Advances in lentiviral vector design for gene-modification of hematopoietic stem cells

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Lentiviral vectors are more efficient at transducing quiescent hematopoietic stem cells than murine retroviral vectors. This characteristic is due to multiple karyophilic components of the lentiviral vector pre-integration complex. Lentiviral vectors are also able to carry more complex payloads than murine retroviral vectors, making it possible to deliver expression cassettes that direct either constitutive or targeted expression in various hematopoietic stem cell progeny.

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Abbreviations

cPPT	central polypurine tract
Eμ	immunoglobulin heavy-chain enhancer
hCMV-IE	human cytomegalovirus immediate-early promoter
hEF1α	human elongation factor 1α
HIV-1	human immunodeficiency virus type 1
HSC	hematopoietic stem cell
LCR	locus control region
LTR	long terminal repeat
MAR	matrix-attachment region
MMLV	Moloney murine leukemia virus
PGK	phosphoglycerate kinase
PIC	pre-integration complex
RCL	replication-competent lentivirus
RRE	Rev-responsive element
SCID	severe combined immunodeficiency
SIN	self-inactivating

Introduction

The stable introduction of genes into hematopoietic stem cells (HSCs) holds the promise of curing congenital gene deficiencies and might provide potential therapies for human immunodeficiency virus infection and cancer. HSCs are attractive targets for gene therapy approaches to these diseases, because they can be genetically modified *ex vivo* and upon transplantation contribute to hematopoiesis for long periods of time, possibly for the lifespan of the recipient. Retroviral and lentiviral vectors are useful tools for the genetic modification of HSCs, owing to their ability to stably integrate into the host-cell genome. Successful integration of a retroviral vector bearing an expression construct into the genome of an HSC has the potential to be expressed in all progeny of the HSC or, with the use of specific control elements in the vector, targeted expression in a subset of the HSC progeny is possible. Although lentiviral vectors have not yet been used for therapeutic HSC gene-modification in humans, several recent advances in lentiviral vector design will increase the likelihood of their clinical use in the near future. We discuss here recent advances in this area of research, including developments in knowledge about the cell-cycle dependency of HSC transduction, as well as enhancements in the biosafety and expression profiles of lentiviral vector systems.

Properties of lentiviral vectors that facilitate HSC transduction

Over the past decade, retroviral vectors derived from the Moloney murine leukemia virus (MMLV) have been used successfully to transduce HSCs, and this approach has recently demonstrated clinical utility [1•–3•]. Because simple retroviruses like MMLV require cell division and nuclear membrane dissolution to integrate into the target cell genome, the introduction of useful expression constructs using MMLV vectors into quiescent HSCs has proven quite challenging. Lentiviral vectors based on human immunodeficiency virus type 1 (HIV-1) have been convincingly demonstrated to transduce unstimulated HSCs with greater efficiency than MMLV vectors [4–7]. This attribute of HIV-1-based vectors is a topic that has received much attention in gene therapy literature, and has been attributed to several *cis-* and *trans*-acting components of the vector particle.

Trans-acting protein components of the vector particle that remain associated with the pre-integration complex (PIC) after reverse transcription include the matrix and integrase proteins, both of which contain nuclear localization sequences [8,9]. Additionally, viral protein R (Vpr), when included in the packaging construct, also facilitates nuclear targeting of the PIC. Vpr is incorporated into vector particles in the producer cell and contained within the PIC; Vpr causes transient disruptions of the target cell nuclear membrane, which may then allow access to the nucleus by the PIC [10]. Recently, a *cis*-acting determinant of nuclear import, the central poly-purine tract (cPPT), which is normally found in the *pol* region of the HIV-1 genome, has been demonstrated to be an important factor in the ability of lentiviral vectors to transduce non-dividing cells, including HSCs. Incorporation of the 178-nucleotide cPPT and its associated central termination sequence, dramatically increases the ability of the PIC to cross the nuclear pore complex in intact nuclear membranes [11••,12]. This region directs the formation of a 99-nucleotide plus-strand overlap during reverse transcription, which interacts with currently unknown cellular factors to target the PIC to an afferent nuclear trafficking pathway. Sirven *et al.* [13] studied the influence of the cPPT on the ability of lentiviral vectors to transduce bulk CD34+ and CD34+/CD38− HSCs and found that cPPT inclusion increased transduction of both populations by up to three- to fourfold.

