Amino Acids (2014) 46:499–509
DOI 10.1007/s00726-013-1549-2

MINIREVIEW ARTICLE

Innovative approaches to the use of polyamines for DNA nanoparticle preparation for gene therapy

Veena Vijayanathan · Enzo Agostinelli · Thresia Thomas · T. J. Thomas

Received: 6 May 2013 / Accepted: 26 June 2013 / Published online: 17 July 2013

Abstract Advances in genomic technologies, such as next generation sequencing and disease specific gene targeting through anti-sense, anti-gene, siRNA and microRNA approaches require the transport of nucleic acid drugs through the cell membrane. Membrane transport of DNA/RNA drugs is an inefficient process, and the mechanism(s) by which this process occurs is not clear. A prerequisite for effective transport of DNA and RNA in cells is their condensation to nanoparticles of ~100 nm size. Although viral vectors are effective in gene therapy, the immune response elicited by viral proteins poses a major challenge. Multivalent cations, such as natural polyamines are excellent promoters of DNA/RNA condensation to nanoparticles. During the past 20 years, our laboratory has synthesized and tested several analogs of the natural polyamine, spermine, for their efficacy to provoke DNA condensation to nanoparticles. We determined the thermodynamics of polyamine-mediated DNA condensation, measured the structural specificity effects of polyamine analogs in facilitating the cellular uptake of oligonucleotides, and evaluated the gene silencing activity of DNA nanoparticles in breast cancer cells. Polyamine-complexed oligonucleotides showed a synergistic effect on target gene inhibition at the mRNA level compared to the use of polyamines and oligonucleotides as single agents. Ionic and structural specificity effects were evident in DNA condensation and cellular transportation effects of polyamines. In condensed DNA structures, correlation exists between the attractive and repulsive forces with structurally different polyamines and cobalt hexamine, indicating the existence of a common force in stabilizing the condensed structures. Future studies aimed at defining the mechanism(s) of DNA compaction and structural features of DNA nanoparticles might aid in the development of novel gene delivery vehicles.

Keywords Polyamines · Nanoparticles · Gene therapy · DNA condensation · Gene delivery · Reactive oxygen species

V. Vijayanathan · T. Thomas · T. J. Thomas (✉)
Departments of Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ 08901, USA
e-mail: thomastj@rwjms.rutgers.edu

Present Address:
V. Vijayanathan
Albert Einstein College of Medicine, Yeshiva University, 1300 Morris Park Ave, Bronx, NY 10461, USA

E. Agostinelli
Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Biochemical Sciences "A. Rossi Fanelli", SAPIENZA University of Rome and CNR, Institute of Biology and Molecular Pathology, P.le Aldo Moro 5, 00185, Rome, Italy

T. Thomas
Environmental and Community Medicine, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ 08901, USA

Present Address:
T. Thomas
40 Caldwell Drive, Princeton, NJ 08540, USA

Introduction

DNA condensation by polyamines has been an active area of research for the past 4 decades due to its biological implication in the packaging of DNA in virus head and bacteriophages and to understand the energetic forces involved in the collapse of DNA (Bloomfield 1996; Grosberg and Kuznetsov 1992; Korolev et al. 2010). In recent years, DNA collapse from its random coiled

