Considered by some to be among the simpler forms of life, viruses represent highly evolved natural vectors for the transfer of foreign genetic information into cells. This attribute has led to extensive attempts to engineer recombinant viral vectors for the delivery of therapeutic genes into diseased tissues. While substantial progress has been made, and some clinical successes are over the horizon, further vector refinement and/or development is required before gene therapy will become standard care for any individual disorder.

Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics

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Converting a virus into a vector

The viral life cycle can be divided into two temporally distinct phases: infection and replication. Infection results in the introduction of the viral genome into the cell. This leads to an early phase of gene expression characterized by the appearance of viral regulatory products, followed by a late phase, when structural genes are expressed and assembly of new viral particles occurs. In the case of gene therapy vectors, the viral particles encapsulate a modified genome carrying a therapeutic gene cassette in place of the viral genome (Fig. 1). Transduction is defined as the abortive (non-replicative or dead-end) infection that introduces functional genetic information expressed from the recombinant vectors into the target cell (Fig. 2).

The viral genome comprises both genes and *cis*-acting gene regulatory sequences. Although some overlap exists, most *cis*-acting sequences map outside of the viral coding sequences. This spatial segregation of genes and *cis*-acting sequences along the viral genome is exploited in the design of recombinant viral vectors.

To generate a vector, coding genes and *cis*-acting sequences are separated into distinct nucleic acid molecules to prevent their reconstitution by recombination into productive viral particles (Fig. 1). Coding sequences work in *trans*, and the viral genes can be expressed by heterologous plasmids, or even incorporated in the chromatin of producer cells to ensure their stability and limit their remobilization. The viral *cis*-acting sequences linked to the therapeutic gene can then be introduced into the same cell, leading to the production of replication-defective particles able to specifically transduce the new genetic information into target cells. Maintaining the separation of viral genes and *cis*-acting sequences during production is an important factor determining the efficiency and safety of a vector system.

The genetic engineering described above is limited by the degree of organizational complexity of the viral genome. The intact viral genome often ensures an appropriate balance of viral protein production by complex regulatory changes in gene expression. Moreover, *cis*-interactions between the genome and its translation products are lost in an engineered vector-packaging system. These intrinsic limitations of vector design may result in inefficient packaging of vector genomes as compared to wild-type viruses, and in the release of excess defective vector particles that not only are incapable of gene transfer but often interfere with the transduction of biologically active vector particles.

The complex integration of the viral lifecycle with the cellular machinery limits attempts at reconstituting vector particles from synthetic components. Duplicating these processes in an *in vitro* assembly system has proven to be a daunting challenge that would if successful increase the biosafety of viral vectors.

The relative concentration of vectors is measured as a titer expressed as the concentration of viral particles and/or the number of virions that are capable of transduction. The transducing particles usually represent a small percentage of total particles, and can vary between different preparations. Quantification is generally subject to variation resulting from different methods used in different laboratories. This strongly indicates a need for standardized methods for determining the specific activity of vectors. Particle titer and an infectious or transducing titer are both important, because impurities and variations in infectious activity can influence efficacy, toxicity and immunogenicity.

Properties of vectors for gene therapy

For gene therapy to be successful, an appropriate amount of a therapeutic gene must be delivered into the target tissue without substantial toxicity. Each viral vector system is characterized by an inherent set of properties that affect its suitability for specific gene therapy applications. For some disorders, long-term expression from a relatively small proportion of cells would be sufficient (for example, genetic disorders), whereas other pathologies might require high, but transient, gene expression. For example, gene therapies designed to interfere with a viral infectious process or inhibit the growth of cancer cells by reconstitution of inactivated tumor suppressor genes may require gene transfer into a large fraction of the abnormal cells. Gene transfer strategies based on the delivery of tumor-specific toxins or the conversion of prodrugs into toxins may be facilitated by a process referred to as the bystander effect. This allows either the gene product or the converted prodrug to transport between cells, such that therapeutic efficacy may be achieved even when targeting only a fraction of the cells within a tumor[1]. Other gene transfer strategies for cancer based on the induction of immune responses to tumor antigens or the interruption of the tumor vascular supply may require intermediate levels of gene transfer in a cell-type specific subset of the cells within, or from, a tumor. Finally, oncolytic viruses do not contain transgenes but are genetically engineered to allow tumor-specific viral replication resulting in cell lysis, and spread to neighboring malignant cells. All of these approaches are in or near clinical trials[1–6].