An additional benefit of using HIV-1-based vectors is that they can be engineered to deliver complex expression elements including introns or large stretches of genomic sequence by taking advantage of the nuclear export function of the HIV-1 protein Rev, which binds to the Rev-responsive element (RRE) present in the vector [14]. Genomic sequences such as enhancers and promoters, locus control regions (LCRs), and scaffold/matrix attachment regions (S/MARs), which do not normally exist in an RNA form, may contain cryptic splice sites or be unstable as RNA intermediates. To some degree, the constitutive nuclear export pathway used by Rev/RRE may help vector genomes containing these elements to accumulate in the cytoplasm of the vector-producing cell in high enough concentrations to be packaged efficiently into vector particles. Removal of the RRE from most lentiviral transfer vector constructs dramatically reduces vector titer, but Mautino and colleagues [15] have demonstrated that the function of HIV-1 Rev/RRE can be replaced by inserting a constitutive transport element (CTE) from a Simian retrovirus (SRV-1) into the transfer vector. Interestingly, these investigators found that the CTE must be placed in the 3′ end of the vector to efficiently increase nuclear export of the vector genome and thus raise titer. By contrast, studies looking at placement of the RRE at either the 5′ or 3′ end of the vector demonstrated no differences in the efficacy of the RRE at either position [16].

In vitro transduction of HSCs

The optimal conditions for the transduction of HSCs have been an area of extensive investigation. Studies with MMLV vectors demonstrated that HSCs could not be transduced *in vitro* without the addition of cytokines that stimulate cell division [17–19]. Unfortunately, HSC cell division *in vitro* is frequently associated with loss of pluripotency and/or long-term engraftment capabilities. Although HIV-1-based vectors have been demonstrated to transduce the extremely primitive CD34+/CD38−/lo/Thy-1+/Lin− subset of HSCs without stimulation by cytokines [4,6,20], it has been suggested that deeply quiescent HSCs (i.e. those in G_0) may be incapable of being transduced efficiently by either retroviral or lentiviral vectors.

To address this issue, detailed analyses of the transducibility of fresh HSC samples, where attempts were made to differentiate HSCs in G_0 from those in G_1, have been performed with somewhat disparate results. Sutton *et al.* [21] report that HSCs in G_1 or $S/G_2/M$ are three to four times more transducible by lentiviral vectors than HSCs in G_0. In that study, G_0 was defined as cells with 2N DNA (i.e. diploid cells prior to S phase) and low RNA content. Using a different cell-cycle staging technique in which G_0 cells were defined as having 2N DNA and low expression of the proliferation-associated nuclear protein Ki-67, our laboratory has also demonstrated that HSCs in $S/G_2/M$ are significantly more transducible than G_0/G_1 cells; however, we found HSCs in G_0 and G_1 to be equally transducible [6]. Korin *et al.* [22] found that primary peripheral blood

T cells must progress into a G_{1b} state in order for reverse transcription to occur during HIV-1 infection. Although it still needs to be tested experimentally, this finding may have implications for the transduction of HSCs with HIV-1-based vectors, and may explain the discrepancies in previous studies with HSCs using different definitions of G_0 and G_1, wherein G_{1a} and G_{1b} states were not differentiated. If the block to lentiviral vector transduction of G_0 cells is at the level of reverse transcription, it may not be possible to overcome this limitation. The ability of the cPPT to enhance the transduction of unstimulated HSCs has been shown to be attributable to enhancement of nuclear import of the PIC, and not to enhancement of reverse transcription [13]. Presumably, then, vectors bearing the cPPT will still exhibit cell-cycle-dependent transduction rates.

While numerous reports demonstrate transduction of human CD34+ cells without stimulation by recombinant cytokines, our group and others consistently observe higher levels of gene transfer if the CD34+ cells are cultured with recombinant cytokines, such as Flt-3 ligand, c-Kit ligand and thrombopoietin. Although the presence of recombinant fibronectin does not increase gene transfer with vesicular stomatitis virus G (VSV-G)-pseudotyped lentiviral vectors to the high extent seen with retroviral-envelope pseudotyped retroviral vectors, the presence of fibronectin during *ex vivo* culture of CD34+/CD38− cells has been shown to significantly maintain HSC engraftment activity [23]. Additionally, our laboratory has found that at any given multiplicity of infection (MOI), the level of transduction achieved is governed by the vector concentration; however, transduction levels plateau when vector supernatants contain 10^8 IU/mL or greater [24]. A similar plateau in the level of HSC transduction has been seen in other studies [21,25]. These findings indicate that a subset of CD34+ HSCs are not permissive to transduction with lentiviral vectors, and may be related to the cell-cycle issues discussed above. Thus, in summary, our favored conditions for the most efficient transduction of human CD34+ cells by lentiviral vectors include the use of recombinant cytokines and fibronectin in serum-free medium, with the addition of a relatively high concentration of lentiviral vector particles (e.g. $\geq 10^7$ IU/ml).

