

REVIEW

Viral vectors for gene delivery and gene therapy within the endocrine system

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Abstract

The transfer of genetic material into endocrine cells and tissues, both *in vitro* and *in vivo*, has been identified as critical for the study of endocrine mechanisms and the future treatment of endocrine disorders. Classical methods of gene transfer, such as transfection, are inefficient and limited mainly to delivery into actively proliferating cells *in vitro*. The development of viral vector gene delivery systems is beginning to circumvent these initial setbacks. Several kinds of viruses, including retrovirus, adenovirus, adeno-associated virus, and herpes simplex virus, have been manipulated for

use in gene transfer and gene therapy applications. As different viral vector systems have their own unique advantages and disadvantages, they each have applications for which they are best suited. This review will discuss viral vector systems that have been used for gene transfer into the endocrine system, and recent developments in viral vector technology that may improve their use for endocrine applications – chimeric vectors, viral vector targeting and transcriptional regulation of transgene expression.

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Introduction

Gene delivery into the endocrine system poses a series of challenges, which include the expression of transgenes within post-mitotic or very slowly proliferating cells, the need to restrict gene expression to pre-determined cell types in order to avoid non-specific effects to neighbouring cells, the need to prevent vector spread to non-target structures, and the need to extend the duration of transgene expression to desired time intervals (Lowenstein *et al.* 1999). To reduce non-specific diffusion of vectors, local delivery using direct injection into the target tissues is the method of choice. This constraint has favoured the use of viral vectors for gene delivery, rather than non-viral systems such as naked DNA- or liposome-mediated gene transfer. Recent developments have made possible enhanced and sustained transgene expression from Epstein–Barr Virus (EBV)-based mini-chromosome vectors (Saeki *et al.* 1998b, Tsukamoto *et al.* 1999).

This review concentrates on the main viral vector systems that have the potential for gene transfer applications in the endocrine system. The advantages and disadvantages of each system will be discussed, as will the ability to restrict transgene expression to predetermined cell types within endocrine tissues. Transcriptional target-

ing of transgenes within mammalian cells is an area of rapid development. Viral promoters, which have been used routinely to drive transgene expression, can be down-regulated with time, especially *in vivo*. Conversely, cell-type specific mammalian promoters are several-fold weaker than their viral counterparts. Thus hybrid and synthetic promoters are being developed in order to retain the cell-type specificity and provide high levels of transgene expression. The possibility of regulating levels of transgene expression is also a very attractive prospect and, to achieve this, synthetic regulatory elements from prokaryotes and mammalian systems are being developed. These systems can be regulated using small molecules, the most common of which is derived from the bacterial tet operon. This system will be discussed and examples of its use within the endocrine system will be highlighted. New developments in viral and non-viral gene delivery systems occurring in the future will dramatically improve the potential for therapeutic gene transfer into the endocrine system.

Retrovirus vectors

Retroviruses are enveloped single-stranded RNA viruses, which have been widely used in gene transfer protocols.

Retroviruses have a diploid genome of about 7–10 kb, composed of four gene regions termed *gag*, *pro*, *pol* and *env*. These gene regions encode for structural capsid proteins, viral protease, integrase and viral reverse transcriptase, and envelope glycoproteins, respectively. The genome also has a packaging signal (Ψ) and *cis*-acting sequences, termed long-terminal repeats (LTRs), at each end, which have a role in transcriptional control and integration. The most commonly used retroviral vectors are based on the Moloney murine leukaemia virus (Mo-MLV) and have varying cellular tropisms, depending on the receptor binding surface domain (SU) of the envelope glycoprotein. Mo-MLV vectors with a strictly murine host range are termed ecotropic (Mo-MLV-E), the native receptor for Mo-MLV-E being the cationic amino acid transporter, CAT-1 (Weiss & Tailor 1995). Mo-MLV vectors with a murine and human host range are termed amphotropic (Mo-MLV-A), and the native receptor for Mo-MLV-A is the transmembrane phosphate transporter, RAM-1 (Weiss & Tailor 1995).

After binding to its extracellular receptor, a conformational change within its envelope glycoprotein enables the retroviral envelope to fuse with the cell membrane and allows the release of the capsid core into the cytoplasm. Once inside the cytoplasm, the single-stranded RNA genome is reverse-transcribed into a double-stranded DNA proviral genome by the viral reverse transcriptase inside the capsid. The proviral genome then forms a pre-integration complex with the viral integrase and this is transported to the nuclear membrane. At the nuclear membrane, mitosis must occur to enable the pre-integration complex to enter the nucleus as the nuclear membrane becomes disrupted (Roe *et al.* 1993). Having entered the nucleus, the viral integrase can randomly integrate the proviral genome into the host chromosomal genome, where the host's transcriptional machinery gives rise to expression of viral genes (see Fig. 1A for retrovirus life cycle).

Recombinant retroviral vectors are deleted of all retroviral genes, which are replaced with marker or therapeutic genes, or both. The LTRs and Ψ sequence are the only viral sequences that remain in the vector and, because of this, infected cells are unable to express any viral proteins. To propagate recombinant retroviruses, it is necessary to provide the viral genes, *gag*, *pol* and *env* in *trans* (Fig. 1B). This is possible by creating packaging cell lines that express these genes in a stable fashion. With this system, it is possible to produce viral titres of 10^5 – 10^7 colony forming units/ml (Palmer & Gage 1996).

The disadvantages of retroviral vectors include: the random insertion into the host genome, which could possibly cause oncogene activation or tumour-suppressor gene inactivation; the limited insert capacity (8 kb); the low titres; their inactivation by human complement; the inability to transduce non-dividing cells and the potential shut-off of transgene expression over time. Their

advantages include the ability to stably transduce dividing cells, the inability to express any viral proteins that could be immunogenic and the ability to achieve long-term transgene expression (over 2 years) in humans (Bordignon *et al.* 1995).