Some forms of gene therapy will require regulated gene expression. In the case of diabetes, exogenous expression of insulin will need to be tightly regulated based on rapid changes in glucose concentrations and metabolic perturbations. This situation will require appropriate post-translational processing that is responsive to these metabolic cues. In other cases such as anemia, the hematocrit might be regulated by turning the erythro-

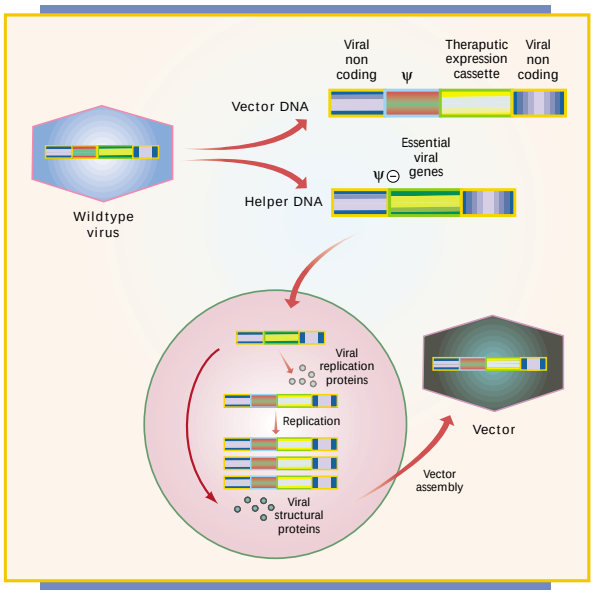

Fig. 1 Generic strategy for engineering a virus into a vector. The helper DNA contains genes essential for viral replication placed in a heterologous/unrelated DNA context that can be delivered as a plasmid, helper virus or stably inserted into the host chromosomal DNA of the packaging cell. The helper DNA can be delivered as a single molecule or in some cases split into different DNA molecules for safety reasons (see text). The helper DNA lacks the packaging domain (ψ) so it itself or its RNA cannot be packaged into a viral particle. The helper DNA of some vectors also lacks additional transfer functions, to increase safety. The vector DNA contains the therapeutic expression cassette and non-coding viral *cis*-acting elements that include a packaging domain. Some vectors contain viral genes that are relatively inactivated (not transcriptionally active at the same level as in a wild-type infection) due to the absence of other viral genes. The viral proteins required for replication of the vector DNA are produced, leading to the synthesis of many copies of the vector genome (RNA or DNA, depending on the type of vector). Viral structural proteins recognize the vector (psi plus) but not the helper (psi negative) nucleic acid to result in packaging of the vector genome into a particle.

poetin gene on or off by the administration of oral drugs (for example, tetracycline derivations) that regulate a specific transactivator that activates or represses a specific promoter[7].

As the expression of viral genes is responsible for most pathological and immunological consequences of viral infection, gene transduction by recombinant vectors is often well tolerated. Problems that may be observed with gene transfer vectors include acute toxicity from the infusion of foreign materials, cellular immune responses directed against the transduced cells, humoral immune responses against the therapeutic gene product and the potential for insertional mutagenesis by certain integrating vectors.

Whereas humoral immunity directed against the viral vector particle is generally observed, it becomes a problem if the effects of gene transfer are short-lived, necessitating repetitive administration of the vector. Though there is a theoretical risk that antibody–vector complexes could be harmful, no such events have been described to date. Even with purified vector preparations, the risk of inducing an immune response (or autoimmunity) to a gene product never seen by the recipient's immune system is not known. It is also possible that gene transduction into antigen-presenting cells (APCs) may break tolerance to its product, because of presentation in the context of class I versus class II major histocompatibility complex (MHC). As most studies have been performed in inbred animal strains with non-species-specific transgene products, accurate predictions of these types of immune responses in humans have been difficult to make. Moreover, the immune responses are likely to be influenced by polymorphic variation in host immune-relevant genes, the transgene product, the vector used for gene transfer and the target organ.

