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# Gene therapy of cystic fibrosis (CF) airways: A review emphasizing targeting with lactose

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**Cystic fibrosis is a disease for which a number of Phase I clinical trials of gene therapy have been initiated. Several factors account for the high level of interest in a gene therapy approach to this disease. CF is the most common lethal inherited disease in Caucasian populations. The lung, the organ that is predominantly responsible for the morbidity and mortality in CF patients, is accessible by a non-invasive method, the inhalation of aerosols. The vectors employed in the Phase I trials have included recombinant adenoviruses, adeno-associated viruses and cationic lipids. While there have been some positive results, the success of the vectors until now has been limited by either immunogenicity or low efficiency. A more fundamental obstacle has been the absence of appropriate receptors on the apical surface of airway epithelial cells. Molecular conjugates with carbohydrate substitution to provide targeting offer several potential advantages. Lactosylated polylysine in which 40% of the lysines have been substituted with lactose has been shown to provide a high efficiency of transfection in primary cultures of CF airway epithelial cells. Other important features include a relatively low immunogenicity and cytotoxicity. Most importantly, the lactosylated polylysine was demonstrated to give nuclear localization in CF airway epithelial cells. Until now, most non-viral vectors did not have the capability to provide nuclear localization. These unique qualities provided by the lactosylation of non-viral vectors, such as polylysine may help to advance the development of molecular conjugates sufficiently to warrant their use in future clinical trials for the gene therapy of inherited diseases of the lung.**

***Keywords:*** **lactosylated poly-L-lysine, gene transfer, non-viral vector, cystic fibrosis airway epithelial cells, nuclear translocation, cystic fibrosis, gene therapy**

***Abbreviations:*** **CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; NLS, nuclear localization signal; NPC, nuclear pore complex; PEI, polyethylenimine; WGA, wheat germ agglutinin.**

## Introduction

Gene therapy offers the potential of correcting the underlying cause of monogenetic diseases for which the responsible gene is known. Cystic fibrosis is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encodes for a chloride channel residing in the epithelium of multiple affected organs [1]. Since CF is a recessive monogenetic disorder, introduction of a normal copy of the gene into the host cell should restore the normal $Cl^-$ transport function. A variety of epithelial tissues of organs are affected in this disease, including airways, pancreas, sweat ducts and the gastrointestinal system. However, CF lung disease, characterized by thick mucous, inflammation and bacterial infection, is the major cause of morbidity and mortality [2]. Therefore, most CF gene therapy studies target the airway epithelium. Moreover, it is believed that the human airways can be selectively targeted by non-invasive methods such as aerosol and direct instillment [3,4].

The first step of gene transfer in general is the attachment of the delivery vehicle to the surface of target cells. Subsequently, the attached vehicle is taken up by endocytosis. Non-viral gene delivery systems rely on normal cellular uptake mechanisms. The specificity of gene expression can be increased by the addition of a targeting ligand to the surface of the DNA delivery vehicle. After endocytosis the DNA-containing particles are largely retained in perinuclear endosomes/lysosomes [5]. The normal fate of early endosomes to lysosomal vesicles includes acidification, which provides a milieu for the activity of hydrolytic catabolic enzymes. In order to avoid the degradation of

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the therapeutic DNA, gene delivery systems must escape from endosomes [6] and reach the nucleus. Pharmacological agents such as chloroquine can be used to disrupt the internal routing of the delivery vehicle/cDNA complex from the endosomes to the lysosomes [7]. Once released from endosomes the cDNA has to reach the nucleoplasm where transcription can take place. The nuclear envelope consists of an outer nuclear membrane and an inner nuclear membrane, connected to each other by the nuclear pore complex, a protein structure [8]. Nuclear pore complexes form aqueous channels through the double membrane of the nucleus [9] and thus create passageways for proteins and genetic material [10].

## Vectors for gene delivery

A number of different gene delivery systems either have been or are currently under evaluation for use in therapy for CF. These delivery vehicles fall into two classes. First those based on viral vectors such as recombinant retrovirus [11], adenovirus [12] and adeno-associated virus (AAV) [13], and second those that are synthetic self-assembling systems such as cationic lipids [14] or molecular conjugates [15–17]. Characteristics of each gene delivery system are summarized in Table 1. However each delivery system has to overcome considerable obstacles and to date none has proven to be completely satisfactory [4].

