

# 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/](http://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 enhancers<sup>1</sup>, 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<sup>2,3</sup>, 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 cost<sup>5</sup>. 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 reviews<sup>6</sup>.

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),

# Synthetic Dna Delivery Systems

**Gretchen Marie Larson**



## **Synthetic Dna Delivery Systems:**

*Synthetic DNA Delivery Systems* Dan Luo, W. Mark Saltzman, 2003-09-30 DNA delivery into cells is a rapidly developing area in gene therapy and biotechnology. Moreover, it is a powerful research tool to determine gene structure, regulation, and function. Viral methods of DNA delivery are well characterized and efficient, but little is known about the toxicity and immunogenicity of viral vectors. As a result, non-viral transfection methods of DNA delivery are of increasing interest. *Synthetic DNA Delivery Systems* is a comprehensive and current resource on DNA transfection. The use of histidine-rich peptides and polypeptides as DNA delivery systems and self-assembled delivery systems based on cationic lipids and polymers are discussed. Targeted delivery to organelles, tumor cells, and dendritic cells comprise an important topic.

**Advanced Gene Delivery** Alain Rolland, 2003-09-02 A practical resource for everyone involved in the gene therapy field and in the design of effective gene delivery systems, this volume presents an overview and update of recent advances in the field of non-viral methods for the *in vivo* transfer of therapeutic genes to biological targets using conventional routes of administration. Methods to control the spatial and temporal modulation of gene function *in vivo* as well as the level, duration, specificity, and fidelity of gene expression are described. The rational design and the applications of a variety of non-viral gene delivery systems such as cationic lipid polymer and poly-peptide based systems are exemplified for the control of location of therapeutic genes administered by various routes. Current and potential clinical applications of gene-based medicines are presented for the prevention, correction, or modulation of diseases. Examples of current applications of plasmid-based systems for genetic vaccination, treatment of genetic disorders such as cystic fibrosis, and treatment of acquired diseases such as cancer are also provided.

**An Investigation of Sulfhydryl Cross-linked DNA Delivery Systems for Liver Targeted Gene Therapy** Kai Yu Kwok, 2001

**Nutrition and Biotechnology in Heart Disease and Cancer** John B. Longenecker, David Kritchevsky, Marc K. Drezner, 2012-12-06 There is a unique nutritional commonality developing in research relating to coronary heart disease and cancer. The primary aim of this conference was to provide a forum for the leading researchers, clinicians, educators, and administrators in these two fields to present a program on heart disease and cancer which included: a) the major historical milestones; b) the present areas of greatest interest in research and therapy; c) the latest nutritional, molecular, and biotechnological advances; and d) a perspective on the most promising areas for future research and therapy. Scientists have long contended that research marches on the feet of methodology. Thus, there are numerous examples of research fields opening secondary to methodological advances. Some examples are: 1) thin layer and gas liquid chromatography which, along with high pressure liquid chromatography, have broadened the line of advances in lipid research; and 2) PCR and the resultant impact on molecular biological approaches to several fields of science. The organizers of this conference thought the time was propitious for bringing together knowledge on newer aspects of molecular biological research with current advances in the two major areas of degenerative disease: coronary heart disease and cancer.

Our knowledge of these killer diseases has expanded greatly in the past few years and the advance has been catalyzed by use of an array of molecular biological techniques Thanks to these medical thinking in these areas is changing from considerations of treatment to strategies for prevention      Advanced Vaccination Technologies for Infectious and Chronic Diseases Vasso Apostolopoulos,Lalitkumar K. Vora,Vivek P. Chavda,2024-03-25 The role of vaccines is emerging and even critical to ending infectious and chronic diseases and pandemics alike The design and development of new vaccines could lead to improved health Handbook on Advanced Vaccination Technologies for Infectious and Chronic Disease discusses these new developments and introduces the reader to the current state of the science and the outlook going forward from the discovery of vaccines to the clinical trials of personalized vaccines Handbook on Advanced Vaccination Technologies for Infectious and Chronic Diseases is a valuable reference for occupational health professionals whose role involves supervision of immunization programs such as those working in the National Health Service some sectors of higher education and the pharmaceutical industry Offers comprehensive coverage of different vaccine platforms and their development Includes information on the regulatory perspective of vaccine development Describes different delivery approaches for vaccinology Explains the clinical development of vaccines along with novel platforms Covers all recent developments of vaccine production technologies new types of vaccines and ongoing research that could prevent future pandemics      **Synthesis of Dendrimer-like DNA and Its Application as a Nanobarcode Scaffold for DNA Detection** Yougen Li,2005

