Progress towards a synthetic virus for systemic gene therapy

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Here we discuss recent progress in non-viral gene therapy with an emphasis on developments which specifically attempt to address the limitations of current systems and their inability to overcome the first barrier to systemic gene delivery, delivery to the disease site and the target cell. Other issues associated with the efficiency of transfection, such as intracellular delivey, the endosomal release of vectors internalized through endocytosis and nuclear delivey are also discussed.

Introduction

Gene therapy, once regarded as an experimental treatment for inherited genetic disorders, is now considered a candidate for front line therapy of acquired disease. The future success of gene-based drugs will be measured by their ability to compete with the clinical utility of conventional therapeutics. Accordingly, the areas of medicine most suited to gene therapy are those that have failed to respond to existing treatments, such as oncology and chronic inflammation.An attribute that is shared by these indications is the disseminated nature of the disease. As such, treatment with local or regional therapeutics is unlikely to affect clinical outcome or overall disease progression. Relevant gene delivery systems must be able to access distal disease sites following systemic administration. Gene delivery systems are based on one of two predominant platforms, viral or non-viral. Vii vectors are limited by their inability to access disease sites upon systemic administration. Until significant progress has been made in improving the pharmacokinetics and biodistriiution of viral vectors, their utility is likely to be limited to local or regional applications. Non-viral systems offer specific advantages for systemic gene therapy. Because they use synthetic or purified components, they are chemically defined and free of adventitious agents. In spite of this advantage, progress in the development of systemic non-viral vectors has been slow. Non-viral vectors currently suffer from rapid clearance from the circulation as a result of their inabiity to bypass 'first pass' organs such as the lung, liver and spleen, leading to poor delivery to the disease site. The primary goal of the research reviewed here is to overcome this limitation and thereby further the development of a non-viral gene delivery system for systemic gene therapy.

Properties of an ideal vector for systemic gene therapy

The ideal vector for systemic gene therapy will have the following properties: (i) it must be safe and well tolerated upon systemic administration; (ii) it must have appropriate size and pharmacokinetic attributes to ensure delivery to disease sites; (iii) it must deliver intact DNA to target tissue and mediate transfection of that tissue; (iv) it must be non-immunogenic; and (v) it must be stable upon manufacture so that large batches can be prepared with uniform reproducible specifications. An ideal vector must access target cell populations at levels that are adequate to effect the levels of transfection required in order to obtain the maximal therapeutic benefit. It is of particular importance to take advantage of the phenomenon of 'disease site targeting' or 'passive' targeting. Tumor growth and inflammatory disorders are associated with changes in vascular permeability that favor the local accumulation of vectors with small size and long circulation lifetimes. Normal vascular endothelium is characterized by intact intracellular junctions which permit only the passage of small molecules. However, the capillaries in tumors and sites of inflammation develop fenestrae or gaps in the endothelial layer ranging in size from 30 to 500 nm [1•]. These discontinuous capillary beds expose the underlying tumor cells to the circulation. Vectors which aim to take advantage of this unique physiology should have circulation timesofhoursorlongerinmurinemodelsandexhibita homogeneous small size with mean diameter of <1OOnm. This is small enough to exit the fenestrated epithelia and well within the size limit for receptor-mediated endocytosis and intracelhrhu delivery. The benefits of disease site targeting can be profound. In the case of delivery of chemotherapeutic drugs to distal tumor sites, for example, encapsulation in small long circulating liposomes can result in 50-to100-fold enhancements in the amount of drug delivered to the disease site [2-4].