### Viral vectors

Viral based vectors pose concerns regarding their immunogenicity and toxicity. Alveolar tissue is sensitive to adenoviral vectors [18], and major adverse events during clinical trials have been described [19]. In addition to immunogenicity and toxicity the present use of viral gene delivery systems is limited to low transfection efficiency *in vivo* due to the complex barriers airway epithelia created to prevent penetration of lumenally delivered materials. A well-defined mucus layer may bind and clear vectors; a glycocalyx may inhibit vectors from binding to their receptor and most importantly the apical membrane lacks a receptor for adenoviral vectors [20,21]. Although a receptor (heparan sulfate) for AAV vector has been located on the basolateral membrane of airway epithelial cells [22], it has been demonstrated that the apical membrane contains abundant high affinity receptor for AAV5 and AAV4. Furthermore the binding requires sialic acid in an $\alpha$ 2,3 linkage but the vectors differ in sialic acid linkage specifity [23,24]. Moreover, transfection with AAV from the apical membrane may also be limited by endosomal processing [25]. However understanding these obstacles will help in the development of viral-based gene delivery systems that may circumvent these problems.

Retroviral vectors under investigation for CF gene therapy were reviewed [26]. Lentiviral vectors and recombinant AAV address the problem of poor persistence by integration into the host DNA [27]. However the drawback is the possible risk of mutagenesis. The risk is low but leukemia induction in an animal model of retroviral gene marking was observed [28]. Another obstacle is the failure of retro- and lentiviral vectors to transfect airway epithelia when administrated to the apical surface of polarized airway epithelial cells. This may be overcome by the development of either methods that increase access to the basolateral membrane or better apical membrane-binding pseudotypes [29]. A filovirus-pseudotyped HIV vector has been described to efficiently transfect murine airway epithelium *in vivo* [30].

### Non-viral vectors

Non-viral vectors may overcome the current problems in safety, immunogenicity and mutagenesis associated with viral vectors [31]. Cationic-lipid based gene delivery systems are lipid:DNA complexes which have been proven to transfect airway epithelium *in vivo* [32]. Initial clinical trials have indicated that liposomes have a relatively low toxicity profile for administration to nasal epithelium [33–35] even when administrated repetitively [36]. However when administrated to human lungs mild influenza-like symptoms were described which may be related to the DNA [14]. In addition cationic lipid/DNA complexes have generally shown to be less efficient than viral vectors [37].

**Table 1.** Gene delivery systems for CF airway cells

| *Gene delivery system* | *Toxicity and immunogenicity* | *Efficiency* | *Specificity* | *Packaging size* | *Risk of insertional mutgenesis* | *Clinical trials* |
|---|---|---|---|---|---|---|
| Adenovirus | Dose related immune response | Moderate | CAR receptor | Limited | Potential risk present | [12,19,87,88,89] |
| AAV | Non-pathogenic | Moderate | Serotype dependent | Small | Integrates into host genome | [13] |
| Retrovirus | Potentially toxic and immunogenic | Low | Serotype dependent | Limited | Integrates into host genome | None |
| Liposomes | Low but varied toxicity | Low | Requires targeting ligand | Large | No | [33,34,35,36] |
| Molecular conjugates | Toxicity varies | Low | Requires targeting ligand | Large | No | None |

However the composition of lipids in lipid based gene delivery vehicles varies and is critical to vector targeting and efficiency. Moreover, additional components can both increase efficiency and promote selective targeting [38].

## Glycosylation for targeting of non-viral vectors

Non-viral gene delivery using cationic polymers or cationic lipids based carriers continues to be of interest due to the safety advantages. However there are many problems to overcome, notably their comparatively low efficiency and lack of capability to target gene expression to the area of pathology [39]. The specificity can be modified by the addition of a targeting ligand to the carrier [40].

