 helper-free cell line). Subsequent selection on 6-thioguanine allowed isolation of mutants lacking HPRT enzymatic activity. Clonal subpopulations of these mutant cells were then used to generate chimaeric animals (male and female) in which mutant cells have contributed to the germline. Male chimeras provide a potentially invaluable model for the investigation of underlying biochemical mechanisms in this X-linked human disorder. The retroviral marker eliminated the need to culture fibroblasts and assay for enzymatic activity, as is usually required in the human disease, and as was done in the other model system, which consists of chimaeric animals generated from spontaneously mutated HPRT deficient mouse stem cells (Hooper et al., 1987). A big advantage of this method compared with embryonic infection is that cells can be genetically manipulated extensively in culture, and specific features selected, prior to the generation of the transgenic animals.

Transduced Genes in Lineage Analysis Studies

In the past, development has been studied by anatomical, physiological, and biochemical methods. In vitro culture techniques and biochemical examination for specific markers have allowed for analysis of cellular lineage in several systems, including the central nervous system of mammals. A genetic marker that could be easily followed would greatly facilitate such studies.

About 10 years ago the wild-type retrovirus Mo-MuLV was used to infect mouse embryos at midgestation, by microinjection. As mentioned above, infected preimplantation embryos de-

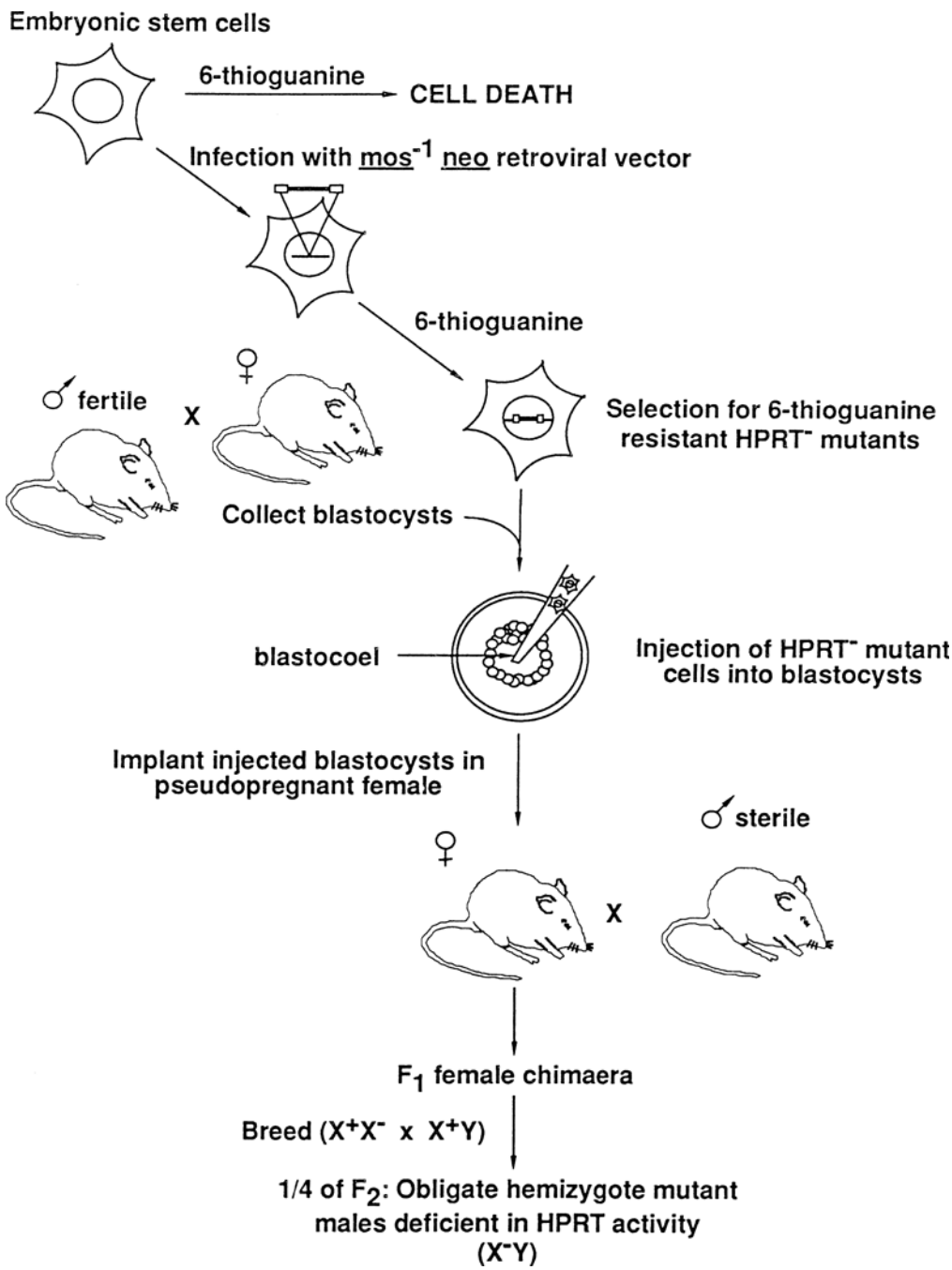

Fig. 6. An animal model for Lesch-Nyhan syndrome: construction of HPRT deficient chimaeric animals by retroviral mediated insertion mutation (Kuehn et al., 1987).

veloped normally, without apparent expression of the viral sequences (Jaenisch et al., 1975; Jaenisch, 1976). However, infected postimplantation, or midgestion, embryos developed viral expression in all tissue types studied, most became viremic, and these succumbed to leukemia between the fourth and sixth months of life. In contrast, infected newborn mice displayed viral integration in only the spleen and thymus. These, too, subsequently died of leukemia. This seems to indicate that it is the stage of cellular differentiation that determines the tissue specificity of infection, and that the earlier an embryo is infected, the wider the viral distribution in postnatal tissues will be. Limiting factors on these studies included the lack of viral expression in preimplantation embryos, and the fact that all infected individuals expressing virus died at a fairly early age, limiting studies on development of lineage relationships in tissues of adult animals (Jaenish, 1980).