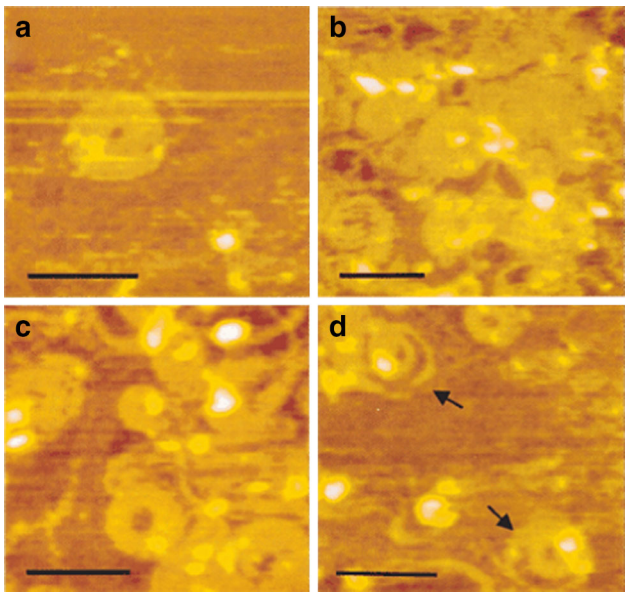

Fig. 1 Scanning force microscopic images showing toroid structures of pGL3 plasmid DNA formed by incubation with 25 μM spermine (**a**), 5 μM 3-3-3-3 (**b**), 2 μM 3-4-3-4-3 (**c**), and the partly formed toroids, observed in the presence of 2 μM 3-4-3-4-3 (**d**). *Scale bar* is 200 nm. (Vijayanathan et al. 2004; reproduced by permission of the publisher, Oxford University Press Journals)

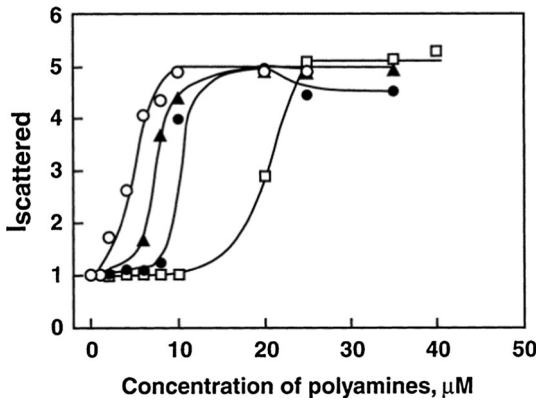

Fig. 2 Typical plots of the relative intensity of scattered light at 90° plotted against the concentrations of spermine (*open circles*), 3-10-3 (*filled triangles*), 3-11-3 (*filled circles*), and 3-12-3 (*squares*). The λ-DNA solution had a concentration of 1.5 μM DNA phosphate, dissolved in 10 mM sodium cacodylate buffer, pH 7.4 (Vijayanathan et al. 2001; reproduced by permission of the publisher, American Chemical Society)

conformation to ordered nanometric particles has been extensively studied to develop non-viral vehicles for gene delivery applications (Behr 2012; Luo and Saltzman 2000; Mintzer and Simanek 2009; Vijayanathan et al. 2002). DNA transport in cells is restricted by cell membrane barriers; however, DNA nanoparticles undergo facile transport through the cell membrane. A large number of cationic molecules varying in size, shape and cationicity, including cationic lipids, polyaminolipids, dendrimers, polyethyleneimines and neutral polymers, are currently under development as non-viral gene delivery vehicles (Blessing et al. 1998; Schaffer and Lauffenburger 2000). In the presence of sufficient concentrations of multivalent cations, dilute solutions of DNA undergo monomolecular condensation to compact nanometric particles such as toroids, spheroids and rod-like structures (Fig. 1) (Eickbush and Moudrianakis 1978; Thomas and Bloomfield 1985). Extensive research on DNA condensation has been carried out using natural polyamines, spermine and spermidine, and the inorganic cation, cobalt hexamine (Widom and Baldwin 1980; Thomas and Bloomfield 1983). In vitro, polyamines were shown to interact with proteins and nucleic acids, stabilizing duplex and triplex structures and protect DNA from external radiation and reactive oxygen species (Thomas and Bloomfield 1984; Thomas and Thomas 1993; Beauchemin et al. 2007; Dubeau et al. 2010; Nayvelt et al. 2010; Essemine et al. 2011; Beck et al. 2013). Polyamine functions are not only confined to their interaction with nucleic acids inside the cell, but they also have important roles in modulating gene expression, cell signaling and cell growth (Thomas and Thomas 2001; Ouameur et al. 2010; Agostinelli et al. 2010a; Agostinelli 2012). As a part of our ongoing research program in developing non-viral gene delivery vehicles, we synthesized a series of polyamine analogs differing in chemical structure and charge density and studied their ability to condense oligonucleotides, plasmids and genomic DNA to nanometric particles (Vijayanathan et al. 2001, 2004, 2005; Nayvelt et al. 2010).