Lectins are proteins that bind to specific carbohydrate structures [41]. The natural ligands for most lectins are typically complex glycoconjugates that carry clustered arrays of the cognate carbohydrate, thus cooperating with clustered lectin-binding sites to generate high-avidity binding [42]. Some membrane-bound lectins are internalized upon binding to ligands, followed by delivery to internal acidic compartments [43]. Therefore glycosylated gene delivery systems may take advantage of membrane bound lectins to enter a specific cell through receptor-mediated endocytosis. Currently several carbohydrates are under investigation for their application in gene transfer, including galactose, mannose and lactose (Table 2).

### Galactosylation

The asialoglycoprotein located on the hepatocyte membrane specifically recognizes terminally linked $\beta$-galactose or GalNAc residues on circulating glycoproteins and cells [44]. In one of the first gene transfers experiments asialoorosomucoid covalently bound to polylysine was used as a ligand for the transfection of HepG2 cells [45] followed by transfection of the liver in a rat model [46]. In addition galactosylated polylysine was used to deliver genes to the rat liver [47], and biochemical and functional characterization of the galactosylated polylysine/DNA complex was reported [48]. Galactosylated PEI was used to transfect hepatocytes *in vitro.* An advantage over other polycation polymer-DNA carriers is that PEI is capable of endosomal escape without the aid of endosome disruptive additives [49]. Bettinger et al. [50] demonstrated that size reduction of galactosylated PEI/DNA complex improved the gene transfer. When galactosylated poly-L-ornithine was used to transfect the mouse liver, it was demonstrated that administration of galactosylated albumin prior to transfection reduced reporter gene expression [51]. Kawakami et al. [52] demonstrated that galactosylated liposome/DNA complex transfected the mouse liver through asialoglycoprotein receptor-mediated endocytosis. Thus a variety of DNA carriers are in development using galactose as a tageting ligand for the hepatocyte asialoglycoprotein receptor taking advantage of receptor-mediated endocytosis to both enhance efficiency and specificity of gene transfer.

**Table 2.** Glycosylated non-viral gene delivery systems

| *Targeting ligand* | *Carrier* | *Target cells* | *References* |
|---|---|---|---|
| Lactose | Polylysine | Airway cells in culture | [15,16,67,70] |
| | PEI | Isolated hepatocytes | [65] |
| | Liposome | Kupffer cells and hepatocytes *in vivo* | [64] |
| Galactose | Polylysine | Rat hepatocytes *in vivo* | [47,48,49] |
| | PEI | Hepatocytes *in vitro* | [50] |
| | Liposome | Murine hepatocytes *in vivo* | [52] |
| | Poly-L-ornithine | Murine hepatocytes *in vivo* | [51] |
| Mannose | Polylysine | Macrophage *in vitro* | [55] |
| | | Murine macrophages *in vivo* | [51,56,57] |
| | Liposomes | Murine macrophages *in vitro* and *in vivo* | [61,62] |
| | Liposome-PEI | Murine macrophages *in vitro* and *in vivo* | [63] |
| Asialoorosomucoid | Polylysine | Hepatocytes *in vitro* | [45] |
| | | Rat hepatocytes *in vivo* | [46] |

### Mannosylation

The mannose receptor located on a variety of macrophage subtypes [53] recognizes glycoproteins with mannose, glucose, fucose and *N*-acetylglucosamine residues in exposed, nonreducing positions [54]. Mannosylated poly-L-lysine was the most efficient in gene transfer into human monocyte-derived macrophages when compared to other glycosylated poly-L-lysine based DNA carriers [55]. In addition mannosylated poly-L-lysine was used to transfect murine macrophages *in vivo* successfully [56,57]. Macrophages are targets for gene therapy of diseases as Gaucher's disease [58] and HIV infection [59] but may also be a target cell for expressing an exogenous gene with therapeutical effects [60]. When the biodistribution of mannosylated liposomes in mice was studied, it was found that intravenously administered mannosylated liposomes were taken up mainly by non-parenchymal cells in the liver [61] and transfected mouse peritoneal macrophages *in vitro* efficiently. *In vivo* expression of the reporter gene was detected in the non-parenchymal cells of the liver and was reduced by predosing with mannosylated bovine serum albumin [62]. Expression was further enhanced by the incorporation of PEI

into the liposome complex [63]. Therefore mannosylated DNA carriers transfect macrophages efficiently *in vitro* and *in vivo* and is thought to employ the mannose receptor for endocytosis.