Because of the low mitotic rates of most endocrine cells, retroviral vectors based on Mo-MLV are not ideal for direct *in vivo* gene transfer to the endocrine system. Alternatively, *ex vivo* techniques, in which endocrine cells are transduced *in vitro* and then transplanted back into the host, may be of use. Recombinant retroviruses have been used to transduce anterior pituitary AtT20 cells (Wolf *et al.* 1988, Horellou *et al.* 1989), thyroid cells (O'Malley *et al.* 1993, O'Malley & Ledley 1993, Ivan *et al.* 1997) and pancreatic cells (Leibowitz *et al.* 1999). However, transduction efficiency is low (\sim 3–30%). Retroviruses may be of more use for endocrine cancer, in which the tumour cells can be transduced as they undergo mitosis. This approach has been used by Braiden *et al.* (1998), who were able to kill rat and human thyroid carcinoma cells, using a retrovirus carrying the suicide gene, herpes simplex virus thymidine kinase (*HSV-TK*), under the control of the thyroglobulin promoter. The difficulty of this approach, however, is the need to target all tumour cells to yield an effective treatment.

Lentiviruses are complex retroviruses that have the ability to infect and express their genes in both mitotic and post-mitotic cells. They are able to do this because they have two virion proteins (matrix and Vpr) that interact with the nuclear import machinery and can transport the pre-integration complex across the nuclear membrane from the cytoplasm to the nucleus in the absence of mitosis (Naldini 1998). The most commonly known lentivirus is the human immunodeficiency virus (HIV). Naldini *et al.* (1996) have constructed a viral vector system, based on HIV, which uses the envelope glycoproteins of other viruses (vesicular stomatitis virus (VSV-G) and Mo-MLV-A) to target a broad range of cell types. The system used to generate these lentiviral vectors requires a three-plasmid co-transfection in 293 cells. The first plasmid, which has no packaging signal Ψ , provides all the viral genes in *trans*, with the exception of *env* and some accessory proteins; the second plasmid carries the gene for the pseudotyped envelope protein; the third plasmid carries the transcription unit of choice in addition to the *cis*-acting sequences of HIV needed for packaging, reverse transcription and integration. This HIV-derived vector system is unlikely to generate replication-competent retrovirus (RCR), as two independent homologous recombination events would have to occur. The viral sequences, split onto three separate plasmids, are unlikely to recombine, as they have been generated to have low sequence homology (Naldini *et al.* 1996, 1997, Naldini 1998).

This lentiviral vector system has all the advantages of Mo-MLV-based retroviral vectors, alongside the added

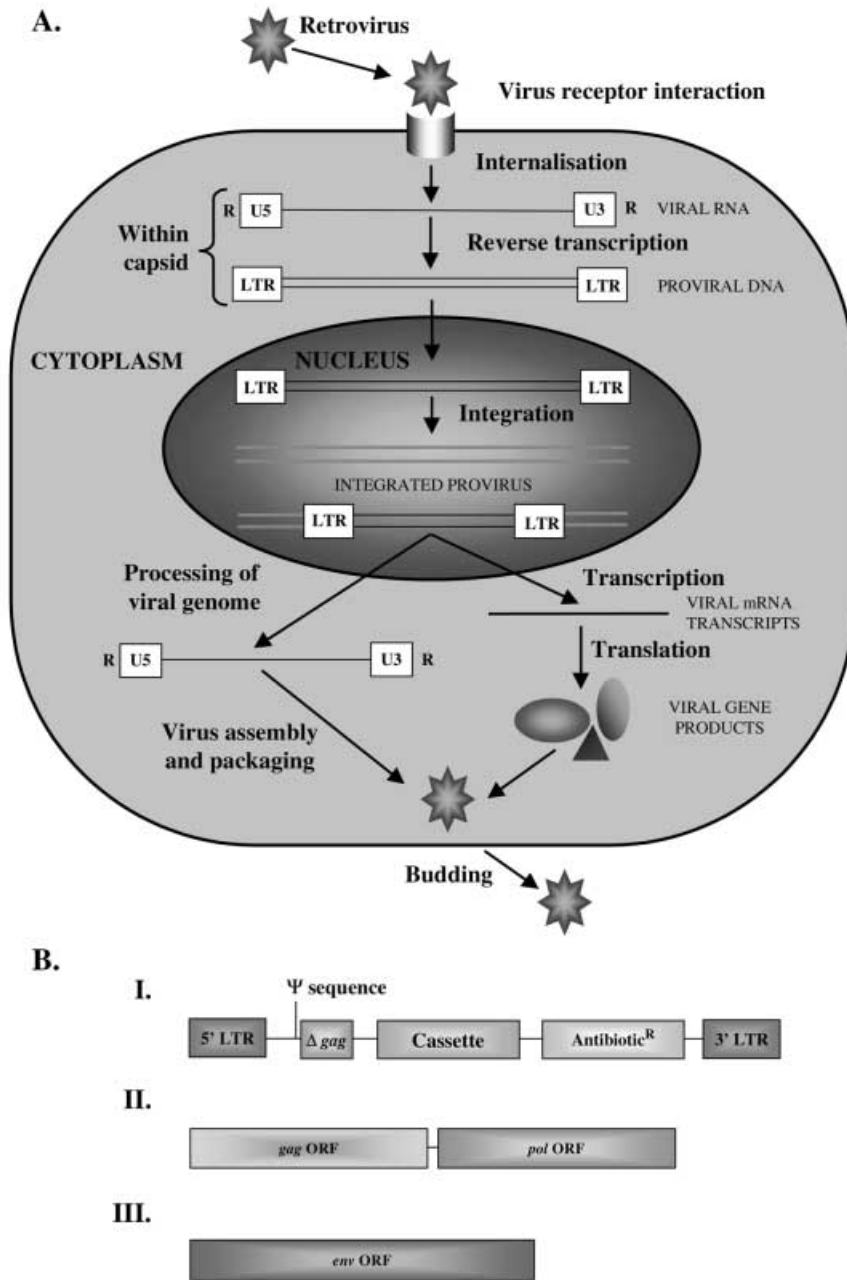


Figure 1 (A) Schematic representation of the retrovirus life cycle, showing internalisation, reverse transcription, integration, virus assembly and budding. (B) Representation of the constructs used to make Mo-MLV-derived retroviral vectors. Construct I is transfected into a cell line, which stably expresses constructs II and III. After transfection, the cells are placed under antibiotic selection. Individual clones are then selected and assessed for their ability to produce recombinant retrovirus vectors. Within these retroviral producer cells, constructs II and III produce the packaging functions *in trans* and the genome is derived from construct I, which contains the packaging signal Ψ and LTR regions. The viral integrase and reverse transcriptase, produced by the *pol* ORF of construct II, are also packaged into infective virions.

ability to transduce post-mitotic cells. These vectors have been shown to transduce neurones *in vivo*, where the transgene expression is effective for up to 6 months (Blomer *et al.* 1997). Questions about the biosafety of lentiviral vectors remain outstanding, and further safety issues concerning RCR formation are currently receiving attention. Lentiviral vectors have been used for transduction of pancreatic islet cells and display promising results when compared with retroviral vectors (Ju *et al.* 1998, Leibowitz *et al.* 1999).

