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Intracellular routing of plasmid DNA during non-viral gene transfer

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Abstract

Gene transfer using non-viral vectors is a promising approach for the safe delivery of therapeutic DNA in genetic and acquired human diseases. Whereas the lack of specific immune response favors the use of plasmid–cationic polymer complexes, the limited efficacy and short duration of transgene expression impose major hurdles in the application of non-viral gene delivery techniques. Here, we review the major cellular, metabolic and physico-chemical impediments that non-viral vectors encounter before plasmid DNA enters the nucleus. Following endocytosis of DNA–polycation complexes, a large fraction of the DNA is targeted to the lysosomes. Since the cytosolic release of heterologous DNA is a prerequisite for nuclear translocation, entrapment and degradation of plasmid DNA in endo-lysosomes constitute one of the major impediments to efficient gene transfer. Plasmid DNA that escapes the endo-lysosomal compartment encounters the diffusional and metabolic barriers of the cytoplasm, reducing greatly the number of intact plasmids that reach the nucleosol. Nuclear translocation of DNA requires either the disassembly of the nuclear envelope or active nuclear transport via the nuclear pore complex. A better understanding of the cellular and molecular basis of non-viral vector trafficking from the extracellular compartment into the nucleus may provide strategies to overcome those obstacles that limit the efficiency of gene delivery.

Keywords: Lipoplex; Plasmid DNA degradation; Endocytosis; Endo-lysosome; Cytoplasm; Nuclear targeting; Diffusional mobility

Contents

Abbreviations: DOPE, dioleoylphosphatidylethanolamine; MT, microtubule; MTOC, microtubule-organizing center; NPC, nuclear pore complex; NLS, nuclear localization sequence; PEI, polyethylenimine; POL, polylysine; PAM, poliamidoamine; WGA, wheat germ agglutinin; NPC, nuclear pore complex.

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1. Introduction

Besides limitations on the size of therapeutic genes, random integration in the human genome, insertional mutagenesis and immunogenicity of viral vectors are potentially serious concerns in the application of viral vectors, which at present represent the most efficient delivery vehicles of therapeutic genes. These considerations favor the utilization of synthetic vectors over viral delivery systems for gene therapy of genetic or acquired human diseases. Although synthetic vectors largely lack the adverse effects of viral delivery systems, their severely limited in vivo transduction efficiency has limited clinical applications.

One of the most widely used synthetic DNA delivery systems is comprised of an expression cassette, inserted into a plasmid and complexed with cationic polymer (polyplex), cationic lipid (lipoplex) or a mixture of these (lipopolyplex). The positively charged DNA complex is taken up from the extracellular compartment by endocytosis and transferred into the nucleus of the target cell, a prerequisite for gene expression. While the accessibility and specific characteristics of the target organ may impose additional hurdles to gene delivery, phospholipid membranes of various cellular compartments, delimiting the cytosol, endosomes and the nucleosol, appears to constitute major obstacles along the nuclear delivery pathway of therapeutic genes.

Investigations of the cellular itinerary of plasmid DNA, complexed by synthetic molecules or phospholipids, have provided insight into the nature of potential barriers to gene transfer. Once internalized, plasmid DNA must overcome endo-lysosomal entrapment, cytosolic sequestration and nuclear exclusion of the DNA. In addition to these physical barriers, the DNA is subjected to metabolic degradation in the endo-lysosomal compartment and in the cytoplasm, further reducing the efficiency of gene transfer. Individual evaluation of these barriers provided some explanations for the enormous reduction in the number of plasmid molecules that reach the nucleus relative to that exposed to the cell. It is estimated that at least 10^5 plasmids per cell are required in the extracellular compartment to ensure that a few DNA molecules are taken up into the nucleus of non-mitotic cells. Here, we review the intracellular trafficking pathway of plasmid DNA, with focus on barriers that reduce the nuclear accumulation of plasmid DNA.

2. Internalization and degradation of DNA in the endo-lysosomal compartment

Endocytosis results in the internalization of specialized regions of the plasma membrane as well as small volumes of extracellular fluid [1]. The best known form of endocytosis is initiated by clathrin-coated pit formation and involves the localized accumulation of cargo molecules and AP-2 adaptor complexes, producing nucleation of clathrin chains into clathrin coated pits. Association of the dynamin GTPase ensures the fission of the clathrin-coated vesicles, followed by rapid un-coating and fusion of the vesicles with early endosomes [2]. Others forms of endocytosis are caveolae-mediated, clathrin-independent and adsorptive internalization [3]. Finally, some cell types are capable of internalizing extracellular fluid via macropinocytosis and large particles via phagocytosis [4]. In non-polarized cells, internalized material is first targeted to tubulovesicular sorting