DNA condensation is an example of polymeric coil–globule transition that occurs when approximately 90 % of the polyanionic charge is neutralized by multivalent cations (Wilson and Bloomfield 1979; Thomas and Bloomfield 1983; Korolev et al. 2010). When followed by light scattering techniques, DNA condensation is associated with an abrupt increase in the intensity of scattered light (excess scattering above uncondensed DNA) over a narrow concentration range of the condensing agent. Figure 2 shows a typical light scattering profile of λ-DNA condensation in the presence of different polyamines (Vijayanathan et al. 2001). A measure of the efficacy of an agent to condense DNA can be quantified by the cation concentration at which 50 % increase in scattered light intensity occurs (EC_{50} value). Multivalent cations mediate attraction between the DNA helices by neutralizing the DNA phosphate charges or by rendering DNA-solvent interactions less favorable (Bloomfield 1996).

Energetics of DNA condensation

Utilizing the counterion condensation theory developed by Manning (1978) and Record et al. (1978), Wilson and

Bloomfield (1979) analyzed the significant role of electrostatic interactions in DNA condensation by polyamines. They observed a striking unity among different condensing ions, with collapse of the extended DNA chain occurring at 89–90 % DNA phosphate charge neutralization. Multivalent cations mediate an attractive interaction between negatively charged DNA chains. However, DNA charge is not completely reduced to zero value in the condensed structures and ~10 % uncompensated negative charge can generate a significant repulsive force between DNA chains. Therefore, long range attractive and repulsive forces exist in condensed structures, and the DNA double helix remains separated by 0.7–1.2 nm of water-filled space (Rau and Parsegian 1992). Several theories have been put forward to explain the magnitude of attractive forces that hold the condensed structures together. It has been postulated that in addition to electrostatic forces, counterion correlations, screened Debye–Huckel interactions and hydration forces contribute to drive DNA condensation to nanometric particles. It was suggested that one possible source of attraction is the reduction of electrostatic repulsion by counterion condensation and correlation in the motion of the loosely associated ions, resulting in net attraction between DNA segments (Oosawa 1971). Utilizing the concept of correlated fluctuations, Rouzina and Bloomfield developed a theory in which the character of the counterion distribution close to the highly charged DNA surface played a significant role and the magnitude of interaction was determined by surface charge density and solution dielectric constant (Rouzina and Bloomfield 1997). Since condensation of DNA was accompanied by the rearrangement of water molecules around macromolecular structures, Rau and Parsegian postulated the existence of hydration forces when the interhelix distance was smaller than 3.0–3.5 nm (Rau and Parsegian 1992).

In an attempt to distinguish attractive and repulsive forces, Todd et al., combined single molecule magnetic tweezers with osmotic stress on DNA assemblies and measured the free energy change involved in DNA condensation for different polyamines (Todd et al. 2008). Direct measurement of these forces showed that the attractive component of free energy was always 2.3 ± 0.2 times larger than the repulsive component and repulsive forces decayed exponentially with 2.3 ± 0.2 characteristics to decay length. These results showed a striking correlation between attractive and repulsive forces with cobalt hexamine and structurally different polyamines, suggesting a common underlying force in stabilizing condensed DNA structures, which probably involved polarization of water molecules by surface polar groups. Another force that can possibly influence the stabilization of condensed DNA structures is the ability of condensing counterions to undergo inter- and intra-molecular cross-linking between DNA chains. Investigations on the influence of spermidine on the persistence length of DNA indicated that polyamine-mediated cross-linking was required for DNA collapse (Baase et al. 1984). Cationic silanes possessing both the condensing properties of polyamines and cross-linking properties of silanes could also condense DNA to well-defined toroidal and rod-shaped structures (Fang and Hoh 1999).