Vector systems and biosafety

The prospect of using HIV-1-based vectors for gene therapy in humans is, without question, a scenario that will need to be approached with caution. It is imperative to develop a vector-production system that minimizes the risk of reconstituting a replication-competent lentivirus (RCL). Because of the difficulties encountered when generating packaging cell lines for lentiviral vector production (discussed below), the vast majority of lentiviral vector studies continue to utilize transient cotransfection of plasmids separately expressing the transfer vector genome, the viral structural components, and a heterologous envelope protein, with the VSV-G protein being most commonly used to pseudotype vector particles (Figure 1a) [26]. Each of these

Figure 1
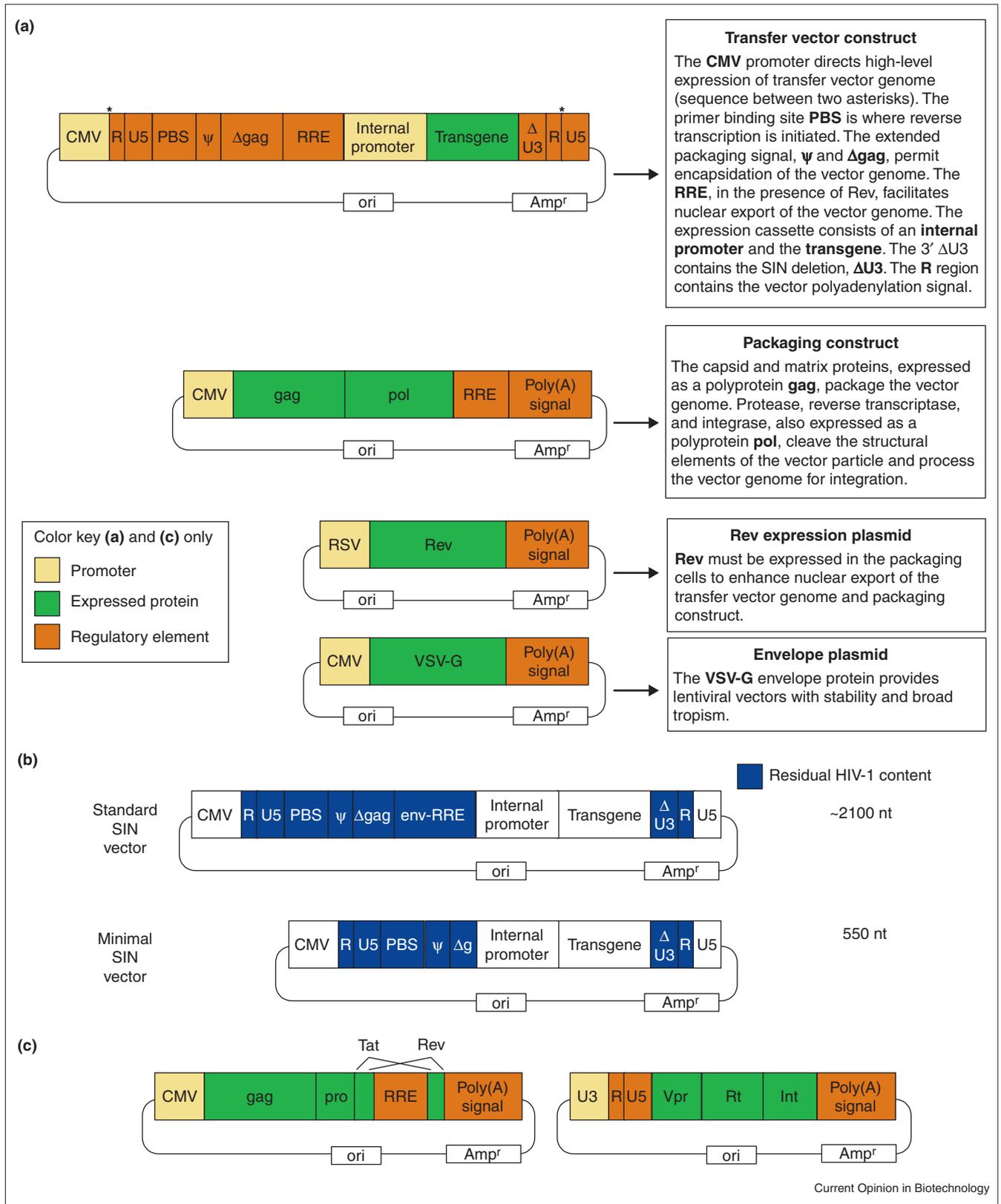
Plasmid systems for the production of lentiviral vectors. **(a)** Standard plasmid system for lentiviral vector production using transient transfection [26]. **(b)** Comparison of standard SIN vector and minimal SIN vector [32•]. **(c)** Split-packaging construct [33••]. (Diagrams not to scale.)