### Lactosylation

It was demonstrated that when bound to low density lipoprotein the lactose residue can target both Kupffer cells and parenchymal cells of the liver. The liver expresses two types of galactose receptors, one specific for parenchymal cells and one for Kupffer cells. The specificity depends on the degree of lactosylation. At high substitution of lactose the Kupffer cells mainly take up lactosylated low-density protein. At low substitution of lactose parenchymal cells are the main site of uptake [64]. Also lactosylated PEI was used to deliver RNA/DNA oligonucleotides in rat hepatocytes [65]. The gene transfer efficiency of glycosylated poly-L-lysines in CF airway epithelial cells was determined using a spectrum of carbohydrates to derivatize poly-L-lysine. It was found that lactosylated poly-L-lysine was the most efficient for transfection in CF airway epithelial cells in primary culture (Figure 1) [15] and in tracheal serous glands *in vitro* [66]. Since it was demonstrated that lactosylated bovine serum albumin was bound to airway epithelial cells and that this binding was inhibited by 0.1 M lactose [15], lactose may provide a targeting ligand for both airway and liver cells.

## Lactosylated poly-L-lysine for targeting CF airway cells

Lactosylated poly-L-lysine was formulated on a homopolymer of 190 residues of L-lysine, substituted with lactosyl residues on approximately 40% of the $\varepsilon$-amino groups of lysine [67]. The substitution of poly-L-lysine with carbohydrate groups diminishes the immunogenicity and the toxicity of poly-L-lysine [67–69].

### High efficiency of transfection with lactosylated poly-L-lysine

Lactosylated poly-L-lysine when complexed to cDNA proved most effective in gene transfer into CF airway epithelial cells in primary culture when compared to other glycosylated polylysines. The transgene expression can be enhanced by using a combination of agents. The combination of chloroquine and E5CA peptide, a fusogenic peptide, increased the reporter gene expression in immortalized cells 6 fold, when compared to transfection with chloroquine alone [67]. The combination of glycerol and E5CA peptide proved to give the highest reporter gene expression in airway epithelial cells in primary culture [16]. The role of these agents, which are thought to be endosomolytic are discussed [60,67].

Transfection with lactosylated polylysine not only yielded high reporter gene expression levels but also proved to be very efficient regarding the percentage of cells transfected. When immortalized CF airway epithelial cells were transfected with lactosylated polylysine/cDNA complex in the presence of chloroquine and E5CA peptide, 90% of the cells showed reporter gene

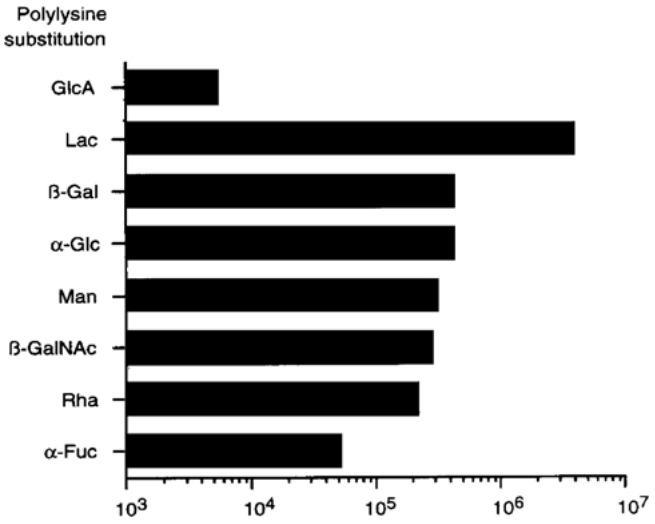


**Figure 1.** Reporter gene expression by CF airway epithelial cells in primary culture using glycosylated polylysine as vectors. Glycoslylated polylysines were complexed to pCMV*Luc* (ratio wt:wt 3:1) and added to CF airway epithelial cells in primary culture for 4 hours in the presence of 100 $\mu$M chloroquine. Luciferase activity was measured 48 hrs post transfection [15]. Used by permission.

expression [67]. It was further shown that lactosylated poly-L-lysine is a highly efficient (80–90%) vector for transferring the CFTR gene into airway epithelial cells in primary culture and into immortalized cell lines [16]. The transfection efficiency *in vitro* of lactosylated polylysine is comparable to that of viral vectors.