The advent of recombinant retroviruses, and use of these for lineage analysis studies, has eliminated most problems of lethal viral proliferation and untimely death of experimental animals. Sanes and coworkers (Sanes et al., 1986) used a construct consisting of the Mo-MuLV backbone with a fragment of the *E. coli lacZ* (LZ1) gene that encodes for β-galactosidase. Infected cells can be visualized by a histochemical stain for the enzyme. Infection of a variety of cell lines in culture demonstrated that integration had no deleterious effects on viability or growth. Clones of infected cells were detected in the skin, skull, meninges, brain, visceral yolk sac, and amnions of infected midgestation mouse embryos. Previously established lineage relationships were confirmed in yolk sac and skin. Evidence for pluripotential ancestors and progressively restricted cell fates was obtained for each tissue.

Another interesting application of this technology has been in studies of lineage relationships within the hematopoietic system. Stem cells infected with a recombinant retrovirus carrying the bacterial *neo* gene, which confers resistance to G418 in mammalian cells, have been introduced into anemic W/W^v mutant mice. Bone marrow was reconstituted and produced the full complement of myeloid and lymphoid cells all expressing *neo* (Dick et al., 1985). Bone marrow stem cells were infected with a similar retrovirus carrying *neo* and the DHFR gene as markers. Lethally irradiated mice were salvaged by the infected bone marrow cells, which expressed *neo* and DHFR (Williams et al., 1984). These studies served to confirm previously suspected lineage patterns within hematopoietic tissue. Dog, sheep, monkey, and human bone marrow tissues have been successfully infected in vitro (Gilboa et al., 1986; Hock and Miller, 1986; Kantoff et al., 1986). Bone marrow of sheep and nonhuman primates has been successfully infected in vivo, with resultant recombinant viral genome expression (Giboa et al., 1986).

The complexity of the central nervous system and the nature of the cells that comprise it pose special problems for development and lineage analysis studies. Unlike in the hematopoietic system, progenitor cells cannot be effectively isolated from adult CNS tissues. In addition, neuronal cells cannot be propagated in tissue culture. During normal development there is excessive neuronal proliferation, followed by massive cell death. Some neurons migrate, differentiate, and form synapses, resulting ultimately in the complex architecture required for normal brain function. A stable genetic marker, such as a retrovirus, which could be introduced into progenitor cells during very early stages of development, would provide a powerful tool for the study of these complex events.