components have undergone several refinements in recent years, with due emphasis being put on developing vector systems capable of producing high titer supernatants, but also having improved biosafety profiles.

Perhaps the most significant modification to the transfer vector construct has been the deletion of most of the U3 region of the long terminal repeat (LTR), producing self-inactivating (SIN) vectors. The deletion most commonly used by different investigators, which removes nucleotides –418 to –18 relative to the U3–R junction, renders the LTR largely transcriptionally inactive [27]. This provides two important biosafety features to SIN vectors: decreased chance of insertional mutagenesis due to transcription from the 3′ LTR of the integrated provirus, and decreased production of vector transcripts which contain the packaging signal ψ. The importance of the latter point is illustrated by studies that demonstrate interactions between lentiviral vectors and wild-type HIV-1. Evans and Garcia [28] found that lentiviral vectors with complete HIV-1 LTRs could be mobilized from T-cell lines and primary human lymphocytes after infection with HIV-1. This is particularly relevant when considering the transduction of HSCs, as several subsets of HSC progeny are susceptible to infection with HIV-1. SIN vectors reduce, but do not completely eliminate the chance of vector mobilization by HIV-1. Although LTR-directed transcription in SIN vectors is diminished enough that LTR-driven transcripts cannot be detected by northern analysis [27], we have been able to detect transcripts arising from the LTR of SIN vectors in several target cells using a sensitive RT–PCR-based assay (AC Logan and DB Kohn, unpublished results). Additionally, although two groups have been unable to demonstrate mobilization of SIN vectors [29–30], Xu *et al.* [31] were able to detect very low levels of SIN vector mobilization. These results indicate that there may be some room for improvement in the design of SIN lentiviral vectors.

Another area of concern is the development of recombinant vector genomes, as they may lead to the production of RCL. In fact, there are no published reports of the generation of RCL by any investigators using standard HIV-1-based vector production systems. Nevertheless, efforts have been made to reduce the generation of even partial recombinants by modifying the transfer vector or the packaging construct to reduce areas of overlap between the two. Transfer vector modifications include attempts to make large deletions in the lentiviral backbone that go far beyond the deletions found in standard SIN vectors. The rationale for this approach is to remove as much of the native HIV-1 genome sequence as possible from the transfer vector construct. Iwakuma *et al.* [30] found that the U3 region can tolerate a slightly larger SIN deletion than commonly used without seriously impairing titer, but that any deletions in U5 significantly impair vector function. This may indicate that the minimal HIV-1 LTR that can be used in a vector will need to consist of a small portion of U3 containing the left attachment (*attL*) site required for

integration, and the entire R and U5 regions. Nevertheless, the area between the end of U5 and the internal promoter contains a long stretch of residual HIV-1 genome, including portions of *gag* and the RRE, which are attractive targets for removal. Cui *et al.* [32•] analyzed a series of deletions that removed the RRE and the residual portions of *gag* in the vector, and demonstrated that respectable titers can be maintained after deletion of the RRE only if the *cis*-repressive elements found in *gag* are also removed. These deletions resulted in a 'minimal lentiviral vector' with only 550 residual HIV-1 nucleotides, compared with over 2 kb remaining in standard SIN vectors (Figure 1b). Similar observations have been made by other groups, and further demonstrate that the waning titers seen with vectors from which the RRE have been deleted can be reversed by inserting a strong polyadenylation signal from the SV40 virus into the U3 region of the vector LTR (P Cannon, personal communication). It remains to be determined whether such minimal lentiviral vectors will have the ability to carry large or intron-containing expression cassettes without demonstrating vector instability or loss of titer.