In addition when immortalized CF airway epithelial cells were transfected using lactosylated polylysine complexed to a GFP-CFTR fusion gene, patch clamp electrophysiology demonstrated the functional correction of $Cl^-$ channel activity of CFTR. Lactosylated polylysine therefore may provide the potential for future therapeutic use [16].

## Intracellular trafficking of lactosylated polylysine/cDNA complex

The uptake and intracellular path of lactosylated polylysine/cDNA complex with time was investigated using fluorescent markers and confocal microscopy. It was recently shown that the binding of lactosylated polylysine/cDNA complex is inhibited by both lactose and lactosylated polylysine (Figure 2). Lactosylated polylysine/cDNA complex appears to be internalized via receptor mediated endocytosis utilizing lactose binding moieties (Manuscript in preparation).

### Cellular uptake of lactosylated polylysine/cDNA complex

Thirty minutes after the addition the complex was detected by optical sectioning with confocal microscopy in intracellular vesicles [70]. Escape from endosomes and avoidance of lysosomal degradation is key for transport by gene delivery vehicles

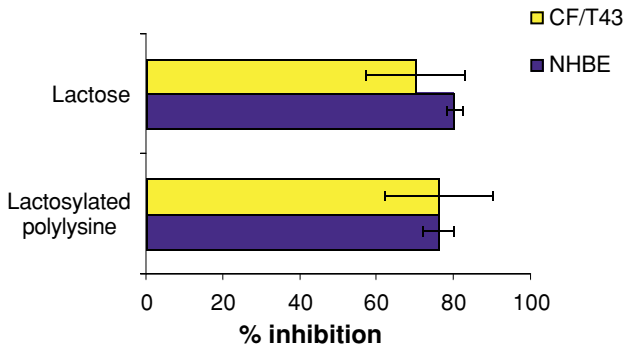


**Figure 2.** Lactose and lactosylated poly-L-lysine both inhibit binding of lactosylated poly-L-lysine/cDNA complex to airway epithelial cells. CF/T43 cells and airway epithelial cells in primary culture (NHBE) were incubated with 1 $\mu$g rhodamine-pCMV*LacZ* complexed to 4 $\mu$g FITC-lactosylated poly-L-lysine. All incubation took place at 4°C in either DMEM alone or with the addition of 0.1 M lactose, 0.1 M GlcNAc or lactosylated poly-L-lysine (200 $\mu$g). After fixation of the cells, the complex was visualized with fluorescent microscopy and digitally merged with the phase contrast image of the same field. Bars represent means $\pm$ standard deviation ($n = 5$ experiments in duplicates for CF/T43; $n = 2$ experiments in triplicates for NHBE).

into the nucleus. Chloroquine is a weak base and has an affinity for lysosomes. It was proposed that it accumulates in acidic compartments of endocytosed material, raising the luminal pH, inducing a reduction of the delivery to lysosomes and of the intravesicular degradation of endocytosed material [60]. Therefore chloroquine, by preventing lysosomal degradation ensured that the lactosylated poly-L-lysine/plasmid complex reached the perinuclear region intact where it was then transported into the nucleus. One hour after the addition of the transfection medium, optical sectioning of the cell nucleus demonstrated the presence of intact lactosylated poly-L-lysine/cDNA complex within the nucleus itself. Six hours post transfection the nuclear accumulation of complex could be observed (Figure 3A). Similar results were observed when cells were transfected in the presence of glycerol or E5CA peptide. In comparison mannosylated poly-L-lysine (Figure 3B) or unsubstituted poly-L-lysine (Figure 3C) complexed to plasmid were less efficient for nuclear accumulation [70].