Structural specificity effects of polyamines

Studies on DNA condensation using isovalent cations such as cobalt hexammine and spermidine showed that cobalt hexamine was fivefold more efficacious than spermidine in condensing DNA, and the size of condensates was smaller with cobalt hexamine than that formed with spermidine (Thomas and Bloomfield 1983). Structurally modified polyamines, including polyamines with differences in the number of methylene groups between nitrogen atoms, regiochemical distribution, terminal N substitution and conformational restrictions, have been prepared and tested for their ability to condense DNA. Allison et al., used simple triamines to demonstrate the structural specificity effects of polyamines in provoking DNA condensation (Allison et al., 1981). Vijayanathan et al. used spermine and a series of its homologs, $H_2N(CH_2)_3NH(CH_2)_{n=2-12}$ $NH(CH_2)_3NH_2$ ($n = 4$ for spermine) to elucidate the structural features of polyamines governing DNA condensation and found significant differences in the ability of homologs to condense DNA to nanoparticles (Vijayanathan et al. 2001). Investigations on the ionic and structural effect of polyamines on provoking DNA condensation indicated that higher valent polyamines were more efficacious than spermine. Hydrodynamic radii decreased with increasing cationicity and atomic force microscopy (AFM) studies indicated the presence of toroidal particles (Vijayanathan et al. 2004). Significant differences were also observed in the ability of spermine analogs to aggregate oligonucleotides and genomic DNA (Saminathan et al. 1999). Geall et al., synthesized a series of polyamine carbamates of cholesterol, where both charge and regiochemical distribution were varied along the polymer chain of the head group. They observed that polyamine head group had a profound influence on its ability to bind DNA and induce conformational changes (Geall et al. 1999). Polyamines exhibited distinct differences in their binding to nucleic acids depending on their structure and charge density (N'soukpoé-Kossi et al. 2008, 2009). It has been postulated that while the tetramethylene spacing is suitable to bridge between different strands of DNA, trimethylene spacing between the amino groups is suitable to interact with adjacent phosphate charges within the same strand. Rowat

and Williams investigated the strength of polyamine binding to DNA and observed that the presence of butylene rather than propylene group was more suitable for establishing direct contact with every phosphate group of DNA (Rowat and Williams 1992).

Chen et al., synthesized polyamine conjugates by substituting one of the primary nitrogen atoms of spermine or putrescine with a pteridine ring and studied their DNA condensing ability. Since this structural modification conferred lower cationicity compared to the parent compound, these molecules aided in the collapse of DNA to loosely folded toroidal condensates (Chen et al. 2005). In addition to geometric effects, chiral effects of polyamines were also reported on DNA condensation. Nayvelt et al., used stereoisomers of α-methylated spermine analogs and observed differences in threshold concentration required for DNA condensation (Nayvelt et al. 2010). Yoshikawa et al., conferred rigidity and chirality to spermine by incorporating *trans*-cyclopentane units. The resultant molecule possessed a total of four stereogenic centers and presented differences in their ability to induce DNA compaction (Yoshikawa et al. 2013). Bifunctional molecules containing macrocyclic polyamines and naphthyl moieties condensed DNA and the presence of both units in the same molecule proved to be necessary for facilitating condensation (Yan et al. 2012).

Oligo- and poly-peptides of different length, sequence and presentation were synthesized and tested for their DNA condensing ability (Korolev et al. 2012; Nayvelt et al. 2007; Derouchey et al. 2013). Mann et al., compared DNA condensing ability of oligolysine and oligoarginine homopeptides and observed that arginine homopeptides predominantly formed multimolecular complexes, whereas oligolysines formed both mono- and multi-molecular complexes (Mann et al. 2011). Since arginine possesses a guanidine group having three amino groups, the charge density of the homopeptides differs with differences in DNA binding mechanism. Nayvelt et al., found such differences in the condensation of DNA by oligo- and polylysines (Nayvelt et al. 2007). Oligopeptides containing more than 13 lysine residues led to the formation of tight, small polyplexes sizing between 50 and 200 nm, whereas shorter peptides containing eight or less lysine residues formed large condensates ranging from 0.7 to 3 μm that weakly bound to DNA (Wadhwa et al. 1997). Several peptides differing in the number of cysteine residues condensed DNA with a size range of 40–50 nm. Lysine containing peptides with aromatic tyrosine and tryptophan groups were also studied and the results showed that aromatic residues had minimal influence on DNA binding, condensation and transfection efficiency (McKenzie et al. 1999). In an attempt to model protamine-mediated DNA compaction, that is involved in spermatogenesis, arginine rich peptides were used to compact DNA and the results showed that the DNA surface-to-surface separation was significantly shorter with poly-arginine compared to polylysine (Derouchey et al. 2013).