Currently used vectors that integrate do so in a random manner. Integration is a mutagenic event with a well-established potential for disruption and transcriptional activation of cellular genes, including oncogenes. Nonetheless, integration is well-tolerated by most transduced cells, and instrumental to ensure stability of the newly introduced genetic information in the recipient. It should be noted, however that human trials, to date, have used vectors that integrate into a relatively small proportion of cells within a target tissue. With the generation of more efficient vectors capable of targeting a wider spectrum of cells, including stem cells capable of self-renewal and massive clonal outgrowth, the risks of viral integration might need to be reconsidered. An additional undesirable potential effect is inadvertent transmission of vector sequences into germ cells. Though the risk of this happening with an integrating vector is negligible, any such germline event raises important safety and ethical issues.

Desirable vector properties that could mitigate some of these potential risks include the ability to infect selectively a specific target cell or tissue, such that after parenteral administration, tissue-specific uptake occurs without widespread tissue dissemination of a therapeutic gene that is toxic or antigenic when expressed from the "wrong" tissue. Though attempts to achieve tissue-specific targeting continue to receive much attention, there has been little practical success. Current methods to circumvent some of the problems of promiscuous transduction include the use of tissue-specific promoters to drive expression of the transduced gene and the modification of the surface recognition elements of recombinant viral particles to change their cell-recognition properties. Another formidable challenge is the attempt to engineer vectors that can integrate into predetermined sites within the genome. This would avoid random integration into potentially harmful sites that might result in detrimental events as discussed above.

Retroviruses

Retroviruses are lipid-enveloped particles comprising a homodimer of linear, positive-sense, single-stranded RNA genomes of 7 to 11 kilobases. Following entry into target cells, the RNA genome is retro-transcribed into linear double-stranded DNA and integrated into the cell chromatin. This family of viruses includes several varieties being exploited for gene therapy: the mammalian and avian C-type retroviruses (hereafter also referred to as oncoretroviruses), lentiviruses (such as HIV and other immunodeficiency viruses) and spumaviruses. They tend to establish chronic infection that is usually well tolerated by the host but may also cause latent diseases ranging from malignancy to immunodeficiency[8].

All retroviral genomes have two long terminal repeat (LTR) sequences at their ends. LTR and neighboring sequences act in *cis* during viral gene expression, and packaging, retro-transcription and integration of the genome. The LTR sequences frame the tandem *gag*, *pol* and *env* genes encoding the structural pro-

teins, nucleic-acid polymerases/integrases and surface glycoprotein, respectively. Lentiviruses have a more complex genome; in addition to the *gag*, *pol*, and *env* genes, they encode two regulatory genes, *tat* and *rev*, essential for expression of the genome, and a variable set of accessory genes. Spumaviruses also contain *bel-1*, an essential gene regulating expression of the genome, and other genes of unknown functions.

The location of most *cis*-acting sequences in the terminal regions has enabled simple and effective retrovector design, making them the most widely used vector system in gene therapy clinical trials to date[9]. Up to eight kb of exogenous DNA can be inserted and expressed in place of the viral genes, which are expressed by heterologous transcriptional signals from two separate constructs lacking most viral *cis*-acting sequences and stably incorporated in packaging cell lines[10]. The split construct design improves the biosafety of the vector by increasing the number of recombination events that would be required to reconstitute a replication-competent genome[11,12]. Studies in non-human primates did not detect pathological consequences of an exposure to an amphotropic replication-competent retrovirus[13] (RCR). Infusion of vector, however, contaminated by a large amount of such an RCR into immunocompromised primates resulted in lymphomas in some of the animals[14]. The more recent packaging cell lines yield titers above 1×10^7 transducing particles (t.u.)/ml in the culture medium and can be conveniently banked and scaled-up for manufacturing large amounts of vector free from RCR.