### WGA inhibits the nuclear uptake of lactosylated poly-L-lysine/cDNA complex

Optical sectioning by confocal microscopy of the nucleus demonstrated that the lactosylated poly-L-lysine/cDNA complex was translocated into the nucleus intact (Figure 3A). The nucleus is bound by the nuclear envelope, which poses a considerable hydrophobic barrier to macromolecular transport [71]. The nuclear envelope consists of an outer nuclear membrane and an inner nuclear membrane, connected to each other by NPCs [8]. NPC form aqueous channels through the double membrane of the nucleus [9] and permit entry of proteins and genetic material [10]. WGA binds to *N*-acetylglucosamine residues present on a class of NPC proteins and so blocks transport through the NPC [72].

CF airway epithelial cells were incubated with FITC-labeled WGA for 60 minutes and subsequently transfected. WGA inhibited the nuclear transfer of lactosylated poly-L-lysine/cDNA complex (Figure 4). WGA added alone was internalized and accumulated around the nucleus within 60 minutes (Figure 4A), and 6 hours later it was still present around the nucleus in small

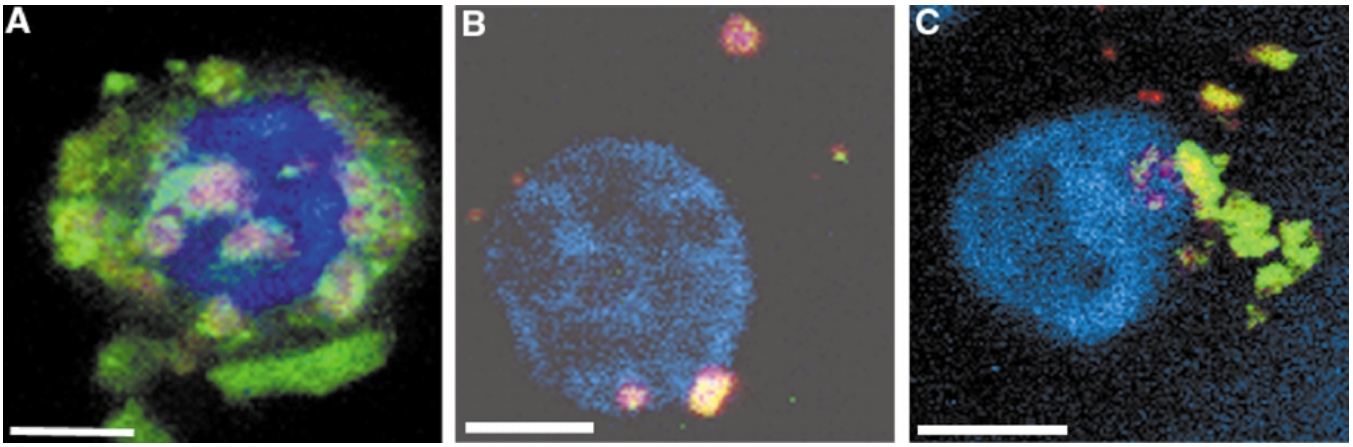


**Figure 3.** Nuclear localization of FITC-labeled glycosylated and unsubstituted polylysine/rhodamine-labeled cDNA complex in airway epithelial cells in primary culture in the presence chloroquine. Cells were grown on cover slips for 48 hrs. The cells were transfected with FITC-labeled glycosylated or unsubstituted polylysine complexed to rhodamine labeled pCMV*LacZ* in the presence 50 $\mu$M chloroquine. Six hours after addition, transfection medium was removed and cells were washed. Subsequently the cells were fixed in methanol and the nuclei were stained with Hoechst reagent. A Leica TCS4D confocal microscope was used for optical sectioning. Rhodamine was visualized in red, FITC was visualized in green and Hoechst was in blue. Yellow fluorescence represents the co-localization of the rhodamine and FITC signal. (A) Lactosylated polylysine/cDNA complex; (B) mannosylated polylysine/cDNA complex; (C) unsubstituted polylysine/cDNA complex. Original magnification $\times$100. *Size bar*, 10 $\mu$m [70]. Used by permission.