Since the major force governing DNA condensation is electrostatic in origin, cationicity of the condensing agent and ionic conditions of the medium play important roles in mediating DNA condensation (Wilson and Bloomfield 1979; Thomas and Bloomfield 1983). Higher valent polyamines are superior to lower valent molecules in condensing DNA (Vijayanathan et al. 2004). It has been demonstrated by us and others that the critical concentration at which polyamines condense DNA increased with increasing monovalent salt concentration, with a straight line relationship between log[EC_{50} of polyamine] versus log[Na^+] (Vijayanathan et al. 2001; Korolev et al. 2010). The slope value is a quantitative measure of the concentration dependence between multivalent and monovalent cations in condensing DNA. Interestingly, homologation of the methylene groups in the central region of spermine resulted in a periodic oscillation of the slope values between odd and even number of methylene groups, indicating the importance of regiochemical distribution of cationic charges along the polyamine chain (Vijayanathan et al. 2001).

Morphology of DNA condensates

Detailed research using electron microscopy (EM), atomic force microscopy (AFM) and X-ray diffraction revealed the presence of toroids, spheroids and rod-like DNA structures depending on the polyamine structure and solution conditions used for DNA condensation (Hud and Downing 2001; Thomas and Bloomfield 1985; Vijayanathan et al. 2004). Toroid size and structure was dependent on the topology of DNA, solution conditions and nature of the cation. The average toroid size was unaffected by the size of DNA, indicating that toroids could be mono- or multi-molecular. Interesting intermediate morphologies were also observed using AFM, indicating that several inter- and intramolecular contacts were involved during the early stages of DNA condensation (Fang and Hoh 1999). Structural arrangement of DNA precipitated by spermidine homologs had a strong dependence on the chemical structure of polyamines. The Bragg spacing and inter helical distance varied systematically with the length of the methylene bridging region (Schellman and Parthasarathy 1984). The effective concentration of spermine homologs in condensing λ-DNA and the size of condensates were dependent on polyamine structure. AFM studies showed that individual DNA strands in a toroid were separated by 3.2 nm (Dunlap et al. 1997). X-ray diffraction and polarizing microscopic

