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Review

Molecular modeling of polynucleotide complexes

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ABSTRACT

Delivery of polynucleotides into patient cells is a promising strategy for treatment of genetic disorders. Gene therapy aims to either synthesize desired proteins (DNA delivery) or suppress expression of endogenous genes (siRNA delivery). Carriers constitute an important part of gene therapeutics due to limitations arising from the pharmacokinetics of polynucleotides. Non-viral carriers such as polymers and lipids protect polynucleotides from intra and extracellular threats and facilitate formation of cell-permeable nanoparticles through shielding and/or bridging multiple polynucleotide molecules. Formation of nanoparticulate systems with optimal features, their cellular uptake and intracellular trafficking are crucial steps for an effective gene therapy. Despite the great amount of experimental work pursued, critical features of the nanoparticles as well as their processing mechanisms are still under debate due to the lack of instrumentation at atomic resolution. Molecular modeling based computational approaches can shed light onto the atomic level details of gene delivery systems, thus provide valuable input that cannot be readily obtained with experimental techniques. Here, we review the molecular modeling research pursued on critical gene therapy steps, highlight the knowledge gaps in the field and providing future perspectives. Existing modeling studies revealed several important aspects of gene delivery, such as nanoparticle formation dynamics with various carriers, effect of carrier properties on complexation, carrier conformations in endosomal stages, and release of polynucleotides from carriers. Rate-limiting steps related to cellular events (i.e. internalization, endosomal escape, and nuclear uptake) are now beginning to be addressed by computational approaches. Limitations arising from current computational power and accuracy of modeling have been hindering the development of more realistic models. With the help of rapidly-growing computational power, the critical aspects of gene therapy are expected to be better investigated and direct comparison between more realistic molecular modeling and experiments may open the path for design of next generation gene therapeutics.

1. Introduction

Gene therapy aims to treat a wide range of disorders by altering gene expression with the delivery of genetic materials (polynucleotides). The initial impetus behind gene therapy was the desire to synthesize therapeutic proteins *in situ* with functional DNA expression vectors. Exogenous DNA has to reach cell nucleus and produce mRNAs for desired proteins by recruiting the appropriate transcription factors. With the discovery of RNA interference (RNAi) [1] the scope of gene therapy was expanded. In RNAi, relatively long double-stranded RNAs (dsRNAs) are cleaved by the enzyme Dicer into short (21–22 nucleotide) RNAs. The guide strand in truncated dsRNAs, after dissociation, gets incorporated into RNA-induced silencing complex (RISC) to identify complementary sequence in mRNAs, leading to mRNA cleavage. The therapeutic use of RNAi relies on short interfering RNAs (siRNAs), synthetic 22 nucleotide dsRNAs. The challenges in the delivery of polynucleotides, however, have dampened the great interest in DNA and siRNA therapeutics.

The anionic polynucleotides cannot efficiently cross hydrophobic and anionic lipid bilayers of cell membranes. This limitation