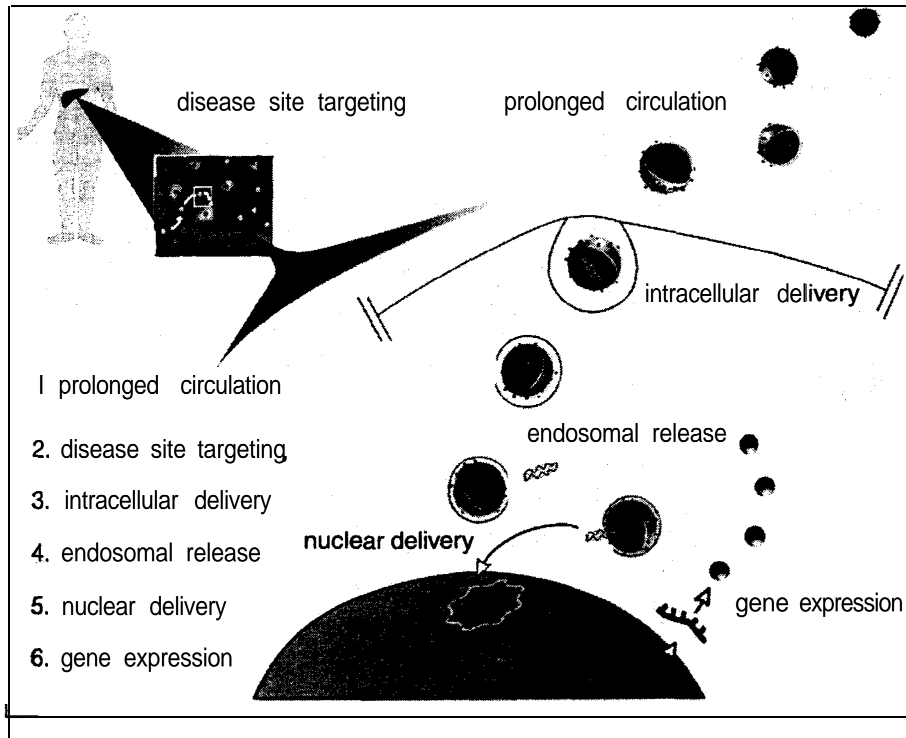
other barriers to transfection, common to local, regional and systemic gene therapy applications, include cytoplasmic and nuclear delivery (Figure 1). It is unlikely that one single approach will address all of these barriers. In fact, those properties which have been shown to be required for systemic disease site targeting can also inhibit intracellular delivery. Furthermore, nuclear delivery, thought to be one of the most inefficient aspects of the transfection process, may be inhibited by carrier components required to facilitate cellular uptake. This supports the concept of a modular solution for systemic gene therapy, a vector with individual components fulfilling different functions in the transfection process. Such a system can be thought of as an artificial virus.

Vector systems for systemic gene therapy

Lipid complexes

Although lipid-mediated systemic gene delivery and expression was reported by Zhu et *al in* 1993 [5••], progress in developing systems capable of delivering plasmid DNA to distal disease sites has been slow. Early studies on the biodistribution and pharmacokinetics of DC-Chol:DOPE complexes [6,7] determined that cationic liposomes were cleared from the circulation within minutes of intravenous (iv) administration. The majority of lipid label accumulates immediately in the lung, with the remainder being distributed in the spleen, heart and liver. Although several factors amtriiute to clearance, size and surface charge are thought to be most critical The sires of non-viral gene transfer

Figure 1. Stages of systemic gene delivery.



systems are determined by the physical chemistry of the self-assembly process. Control of self-assembly is therefore critical in order to prevent aggregation. Complex formation occurs spontaneously upon addition of plasmid DNA to cationic liposomes. Although theoretically it should be possible to standardize this process, in practice the results are often a heterogeneous mixture of complexes of lipid and plasmid DNA that range in size from 100 to 1000 nm in diameter. Rapid pulmonary clearance occurs primarily because the lungs, the first major organ encountered upon iv administration, have a large capillary bed with internal diameters < 10 μm. Large cationic complexes interact electrostatically with the pulmonary epithelium and deposition presumably ensues. Attempts to overcome the inappropriate pharmscokinetics of lipid complex systems by escalating the delivered dose typically leads to dose-limiting toxicities [8]. Despite alterations in plasmid:liposome charge ratios or the nature of the cationic lipid or co-lipids in the cationic liposomes, the pharmacokinetic limitations of these systems have not been overcome.