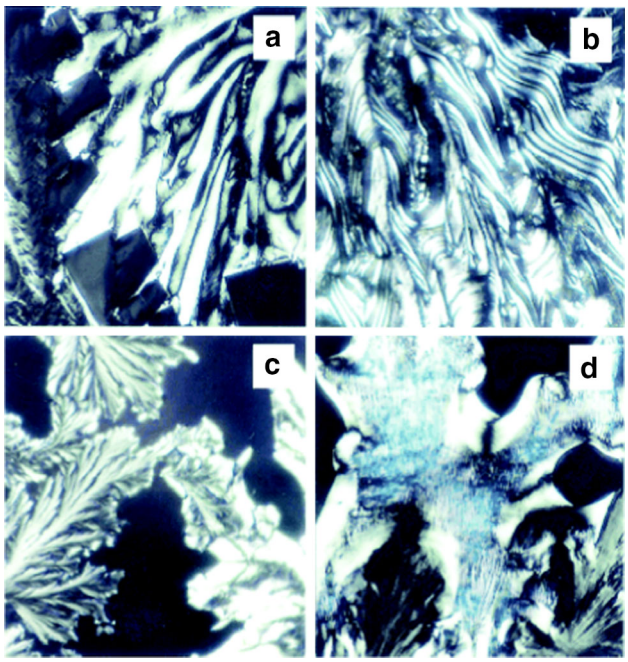

Fig. 3 Effects of higher homologs of spermine on the liquid crystalline phase transitions of calf thymus DNA. **a** DNA (25 mM in Na cacodylate buffer) was incubated with 1 mM 3-5-3 for 24 h at 37 °C. A crystalline phase is observed (×100). **b** A myelin like growth, which developed a striped appearance, is found after incubation of DNA with 1 mM 3-6-3 for 12 h at 37 °C (×360). **c** A crystalline phase was observed on further incubating the DNA for 24 h at 37 °C (×90). **d** Fingerprint textures are obtained on incubating DNA with 1 mM 3-9-3 for 12 h at 37 °C (×200) (Saminathan et al. 2002; reproduced by permission of the publisher, Oxford University Press Journals)

studies further showed local ordering of DNA with interaxial spacing of 2.5–3.0 nm (Ha and Liu 1998). Topologically constrained DNA such as supercoiled plasmids and DNA with AT tracts generally yielded smaller toroids compared to those formed from linear DNA (Conwell et al. 2003).

Liquid crystalline textures, including fingerprint textures of the cholesteric and columnar hexagonal phase have been identified in DNA–polyamine complexes (Livolant and Leforestier 1996; Lin et al. 1998; Saminathan et al. 2002). Upon DNA condensation, the excluded volume is minimized, thereby increasing the local concentration of DNA resulting in a highly ordered liquid crystalline cholesteric phase. With increasing DNA concentration, the cholesteric phase can gradually transform to columnar hexagonal two-dimensional phase and then to three-dimensional crystals. Pelta et al., observed the organization of spermidine-DNA condensates into two different phases, a liquid crystalline cholesteric phase with a larger helical pitch and a columnar hexagonal phase with restricted mobility (Pelta et al. 1996). The formation of these phases was dependent on the concentration of spermidine and NaCl. Circular dichroism studies also showed cholesteric helical supramolecular ordering of DNA called the Ψ-DNA in the presence of spermine (Becker et al. 1979). Liquid crystalline DNA textures were also observed in the presence of natural and synthetic polyamines (Saminathan et al. 2002). While DNA exhibited characteristic finger print textures of cholesteric phase with a helical pitch of 2.5 μm in the presence of spermidine and spermine, DNA adopted dendrimeric and crystalline phases in the presence of spermine homologs and bisethyl derivatives (Fig. 3).

Polyamines as gene delivery vehicles

The past decade has seen an intense effort to develop gene delivery vehicles using a wide spectrum of DNA compaction agents, including polyamines, peptides, proteins, lipids and combinations thereof (Grandinetti and Reineke 2012; Vijayanathan et al. 2002). The polyamine backbone provides an excellent platform to synthesize gene delivery vehicles because of the presence of the flexible methylene bridging region, presence of amino and imino groups that are amenable to modifications and attachments, and the ability of the resultant molecules to condense DNA and RNA to nanoparticles (Blagbrough et al. 2003; Vijayanathan et al. 2002; Jeong et al. 2007; Park et al. 2006). Compared to viral vectors, synthetic vectors do not elicit immunogenic response and impose restrictions on the size of the encapsulated genetic material. However, success in this area is hampered by factors such as toxicity of delivery vehicles, uptake by the reticuloendothelial system and lack of selectivity toward target cells (Li and Huang 2000). Therapeutic efficacy of polyplex-mediated gene delivery strategies depends on several factors, including effectiveness to condense DNA, ability to deliver therapeutic genes to cellular targets, endosomal escape, efficient unpacking and nuclear transport of genetic payload to maintain sustained gene expression.

Liposome-mediated gene transfer is one of the earliest methods used for gene transfection studies. Cationic lipids used in gene delivery applications generally possess a quaternary ammonium salt or a polyamine head group connected to the lipid moiety through hydrophobic spacers (Byk et al. 1998). Commercially available lipid reagents differ in charge density of the polyamine headgroup and are linked to a hydrophobic tail through a linker. The lipopolyamines readily form complexes with DNA and are easily internalized into cells. However, they lack specificity and yield heterogeneous complexes.