In addition to deleting unnecessary HIV-1 genome content from the transfer vector, it is also possible to reduce the incidence of recombination by modifying the packaging construct, which must encode the Gag and Pol polyproteins. Wu *et al.* [33••] reported that partial recombinants between portions of lentiviral vectors and the *gag/pol* sequences of packaging plasmids are indeed produced during standard vector production using transient transfection. By sequencing the recombinants, the authors demonstrate that the recombination occurred due to template switching during reverse transcription. The evidence for this was the presence of part of the poly(A) tail from the packaging construct in the recombinant just upstream of the 3′LTR [33••]. These partial recombinants can be spread to other cells if an envelope protein is provided, but are not themselves replication-competent. To eliminate the production of these partial recombinants containing the vector LTRs and the complete *gag/pol* reading frames, Wu *et al.* [33••] produced a 'split-packaging' system in which segments of the *gag* and *pol* genes were placed on separate plasmids (Figure 1c). It is still a subject of debate as to whether partial vector recombinants, which are absolutely replication-defective by themselves, are biohazardous and need to be considered in safety certification of lentiviral vectors for clinical usage.

One obstacle to translating all of the vector production systems mentioned above to clinical use is that they rely on transient transfection of plasmids encoding all vector components into producer cells. Vector generation by transient transfection is not suitable to scale up for producing clinically useful quantities of vector, nor is it likely to be looked upon favorably by regulatory agencies seeking extremely thorough characterization of vector producer cells. Thus, it is highly desirable to develop lentiviral packaging cell lines analogous to those used to produce MMLV

vectors for clinical usage. For many years, attempts to generate lentiviral vector packaging cell lines that stably express vector-packaging elements have not been successful. In large part, these failures were due to constitutive expression of the HIV-1 packaging and VSV-G envelope proteins in the cells. Recently, two groups have reported the successful generation of lentiviral vector packaging cell lines by putting the expression of all HIV-1 proteins and VSV-G under control of the tetracycline (tet)-responsive element [31,34•]. Both groups have used a tet-off system, such that expression of toxic packaging proteins such as VSV-G and HIV-1 protease are suppressed in the presence of tetracycline or doxycycline, but are induced upon drug removal. In their current format, however, both of these systems will probably be unsuitable for the production of clinically useful lentiviral vector supernatants, because they both rely on infection of the packaging cells with a transfer vector (produced by transient transfection) containing an LTR with transcriptional activity. Xu *et al.* [31] have taken an interesting approach by putting the tet-responsive element in the LTR of the transfer vector construct, so that full-length vector transcript can only be produced in cells with the tet-regulated transactivator (tTA) (i.e. the LTRs express in the tTA⁺ packaging cells, but not in target cells — what the authors call a conditional SIN vector). Further work will be needed to develop mechanisms for producing standard SIN vectors in the context of a stable packaging cell line.

Enhancing expression from lentiviral vectors

As lentiviral vector constructs and packaging systems mature, many investigators have begun designing lentiviral vectors that can achieve desired levels and patterns of expression. The goals of the many investigators currently developing lentiviral vectors for use in transducing HSCs vary widely. Some wish to introduce expression cassettes that will be active not only in the HSC, but also in each of its widely divergent progeny; others require their vectors to express specifically in a particular lineage.

The first widely distributed lentiviral transfer vector construct possessed an internal human cytomegalovirus immediate-early (hCMV-IE) promoter [35]. Although this promoter does work extremely well in cell lines used frequently in the laboratory to test vector constructs for viability and titer (such as HEK293 fibroblasts and HeLa cervical carcinoma cells), it is not well-suited for robust expression in many hematopoietic lineages. To improve the levels and stability of expression in HSCs and their progeny, several investigators have replaced the internal hCMV-IE promoter with heterologous enhancer/promoters from viruses, human and murine cellular genes or synthetic promoters. Studies with viral promoters have shown high-level expression in hematopoietic and lymphoid cells using the U3 regions of murine retroviral LTRs, including murine stem cell virus (MSCV), myeloproliferative sarcoma virus (MPSV), Gibbon ape leukemia virus (GALV) and spleen focus forming virus (SFFV) [36–39]. Cellular promoters for 'housekeeping' genes such as the human elongation factor 1α (hEF1α) and human or murine phosphoglycerate kinase (PGK) have successfully been adapted to expression from lentiviral vectors. Additionally, a composite CAG promoter consisting of the hCMV-IE enhancer and the chicken β-actin core promoter has been used successfully.