Adenovirus vectors

Adenoviruses are non-enveloped double-stranded DNA viruses, which are usually associated with mild human infections (Shenk 1996), including upper respiratory tract infections, keratoconjunctivitis and gastroenteritis. They efficiently infect and express their genes in a wide variety of cell types, including dividing and non-dividing cells. The adenovirus genome is 36 kb in length and is easy to manipulate using classical recombinant DNA techniques (Graham & Prevec 1991). It has inverted terminal repeat (ITR) sequences at both ends, and the gene transcripts can be divided into two phases of gene expression: early genes (E), expressed before the onset of viral DNA replication, and late genes (L), expressed after the onset of viral DNA replication. An essential protein in this viral replication process is the E1A gene product. E1A is the first gene to be expressed after infection and has a key role as transactivator of all other adenoviral genes.

The process of virus entry into the cell is initiated by the attachment of the fibre knob protein to a high-affinity cell-surface receptor called the Coxsackie and Adenovirus Receptor (CAR) (Bergelson *et al.* 1997). Entry of the virus into the cell is then further mediated by an interaction between cell-surface integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ with the capsid penton base (Wickham *et al.* 1993). After these initial extracellular interactions, the adenovirus virion becomes endocytosed through a clathrin-coated pit into an endosome. A subsequent decrease in pH within the endosome causes a conformational change to the virion capsid proteins, resulting in the release of the viral capsid into the cytoplasm. From here, the capsid is then transported to the nucleus of the cell, where the viral genome is released and undergoes replication and transcription from an episomal location (Fig. 2A, adenovirus life cycle).

First-generation recombinant adenovirus vectors (rAds) are mostly based on the human adenovirus serotypes 2 and 5, and have been rendered replication-defective through deletion of the E1 gene region. The deletion of the E1 region allows the insertion of foreign DNA into the adenoviral genome. To allow the insertion of larger transgenes, first-generation rAds may also carry deletions in the E3 gene region (Bett *et al.* 1994). Because an adenoviral vector without the E1 gene region cannot

replicate, rAds have to be propagated in a cell line stably expressing a copy of the E1 region (Fig. 2B). Several cell lines, including HEK 293 (Graham *et al.* 1977) and 911 (Fallaux *et al.* 1996), have been generated that are able to propagate replication-defective rAds in the presence of an E1-deleted genome. The resulting rAd vectors produced from these cell lines can be grown to titres as high as 10^{12} plaque forming units per ml (Graham & Prevec 1991, Lowenstein *et al.* 1996).

One of the main drawbacks of rAds *in vivo* is the immune response elicited against the viral vector, vector-encoded proteins and infected cells expressing these proteins. This immune response can be separated into two phases, comprising an early innate inflammatory response and a later acquired immune response. The initial inflammatory response is believed to be directed against the viral capsid proteins. It is characterised by up-regulation of proinflammatory cytokines (Cartmell *et al.* 1999) and activation of innate inflammatory cell types, including macrophages, neutrophils and natural killer cells (Worgall *et al.* 1997). The acquired immune response, consisting of cellular and humoral components, is believed to be directed against adenoviral gene products, which are expressed at low levels in infected cells even in the absence of the E1A gene product (Yang *et al.* 1994a, b, 1995), or the transgene (Yang *et al.* 1996, van Ginkel *et al.* 1997, Michou *et al.* 1997). As a consequence of this immune response transgene expression from E1-deleted rAds has been shown to be relatively short in the periphery, being optimal at 3–4 days and disappearing around 1–2 weeks, although, within the central nervous system (CNS), elimination of rAd-infected cells is much slower, with gene transfer present after at least 3 months (Byrnes *et al.* 1995, 1996, Geddes *et al.* 1997b, Dewey *et al.* 1999, Thomas *et al.* 1999).

Although the process of transgene elimination may not be a problem in cancer or transplantation gene therapy, it may hamper clinical applications such as the treatment of hereditary genetic disorders or neurodegenerative diseases, which require long-term transgene expression. Furthermore, the generation of adenovirus-neutralising antibodies by the humoral arm of the acquired immune response, coupled with the likely pre-exposure to wild type adenovirus by most of the population, means that re-administration of rAds is likely to be unsuccessful without prior tolerization (Dai *et al.* 1995).

Newer rAd vectors, which are less immunogenic than the existing first-generation vectors, have more recently been developed. These vectors, which are deficient or defective in the E2, E3 or E4 gene regions, can be propagated on *trans*-complementing cell lines (Englehardt *et al.* 1994a, b, Yang *et al.* 1994c, Brough *et al.* 1996). Vector systems have also been generated in which the rAd is deleted of all viral genes. These 'gutless' vectors – in which the genome contains only the ITRs and packaging signal of the wild type virus – can, theoretically,