Very recently, Sanes and coworkers have successfully used the same recombinant retrovirus (LZ1) described above, as well as other similar constructs, in cell lineage studies in the developing nervous system. Viral concentrate was injected into the telencephalon of rat embryos *in utero*. When serial sections of intact

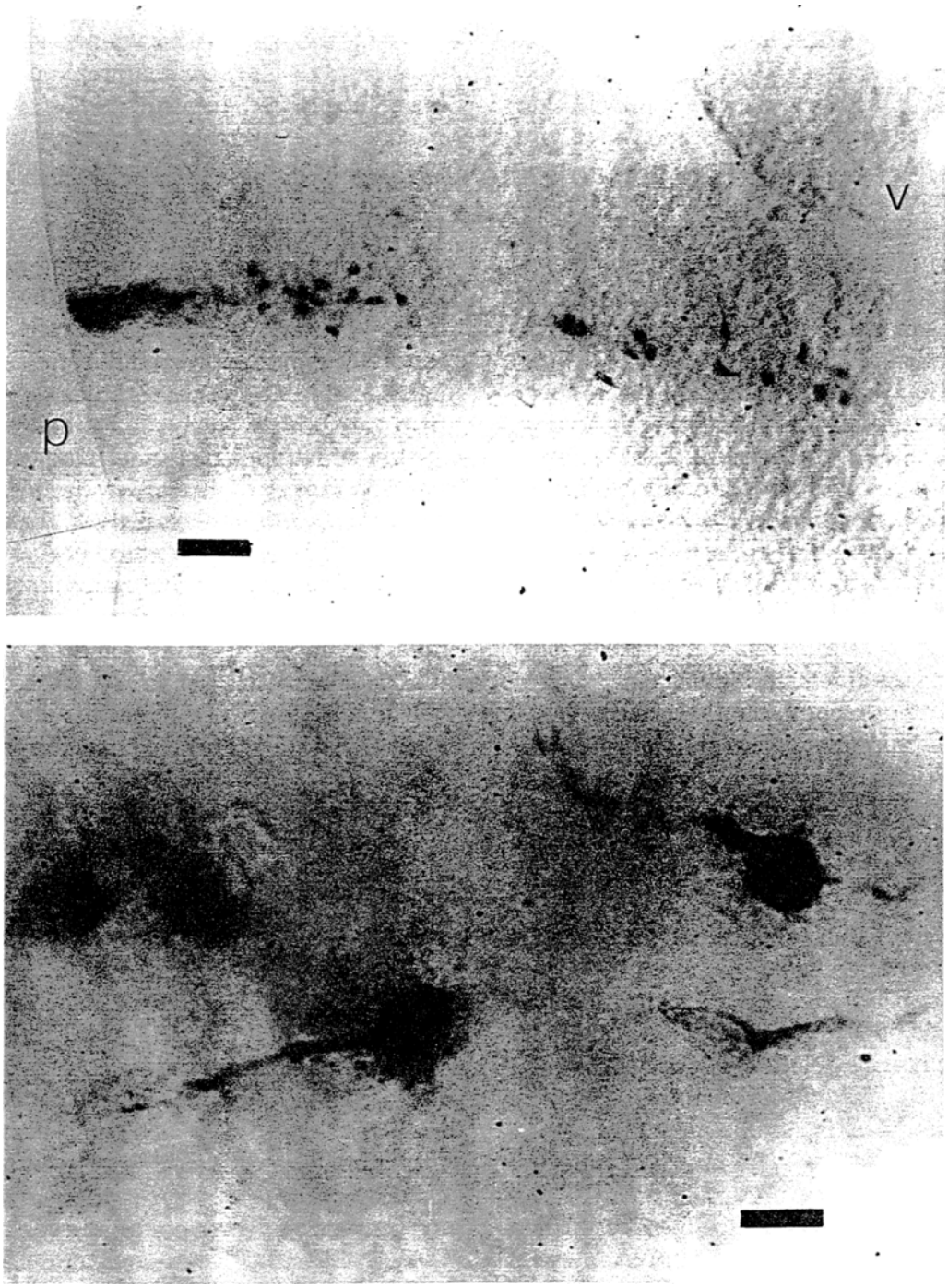

Fig. 7. Lineage analysis in developing chick frontal cortex. A. Thick handcut section of chick optic tectum at embryonic stage 33, following injection of recombinant retrovirus into the tectal ventricle during stage 16, a period of active cellular proliferation. Section extends from the pia (P) to the ventricle (V). A clonal population of about 30 cells stains dark blue in the assay for β-galactosidase activity. The columnar arrangement of the cortical laminae is demonstrated (Glover et al., 1987). Bar = 60 μm. B. High power view of single cells from the clonal population shown in A, demonstrating that discrete cells express the recombinant virus. Bar = 10 μm. Photos kindly provided by J. R. Sanes.

postnatal brains were examined, *lac Z* positive clones consisting of 2-10 cells were identified in cerebral cortex and several other brain regions (Luskin et al., 1987). Likewise, in developing chick nervous system, when retrovirus was injected into the tectal ventrical *in ovo, lac Z* positive clones were subsequently identified in later stages of embryonal development (Glover et al., 1987) (Fig. 7).

Although retroviral infection of postnatal tissues is more difficult to accomplish, it has been done successfully in rat retina and primary neural cultures, including cerebral cortex. Injection of neonatal rat retinas with, again, a recombinant Mo-MuLV carrying the β-galactosidase gene, resulted in the identification of cell clones in the retina consisting of either single or multiple cell types. Rod, bipolar, amacrine, and Müller glial cells were identified, all four types being postnatally mitotic for one week. It was demonstrated that rods, bipolar cells, and amacrine cells have a common lineage until late, that rods, bipolar cells, and Müller glia share a lineage, and that rods and Müller glia can have a common progenitor as late as to the final mitotic event (Price et al., 1987; Turner and Cepko, 1987).