In a study comparing expression from the hCMV-IE, hEF1α and mPGK promoters, Salmon *et al.* [25] found the hEF1α promoter to direct the highest levels of expression in human CD34⁺ cells and this high level of expression was maintained after *in vitro* differentiation into several subsets of the erythromyeloid lineage. Activity of the hEF1α promoter was also shown to be high in primary human T lymphocytes isolated from adult peripheral blood [25]. Using the CD34⁺ hematopoietic precursor cell line KG1a, Ramezani and colleagues [36] found that the greatest levels of expression were directed by the hEF1α and CAG promoters, with both directing three- to fourfold increases over hCMV-IE, whereas vectors containing the MSCV U3, the GALV U3 or hPGK promoters maintained levels of expression at least twofold greater than hCMV-IE. The same group also found that the MSCV U3 promoter directed stable, robust expression in human CD34⁺ SCID (severe combined immunodeficiency)-repopulating cells, including both myeloid and lymphoid progeny, indicating that this promoter element can be used in the lentiviral setting without inducing silencing of transgene expression. In our laboratory, we have also found that the MPSV U3 region directs significantly higher levels of expression in HSCs and several hematopoietic lineages [37]. Using a different approach, two groups have demonstrated that murine retroviral U3 promoters can be incorporated into the LTR of the HIV-1 vector, permitting high levels of expression from the hybrid HIV/MSCV LTR [40,41]. Interestingly, both groups found the MSCV U3 to direct fourfold higher levels of expression in the context of the hybrid LTR as compared to lentiviral vectors with MSCV U3 placed as an internal promoter. Unfortunately, such vectors might be deemed unsuitable for clinical use owing to the production of full-length LTR-driven transcripts.

Regulated transgene expression

Constitutive transgene expression from lentiviral vectors like those described above will probably be tolerated for therapeutic applications in deficiencies of 'housekeeping' genes, such as enzymes required for lysosomal storage and nucleotide metabolism. For other disorders in which the deficient gene is involved in tightly regulated cellular processes, it will be necessary to utilize vectors capable of recapitulating the normal expression patterns of the gene in question. Constitutive expression of a gene that is required in specific hematopoietic lineages may be undesirable or toxic in other cell types. For example, studies in our laboratory have demonstrated that expression of the Wiskott–Aldrich syndrome protein from a strong constitutive promoter is toxic to fibroblasts, but necessary for normal T-lymphocyte function [42].

Targeted expression strategies can take advantage of the SIN lentiviral backbone, from which transgene expression is directed by an internal promoter or other regulatory elements. One approach to regulated transgene expression is to express the transgene from an inducible promoter, such as tet-responsive promoters [43,44]. Upon induction, this system can provide up to a 500-fold increase in transgene expression; however, a basal 'leaky' level of expression is typically observed from these vectors that may be undesirable for some applications.

Another approach to regulating transgene expression is to construct vectors with the transgene expressed from the promoter of a gene normally expressed in the target tissue. An example of this approach is found in the design of vectors that will express a transferred globin gene in erythrocytes at a level sufficient to correct sickle cell disease or thalassemia. Previous efforts to achieve high-level, erythroid-specific expression of a globin gene transferred by retroviral vectors into HSC were repeatedly frustrated by the instability of vectors carrying the sequences from the β-globin gene complex that were necessary to attain appropriate expression patterns in transgenic mice. Using lentiviral vectors, Sadelain and colleagues [45•,46] have cured mice with β-thalassemia by expressing the normal human β-globin gene from the β-globin promoter and intragenic enhancer with a full set of the minimal LCR elements required for suitable expression control in transgenic mice. In sharp contrast to previous findings with retroviral vectors, the lentiviral vector did not suffer from instability. These results comprise one of the most promising preclinical advances toward clinical trials for the hemoglobinopathies.