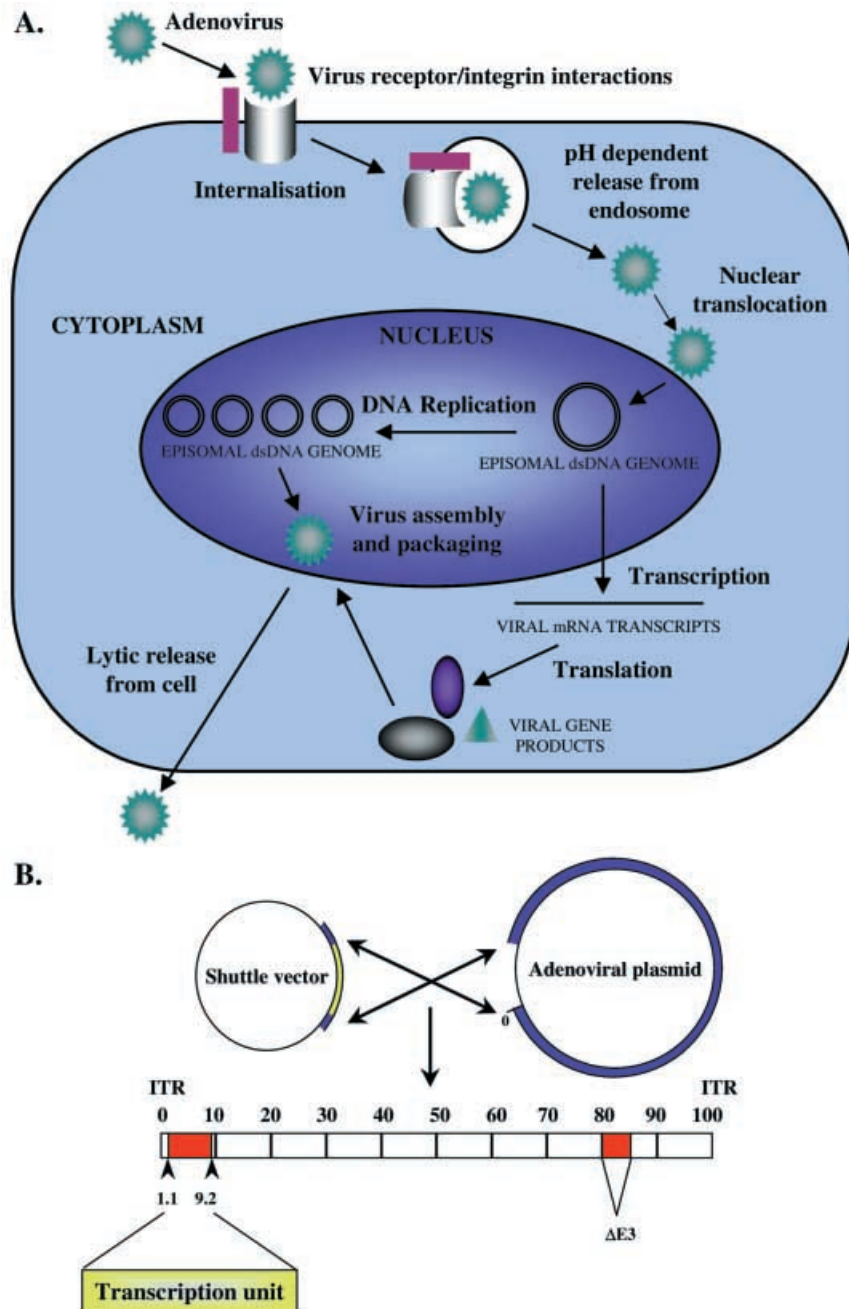


Figure 2 (A) Adenovirus life cycle, indicating internalisation, replication, virus assembly and release. (B) Schematic representation of rAd generation. The shuttle vector, containing the transcription unit (yellow) flanked by adenoviral sequences (blue), is co-transfected with the adenoviral genome plasmid into 293 cells. The adenoviral genome plasmid pJM17, containing the full viral genome with a deletion in E3 and the bacterial plasmid pBRX inserted in E1, and the shuttle vector undergo homologous recombination. The resultant adenoviral genome is deficient in E1 and E3 (red) and has the transcription unit inserted in E1. This genome can then be packaged into virions, which are produced by the adenoviral genome in 293 cells, which express the E1A adenoviral transactivator *in trans*.

accommodate up to 35 kb of foreign DNA (Kochanek *et al.* 1996, Parks *et al.* 1996, Schiedner *et al.* 1998). In order to be propagated, the gutless vector needs a helper virus to provide, in *trans*, all the genes needed for virion assembly (Parks *et al.* 1996). To avoid helper virus contamination, Lox P sites have been placed on either side of its viral packaging signal. When gutless viruses are propagated in 293 cells expressing the enzyme Cre-recombinase (Cre 293 cells), the packaging signal of the helper viral genome is excised by Cre and the resulting helper genome cannot be packaged. Gutless viruses prepared in this way can reach 10^{10} transducing virions/ml and contain as little as 0.01% helper virus contamination (Parks *et al.* 1996). Gutless adenoviruses have also been shown to give rise to prolonged transgene expression compared with first-generation adenoviruses (Morsy *et al.* 1998)

rAds have been used for gene transfer into a variety of endocrine cells, including pituitary cells, pancreatic beta cells and thyroid cells. In this laboratory, Castro *et al.* (1997) have demonstrated the ability of rAds to transduce normal and neoplastic anterior pituitary cells, opening the possibility for using rAds to treat pituitary diseases (Castro 1999). Furthermore, rAds carrying the prodrug-activating enzyme, HSV-TK, under the control of cell-type specific (Castro *et al.* 1999b, Lee *et al.* 1999, Windeatt *et al.* 1999a) and ubiquitous (Windeatt *et al.* 1999b) promoters, are able to kill anterior pituitary tumour cells. Riley *et al.* (1996) successfully treated pituitary tumours in Rb+/- mice with a rAd expressing the retinoblastoma (Rb) gene. Freese *et al.* (1996) reported a decrease in prolactin release from human lactotrophic adenoma cells *in vitro*, using a rAd encoding the enzyme, tyrosine hydroxylase. Using rAd transduction in normal pituitary cells, Neill *et al.* (1999) have recently illustrated the role of G protein-coupled receptor kinases (GRKs) in gonadotrope responsiveness. Normal pituitary cells were infected with a rAd encoding GRK2 and showed decreased gonadotrophin-releasing hormone (GnRH)-stimulated luteinising hormone secretion and inhibited GnRH-stimulated inositol triphosphate production. A rAd carrying the gene for murine interleukin-2 was used to treat medullary thyroid carcinoma in mice and rats without significant toxicity to other organs (Zhang *et al.* 1998a,b, 1999). Androgen receptor function in genital skin fibroblast cultures of patients suffering from a defect in virilisation has been assessed using a rAd containing an androgen-responsive reporter gene (McPhaul *et al.* 1993, 1997). A rAd encoding p53 has inhibited cell growth of prostatic cell lines *in vitro* and decreased prostate tumour growth in mice (Ko *et al.* 1996). Incubation of oestrogen receptor (ER)-negative breast carcinoma cells with a rAd encoding ER renders them sensitive to hormone treatment and suppresses their proliferation in the presence of oestradiol (Lazennec & Katzenellenbogen 1999).