Molecular Mechanisms Involved in Neurotoxicity

Our laboratory has used retroviral mediated gene transfer to investigate neurotoxicity at a molecular level. More specifically, we have chosen to infect cells and then select a specific mutant phenotype and search for viral integration into common chromosomal regions, which if present may reflect mutations in areas of DNA crucial to the selected phenotype. We have used a rat pheochromocytoma line, PC12, which resembles sympathetic neurons in many ways, and possesses high affinity catecholamine uptake (Greene and Tischler, 1982).

N-Methyl-4-phenyl-1,2,3,6-tetrahydopyridine (MPTP) is highly neurotoxic, causing neuropathologic changes in humans often resulting in an illness resembling Parkinson's disease (Davis et al., 1979; Langston et al., 1983). The etiology of MPTP neurotoxicity involves the production of 1-methyl-4-phenyl pyridine (MPP^+) by monoamine oxidase B in brain mitochondria (Chiba et al., 1984; Markey et al., 1984). MPP^+ is avidly accumulated in both dopamine and norepinephrine neurons by the catecholamine uptake system, whereas MPTP is not apparently accumulated (Javitch et al., 1985). MPP^+ is concentrated in these specific neurons to a lethal level, causing the selective destruction of catecholaminergic neurons in the central nervous system (Javitch et al., 1985). Both MPTP and MPP^+ are cytotoxic to pheochromocytoma (PC12) cells, the latter being more potent (Snyder and D'Amato, 1986; Denton and Howard, 1987). This toxicity is mediated by uptake of MPP^+ via the catecholamine uptake site (Snyder and D'Amato, 1986). Some PC12 mutants resistant to MPTP have arisen spontaneously (Denton and Howard, 1984). These mutants are apparently defective in many of their usual neuronal properties; for example, they have markedly reduced levels of tyrosine hydroxylase and choline acetyl transferase activities, negligible dopamine content, and no sodium dependent uptake of dopamine. These cells are also not responsive to added NGF and become "fibroblast-like," although they remain tumorogenic (Bitler et al., 1986).

