

Synthetic DNA delivery systems

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The ability to safely and efficiently transfer foreign DNA into cells is a fundamental goal in biotechnology. Toward this end, rapid advances have recently been made in our understanding of mechanisms for DNA stability and transport within cells. Current synthetic DNA delivery systems are versatile and safe, but substantially less efficient than viruses. Indeed, most current systems address only one of the obstacles to DNA delivery by enhancing DNA uptake. In fact, the effectiveness of gene expression is also dependent on several additional factors, including the release of intracellular DNA, stability of DNA in the cytoplasm, unpackaging of the DNA-vector complex, and the targeting of DNA to the nucleus. Delivery systems of the future must fully accommodate all these processes to effectively shepherd DNA across the plasma membrane, through the hostile intracellular environment, and into the nucleus.

Keywords: nonviral gene delivery, transfection, gene therapy, DNA, intracellular transport

Over the past 30 years, DNA delivery, especially via the nonviral route (i.e., transfection), has become a powerful and popular research tool for elucidating gene structure, regulation, and function. Indeed, a recent search of the keyword "transfection" using the National Center for Biotechnology Information's (NCBI, Rockville, MD) MEDLINE database (www.ncbi.nlm.nih.gov/PubMed/) yielded more than 30,000 papers. DNA delivery has also been pivotal in developing new approaches (e.g., gene therapy and DNA vaccination) for treating and controlling diseases that are likely to impact clinical medicine and biotechnology over the next few years. Before such applications can be realized, however, the relative inefficiency and cytotoxicity of modern synthetic DNA delivery systems must be addressed.

Crucial to the success of DNA as a pharmaceutical or a basic research tool is transfection efficiency: in general practice, too few cells receive and express the exogenous DNA. Efficiency of transfection is dependent on both the efficiency of DNA delivery (i.e., fraction of DNA molecules getting into the nucleus) and the efficiency of DNA expression (i.e., fraction of nuclear DNA molecules that undergo transcription). Although a greater efficiency of expression can usually be achieved with strong promoters and enhancers¹, improvements in the efficiency of DNA delivery per se have been difficult to achieve; thus, the number of cells receiving DNA in their nucleus is usually small. In addition, transfection efficiency *in vitro* and *in vivo* do not always correlate^{2,3}, making translation of positive results in cell culture into animals even more difficult.

Therefore, the challenge of DNA delivery is to develop a system that is both highly efficient in delivery/expression and applicable to basic research as well as clinical settings. This paper reviews current methods of nonviral DNA delivery, highlighting systems that exploit our understanding of basic mechanisms of DNA transport and metabolism.

Defining the problem

Traditionally, DNA delivery systems have been classified as viral vector-mediated systems and nonviral vector-mediated systems (the majority of which are synthetic systems). Currently, because of their highly evolved and specialized components, viral systems are by far the most effective means of DNA delivery, achieving high efficiencies (usually >90%) for both delivery and expression. In fact, around 75% of recent clinical protocols involving gene therapy use recombinant virus-based vectors for DNA delivery (<http://www.wiley.com/genetherapy/clinical/vectors.html>). As yet, however

(except for a few anecdotal reports of success in individual patients), no definitive evidence has been presented for the clinical effectiveness of any gene therapy protocol (for a review, see ref. 4). The impotence of current methodology is attributable to the limitations of viral-mediated delivery, including toxicity, restricted targeting of specific cell types, limited DNA carrying capacity, production and packaging problems, recombination, and high cost⁵. Furthermore, the toxicity and immunogenicity of viral systems also hamper their routine use in basic research laboratories. For these reasons, nonviral systems, especially synthetic DNA delivery systems, have become increasingly desirable in both basic research laboratories and clinical settings.

Most DNA delivery systems operate at one of three general levels: DNA condensation and complexation, endocytosis, and nuclear targeting/entry. Negatively charged DNA molecules are usually condensed and/or complexed with cationic transfection reagents before delivery. These complexes are taken up by cells, usually through endocytosis, the route of uptake determining subsequent DNA release, trafficking, and lifetime in the cell. Endocytosis is a multistep process involving binding, internalization, formation of endosomes, fusion with lysosomes, and lysis. The extremely low pH and enzymes within endosomes and lysosomes usually bring about degradation of entrapped DNA and associated complexes. Finally, DNA that has survived both endocytotic processing and cytoplasmic nucleases must then dissociate from the condensed complexes either before or after entering the nucleus. Entry is thought to occur through nuclear pores (which are ~10 nm in diameter) or during cell division. Once inside the nucleus, the transfection efficiency of delivered DNA is mostly dependent on the composition of the gene expression system, which has been addressed in other recent reviews⁶.

The low efficiency of DNA delivery from outside the cell to inside the nucleus is a natural consequence of this multistep process. As a result, the number of DNA molecules decreases at each step of the journey to the nucleus. Therefore, identifying and overcoming each hurdle along the DNA entry pathways can improve DNA delivery, and hence overall transfection efficiency, dramatically. There are three major barriers to DNA delivery: low uptake across the plasma membrane, inadequate release of DNA molecules with limited stability, and lack of nuclear targeting (see Fig. 1). The effect of delivery systems on these three major barriers is the focus of this review. In the following sections, we describe the use of different methods for enhancing DNA penetration of the plasma membrane (see Table 1),

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