In vitro and *ex vivo* transduction of pancreatic islets from adult rats or new-born mice that had islet integrity before

transduction has been described after incubation with rAds encoding hexokinase I or β -galactosidase (Becker *et al.* 1994, Csete *et al.* 1995). In rats, a direct *in vivo* approach has been developed by rAd delivery of the pancreaticobiliary duct (Raper & DeMatteo 1996). This method induces transduction into the ductal epithelium, acinar cells and islets of Langerhans; however, it has been associated with inflammation (severe pancreatitis) and achieved only transient transgene expression. Despite normal islet function (insulin secretion, glycaemia, serum amylase concentration), both *ex vivo* (before graft) and direct *in vivo* rAd-mediated gene transfer induce inflammation (McClane *et al.* 1997, Sigalla *et al.* 1997) and antibody production against the transgene (Sigalla *et al.* 1997). Muzzin *et al.* (1996) used a rAd carrying the leptin gene to correct the phenotype of the ob/ob mouse, in which the leptin gene mutation causes a syndrome resembling non-insulin-dependent diabetes mellitus in humans. This treatment normalised food intake, body weight, serum insulin concentrations and glucose tolerance, demonstrating a possible use of rAds to treat non-insulin-dependent diabetes mellitus. Furthermore, Wang *et al.* (1997a,b) have shown restoration of islet function after perfusion of the Zucker rat pancreas with a rAd encoding the leptin receptor. In diabetic mice, intravenous injection of a rAd containing a modified proinsulin cDNA resulted in functional insulin expression and normalisation of glycaemia (Short *et al.* 1998). In Brattleboro rats, diabetes insipidus has been reversed after injection of a rAd encoding arginine vasopressin into the hypothalamus (Geddes *et al.* 1997b). Geddes *et al.* (1997a) have shown that delivery of rAd into the hypothalamus induced efficient stable transgene expression with limited immune responses. Treatment with cyclosporin further reduced this immune response.

In summary, rAds have the following advantages: the ability to infect a wide variety of cell types, infection of dividing and non-dividing cells, high efficiency of gene transfer, no integration into the host genome, relatively large transgene capacity, easy manipulation, and high titres. Their drawbacks include limited duration of transgene expression and immunogenicity *in vivo*.

Adeno-associated vectors

Adeno-associated viruses (AAV) are members of the parvoviridae family. In humans, AAVs are not associated with any disease. Their structure is simple: a short, linear, single-stranded DNA genome (Rose *et al.* 1969) composed of two open reading frames (ORFs), rep (regulation) and cap (structure), and two small (145 bp) inverted terminal repeats (ITRs) (Srivastava *et al.* 1983). The ITRs are the only *cis*-acting elements necessary for efficient encapsidation and integration of the viral genome into the genome of the host cell (Samulski *et al.* 1989).

The AAV genome is able to integrate stably into a specific locus on human chromosome 19 (Kotin & Berns 1989). AAV has the ability to transduce both mitotic and post-mitotic cells after its interaction with the host cell, through heparan sulphate proteoglycans and integrins (Summerford & Samulski 1998, Summerford *et al.* 1999). However, AAVs are unable to replicate without sequences provided *in trans* by a helper virus, such as an adenovirus or HSV (Fig. 3A, AAV life cycle).

Recombinant AAV (rAAV) vectors are constructed by co-transfection of two plasmids (Fig. 3B). The first contains the transcription unit of interest flanked by the ITRs, and the second contains the rep and cap ORFs. In order to propagate rAAVs, infection with a helper virus (classically an adenovirus) is required. This technique (Samulski *et al.* 1989) enables the production of rAAVs with relatively high titres (10^{10} infectious particles/ml). However, these preparations are contaminated with helper virus, demanding purification steps involving heating and caesium chloride gradient centrifugation. An alternative strategy to provide helper virus functions involves a plasmid containing a mini-adenovirus genome to supply helper virus-like functions *in trans* (Xiao *et al.* 1998).

Apart from the complex procedures needed to obtain rAAVs, these vectors also have the drawback of a limited packaging capacity for the transgene (4.7 kb). As rAAVs are deleted of all viral genes, these vectors are believed to be relatively non-immunogenic. After *in vivo* gene transfer of rAAVs, no evidence of a cellular immune response has been shown; however, specific circulating antibodies to rAAVs have been detected (Xiao *et al.* 1996), limiting their potential re-administration. Despite this, transgene expression from rAAV vectors has been shown to continue for up to 10 months in the periphery (Rivera *et al.* 1999) and 15 months in the CNS (Lo *et al.* 1999).

AAV has been used to treat some endocrine dysfunctions in ob/ob mice. Murphy *et al.* (1997) have shown that a single intramuscular injection of a rAAV vector carrying the mouse leptin gene produces long-term correction (6 months) of some endocrine and metabolic defects. These include body weight, food intake, leptin concentrations, serum insulin and glucose concentrations, and glucose tolerance test. Sugiyama *et al.* (1997) have shown that diabetic mice infected in the liver with an rAAV vector carrying the rat insulin gene have significantly decreased serum glucose concentrations.

Herpes simplex virus vectors

HSV-1 is an enveloped, double-stranded DNA virus with a genome of 152 kb encoding more than 80 genes. Its wide host range is due to the binding of viral envelope glycoproteins (gB & gC) to the extracellular heparan

sulphate molecules found in all cell membranes (WuDunn & Spear 1989). Internalisation of the virus then requires envelope glycoprotein gD and fibroblast growth factor (FGF) receptor (Kaner *et al.* 1990). HSV is able to infect cells lytically or can establish latency. HSV vectors have been used to infect a wide variety of cell types, including neurones (Lowenstein *et al.* 1994, 1995), muscle (Huard *et al.* 1995), brain tumours (Boviatsis *et al.* 1994), liver (Miyanochara *et al.* 1992), pancreatic islets (Liu *et al.* 1996) and pituitary cells (Goya *et al.* 1998).

