

Can painting human cells with exogenous maltoporin enable efficient therapeutic gene transfer by bacteriophage lambda vectors?

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Many gene therapy strategies require transfer of high-molecular weight DNA into human cells. To enable clinical trials, these vectors need to be produced on a large scale and at low cost. The production of effective high-capacity vectors like HSV-amplicons and helper-dependent adenoviral vectors is difficult to up-scale, so new inexpensive vectors are needed for the efficient delivery of high-molecular weight DNA to human cells. Bacteriophage lambda vectors can accommodate up to about 46 kb of therapeutic DNA and can be easily produced in an industrial setting. However, the lambda vectors transfer DNA into mammalian cells with only a low efficiency. It was shown that bacteriophage lambda virions ejected their DNA in the presence of the purified receptor for bacteriophage lambda, maltoporin (LamB protein), encoded by the *malB* gene of *Shigella sonnei* 3070. This property of *S. sonnei* maltoporin was exploited for the bacteriophage injection-driven DNA loading of liposomes and other polymer nanocontainers displaying maltoporin. Relying on the above evidence I hypothesize that the efficient gene transfer by industrially produced bacteriophage lambda vector virions, such as cosmid transducing particles, to human cells can be accomplished after incorporation (protein painting) of the purified *S. sonnei* maltoporin into the human plasma membrane.

Background

Gene therapy strategies involving delivery of large therapeutic cDNAs or large cell-specific promoters require vectors capable of efficient transfer of high-molecular weight DNA to human cells (Hibbitt et al. 2007). To enable gene therapy clinical trials, these vectors need to be produced on a large scale and at low cost. However, effective high-capacity gene vectors such as helper-dependent adenoviral vectors and HSV-amplicons are cumbersome to manufacture, especially when a large vector output is required (Palmer and Ng, 2003; Zauza et al.

2003). Therefore, there is a need for new methods of gene delivery that can allow efficient transfer of high molecular weight DNA to human cells with inexpensive gene vectors generated in an industrial setting.

Gene vectors based on bacterial viruses, bacteriophages, can package up to about 170 kb of double stranded DNA and are easily produced on a large scale. However, bacteriophage vectors require bacterial receptors to deliver their genetic cargo into the cells and so can mediate genetic transfer to mammalian cells with only a low efficiency (Volcy and Dewhurst, 2009). One of the best-studied bacteriophages is bacteriophage lambda of *Enterobacteriaceae* which injects its DNA into recipient cells after attachment to its cognate bacterial receptor maltoporin (LamB protein). The bacteriophage lambda genome size is 48.5 kb, which sets the maximum capacity of stable lambda vectors for therapeutic DNA at about 46 kb. It was shown that bacteriophage lambda particles ejected their DNA in the presence of the purified maltoporin receptor, encoded by the *malB* gene of *Shigella sonnei* 3070, while maltoporin of *Escherichia coli* was not sufficient for DNA ejection without addition of toxic organic solvents (Roessner et al. 1983; Roessner and Ihler, 1987). This property of maltoporin from *S. sonnei* was exploited for bacteriophage injection-driven DNA loading of liposomes and other polymer nanocontainers displaying maltoporin (Roessner et al. 1983; Roessner and Ihler, 1987; Graff et al. 2002). It is a straightforward assumption that therapeutic liposomal gene vectors can be produced by injecting therapeutic DNA into liposomes using purified bacteriophage lambda transducing particles, that is, lambda virions containing non-lambda DNA (Roessner et al. 1983; Lambert and Rigaud, 2003). Both replication-competent lambda vectors and cosmids, bearing just a small fragment of the lambda genome with the lambda's packaging sequence, can be considered for injection-driven DNA entrapment in the liposomal vectors.

Injection-assisted DNA loading of maltoporin-bearing liposomes using bacteriophage lambda virions occurs via a process that does not precisely coincide with the mechanism existing *in vivo* during bacteriophage infection of bacteria (Esquinas-Rychen and Erni, 2001). Notably, *in vivo* bacteriophage lambda DNA injection is always complete, while intra-liposomal injections can be occasionally only partial (Roessner et al. 1983). Reproducible complete ejection of DNA from bacteriophage particles can be achieved by the addition of polycations to the interior compartment of the

proteoliposomes (Lambert and Rigaud, 2003) with polycations likely to be providing a pulling force for the entering negatively charged DNA (Inamdar et al. 2006). However, the employment of polycations in therapeutic applications can be undesirable because they are often toxic (Hunter 2006). In addition, in comparison to existing methods of DNA entrapment within liposomes (Szelei and Duda, 1989; Fenske and Cullis, 2005), bacteriophage injection-driven liposome loading appears fairly cumbersome. So far it was never used to prepare of liposomal vectors with a therapeutic genetic cargo.