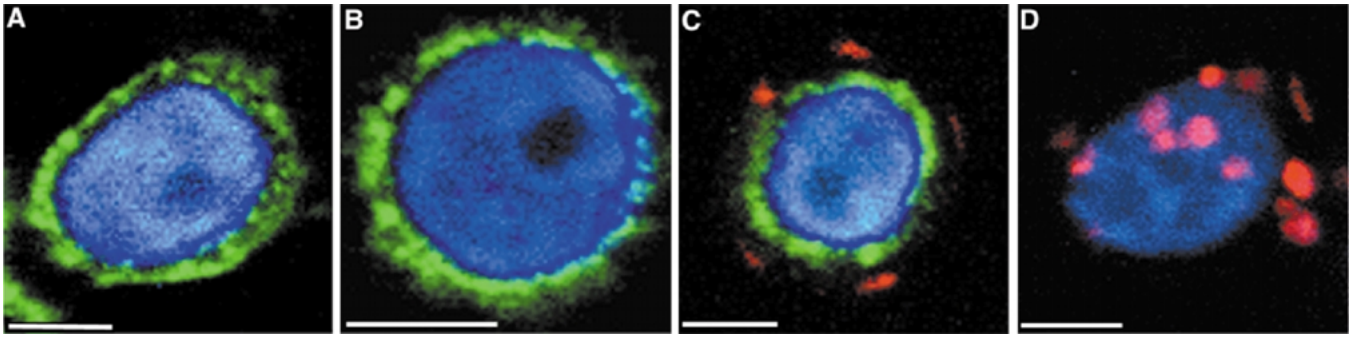


**Figure 4.** FITC-labeled WGA (green) inhibits nuclear accumulation of lactosylated polylysine/rhodamine-cDNA complex (red) in airway epithelial cells in primary culture. Cells were incubated with 300 $\mu$l DMEM containing 40 $\mu$g/ml FITC-WGA for (A) 1 hour. After 1 hour the FITC-WGA was removed and cells were refed with growth medium for (B) an additional 6 hours. After 1 hour incubation (C) with FITC-WGA or (D) DMEM cells were transfected with lactosylated polylysine/rhodamine-cDNA complex for 6 hours. See legend to Figure 3 for details. Original magnification $\times$100. *Size bar*, 10 $\mu$m. [70]. Used by permission of publisher.

patches (Figure 4B). When primary airway epithelial cells pretreated with WGA were transfected with lactosylated poly-L-lysine/rhodamine-labeled cDNA complex, no nuclear accumulation of complex was observed (Figure 4C). In contrast at the same time transfected cells, which were not pretreated with WGA showed nuclear accumulation of complex as expected (Figure 4D) [70].

WGA clearly inhibits the entry of lactosylated polylysine/cDNA complex into the nucleus (Figure 4). It is still unknown how DNA of therapeutic relevant sizes (up to 10 kbp) can pass through the NPC [71]. It was reported that plasmid DNA up to 14.4 kbp localized in the nucleus in the absence of cell division. Its nuclear import required energy, cytoplasmic factors and was blocked by WGA [73].

The nuclear entry of the lactosylated polylysine/cDNA complex may occur through a receptor-mediated event for which the lactosyl residues provide the ligand. The specific nuclear import of glycoconjugates could be related to the presence of lectins in the cell nucleus [74]. Galectin-3 a galactose/lactose-specific lectin was located in the nucleus [75,76]. The nuclear import and retention of galectin-3 has been ascribed to the carbohydrate recognition domain [77]. Galectin-3 has been shown to facilitate transfer of spliceosomes out of the nucleus [78].

The entry of many large molecules into the nucleus depends on a short stretch of basic amino acids called nuclear localization signal [79]. Moreover, another mechanism can be proposed from the recent description of the Chrp protein [80]. This cysteine and histidine rich protein binds galectin-3 at a site other than the carbohydrate recognition domain and is found in both the cytoplasm and the nucleus. We hypothesize that this protein translocates galectin-3 to the nucleus and that galectin-3 binding to the lactose residues on lactosylated polylysine allows translocation of the lactosylated polylysine/plasmid complex to the nucleus (Figure 5).