The viral envelope glycoprotein dictates the host range of retroviral particles through its interaction with receptors on target cells. The post-translational modification of the viral-envelope glycoproteins is a signature of the type and species of producer cell, and influences the stability of the particle when delivered into a specific species. The mechanism of particle assembly allows for the substitution of one viral Env by one from a different virus in a process referred to as pseudotyping. Such an approach can expand the host-range of retroviral vectors by incorporating sequences from unrelated viruses; the application to lentiviral vectors is discussed below. For example, vectors pseudotyped with the G glycoprotein of the vesicular stomatitis virus (VSV-G), can infect most cells, are particularly stable, and can be concentrated to titers exceeding 1×10^{10} t.u./ml (ref. 15).

A useful property of retroviral vectors is the ability to integrate efficiently into the chromatin of target cells. Although integration does not guarantee stable expression of the transduced gene, it is an effective way to maintain the genetic information in a self-renewing tissue and in the clonal outgrowth of a stem cell.

Disruption of the nuclear membrane is required for the preintegration complex to gain access to the chromatin[16], and productive transduction by retroviral vectors is strictly dependent on target cell mitosis shortly after entry[17]. Because only a fraction of cells pass through mitosis at any given time, this severely limits the applications of retroviral vectors in gene therapy to selected targets *ex vivo* such as lymphocytes and hematopoietic progenitor cells[18]. However, some encouraging clinical results of gene therapy have been obtained with these vectors. The suicide gene HSV-TK was transferred into donor lymphocytes to control graft-versus-host disease developing in an allogeneic graft-versus-leukemia response[19]. More recently, the cDNA of the common cytokine receptor γ-chain was transduced into the bone marrow stem cells of two children affected by severe combined immunodeficiency (SCID)-X1 (ref. 20). After infusion of the transduced cells, infants previously requiring protective isolation have reconstituted immune function and done well in a normal environment. Optimized conditions of *ex vivo* transduction and *in vivo* selection of transduced cells were most likely crucial factors for these rare successes among the failures of most other, earlier gene transfer trials.

Lentiviruses

Lentiviruses are promising vectors currently under preclinical development for gene therapy[21]. Unlike retroviruses, they rely on active transport of the preintegration complex through the nucleopore by the nuclear import machinery of the target cell[22]. The lentiviral strategy for nuclear targeting enables infection of non-dividing cells, an attractive attribute for a gene therapy vector. Replication-defective vectors were originally derived from HIV-1 to transduce lymphocytes, but it was a VSV-G pseudotyped lentiviral vector with expanded tropism[23] that spurred applications for gene therapy. The genetic information required to package a functional lentiviral core in the vector was then found to be only a fraction of the parental genome[21]. As the non-required genes are critical for viral pathogenesis, new generations of "minimal" packaging constructs have been adopted to increase vector biosafety[24]. An important approach to alleviate such concerns is the use of self-inactivating transfer vectors[25,26]. These vectors contain a deletion in the downstream LTR that when transduced into target cells, results in the transcriptional inactivation of the upstream LTR and diminishes substantially the risk of vector mobilization and recombination[27].

Hybrid lentiviral vectors have also been derived from non-human lentiviruses (for example, simian, equine, feline, caprine and bovine) following similar approaches to those used for HIV-derived vectors, on the rationale that they would be more acceptable for clinical application because the parental viruses are not infectious to humans[28–30]. The advantages and potential disadvantages of non-primate vectors as compared to HIV-derived ones are discussed elsewhere[21]; however, these issues need to be addressed by *in vivo* testing in appropriate animal models.

VSV-pseudotyped lentiviral vectors can be delivered directly *in vivo*. They efficiently transduce the neurons and glial cells of the central nervous system (CNS) of rodents[31] and non-human primates[32]. Stable, long-term transgene expression was observed without detectable pathological consequences ascribed to the vector. Long-term therapeutic efficacy of lentivirus-mediated gene transfer into the CNS has been reported in animal models of retinal photoreceptor degeneration[33], type VII mucopolysaccharidosis[34], Parkinson disease[35] and metachromatic leukodystrophy[36]. Lentiviral vectors efficiently transduce several non-dividing, differentiated epithelial tissues of rodents, humans and other species, isolated or dissociated *ex vivo*[21]. Direct transduction *in vivo* appears to be more sensitive to tissue barriers limiting vector access, as in the case of respiratory mucosa[37] and to intracellular conditions (for example, cell cycle status) as in the case of hepatocytes[38]. The actual potential and limits of lentiviral vector-mediated gene delivery *in vivo* still need to be defined[37–40].