There are two types of HSV vectors, called the recombinant HSV vectors and the amplicon vectors (for reviews, see Wilkinson *et al.* 1994, Laquerre *et al.* 1999). Recombinant HSV vectors are generated by the insertion of transcription units directly into the HSV genome, through homologous recombination events (Fig. 4A). The amplicon vectors are based on plasmids bearing the transcription unit of choice, an origin of replication and a packaging signal (Fig. 4B). The plasmid is transfected into a cell line, which is subsequently infected with a helper virus. The helper virus provides replication and packaging functions *in trans*, enabling the amplicon to be packaged into infectious HSV-1 virions. As HSV DNA replication occurs via a rolling circle mechanism, amplicon vectors contain multiple copies of the plasmid up to the upper packaging limit of HSV virions (Leib & Olivo 1993). HSV has 38 genes that are non-essential for virus growth, hence large deletions can, theoretically, be made within the genome of these vectors, enabling the insertion of large transcription units. Thus, during replication, concatemers of the transcription unit of ~150 kb are packaged into HSV-1 virions. First-generation amplicon systems did contain contaminating helper virus; however, newer amplicons can now be produced that are free of HSV-1 helper virus (Geller *et al.* 1997, Saeki *et al.* 1998a).

Although HSV vectors have the obvious advantages of a large capacity for insertion of foreign genes, the capacity to establish latency in neurones, a wide host range, and the ability to confer transgene expression to the CNS for upwards of 18 months (Carpenter & Stevens 1996), the problem of vector toxicity is currently an important limitation to their use (Lowenstein *et al.* 1994, Laquerre *et al.* 1999). Toxicity has been linked to the host shut-off functions of some viral gene products and the direct toxicity of others. Although recent vector modifications have made HSV vectors less toxic (Glorioso *et al.* 1992, Krisky *et al.* 1998), the potential therapeutic application of HSV vectors, except for cancer gene therapy (Samaniego *et al.* 1998), may be limited.

HSV vectors have been used for endocrine applications. Goya *et al.* (1998) have shown that it is possible to express transgenes in normal and in tumour anterior pituitary cell lines such as GH₃ and AtT20 cells. Liu *et al.* (1996) demonstrated that apoptosis could be prevented in pancreatic beta cells by transducing them with a HSV-1

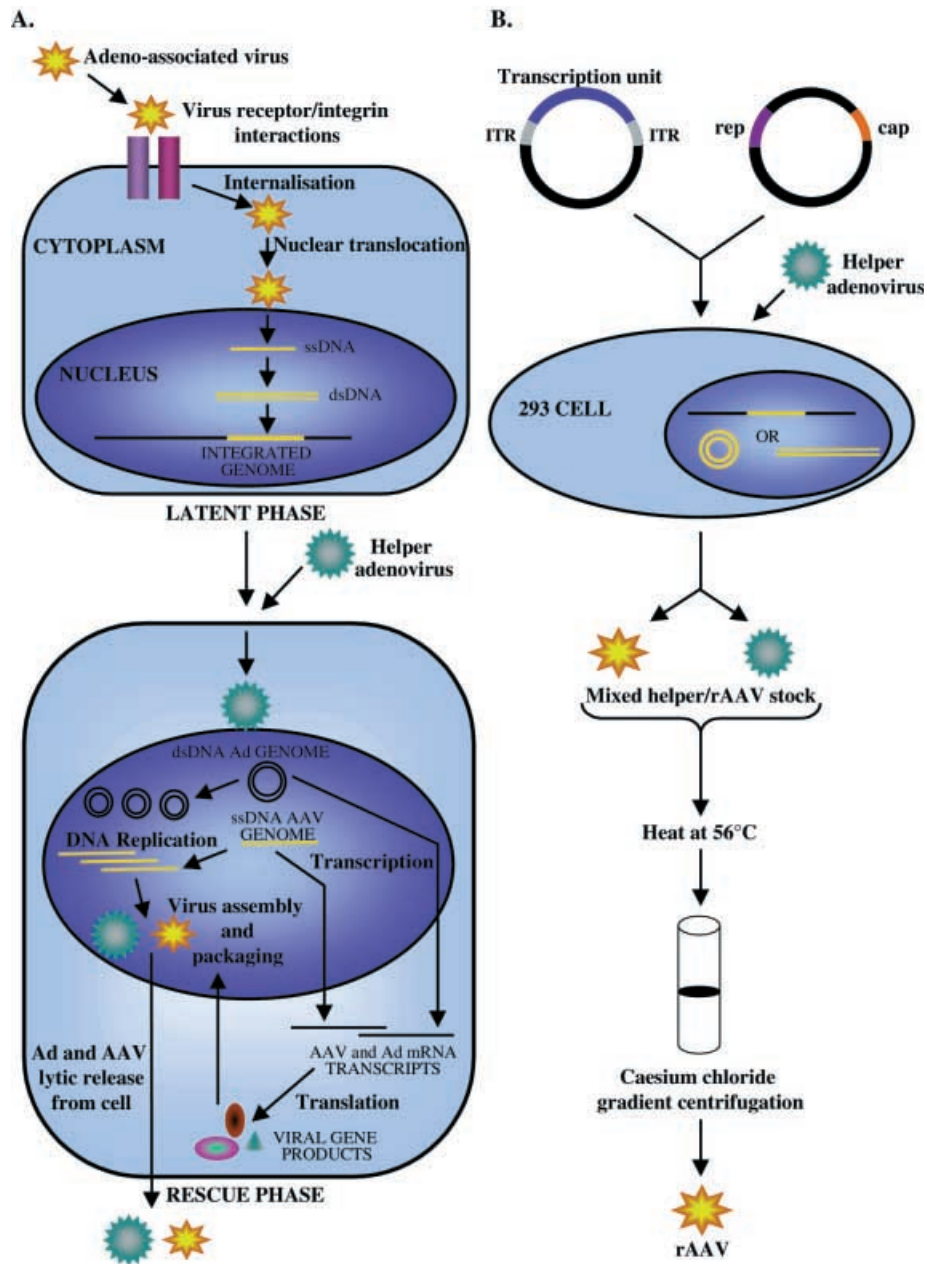


Figure 3 (A) AAV life cycle. Latency is established after AAV infection of the target cell. AAV virions are rescued after subsequent infection with a helper virus, which provides the sequences needed for viral replication in trans. (B) Schematic representation of rAAV production. Permissive cells are co-transfected with a plasmid containing the transcription unit (blue) flanked by the AAV ITRs (grey) and a plasmid containing the AAV genome without the ITRs. Subsequent infection of these cells with a helper adenovirus enables rescue of rAAV virions, following packaging of the ITR-containing plasmid, along with helper virus. Helper and rAAV virions can then be separated after heating at 56 °C by centrifuging on a caesium chloride gradient.