Hofland et al have described a detergent dialysis process which yields stable lipid-DNA complexes after removal of the detergent octylglucoside from a solution containing plasmid DNA, DOSPA and DOPE [9,10]. These particles have substantial gene transfer activity in *vitro* and are stable for > 3 months when stored at 4 °C. Upon intravenous administration these complexes transfected the lung, spleen, heart, muscle, liver and kidney in a dose-dependant manner [10]. The majority of measurable gene expression was again found in the lung. Histological analysis revealed reporter gene expression in endothelial cells lining the alveolar capillaries and in alveolar macrophages. Endothelial cells were the primary site of transfection in all of the other tissues assayed. This expression pattern may again reflect the relatively large size of the complexes formed by this process. Electron microscopy revealed particles of 250 nm diameter, well beyond the size limit for extended circulation life times. Another factor contributing to lung accumulation is the electrostatic interaction between the cationic lipid component (50% of the total lipid dose) and endothelial cells.

Templeton et al have described a novel formulation of stable complexes composed of DOTAP, cholesterol and plasmid DNA [11••]. DOTAP:cholesterol liposomes were formed by hydration of a lipid film, sonication and extrusion through a series of filters of decreasing size. The liposomes form unique structures in which the plasmid DNA is condensed in a space formed by invagination or circling of two lipid bilayers when mixed with plasmid DNA. Dynamic light scattering measurements revealed a mean particle size of 405 nm. Through a series of size fractionation experiments in combination with electron microscopy an homogeneous population was identified (200 to 450 nm diameter) which was responsible for high levels of gene expression observed in vivo. Although the majority of gene expression obtained was in the lung, a portion of this could be redirected to the liver by the incorporation of the targeting ligand, succinylated asialofetuin. Addition of asialofetuin resulted in a 7-fold increase in gene expression. The authors suggest that the improvement in particle stability, in combination with a reduction in surface charge upon incorporation of the targeting ligand, may have reduced the electrostatic interactions responsible for rapid serum clearance and thereby facilitated organ specific targeting.

Polyplex

Polyplex are defined as cationic polymer-nucleic acid complexes formed by the addition of nucleic acid to cationic polymer [12]. Cationic polymer-mediated transfection has become an established approach since polylysine-DNA complexes were first shown to be capable of transfecting mammalian cells [13]. Polylysine, polyethylenimine [14] and other polycations are known to interact electrastatically with plasmid DNA to form toroidal structures 40 to 60 nm in diameter [15,16]. In vitro activity has been found to correlate with small, uniform size and particle stability. Polylysine particles mediate low levels of transfection *in vitro* and are quite prone to aggregation, while polyethylenimine particles appear to be more stable and give higher levels of transfection [15]. It has been suggested that the correlation between high transfection potential and small particle size may be a reflection of the 100 nm diameter of coated pits in receptor-mediated endocytosis and the role of this process in polyplex uptake [16]. In spite of early in *vitro success,* systemic utility has been elusive because of rapid clearance by the reticuloendothelial system and dose-limiting toxicity. In an attempt to address these limitations, some investigators have chosen to focus on the development of polyplex systems which are either less toxic or capable of condensing plasmid DNA into more stable particles than are currently available.

Polyglucosamines are a class of cationic polymers capable of polyplex formation. One such molecule, chitosan, is a derivative of a natural cationic polysaccharide consisting of repeating glucosamine units [17,18]. Upon addition to plasmid DNA, a mixed population of particles is produced. Dynamic light scattering measurements yield particle sizes ranging from 80 to 500 nm with a major population on the order of 100 to 200 nm. Rod-lie particles 20 nm x 50 to 100 nm long can be visualized by transmission electron microscopy Unlike polyethylenimine or many lipid-based systems, chitosan-mediated transfection is not inhibited by the presence of serum *in vitro.* Chitosan is less cytotoxic in vitro than PEI or other polyplex systems [19]. Although the *in vivo* **activity** of chitosan polyplex has yet to be reported, that of a similar molecule, APL-PolyCat57, has been described **[20••]. APL-PolyCat57** is also a synthetic polyamino polymer with a glucose backbone. When used to transfect HT-1080 cells *in vitro* APL-PolyCat57 yielded gene expression that was significantly greater than that obtained with **Lipofectamine™.** APL-PolyCat57-mediated transfection was well tolerated and insensitive to the presence of serum. HT-1080 cells incubated in 60 mg/ml of APL-PolyCat57 demonstrated no cytopathic effects. When the *in vivo* activity of **APL-PolyCat57** polyplex was assessed by direct injection into intracranial tumors, levels of gene expression were comparable to a recombinant adenoviral vector. In the same experiment, **Lipofectamine™** controls yielded undetectable levels of gene expression **[20••].** The pharmacokinetic behavior of polyglucosamine-polyplex has yet to be reported. The challenge will be in controlling particle aggregation and surface charge-mediated serum clearance if polyglucosamines are to overcome the limitations of other polyplex systems.