A possibly unique application of lentiviral vectors is the transduction of the elusive long-term repopulating hematopoietic stem cells (HSC). Short *ex vivo* incubation with lentiviral vector, without need for cytokine stimulation, led to efficient

marking of human and primate HSC originating long-term, multilineage reconstitution in xenogeneic or autologous hosts, respectively[41–43]. The potential of this new approach to improve gene transfer into HSC was dramatically shown in a recent report describing therapeutic correction of a mouse model of β-thalassaemia[44].

The obligatory RNA step in the retroviral lifecycle poses great constraints on the viral genome and on its exploitation for gene transfer purposes. The transgene expression cassette must be of limited size, without introns and internal polyadenylation signals. Together with the exposure to loco-regional differences in the structure and activity of chromatin consequent to random integration, these factors combine to limit expression of the transduced genes.

Replication-competent and replication-defective vectors have also been derived from the human foamy virus[45] (HFV). Their development has lagged behind the ones discussed here but offer some potential advantages. Further studies are required to appreciate their value in clinical applications.

Adenoviruses

The use of recombinant adenoviruses received early attention as a vehicle to transfer genes into the respiratory epithelium for treating diseases such as cystic fibrosis. It was quickly realized, however, that these vectors were a most efficient gene transfer systems in a variety of tissues[46–49].

With over 50 different human adenoviral serotypes, current vectors are primarily derived from those known as 2 and 5—the most common serotypes to which most adults have been exposed[50]. There are now efforts, however, to exploit these other serotypes or even non-human adenoviruses[51] to avoid potential problems related to pre-existing immunity that may preclude or reduce the efficacy of vector administration. Moreover, if and when re-administration of a vector may be required, secondary vector delivery using a different serotyped capsid has been demonstrated in animal models[52,53]. Attempts to alter the cellular tropism or immunological capture by calculated changes in the viral fiber protein responsible for the primary virus–cellular receptor binding may soon be possible[54,55].

The viral life cycle has been recently reviewed[56]. The approximately 36-kilobase viral dsDNA genome contains overlapping transcriptional units on both DNA strands encoding over 50 polypeptides. After cell entry, the viral particle contains proteins that allows for efficient endosomal lysis and escape allowing the genome to enter into the nucleus. The early region 1 (E1) genes are quickly transcribed and serve in part as a master transcriptional regulator that essentially starts the process of viral gene expression leading to genome replication. The E1 genes in combination with the E2 and E4 genes are required for viral genome replication. Late in the life cycle, the viral structural proteins genes are transcribed allowing for encapsidation of the newly replicated genomes. One cell can produce as many as 10,000 virions that are released by viral-induced cell lysis. Purified concentrations of 1×10^{13} vector particles/ml can be routinely achieved. The E3 genes are dispensable for the viral life cycle, and in the wild-type infection play a role in immune surveillance in infected hosts[57]. There have been some suggestions that these genes may offer protection against some of the immune-mediated responses directed against the vector or vector-transduced cells in animal studies, but this remains controversial due to differences in animal species and strains used for the studies[58–60]. The removal, however, of this region allows additional room for larger foreign DNA inserts in the range of 8 kb.

In first generation vectors, the E1 region is removed in order to make room for the therapeutic expression cassette, and to prohibit transactivation of viral genes required for viral replication. To make the vector, the E1-deleted viral genome containing the transgene is added to a cell line that contains a stable E1a expression cassette allowing the added DNA to replicate and be packaged into E1 deleted vectors that are not capable of replication.

It was quickly learned that even in the absence of E1 gene products, low-level transcription of the remaining viral genes occurred resulting in early innate cytokine responses, followed by antigen-dependent immune responses that include cell-mediated destruction of transduced cells, reducing the period of gene expression[61–63]. Later studies included second and third generation vectors containing deletions of E1 and E2 and/or E4 genes[64–68]. Although these vectors gave decreased toxicity profiles in animals, toxicity from an E1/E4 deleted adenovirus vector infused into the hepatic artery of a young man with partial OTC deficiency was the first reported fatality from gene therapy.