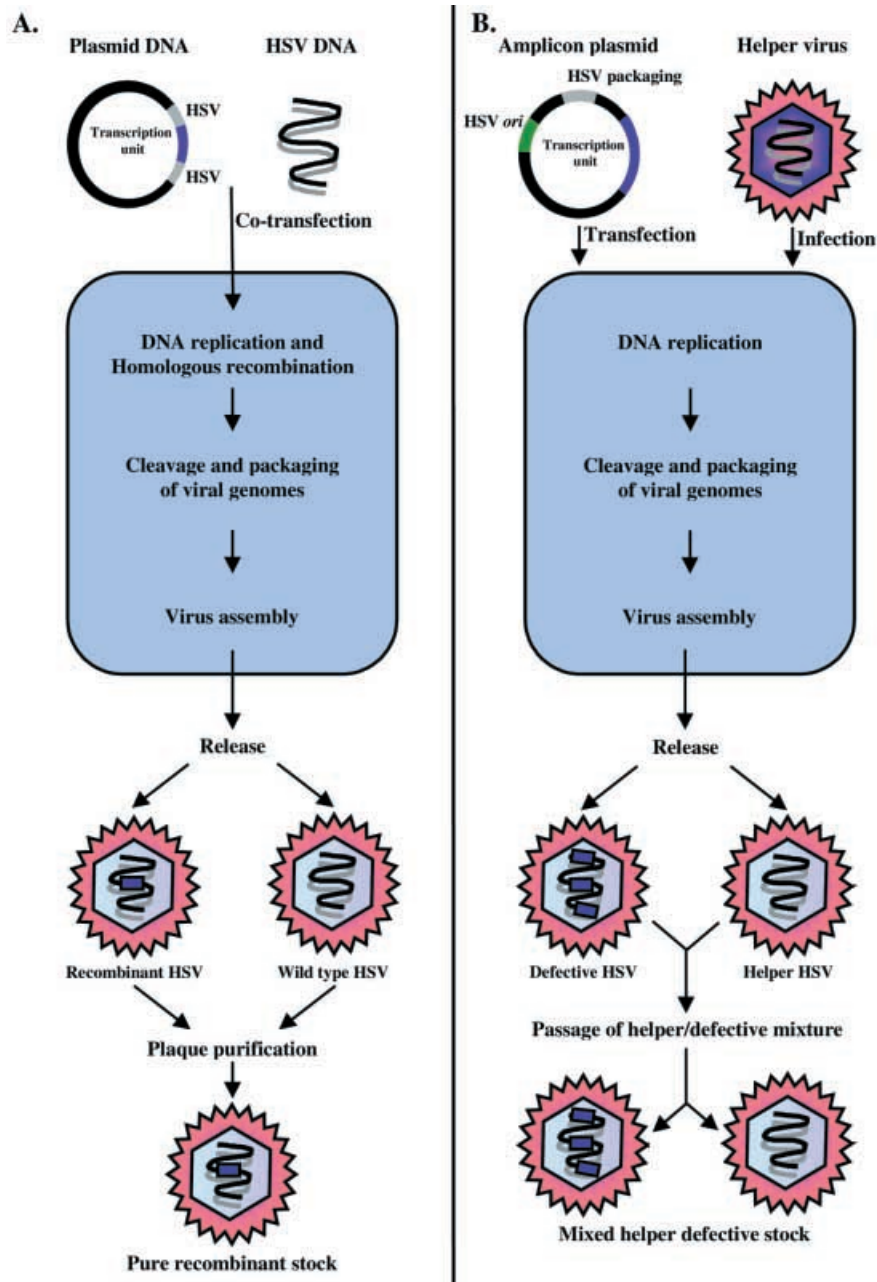


Figure 4 Schematic representation of recombinant and amplicon HSV vector production. (A) Recombinant HSV vectors. HSV genomic DNA is co-transfected into permissive cells along with a plasmid containing the transcription unit (blue) inserted in a fragment of HSV genome (grey). During mitosis, homologous recombination occurs between the HSV sequences within the plasmid and the viral DNA. Replicated and recombined viral genomes are then processed and packaged into new virus particles. Recombinant virus can then be plaque-purified from the parental virus. (B) Amplicon-derived HSV vectors. The amplicon plasmid containing the transcription unit (blue) is transfected into permissive cells, which are subsequently infected with a helper virus that provides all the regulatory and structural genes needed for viral growth. After helper infection, DNA replication is initiated. Helper virus DNA and linear head-to-tail concatemers of the amplicon plasmid are then packaged into separate viral particles, which are both released from the cells. The amplicon-derived virus cannot be physically separated from the helper virus and cannot replicate in the absence of helper virus.

amplicon vector carrying the Bcl-2 gene, showing the potential use of this vector in the treatment of type 1 diabetes mellitus.

Other viral vectors

In addition to the more commonly used viral vector systems previously described, attempts have been made to generate vectors using other viruses. Although not as successful or popular as the more traditional viruses, these vectors may offer benefits in certain gene transfer paradigms.

In the context of cancer gene therapy, the use of replicating viral vectors has been suggested (for review see Kirm & McCormick 1996). One such example is the ONYX-15 adenovirus, which is E1B-55K deficient, and as a result can replicate only in p53-deficient tumour cells (Bischoff *et al.* 1996). Vaccinia viral vectors have also been suggested for use in cancer gene therapy, as they are able to infect a large variety of tumours and have a large cloning capacity (for review, see Peplinski *et al.* 1998). Other vector systems have been based on the Sindbis and Semliki Forest alphaviruses (Polo *et al.* 1999), Sendai virus (Nakanishi *et al.* 1999) and EBV (Robertson *et al.* 1996).