We infected PC12 cells with a recombinant retrovirus (pZIPNEOSV(X)) containing the *neo* gene, which confers resistance to G418 (Cepko et al., 1984). We have selected infected cells in a lethal concentration of the active metabolite, MPP^+, which normally kills PC12 cells (Fig. 8). Several mutants surviving the toxic effects of MPP^+ were isolated, and used for studies to understand what genetic mutations these cells have suffered. As previously described (Barklis et al. 1986; Goff, 1987) the virus provides a useful

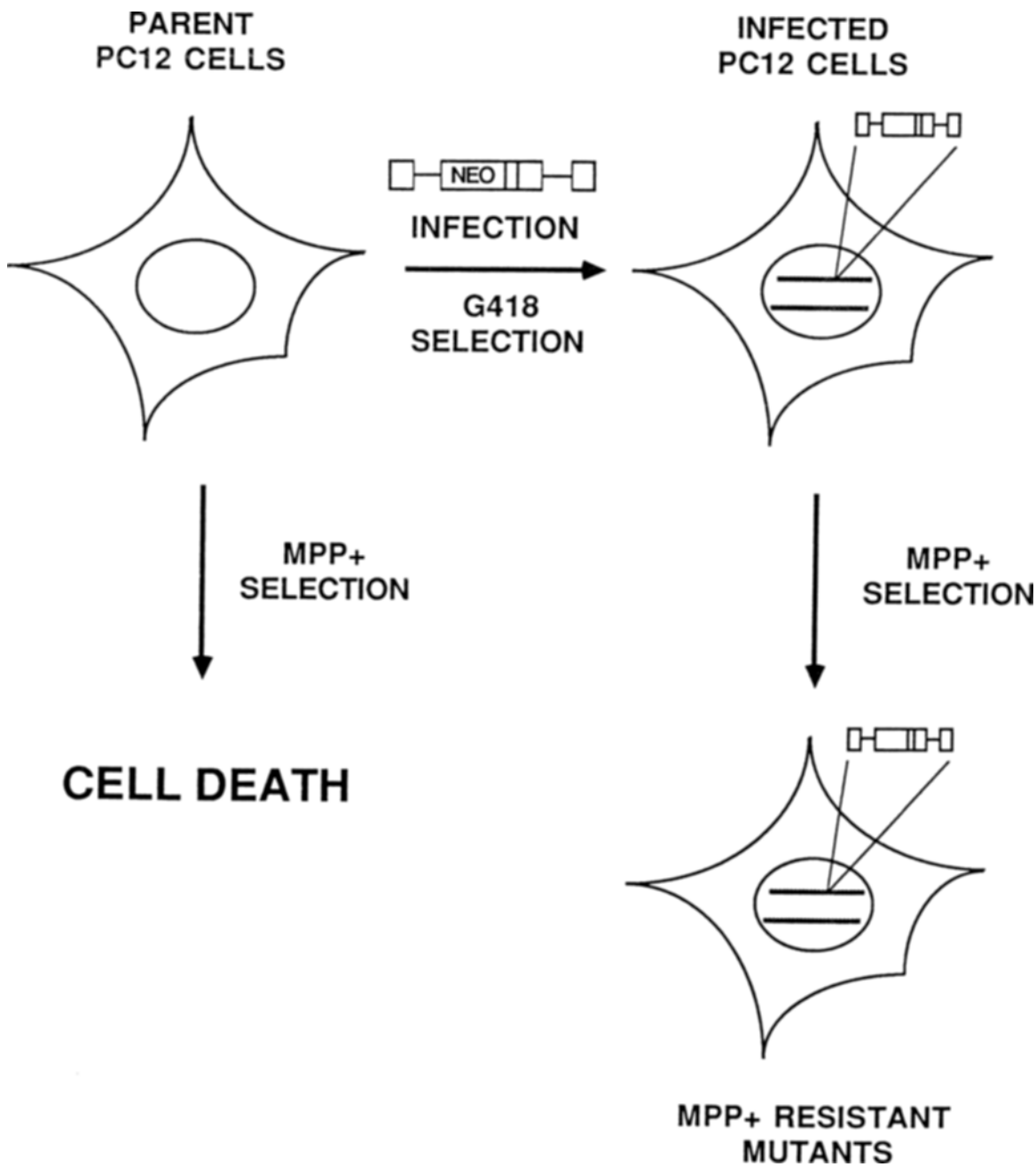

Fig. 8. Studies of MPP^+ neurotoxicity using retroviral insertion mutation. ZIPNEOSV(X) was used to randomly mutate PC12 cells. Successfully infected cells were isolated by selection on G418 (using resistance conferred by *neo*). These were then subjected to selection on the neurotoxin MPP^+. Southern blot analysis of 26 MPP^+ resistant mutant lines showed distinct chromosomal regions of frequent viral integration. This suggests that these host DNA regions contain sequences important in MPP^+ neurotoxicity in PC12 cells.