Another approach to DNA condensation utilizes peptoids, a family of cationic N-substituted glycine oligomers. The chemistry of peptoid synthesis is such that combinatorial techniques can be applied to automate the synthesis of large numbers of compounds of defined composition. Zuchermann et al report the synthesis of a family of 67 peptoid oligomers and describe the biological activity of 24 of these [21]. Only those peptoids consisting of a repeating triplet motif (cationic-hydrophobic-hydrophobic) facilitate the transfection of mammalian cells in *vitro.* Transfection levels were comparable to those obtained with **Lipofectamine™** and were unaffected by the presence of serum. Interestingly, the peptoid with the greatest *in vitro* activity also produced the most regular, spherical plasmid condensates (50 to 100 nm in diameter). Zuchermann *et al* also examined the effects of chloroquine on peptoid-mediated transfection. The addition of chloroquine to transfection medium greatly enhances the activity of polylysine, presumably because of the inability of polylysine to escape the endosome upon internalization, the effect of chloroquine on peptoid-mediated transfection is negligible, implying that peptoid activity is not limited by endosomal release [21]. Although peptoids were shown to effectivly protect DNA from serum nucleases, the behavior of peptiod DNA complexes *in vivo* has not been described.

Lipopolyplex

Lipopolyplex are complexes that contain both polycationic polymers and cationic lipids [12]. Huang et al have improved the potency of lipid complex systems using a strategy which **involved** condensation of plasmid DNA with protamine sulfate prior to the addition of cationic liposomes **[22,23••].** Plasmid condensation results in the formation of a DNA core that becomes coated with a lipid shell The resulting lipopolyplex shows an increased activity when used to transfect cells *in vitro* compared to the corresponding complex [22]. Pm-condensing DNA with protamine sulfate results in an increase in protection from serum *nucleases in vitro*Lipopolyplex yield improved gene expression following systemic **administration** however, due to the large size and charged nature of these particles the majority are rapidly cleared by the lung where the bulk of the gene expression is observed. Huang et al have performed a rigorous analysis of the factors effecting the performance of lipopolyplex particles in vivo **[24••].** They found a charge ratio-dependent association of serum proteins upon *incubation* with mouse serum. This corresponded with a shift in zeta potential from a positive to negative value and a 3- to 4fold increase in particle diameter. Protein binding, zeta shift and increased particle size correlated inversely with transfection potential in *vivo.* These results suggest that the mechanism of lipopolyplex-mediated transfection *in vivo may be very* different from that *in vitro. Serum* protein association may limit lipopolyplex transfection in vivo. Although it has previously been shown that serum protein association affects liposome clearance **[25,26]** the impact of serum pmteins on charged gene delivery vectors has not been fully appreciated. Vectors which incorporate cationic componenta appear to require shielding of their positive charge to facilitate long circulation times and concomitant disease site targeting.

Ptasmid encapsulated in lipid vesicles

Encapsulated plasmid systems are distinguished by the presence of a lipid bilayer surrounding internalized plasmid and are an obvious first step towards **generating a synthetic virus.** Lipid encapsulation of high molecular weight DNA was first demonstrated in the late 197Os, prior to the development of cationic lipid-containing lipoplex [27-29]. Previous attempts to encapsulate plasmid DNA have yielded mostly large multilamellar vesicles with poor transfection efficiency [30-32]. Recent improvements in formulation technology have resulted in the production of cationic lipid-containing particles with a much greater transfection potential.