As currently available viral vectors are unable to provide both targeted, efficient and long-term gene transfer with no adverse immune reactions, several groups are developing systems that incorporate attributes of more than one kind of viral vector (for review see Reynolds *et al.* 1999). One such example is the development of chimeric adenoviral/retroviral systems. In these systems, rAds are generated that contain retroviral vector and packaging sequences. On simultaneous infection of a single cell, the transcription units delivered by these rAds enable the target cell to function as a retroviral producer cell (Feng *et al.* 1997, Duisit *et al.* 1999).

Targeted gene delivery

Modification of viral tropism

For many gene transfer applications, a need to infect or target a specific cell type is desirable. With currently used viruses, this would seem to be a problem, as many of the cellular receptors used for virus attachment/entry are expressed on multiple cell types, giving them widespread tropism. In order to alter this natural tropism many groups are working on methods of modifying the natural virus-receptor interactions, with the aim of producing a modified virus with specificity towards predetermined cell types. The ultimate requirements for these modified, selectively targeted viruses would be: firstly, the virus must not have impaired assembly or propagation; secondly, the

virus must be able to undergo the internalisation steps of infection, and thirdly, the virus must be able to achieve efficient gene transfer after internalisation into target, but not non-target, cells.

Although systems have not yet been developed in which a virus with a modified tropism has been selectively targeted to an endocrine cell population, this section will discuss mechanisms by which the alteration of viral tropism could target a viral vector to pre-determined endocrine cell types.

Methods for enabling the re-targeting of retroviral vectors have relied on the modification of the envelope glycoprotein-cellular receptor interaction. Antibody-directed molecular bridges between envelope glycoproteins and specific cellular receptors have enabled the re-targeting of retroviral vectors (Goud *et al.* 1988, Roux *et al.* 1989, Etienne-Julan *et al.* 1992). The introduction of insertions or substitutions within the receptor-binding domains can also alter the viral tropism (Valsesia-Wittman *et al.* 1994, Matano *et al.* 1995). A more promising strategy has relied upon the addition of targeting-peptide ligands to the N-terminus of the envelope glycoprotein receptor-binding domain (for review see Cosset & Russell 1996). These peptide ligands could be specific for receptors displayed on membranes of specific cell types. As a modification to this strategy, a protease cleavage signal can be included between the N-terminus and the receptor-specific ligand, so that targeted gene transfer will occur only in its presence (Nilson *et al.* 1996, Peng *et al.* 1997, 1998).

Attempts by several groups to alter the tropism of rAds have focused their efforts on understanding and modifying the knob-CAR and penton base-integrin interactions of adenovirus type 5. By pseudotyping the knob domain (Krasnykh *et al.* 1996), directly altering the knob (Dmitriev *et al.* 1998, Krasnykh *et al.* 1998), or attaching targeting motifs to the C-terminus of the knob (Michael *et al.* 1995, Wickham *et al.* 1996a, 1997b), it is possible to alter viral tropism. By modifying the penton-base domain responsible for interaction with cell-surface $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins, viral tropism can also be modified (Wickham *et al.* 1995, 1996b, 1997a). Systems in which the fibre is linked to a specific cell-surface receptor by bispecific ligands have also been developed to alter viral tropism (Douglas *et al.* 1996, Rogers *et al.* 1997, Watkins *et al.* 1997).

Transcriptional targeting

As an alternative strategy to achieve targeted gene expression within specific cells, several groups, including our own, have developed the use of transcriptional targeting. The principle behind transcriptional targeting is that certain promoters can restrict gene expression to a particular cell population (Shering *et al.* 1997). Using this approach, gene delivery from a viral vector can be

widespread, but gene expression will occur only within a predetermined cell population, as a result of control at the transcriptional level. Cell-type-specific promoters have been used to achieve targeted gene expression in many cell populations and tissues, including neurones (Mayford *et al.* 1996, Morelli *et al.* 1999), glia (Morelli *et al.* 1999), heart (Franz *et al.* 1994), lung (Korfhagen *et al.* 1990), liver (Potter *et al.* 1991) and tumours (Hart & Vile 1995, Richards *et al.* 1995, Ring *et al.* 1996).

Cell-type-specific promoters have been used within rAd vectors to target gene expression to cells of the anterior pituitary gland, using the prolactin (Smith-Arica *et al.* 1999, Windeatt *et al.* 1999a) or growth hormone (Lee *et al.* 1999) promoters. Other pituitary-specific promoters that have the potential to achieve transcriptional targeting include the pro-opiomelanocortin (Kraus *et al.* 1993), thyrotropin beta (Haugen *et al.* 1996) or GnRH receptor (Albarracin *et al.* 1999) promoters. Cell-type-specific expression could potentially be achieved in pancreatic cells using the somatostatin (Leonard *et al.* 1993), amylase (DeMatteo *et al.* 1997) and proglucagon (Hussain *et al.* 1997) promoters, or in neuroendocrine cells using the chromogranin A gene promoter (Canaff *et al.* 1998).

In addition to achieving cell-type-specific expression, it would be desirable to achieve regulation of transgene expression in certain gene transfer strategies. Several regulatory systems are currently available, but the one that has attracted the most attention is the tetracycline-inducible system (Gossen & Bujard 1992), which allows gene expression to be regulated by doxycycline (a tetracycline analogue). The tet system has been shown to be functional when expressed from several viral vector systems (Hwang *et al.* 1996, Fotaki *et al.* 1997, Harding *et al.* 1998), enabling tight regulation and inducibility of transgene expression. By combining cell-type-specific promoters with the tet system, expression and inducibility has been restricted to anterior pituitary lactotrophic cell (Smith-Arica *et al.* 1999, Castro *et al.* 1999a) and glial cell (Chen *et al.* 1998, Smith-Arica *et al.* 1999) populations.

Conclusions

Viral gene delivery systems have enabled the transfer and expression of foreign genes in the endocrine system, both *in vitro* and *in vivo*. Gene transfer has been achieved efficiently with varying duration of transgene expression and with acceptable safety margins. The use of tissue-specific promoters has provided transgene expression restricted to specific target-cell populations. Such restricted transgene expression, coupled to the capability to switch transgene expression 'on' and 'off' in response to specific needs, has led to the development of cell-type-specific and regulatable transgene expression systems. The development of

stable, safe, tissue-specific, tightly regulatable and targeted gene transfer vectors will have a major part to play in the implementation of gene therapy strategies for the treatment of endocrine disorders, in which tight spatial and temporal control of transcription are crucial to normal physiology.

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