tool for isolating these disrupted DNA sequences. Proviral integration was found to occur in common chromosomal areas in some mutants. A large fragment containing the provirus and about 12 kb of flanking DNA was rescued from an MPP^+ selected PC12 mutant which had suffered a viral insertion in one of the frequently mutated loci. Coding sequences were detected in three different areas of the rescued genomic DNA. The cDNA for these genes were cloned from a normal PC12 cDNA library. These genes are specifically expressed in PC12 cells and not in two other unrelated neuroblastoma cell lines (NG108 and NCB20). Therefore, MPP^+ can be used to isolate virally infected mutants in which regions of the PC12 genomic DNA, normally encoding for proteins essential for mediating the toxicity, have been disrupted.

In order to ascertain whether resistance to MPP^+ is caused by viral insertion mutation, we measured the number of infected cells in the MPP^+ selected population (1 in 140), and compared it to the infected but unselected population (1 in 10^4) (Kadan and Lo, in preparation). In other words, one is about 100 times more likely to find a virally mutated cell in the MPP^+ selected population than the original unselected population. We believe that the resistance of infected cells (doubly selected with G418 and MPP^+) to MPP^+ can be attributed to viral insertion mutagenesis.

One may wonder about the problem of creating single-copy insertional mutations in cell lines that are genetically diploid, as we have done using PC12 cells. It would seem that such mutations would usually remain silent in such cell lines (unless they are dominant), and that resultant mutant phenotypes would have to be explained by other, perhaps spontaneous, mutational events in the second allele. However, there is now much evidence that the majority of cell lines become functionally haploid after being carried for some time in culture (Ayala and Kreiger, 1984). Therefore, one can attribute phenotypic mutational change to retroviral insertion with a reasonable degree of confidence. Of course, the ultimate proof lies only in the reintroduction of the normal version of the disrupted gene back into the mutant cells for demonstration of reconstitution of normal phenotype.

Future Perspectives

We have reviewed a number of applications of retroviral technology, most from outside the field of neurobiology. These range from the delivery of a cloned gene for studies of its expression, to the less obvious applications of gene transfer, such as use in lineage analysis studies and as tools for insertion mutagenesis.

One thing that has emerged from these studies is that successful gene transfer depends upon construction of a suitable vector. Depending on the goal of the study, various factors need to be considered. When expression of a delivered gene of interest is desired, one needs a strong promoter in the construct, and complications involving cryptic splice sites delivered along with the gene need to be kept in mind. When wanting to study the expression of a gene from its native promoter and enhancer (constructed into the recombinant virus along with the gene of interest), as in the β-globin study above, one must delete the viral U3 region. One must also realize that different insertion sites may cause different levels of expression of the gene of interest, depending on cellular cis-acting factors. In attempts to rescue cellular promoter/enhancer sequences, one must again delete the viral U3 region, and make certain that the construct contains no internal promoters. In addition, one must rule out the possibility that the construct has recombined with the helper virus when cotransfected into the producer cell line, an event that may result in incorporation of a helper virus promoter. When using retroviral insertion for the purpose of causing mutation in

the host cell, one needs to have useful restriction sites in the construct in order to facilitate subsequent mapping and cloning. The possible long-range effects of viral LTRs on distant cellular genes—even those located on other chromosomes—need to be considered as well. Subsequent to mutation, retroviruses can again be used to reintroduce the normal gene back into the mutant cells to check for reconstitution of normal function. Finally, when using retroviral infection to study lineage and development, one needs to incorporate a useful marker that does not interfere with cell or tissue function, and that can be easily assayed.

A very recent improvement in retroviral gene transfer technology is the construction of a helper virus system, called ψ- crip, that eliminates the problem of low pMOVψ- packaging frequency (O. Donos and R. C. Mulligan, in preparation). The system contains two recombinant retroviruses as helpers; one is the packaging mutant pMOVψ- with an additional insertion mutation in the *env* gene, and the other contains only an intact *env* gene but no *gag* or *pol* sequences. Therefore, even if either one happened to become packaged, neither would be capable of subsequently generating any infectious virions. In the producer system, however, they complement each other in their *trans*-functions.