Novel encapsulation methods have been developed in an effort to improve the clearance properties of encapsulated plasmid systems containing high ratios of cationic lipid. Reimer et al describe a process that facilitates the encapsulation of plasmid DNA in liposomes with a reduced cationic lipid content and smaller size [33,34]. It is of obvious interest to extend these procedures to generate plasmid-containing liposomes stabilized in a manner analogous to liposomal drugs. lmprovemenk in *in vivo* stability and improved clearance characteristics should facilitate disease site targeting [35••]. Liposomes which incorporate PEG conjugates in the lipid bilayer have been shown to yield long circulation lifetimes [36,37•,38•]. PEG conjugates are thought to sterically stabilize liposomes by forming a protective hydrophilic layer which shields the hydrophobic lipid layer [39]. This prevents the association of serum proteins and concomitant uptake by the reticuloendothelial system [40•,41]. PEGliposomes containing small molecule drugs accumulate preferentially at tumor sites [42•]. This approach has also been investigated with a view towards improving the stability and pharmacokinetics of complexes [43]. However, complexes incorporating PEG-phophatidylethanolamine demonstrate an improved stability at the expense of transfection activity.

A method of encapsulating plasmid in small, PEG-coated lipid vesicles has recently been described [44••]. These authors employ a detergent dialysis procedure that results in small (approximately 70 nm diameter) 'stabilized plasmid lipid particles. (SPLP) containing one plasmid/vesicle in combination with plasmid trapping efficiencies approaching 70%. The SPLP protocol results in stable particles with low levels of cationic lipids, high levels of fusogenic lipids and high DNA to lipid ratios. SPLP can be concentrated to achieve plasmid DNA concentrations > 1 mg/ml. The properties of small size, serum stability, low levels of cationic. lipid (approximately 6 mol%) and the presence of the PEG coating suggests that SPLP should exhibit extended circulation lifetimes and disease site targeting properties following iv administration, although studies to confirm this have not yet been reported. SPLP compare favorably with previously reported plasmid **encapsulation processes. Plasmid DNA has been encapsulated by a variety of methods including reverse-phase evaporation [45-48], ether injection [49,50], detergent dialysis in the absence of PEG stabilization [47,49], lipid hydration-dehydration techniques [32,51,52] and sonication [53-55], and others [56,57]. The characteristics of these protocols are summarized in Table 1.**

Although PEG-containing SPLP are promising with respect to their ability to deliver intact **plasmid DNA to disease sites** improvements are required in order to increase levels of gene expression. In particular, SPLP exhibit relatively low transfection efficiencies ***in vitro* [44••]. *This is mainly* due to the ability of the PEG coating to inhibit cell-association and uptake of PEG-containing liposomes [58-60]. An ideal carrier would inoxporate PEGlipid conjugates that had the ability to dissociate from the carrier in the blood and transform the SPLP from a stable particle to a transfection-competent entity at the target site. The feasibility of this approach has been confirmed [44••].** A novel series of PEG-ceramide molecules has been incorporated into SPLP. PEG-ceramides differed in the length of the ceramide acyl chain. SPLP consisting of DOPE, DODAC and PEG-ceramide ($CerC_{14}$ or $CerC_{20}$) were prepared and assayed for in vitro transfection activity. **SPLP containing PEG $CerC_{20}$ demonstrated little if any transfection activity** in vitro, consistent with the stability of the PEG in the particle. When the transfection properties of SPLP containing PEG-$CerC_{20}$ were compared to SPLP containing. the PEG-$CerC_{14}$, the SPLP containing the shorter acyl chain **PEG exhibited substantially higher levels of activity than those containing PEG-$CerC_{20}$. This increase in transfection activity correlated with a more rapid dissociation rate when the rate of PEG-ceramide dissociation** *from egg* phosphatidylcholine vesicles was measured ***in vitro*. *The use*** of diffusable PEG-ceramides facilitates the formulation of stable particles containing a high percentage (79 to 84 mol%) of the fusogenic lipid DOPE. As the PEG-ceramide dissociates from the particle it is expected to become increasingly fusogenic. This approach may resolve the two conflicting demands imposed upon carriers for systemic gene therapy. Firstly, the carrier must be stable and circulate long enough to facilitate accumulation at disease sites. Secondly, the carrier must be capable of interacting with target cells in order to facilitate intracellular delivery. The use of PEG coatings which dissociate from the carrier at the disease site provides a potential solution to this problem