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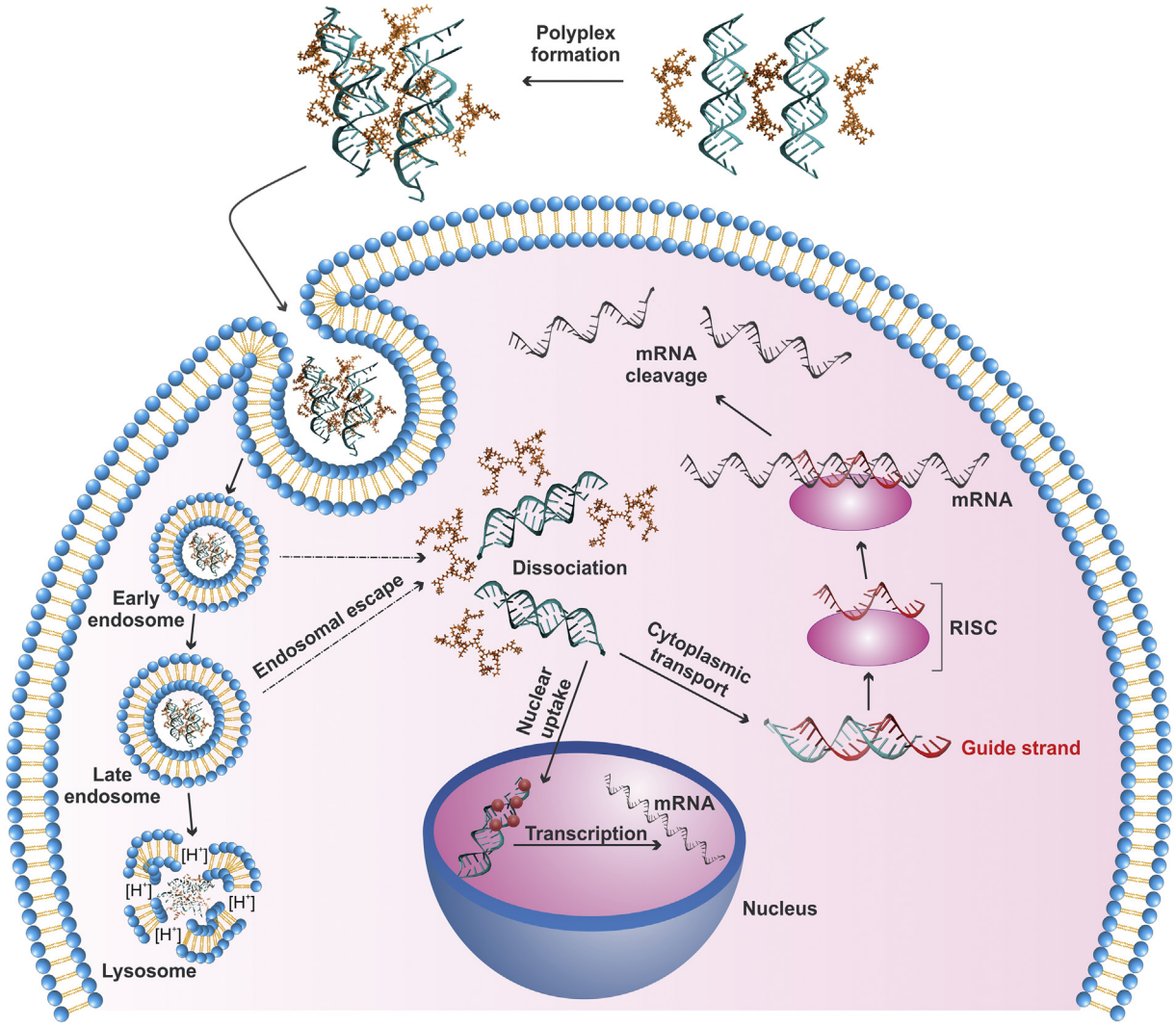

Fig. 1. Main steps involved in gene delivery. While siRNA (in red) gets incorporated into RISC in cytosol, DNA gets trafficked to nucleus to recruit transcription factors (represented as red spheres) to produce desired mRNAs. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

stimulated design of delivery systems (also known as carriers) to neutralize and compact the polynucleotides. Polynucleotides complexed with cationic polymers and lipids are known as 'polyplexes' and 'lipoplexes' (Fig. 1), respectively [2]. Binding of complexes to cell surface is governed by electrostatic interactions between cationic carriers and anionic membrane proteins and/or cell-surface receptors. Endocytosis follows via a variety of mechanisms, such as clathrin- and caveolin-1 independent, clathrin-mediated (CME), caveolae/raft-mediated (CvME) and macropinocytosis [3]. Uptake of the complexes depends on many factors and there have been some contradictory proposals on most effective endocytosis mechanism(s). While some studies proposed CvME to be the most conducive, others suggested CME as well as macropinocytosis for larger complexes that cannot be trafficked with CvME or CME [4].

Intracellular trafficking of complexes starts in early endosomes, which generally fuse into late endosomes (pH ~ 5–6) and lysosomes (pH ~ 4.5). Complexes must efficiently escape into cytosol before lysosomal degradation (Fig. 1). It is possible to facilitate endosomal escape by combining polynucleotides with fusogenic ligands, pH-sensitive carriers, and photosensitive agents [5]. Endosomal escape is also possible through 'proton-sponge effect' if the carriers possess H-buffering properties, such as polyethylenimine (PEI) [6]. Upon release into cytoplasm, polynucleotide dissociation takes place and anionic molecules such as cytoplasmic RNA and heparin-like glycosaminoglaycans are thought to be involved in this process [7]. After release, DNA has to be trafficked to nucleus for transcription and siRNA has to get incorporated into RISC in the cytoplasm to give mRNA cleavage for gene silencing (Fig. 1).

Many issues related to the mechanism(s) of action of carriers cannot be directly addressed due to instrumental limitations at atomic resolution. Molecular modeling is beginning to be employed to overcome some of these limitations. Via computer simulations, motions of individual or groups of atoms are obtained, and physical properties can be extracted from time average of equilibrated systems. Since the first simulation on a biological macromolecule in 1977 [8], molecular modeling has become a unique tool for analyzing complex biosystems. Features of complexes and critical mechanisms in delivery have been explored, placing experimental observations in a better context. An overview of molecular modeling techniques will be first given, followed by a review on modeling of polyplexes and lipoplexes.