The complexity of adenovirus has made removal of all the viral genes from the vector a daunting task because, unlike retroviruses, construction of a packaging cell line has not been possible. Instead, a helper-dependent vector system[69] has been developed in which one virus (the helper) contains all the viral genes required for replication but has a conditional defect in the packaging domain making it less likely to be packaged into a virion. The second vector contains only the viral inverted terminal repeats (ITRs), therapeutic gene sequences (up to 28–32 kb) and the normal packaging recognition signal, which allows this genome to be selectively packaged and released from cells. The helper virus and vector can be further purified by physical means. This process is currently labor intensive and difficult to scale to pharmaceutical levels. Moreover, all of the current methods of vector preparations have contaminating helper viruses, but the amount of these unwanted viruses may approach levels of less than 0.1%. Nonetheless, it appears that these vectors have substantially reduced toxicity and can produce therapeutic quantities of various proteins in animals[52,70,71]. The unresolved issue is how much acute toxicity may occur at high doses from the viral particle itself. Even though transgene expression for the life of a mouse has been achieved, the episomal nature of the vector makes difficult to know the maximal period of persistence that can be achieved in various tissues in cells with different rates of cellular turnover (for example, neurons, hepatocytes and respiratory epithelium).

When given intravenously, most of the adenovirus vector ends up in the liver, but direct injection can transduce most tissues[72]. These vectors have been used in preclinical animal studies to transduce liver, skeletal muscle, heart, brain, lung, pancreas and tumors[73]. Adenoviruses were used early in clinical trials for cystic fibrosis[74] without evidence of clinical efficacy, but more recently they have been primarily used in clinical trials for the treatment of cancer[1–6] in part because of their efficiency of gene transfer but also because cellular toxicity and immunogenicity may actually enhance the anti-tumor effects with specific approaches that are ongoing. Other recent clinical trials include treatment of peripheral vascular and coronary artery disease by delivery of angiogenic promoting factors[75].

Adeno-associated virus

Adeno-associated viruses (AAVs) are human parvoviruses that

normally require a helper virus, such as adenovirus, to mediate a productive infection[76]. They were initially discovered as a contaminant in an adenovirus preparation. There are six known human viral serotypes, each of which may have different tropic properties. Most studies to date have focused on AAV-2. There is no known disease associated with AAV infection, making it an ideal candidate for gene therapy. The development of the virus into a vector and some of its applications have been recently reviewed[76–79]. The viral genome consists of two genes, each producing multiple polypeptides: *rep*, required for viral genome replication; and *cap*, encoding structural proteins. These two genes are flanked by viral ITRs that are 145 nucleotides in length. Each particle contains a single plus- or minus-strand genome. The packaging capacity of AAV is about 5.0 kb, which is a major limitation of this vector system. The wild-type virus in the presence of *rep* has a propensity to integrate into a specific region of human chromosome 19. This property is lost in vectors due to the absence of the *rep* gene.

AAV vectors can be produced by adding separate plasmids containing the ITRs flanking the therapeutic gene cassette, the *rep*/*cap* genes, and the addition of a helper adenovirus or a third plasmid with the essential adenoviral helper genes[80,81]. The latter approach does not require the input of any viruses. The large-scale production of vector is labor intensive but perhaps will become simpler with the recent advances in the development of packaging cell lines and column chromatographic methods of vector purification[82,83].

AAV vectors have been shown to transduce cells both through both episomal transgene expression and by random chromosomal integration[84–86]. After gene transfer in animals there is a slow rise in gene expression, reaching a steady-state level after a period of weeks. This is due, perhaps in part to a requirement for generation of dsDNA genomes by either vector ssDNA annealing, or second strand-synthesis followed by vector genome linking to form concatemers[84,87–92]. The mechanistic process of transduction has been difficult to elucidate because of the numerous and complex genome vector forms found *in vivo* in different tissues. Nevertheless, the discovery of vector genome linkage has allowed different groups to effectively double the limited coding capacity by splitting a gene or expression cassette into two vectors and simultaneously administering them to muscle or liver[93–95].