Clearly, instead of reconstitution in the liposomal membranes, purified maltoporin can be directly used to furnish the plasma membrane of target human cells destined for therapeutic gene transfer with bacteriophage lambda transducing particles. Efficient incorporation of exogenous integral and GPI-anchored proteins into mammalian cells (so-called 'cell painting') was previously described with the proteins being solubilised and delivered to the plasma membrane in a lipid carrier or a conventional mild detergent (Premkumar et al. 2001; Liu et al. 2007).

The hypothesis

Relying on the evidence showing injection-driven DNA transfer by bacteriophage lambda virions into liposomes bearing maltoporin receptor from *S. sonnei* 3070, I hypothesize that efficient gene transfer by the bacteriophage lambda vector virions, such as cosmid transducing particles, into human cells can be accomplished after painting of the cells with the purified *S. sonnei* maltoporin. Transduction of maltoporin-painted human cells by the DNA-ejecting lambda vector particles can be an efficient and up-scalable method for delivery of high molecular weight therapeutic DNA.

Evaluation of the hypothesis

In general, gene therapy can be performed *in vivo* and *ex vivo*. Genetic treatment *in vivo* involves direct administration of the gene vector to a patient. *Ex vivo* gene therapy consists of taking a biopsy from a patient, genetically modifying the cells *in vitro* and then transplanting the modified cells back to the patient. It might be a challenge to confirm the hypothesis in a multi-factored scenario of *in vivo* gene delivery. Therefore, the proposed gene transfer strategy should be initially tested in an *ex vivo* gene therapy setting with better control of the transduction environment *in vitro*. Transplantation of primary human fibroblasts after genetic modification *ex vivo* can be used as a treatment for a number of neurodegenerative and other diseases (Tuszynski et al. 2005). Primary fibroblasts are also simple to grow and, thus, can be used as a model cell population to evaluate the efficiency of the maltoporin painting and subsequent lambda-mediated gene delivery.

A straightforward way to produce *S. sonnei* maltoporin is by its overexpression in a non-pathogenic host such as *E.*

coli K12 harbouring a deletion of its own *malB* gene (Roessner et al. 1983). LamB is an integral membrane protein and its solubilization can be achieved by using sodium cholate, other mild detergents like Triton® X-100 or possibly lysophosphatidylcholine, a known carrier of integral membrane proteins for their efficient incorporation into the cellular plasma membrane (Premkumar et al. 2001).

The cells to undergo genetic treatment are to be furnished with maltoporin from *S. sonnei* with the excess maltoporin thoroughly removed by washing the cells. Maltoporin is likely to be inserted into the plasma membrane in various conformations with only one conformation being a correctly exposed bacteriophage lambda receptor. Maltoporin-painted cells should be incubated with purified bacteriophage lambda vector particles containing therapeutic or marker DNA and the efficiency of gene transfer should be evaluated by standard methods relying on the transgene expression in the treated cells.

Bacteriophages are known for their ability to pester industrial bacterial fermentations and so, turning misfortune into a blessing, the manufacture of replication competent bacteriophage lambda vectors on a large scale is a straightforward process. Non-replicating lambda transducing particles can be produced by cosmid packaging *in vitro* using purified proheads, tails and terminase. A single-strain system for the generation of lambda packaging extract was described by Rosenberg et al. (1985) with inducible phage functions being born on a cos-less helper prophage (Rosenberg et al. 1985). This strain can be adapted for a mass scale *in vivo* packaging of cosmid DNA in industrial fermenters.

The injection of therapeutic DNA by the lambda vector particles into human cells can be complete, perhaps due to the DNA binding factors in the cytoplasm generating a pulling force on the DNA being ejected from the lambda capsids (Inamdar et al. 2006). There might be some differences in the repertoire of the DNA binding factors between various human cell types resulting in a cell-specific variation of the efficiency of lambda injection-mediated gene transfer.

Lambda transducing particles inject into the recipient cells linear double stranded DNA with complementary 12-base single-stranded overhangs (cohesive ends). The linear status of the delivered DNA is compatible with the efficient gene transfer into mammalian cells (Hirata et al. 2007). If ligated in human cells, the cohesive ends might enable circularisation of the transferred DNA and its subsequent replication driven by the Epstein-Barr *oriP*-EBNA1 module or other replicons from circular episomes.

Mutant versions of the maltoporin receptor, which are better adapted to the milieu of the mammalian plasma membrane, can be obtained by directed evolution strategies. In addition, therapeutic lambda virions can be improved by

a capsid display of cell-targeting ligands (Zanghi et al. 2007) and protein moieties mediating evasion of the immune system.

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