Erythrocyte-specific expression with therapeutic benefit has also been demonstrated using lentiviral vectors in murine models of sickle cell disease [47] and erythropoietic protoporphyria [48], the latter using chimeric transcriptional control elements made by combining strong enhancers and promoters for other cellular genes expressed specifically in erythroid cells. Lineage-specific expression has also been demonstrated in T-lymphoid, B-lymphoid and dendritic cells by expressing the transgene from vectors containing the T-cell-specific CD2 locus control region [49], the B-lymphoid-specific immunoglobulin heavy chain enhancer ([50] see below) or the major histocompatibility complex (MHC) class II DR promoter [51], respectively. Therefore, lentiviral vectors can successfully carry transcriptional control elements derived from genes normally expressed in the target tissue to produce patterns of expression that are lineage-specific. These vectors should be useful for clinical applications with genes that are relevant to genetic diseases that are normally expressed in specific lineages.

Minimizing positional effects on gene expression
Because lentiviral vectors integrate randomly into the target cell genome, transgene expression is affected by the local chromosomal environment into which integration has occurred. Local transcriptional activators or repressors can alter transgene expression at each integration site resulting in variable levels of expression. Transgene expression can also be variegated within a clone of cells, but incorporation of genomic regulatory elements such as MARs, LCRs or insulators into vectors can increase the consistency of transgene expression.

The effect of these elements on transgene expression is exemplified in a recent study by our group to develop lentiviral vectors with B-lymphoid-specific transgene expression [50]. Vectors were constructed in which a reporter transgene was expressed from the PGK promoter with and without the immunoglobulin heavy-chain enhancer (Eμ) alone or in combination with associated matrix attachment regions (Eμ-MAR). Transgene expression was examined in a variety of cell lineages. Expression from vectors carrying the Eμ and Eμ-MAR elements was two- to threefold higher in primary human and murine B-lymphocytes than expression from the vector with the PGK promoter alone. An enhancement of expression by the Eμ enhancer was not observed in other hematopoietic or non-hematopoietic cell lineages; however, there were differences in the range of B-lymphoid expression between the two vectors. B-lymphocytes expressing from the Eμ-containing vector had a wide range of expression levels. By contrast, expression from the Eμ-MAR vector was uniformly high in B-lymphocytes but not in other cells, suggesting that MARs can contribute to position-independent, lineage-regulated transgene expression.

Genomic regulatory elements can also be used to produce consistent, position-independent expression from constitutive promoters. Ramezani and colleagues [52] have evaluated expression in transduced cell clones with a series of constitutive promoters with and without the β-interferon MAR and chicken β-globin insulator. Most of the clones transduced with either of the vectors expressed the transgene. However, the percentage of expressing cells within a clone and the level of expression was variable from vectors without the MAR or insulator. Vectors carrying both the MAR and insulator elements expressed the transgene at consistent levels within a clone and between clones, demonstrating position-independent transgene expression. These observations reveal that lentiviral gene-transfer vectors can indeed be designed to express the transgene product in the target cell at a regulated and consistent level.

Notwithstanding the improvements in targeted and consistent expression achieved thus far, there will clearly be limits to the degree of transcriptional control that may be achieved using lentiviral vectors. Genetic elements such as MARs, LCRs, and insulators will diminish, but not totally eliminate, positional effects on expression from randomly integrating vectors. Typical lentiviral vectors have ~6 kb of viral protein encoding sequences removed, which should allow replacement with genes of roughly the same size. Kumar et al. [53•] recently demonstrated that lentiviral vectors were capable of carrying inserts as large

as 18 kb; however, there was a corresponding decrease in vector titers with increasing insert size. In fact, the maximal titers that could be achieved with inserts greater than 6 kb were 100-fold lower than with inserts of less than 3 kb. Thus, the challenge is to identify genomic fragments that contain all necessary *cis*-acting sequences in a compact enough form to permit their inclusion in vector constructs that can still be produced with a high titer. Unfortunately, the design of vectors with optimized expression and which maintain suitable titers is likely to remain somewhat of an empiric undertaking.

Conclusions

All the essential components for the clinical use of lentiviral vectors to gene-modify HSCs are falling into place. Packaging cell lines will permit scale-up of vector supernatant production and characterization in accord with good manufacturing practices. Our growing understanding of the cell-cycle dependency of lentiviral vector transduction, along with further refined HSC culture conditions that do not adversely affect their pluripotency, will permit transduction of long-term engrafting HSCs with increased efficiency. Transfer vector enhancements improve biosafety and will permit high levels of constitutive expression or lineage-specific gene regulation when needed. It is likely that lentiviral vectors will enter into clinical trials of gene therapy using HSCs in the next few years and lead to greater efficacy for a wider array of conditions than has been achieved with murine retroviral vectors.

Acknowledgements

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