Since the AAV vector genome lacks viral coding sequences, the vector itself has not been associated with toxicity or any inflammatory response (except for the generation of neutralizing antibodies that may limit re-administration). The vector particle can be delivered to many different organs (for example, the CNS, liver, lung and muscle) by *in vivo* administration[96] and AAV vectors have been found to efficiently transduce non-dividing cells[97]. Moreover, there have been reports of preclinical efficacy in different animal models of genetic and acquired diseases[77,96]. Clinical trials using AAV for the treatment of cystic fibrosis, hemophilia and muscular dystrophy are underway with early evidence of gene transfer and expression of human clotting factor IX in hemophilia B patients [98–100]. It is likely that this vector will be useful for treating some diseases and more clinical trials with the vector are expected soon.

Herpes simplex virus

Herpesviruses have promise as vehicles for transfer of genes to cells *in vivo* based on their ability to persist after primary infection in humans in a state of latency where disease is absent in human hosts with normal immune status. Herpesvirus gene vectors should not reactivate and produce infectious virus or cause disease even in an immuno-compromised host.

Herpes simplex virus type 1 (HSV-1) is currently the most extensively engineered herpesvirus for purposes of gene transfer. HSV has a large genome composed of 152 kb of linear dsDNA containing at least 84 almost entirely contiguous (unspliced) genes, approximately half of which are nonessential for virus replication in cell culture. These features provide for multiple sites of foreign gene insertion, making HSV a large capacity vector capable of harboring at least 30 kb of non-HSV sequences representing large single genes or multiple transgenes that may be coordinately or simultaneously expressed[101]. Highly defective mutants deleted for the five immediate early (IE) genes do not express the remaining lytic viral functions and are essentially silent except for transgene expression. These vectors can be grown to high titer in complementing cell lines without the production of detectable replication competent virus[102]. The IE gene deletion vectors are non-cytotoxic[102] yet are capable of persisting in a state similar to latency in neurons and other cell types within non-neuronal tissue[103]. A most attractive feature is the efficient infectivity of HSV for a large number of cell types, which results in efficient gene transduction. Efficient infectivity and transduction has made possible repeat vector administration even in immune hosts. Limitations of these vectors include the lack of experience with recombinant herpesviruses in patients, difficulties related to long-term transgene expression in certain tissues including brain and difficulties related to vector targeting, since the mechanism of HSV attachment and entry is complex, involving multiple viral envelope glycoproteins.

HSV amplicon vectors represent an alternative to replication defective, recombinant genomic vectors. Amplicon plasmids are based on defective interfering virus genomes that arise on high passage of virus stocks[104]. They are generally approxi-

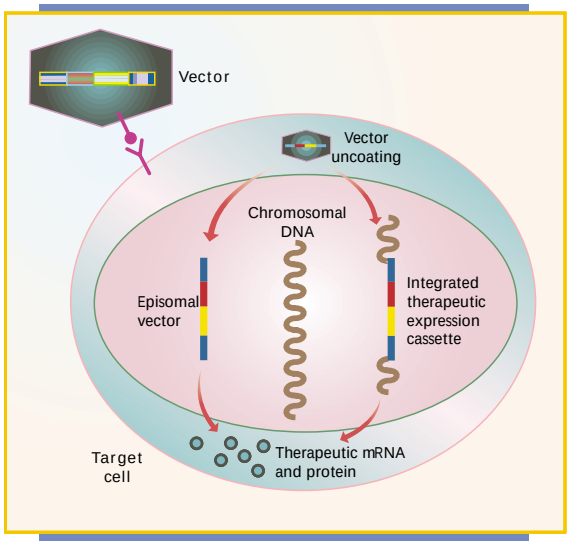

Fig. 2 Transduction of the target cell. The vector particle containing the therapeutic gene sequences binds to a cell, generally through a receptor-mediated process and then enters the cell, allowing the genome to enter the nucleus. The vector genome may go through complex processes but ends up as dsDNA that, depending on the vector, can persist as an episome or become integrated into the host genome. Expression of the therapeutic gene follows.