Helenius and colleagues [81] described the docking and dissociation of the adenovirus capsid followed by DNA entering the nucleus through the nuclear pore accompanied by adenoviral protein VII. We are proposing a similar mechanism in our

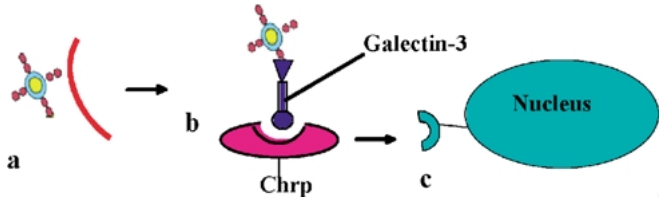


**Figure 5.** Schematic of the hypothetical trafficking of lactosylated polylysine/plasmid complex via galectin-3 and the recently described cysteine and histidine rich protein (Chrp) [80]; (a) the complex entering the cell; (b) the complex binding with galectin-3 which binds to Chrp; and (c) nuclear docking.

hypothesis with the exception that galectins may provide the docking site close to the nuclear pore complex and facilitate binding of the lactosylated polylysine to intranuclear galectins.

## Future directions

Lactosylated polylysine when used *in vitro* as a transfer agent overcomes some of the barriers of gene transfer, namely cellular uptake and nuclear translocation. Although the lungs are readily available for administration through non-invasive methods [3], the airways *in vivo* have evolved a remarkable defense system against foreign material. When administered topically all gene delivery systems, viral and non-viral, have to overcome a layer of mucous-containing liquid, ciliary clearance and phagocytosing macrophages to reach the targeted airway epithelial cells.

Entrapment of transfection agents in the mucous layer in CF lungs could be due to the increased viscosity and increased inflammatory response, therefore mucolytic agents could be used to reduce this difficulty. Moreover, nucleases in the mucus may reduce the activity of the transgene [4]. It is reported however that polylysine prevents DNA degradation [82]. In addition, macrophages may interfere with the gene delivery system and may stimulate an immune response. Macrophage activity may be blocked by pharmacological means such as the use of gadolinium chloride [4].

Cell entry of lactosylated polylysine/cDNA into undifferentiated airway epithelial cells appears to be receptor-mediated (Manuscript in preparation) based on lactose specificity. Preliminary data indicate that lactosylated polylysine also transfers cDNA into polarized airway epithelial cells from the apical side. Agents used for endosome disruption such as chloroquine and fusogenic peptides [83] enhanced transfection *in vitro* using lactosylated polylysine complexed to cDNA. These series of studies will provide clues for investigations *in vivo*.

Most gene delivery systems have problems to be solved before they can be successful *in vivo*, not the least of these are the methods of delivery. Lactosylated polylysine proved to be highly efficient *in vitro* [15] and preliminary data showed lactosylated polylysine was able to transfer cDNA into murine nasal epithelium. Therefore lactosylated polylysine is a promising gene delivery vehicle. Another promising approach may be the addition of lactose to other molecular conjugates, representing a final area of exploration to transfer the CFTR gene into airway epithelial cells.

## Summary

Lactosylated polylysine proved to be a highly efficient vector for transferring genes into CF airway epithelial cells in primary culture. In the presence of enhancing agents the efficiency of transfer (90–100%) is comparable to that of viral vectors [67]. Lactosylated polylysine was used to transfer the CFTR gene into CF airway epithelial cells giving a correction of the chloride channel dysfunction as shown with patch clamp electrophysiology [16]. Moreover, lactosylated poly-L-lysine/cDNA complexes remains intact during cellular internalization and nuclear translocation.

Polylysine, when used unsubstituted as a transfection agent, does not provide nuclear localization [70,84]. Polyethylenimine provided increased expression and nuclear localization after it had been substituted with galactose [49]. These reports, when taken together with the reports of the nuclear galectins [74–78] and the nuclear localization of lactosylated polylysine/cDNA complexes [70] provide support for the concept of a galactose/ lactose binding protein which is involved at least in docking lactosylated polylysine to the nuclear pore complex. The importance of the docking step in nuclear transport was recently emphasized [85].

Anderson [86] cited safety and ease of manufacture as the two most important features of non-viral vectors. These two factors are both characteristics of the glycosylated polylysines. The incorporation of lactose into molecular conjugates can provide both surface membrane and nuclear targeting for gene transfer in CF airway epithelial cells.

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