It is evident from successful studies to date that an additional important requirement is to have a producer cell line generating high titers of infectious recombinant retrovirus. Titers of a good producer line range from 10^5 to 10^6 cfu/mL (colony forming U/mL). Although virus expressed during the transient (epichromosomal) stage can be used, the titers usually range between 10^2 and 10^3 cfu/mL. This may be satisfactory in in vitro studies of gene expression where the target cell is not in limited supply, but in vitro work with primary cultures and in vivo work require a higher virus:target ratio to ensure successful infection. Certainly, in our hands, extremely large numbers of cells need to be infected before mutants can be identified, and very high titer producers are therefore needed. In addition, it has recently become clear that incorporating a portion of the *gag* gene into a recombinant retrovirus enhances its expression (Gilboa et al., 1986; Mulligan, personal communication). However, there are times when a given construct will not produce an adequate titer. This is no doubt owing at least in part to as yet poorly understood molecular regulatory mechanisms.

Clearly retroviruses provide an extremely versatile, powerful tool for the study of a variety of molecular problems. Applications in neuroscience are currently just getting underway, but their use in other fields demonstrates the many exciting possibilities for neurobiological investigations. For example, transgenic animals could be generated in order to search for the molecular basis of hereditary neurological disorders, such as Huntington's disease. Lineage relationships in the various neuronal subpopulations could be studied, as well as differential gene expression in developing and adult cell populations. Mechanisms of neurotoxicity may be elucidated. The cloning of neuroreceptors may be facilitated by means of insertion mutations. Retroviruses may be used to immortalize primary cells of interest to the neuroscientist, as often transformation occurs with few other changes. This has in fact very recently been accomplished in rat optic nerve cells using a Mo-MuLV recombinant containing the SV40-T oncogene and the *neo* gene (Geller and Dubois-Dalcq, in preparation).

Perhaps one of the most exciting future applications, in neuroscience and in other biomedical fields, will be somatic cell gene therapy. The studies discussed above, in which stable expression of a transduced β-globin gene was obtained in the hematopoetic system in vivo, demonstrate that this therapeutic approach may become very feasible in the future.

Retroviruses in CNS Diseases

In addition to the "genetic engineering" applications of retroviruses, the study of retroviral functioning itself promises to provide impor-

tant insights into a number of neurological diseases. There has been much speculation regarding the role of viruses in certain human neurological or neuropsychiatric disorders. However, most of these hypotheses, such as the viral hypotheses of the etiologies of multiple sclerosis, amyotrophic lateral sclerosis, and schizophrenia (Torrey and Peterson, 1976; Weinberger et al., 1983; Stevens et al., 1984), remain to be proven.

One proven neurotropic virus is the AIDS (HIV) virus (Shaw et al., 1985). Manifestations of HIV infection in humans include neurological and psychiatric symptoms, such as generalized hyperreflexia, increased muscle tone, dementia, ataxia, or paraparesis in some individuals. Often the presenting picture of HIV infection is one of decreased concentration and mild memory impairment, which progresses eventually to one of severe encephalopathy involving global cognitive and motor deterioration.

The AIDS virus (HIV) has been found to be closely related to the visna virus (Gonda et al., 1985), a well-studied member of a class of retroviruses, called the lentiviruses, that infects ungulated mammals (Gudnoadottir and Palsson, 1967). Lentiviruses cause persistent infections resulting in degenerative illnesses. The visna virus causes a neurological disease in sheep, resulting in aberrant gait, trembling of the lips, unnatural tilt of the head, blindness, and progressive paralysis of the hind limbs. Histopathologically one sees destruction of the tissues, with cell lysis resulting in inflammation, especially in the periventricular choroid plexus and the meninges. Syncytia formation may be observed in cells infected with the visna virus in vitro (Teich et al., 1984).

An interesting approach that may be taken in the future would be to use components of these naturally occurring, neurological disease producing, viruses for the construction of recombinants, as has been done with the Mo-MuLV. These would provide highly selective tools, in terms of tropism, for primate and human neurobiological studies.

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