mately 15 kb in length and minimally possess a viral origin of replication and packaging sequences. The standard amplicon system requires the functions of helper HSV for particle production and packaging of genome length concatemerized vector DNA. Amplicon vector production has been improved through use of helper virus genome plasmids deleted for packaging signals; the helper genomes are propagated in bacteria as bacterial artificial chromosomes[105]. These preparations are advantaged by being nearly helper-free; however, until the helper DNA is completely devoid of sequences shared with the amplicon vector (for example, origin of replication), recombination between the amplicon and helper DNA will occur raising the possibility of contamination of vector stocks with unwanted recombinants, some of which may be replication competent. Production systems dependent on transfection are also difficult to scale–up, and have not yet produced high titer vector. Thus far, production of replication–competent virus-free genomic vectors using complementing cells results in a 2–3 log higher vector particle yield using a less complicated production system.

HSV vectors have now been successfully applied to treatment of animal models of cancer[106], PNS disease[107,108], certain brain diseases[109–112], spinal nerve injury (J.C. Glorioso and D.J. Fink, unpublished data) and the treatment of pain[113]. Perhaps the most promising current use of these vectors involves gene transfer to sensory neurons. Because wild-type virus has a similar host, HSV is already highly evolved for this purpose. Highly defective vectors can be taken up by sensory neurons following direct inter-dermal injection by sensory neurons where they persist, apparently for life, in nerve cell bodies. The latency promoter can be applied in a separate virus locus for long-term expression of therapeutic genes[103,114].

The coming years will provide an opportunity to test the safety and effectiveness of HSV vectors in the clinical setting. Among these early applications will likely be the treatment of malignant glioma using a multi-modal therapeutic approach exploiting the capacity of HSV vectors to accommodate multiple distinct transgene cassettes. Further vector design improvements should be forthcoming including the development of an HSV packaging line for efficient production of amplicons, and methods to improve transgene control in the HSV vector.

Combining properties of more than one virus

There have been recent efforts to blend properties of vectors to obtain combinational properties of both vectors. One active area is the combination of adenovirus and AAV vectors. Double-stranded AAV genomes in adenoviral capsids containing a combination of AAV and adenoviral ITRs have been shown to transduce and integrate in cells[115]. A different approach involves the placement of AAV vectors into gutless adenoviruses and engineering the vector to transiently express Rep protein to attempt to get site specific integration and avoid insertional mutagenesis[116]. The importance of this approach is still unclear because the chromosome 19 site specific integration may actually occur in the middle of a gene[117] and occurs only in about 50–70% of the integration events[118], which would only decrease the already low risk of insertional mutagensis by two-fold. This risk must be balanced by the potential toxicity of the Rep protein.

Retroviral genomes contained within an adenovirus have been claimed to integrate in the absence of the retroviral integrase activity[119]. Further characterization of this system is required before its value to the gene therapy community can be realized.

Novel methods for site-specific integration into chromosomal DNA using site-specific bacteriophage integrases are in early development[120,121]. Nonetheless, combining properties of different viral vectors perhaps even with non-viral delivery systems will likely have their place in future gene therapy efforts.

Closing remarks

The viral vectors reviewed here are not inclusive, but represent those used in current clinical trials or under advanced preclinical development. Though the vectors described herein have and will continue to make important contributions in clinical applications of gene therapy, a number of different viral and non-viral vectors, some of which have yet to be fully exploited or even discovered, will likely complement the current armamentarium. Other viral vectors under development include those based on SV-40[122], α-viruses[123,124], hepatitis viruses[125], negative strand RNA viruses (for example, influenza and ebola)[126] and Epstein-Barr virus[127].

No single vector system is likely to be optimal for all the potential gene therapy applications. However, for a specific application, a "perfect" vector will be administered by non-invasive delivery routes, target to the desired number of cells within target tissue, and express a therapeutic amount of transgene product with the desired regulation for a defined length of time. Though we are likely to see further gene therapy successes in the near future, the true fruition of gene therapy cannot be realized until the current vectors are perfected, or new vectors developed with properties described above.

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