*McGraw-Hill 2003 Yearbook of Science & Technology* McGraw-Hill,2003-01-21 Expertly edited and well illustrated for ease of understanding the Yearbook satisfies the need of students and scientifically interested general readers as well as professionals to stay up to date on the most important current developments in science engineering and technology It contains approximately 170 articles by the world s leaders in science and technology and provides concise yet authoritative reviews of the progress and significance of research on topics from Agribiotechnology to Zoonoses References to Encyclopedia articles and scientific literature facilitate further study The Yearbook features Approximately 170 articles from leaders in scientific and technical disciplines from astronomy to zoology Up to date coverage in forefront areas such as bio technology cosmology environmental science and technology information technology molecular medicine telecommunications and theoretical physics 300 images and two colour graphics enhance the text Features numerous cross references to the Encyclopedia and bibliographies of key literature after each article An extensive subject index makes finding information a snap      *Artificial Self-assembling Systems for Gene Delivery* Philip L. Felgner,1996 Discusses recent advances in artificial self assembling systems including retrotransposon vectors hairpin ribozymes triple helix forming oligonucleotides liposome mediated transfection and photonic nanostructures Addresses developing efficient synthetic vectors such as modular self assembling systems mimicking important features of viral vectors Presents new developments in synthetic self assembling gene delivery systems including innovations in nonviral systems targeting nucleic acids ligand polylysine mediated transfer

dendrimer mediated transfection cationic liposomes and polylysine DNA complexes      **Atherosclerosis X** J. Davignon, F. Peter Woodford, A. Sniderman, 1995 A summary of the proceedings taken from the 10th International Conference held in Montreal 1994 At this symposium particular emphasis was placed on genetics molecular mechanisms and the clinical relevance of research findings      Kirk-Othmer Encyclopedia of Chemical Technology: Dr-El Arza Seidel, 2004 Presents a wide scope of articles on chemical substances properties manufacturing and uses on industrial processes unit operations in chemical engineering and on fundamentals and scientific subjects related to the field Describes established technology along with cutting edge topics of interest in the wide field of chemical technology      **Wiley Encyclopedia of Chemical Biology, Volume 1** Tadhg P. Begley, 2009 The first major reference at the interface of chemistry biology and medicine Chemical biology is a rapidly developing field that uses the principles tools and language of chemistry to answer important questions in the life sciences It has enabled researchers to gather critical information about the molecular biology of the cell and is the fundamental science of drug discovery playing a key role in the development of novel agents for the prevention diagnosis and treatment of disease Now students and researchers across the range of disciplines that use chemical biology techniques have a single resource that encapsulates what is known in the field It is an excellent place to begin any chemical biology investigation Major topics addressed in the encyclopedia include Applications of chemical biology Biomolecules within the cell Chemical views of biology Chemistry of biological processes and systems Synthetic molecules as tools for chemical biology Technologies and techniques in chemical biology Some 300 articles range from pure basic research to areas that have immediate applications in fields such as drug discovery sensor technology and catalysis Novices in the field can turn to articles that introduce them to the basics whereas experienced researchers have access to articles exploring the cutting edge of the science Each article ends with a list of references to facilitate further investigation With contributions from leading researchers and pioneers in the field the Wiley Encyclopedia of Chemical Biology builds on Wiley's unparalleled reputation for helping students and researchers understand the crucial role of chemistry and chemical techniques in the life sciences

**Gene Therapy Protocols** Joseph LeDoux, 2008-04-24 In this book internationally recognized investigators describe cutting edge laboratory techniques for the study of Production and In Vivo Applications of Gene Transfer Vectors and Design and Characterization of Gene Transfer Vectors Readers will find a comprehensive resource of current and emerging methods for the production of viral and non viral gene transfer vectors as well as detailed protocols for applications in stem cell biology cancer research and infectious disease      **Synthesis of a Taxane Model System and the Development of**

**Strategies for a Total Synthesis of Taxol and Synthesis and Chemistry of Novel 10-membered Ring Eneidyne** Erik Joseph Sorensen, 1995      Functional Materials Technology and Industry Forum IX Xiao Lang Chen, Xin Li Guo, Xiao Long Lu, 2018-02-21 9th China Functional Material Technology and Industry Forum 9th CFMTIF 2017 Selected peer reviewed papers from the 9th China Functional Materials Technology and Industry Forum 9th CEMTIF 2017 August 18 21 2017

Yinchuan China      **Gene Therapy Technologies, Applications and Regulations** Anthony Meager,1999 This text brings together the current data previously separated It combines information on gene therapy technologies and the attendant regulatory requirements for gene therapy products and procedures      Development of Anionic Liposomal Carriers

Containing Listeriolysin O and Red Blood Cell Ghosts as Plasmid DNA Delivery Platforms Gretchen Marie Larson,2004

*Nonviral Vectors for Gene Therapy, Part 1* Leaf Huang,Mien-Chie Hung,Ernst Wagner,2005 The field of non viral vector research has rapidly progressed since the publication of the first edition This new edition is expanded to two separate volumes that contain in depth discussions of different non viral approaches including cationic liposomes and polymers naked DNA and various physical methods of delivery as well as a comprehensive coverage of the molecular biological designs of the plasmid DNA for reduced toxicity prolonged expression and tissue or disease specific genes New developments such as the toxicity of the non viral vectors and recent advances in nucleic acid therapeutics are fully covered in these volumes

Advances in Drug Delivery Systems, 6 James M. Anderson,1994 These papers represent recent research efforts considered to be important to the future development of drug delivery systems They are the result of the Sixth International Symposium on Recent Advances in Drug Delivery Systems held in Salt Lake City in 1993      Bulletin of the Chemical Society of Japan Nihon Kagakkai,1996      *The British National Bibliography* Arthur James Wells,2003

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