Future prospects

The development of well characterized, non-toxic, plasmid-lipid particles with long circulation times and stability in the blood **suggests that** non-viral gene transfer systems are capable of overcoming the first barrier to systemic gene delivery, namely delivery to the disease site and the target cell. In this regard, SPLP are a significant step toward the development of a synthetic virus for systemic gene therapy. Furthermore, they may represent a new generation of non-viral vectors which can be used to test specific theories about the nature of the transfection process in a systemic context The modular nature of SPLP will allow for substitution and evaluation of **individual** carrier componenta which may affect the remaining steps of gene delivery: intracellular delivery, endosomal release, nuclear delivery and gene expression (Figure 1). The use of exchangeable PEG-ceramide coatings has further implications for potentiating transfection. It may be possible to modulate the properties of SPLP through the use of **PEG-ceramides** with varying dissociation rates in order to redirect the SPLP to target tissues. Furthermore, the potential to utilize the SPLP platform to investigate the effects of targeting ligands on **tissue accumulation and transfection is promising. It remains to be seen if the addition of targeting ligands to SPLP serves merely to redirect the accumulation of SPLP particles or if targeting enhances intracellular delivery and gene expression** at **the target site.** The factors affecting intracellular delivery of non-viral vectors are poorly understood. It is believed that both polycations and cationic lipids function by surrounding plasmid DNA with a net positive charge which in turn enables binding of the DNA complex to anionic cell surface molecules. Proteoglycans have been shown to enhance polyplex- and complex-mediated transfection both in vitro [61] and in vivo [62•]. Given that the basis of the interaction between proteoglycans and cationic vectors appears to be electrostatic, differences in charge and charge density between vector systems should yield differences in transfection efficiency. Variations in the cationic lipid content of SPLP formulations may effect intracellular delivery and concomitant transfection invivo.

Table 1. Procedures for encapsulating plasmid in lipid-based systems.

Procedure	Lipid composition	Length of DNA	Trapping efficiency*	DNA-to-lipid ratio*	Diameter
Reverse-phase evaporation [45]	PS or PS:Chol (50:50)	SV40 DNA	30 to 50%	< 4.2 μg/μmol	400 nm
Reverse-phase evaporation [46]	PC:PS:Chol (40:10:50)	11.9 kb plasmid	13 to16%	0.23 μg/μmol	100 nm to 1 μm
Reverse-phase evaporation [47]	PC:PS:Chol (50:10:40)	8.3 kb, 14.2 kbp plasmid	10%	0.97μg/μmol	ND
Reverse-phase evaporation [48]	EPC:PS:Chol (40:10:50)	3.9 kb plasmid	12%	0.38 μg/μmol	400 nm
Ether injection [49]	EPC:EPG (91:9)	3.9 kb plasmid	2 to 6%	< 1μg/μmol	0.1 to 1.5 μm; 0.2 Aug = 230 nm
Ether injection [50]	PC:PS:Chol (40:10:50) PC:PG:Chol (40:10:50)	3.9 kb plasmid	15%	15 μg/μmol	ND
Detergent dialysis [69]	EPC:Chol:stearylami ne (43.5:43.5:13)	sonicated genomic DNA (approximately 250,000 MW)	11%	0.26 μg/μmol	50 nm
Detergent dialysis, extrusion [70]	DOPC:Chol:oleic acid or DOPE:Chol:oleic acid (40:40:20)	4.6 kb plasmid	14 to 17%	2.25 μg/μmol	180 nm (DOPC) 290 nm (DOPE)
Lipid hydration [51]	EPC:Chol (65:35) or EPC	3.9 kb, 13 kb plasmid	ND	ND	0.5 to 7.5 μm
Dehydration-rehydration, extrusion (400 or 200 nm filters) [52]	Chol:EPC:PS (50:40:10)	ND	ND	0.83 μg/μmol (200 nm) 1.97 μg/μmol (400 nm)	142.5 nm (200 nm filter) 54.6 nm (400 nm filter, ultracentrifugation)
Dehydration-rehydration [32]	EPC	2.96 kb, 7.25 kb plasmid	35 to 40%	2.65 to 3.0 μg/μmol	1 to 2 μm
Sonication (in the presence of lysozyme) [53]	asolectin (soybean phospholipids)	1.0 kb linear DNA	50%	0.08 μg/μmol	100 to 200 nm
Sonication [54]	EPC:Chol:lysine-DPPE (55:30:15)	6.3 kb ssDNA 1.0 kb dsRNA	60 to 95% ssDNA 80 to 90% dsRNA	13 μg/μmol ssDNA; 14 μg/μmol dsRNA	100 to 150 nm
Spermidine-condensed DNA, sonication, extrusion [55]	EPC:Chol:PS (40:50:10) EPC:Chol:EPA (40:50:10) or EPC:Chol:CL (50:40:10)	4.4 kb, 7.2 kb plasmid	46 to 52%	2.53 to 2.87 μg/μmol	400 to 500 nm
Ca^{2+}-EDTA entrapment of DNA-protein complexes [57]	PS:Chol (50:50)	42.1 kbp bacteriophage	52 to 59%	22 μg/μmol	ND
Freeze-thaw, extrusion [56]	POPC:DDAB (99:1)	3.4 kb linear plasmid	17 to 50%	ND	80 to 120 nm
SPLP [44]	DOPE:PEG-Cer:DODAC (84:10:6)	4.4 to 10 kb plasmid	60 to 70%	62.5 μg/μmol	75 nm (QELS); 65 nm (freeze-fracture)

*Some values calculated based on presented data.
ND = Not determined.

Future developments will need to address other issues associated with the efficiency of transfection, such as the endosomal release of vectors internalized through endocytosis. One strategy for enhancing the endosomal release of lipid carriers relies on the use of lipid components which render the carrier pH sensitive [63]. When liposomes containing pH sensitive lipids are exposed to the low pH of the late endosome, destabilization of the lipid carrier ensues. Although this strategy has the potential to assist in the release of plasmid DNA from intact SPLP particles, SPLP containing high mol% of fusogenic lipid are expected to be highly fusogenic upon dissociation of PEG-ceramide. Nuclear delivery is generally regarded as one of the least efficient stages of the transfection process. In an effort to improve upon existing gene delivery technology, a number of investigators have directed their attention to 'the last 200 nm' [64] and the factors effecting the nuclear uptake and expression of gene therapy vectors [65•,66-68]. Recently, cationic lipids, but not polyplex, were found to inhibit transgene expression when injected directly into the nucleus

of COS-7 cells [65•]. When injected into the cytoplasm, polyplex were found to enhance the nuclear uptake of DNA independent of charge ratio, implying that nuclear uptake is mediated by DNA compaction rather than net positive charge. These results imply that there may be a benefit to ensuring complete dissociation of the plasmid and lipid components of the delivery system prior to nuclear delivery. They also indicate the potential to enhance the nuclear uptake of DNA in non-viral carriers by incorporation of polycations in the formulation process. There may be an advantage to invoking active transport mechanisms for the uptake of plasmid DNA. Sebestyen et al recently described a significant enhancement in nuclear uptake upon treatment of digitonin permeabilized cells with plasmid DNA covalently modified to contain multiple copies of the SV-40 large T-antigen nuclear localization signal peptide (NLS) [67]. In a separate study, NLS-specific transfection enhancements of 10- to 1000-fold were observed when intact cells were treated with linearized restriction fragments which had been capped with oligonucleotides covalently modified to contain a single copy NLS ([68]. There appears to be considerable potential for improving the nuclear uptake of supercoiled plasrnids through attachment of NLS peptides. It remains to be seen if this can be accomplished in a manner which is compatible with formulation and systemic gene delivery.

Conclusions

Although none of the vector systems described here fully meets the criteria set out in the introduction for an ideal vector for systemic gene therapy, substantial progress has been made towards overcoming the pharmacokinetic limitations of non-viral vectors. Encapsulated plasmid systemshaveshownparticularpromiseinthisarea.The development of SPLP is an outcome of the acknowledgement of the pharmacological principles required to achieve disease site targeting SPLP exhibit small size, high plasmid to lipid ratio, high fusogenic lipid content and the ability to be concentrated to high plasmid DNA concentrations SPLP are stabilized by the presence of a PEG coating that can be designed to dissociate thus increasing the transfection potency of the SPLP at the target site. Those systems which incorporate PEG conjugates in the lipid bilayer are capable of protecting the plasmid DNA from nuclease degredation [44••]. These systems represent a novel platform technology for the development of gene delivery systems for systemic gene therapy. Rational and incremental improvements are possible to proceed towards the systems with ever-increasing resemblance to a 'synthetic virus'.

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of outstanding interest
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