Although spermine and spermidine readily condense DNA to nanoparticles, their interaction with DNA is reversible at high salt concentration because of the small size of natural polyamines and due to ion competition for

negative charges on DNA (Vijayanathan et al. 2005). To circumvent this difficulty, we synthesized pentamine and hexamine analogs of spermine and studied their ability to transport a triplex forming oligonucleotide (TFO) targeted to the c-myc oncogene in MCF-7 breast cancer cells (Thomas et al. 1999). Complex formation with a hexamine analog of spermine, 1,21-diamino-4,9,13,18-tetraazahenicosane ($H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ or 3-4-3-4-3) increased the uptake of the TFO up to sixfold higher compared to the uptake of the un-complexed TFO. In addition, treatment of MCF-7 cells with TFO complexed with 0.5 μM 3-4-3-4-3 suppressed the expression of the c-myc oncogene at the mRNA level by 65 % compared to the level in the absence of the hexamine. The polyamine-complexed TFO/DNA might utilize the well-characterized polyamine transport mechanism for cellular cargo delivery (Sala-Rabanal et al. 2013; Agostinelli et al. 2010a). The higher valent polyamines were shown to provoke distinct DNA nanoparticles, as studied by EM and AFM (Vijayanathan et al. 2004, 2005).

Geall et al., investigated structure–activity relationships of a series of oligopolyamines attached to hydrophobic tail groups, comprising of long chain hydrocarbons of different chain length. They observed that transfection activity depended on the nature of the cationic head group, whereas changes in the tail group had limited effect on gene transfer (Geall et al. 1999). Subtle changes in polyamine headgroup modulated transfection efficiency by several fold. Impact of polyamine polymorphism on gene transfer was studied by using different structural variants of polyamines, including branched, T-shaped, globular and linear polyamines as head groups, and found that linear polyamines were superior to other forms in complexing DNA and facilitating gene transfer (Byk et al. 1998). It appeared that increased steric flexibility of less constrained linear polyamines allowed productive interactions with DNA. Increase in the number of head groups as in the case of gemini surfactants, carrying two cationic head groups connected by a tether, showed enhanced transfection efficiency, thereby suggesting the importance of amine density on DNA compaction and transfection (McGregor et al. 2001).

Polymeric molecules bearing multiple amino groups, such as polylysine, polyethyleneimine, and other polymers are excellent agents in producing stable DNA complexes that resist aggregation and premature de-condensation. Polyethyleneimine (PEI) is the most versatile non-viral delivery vector (Boussif et al. 1995; Behr 2012). Structural variants of PEI, differing from linear to branched to hyperbranched and dendritic structures, exhibit differences in their charge densities and transfection efficiencies (Kircheis et al. 2001; Godbey et al. 1999). Dendrimers with pendant amino groups have also been investigated as gene transfer agents (Vilar et al. 2012; Pettit et al. 2011; Chen et al. 2010).

Mechanism of cellular transport of DNA nanoparticles

A schematic representation of nanoparticle internalization and its subsequent translocation into the cell nucleus is shown in Fig. 4 (Vijayanathan et al. 2002). Cellular internalization of nanoparticles depends on several factors, including size, shape and surface characteristics of the nanoparticles. Excess positive charges enable clustering of the condensates on cell surface via interaction with cell surface proteoglycans. Clustering of these complexes initiates a sequence of events on the cell surface and subsequent internalization of the complex through phagocytosis (Díaz-Moscoso et al. 2010; Petros and DeSimone 2010 and ref. therein). Changing the zeta potential to negative values by passivating the amino groups had a dramatic diminution of particle internalization, suggesting the importance of surface charge in cellular internalization. While size and charge of the particle determine its mode of internalization, geometry of the particle can inhibit or induce its internalization. Inside the cytoplasm, the endosomes are destabilized and release DNA, although this is an inefficient process in many cases.

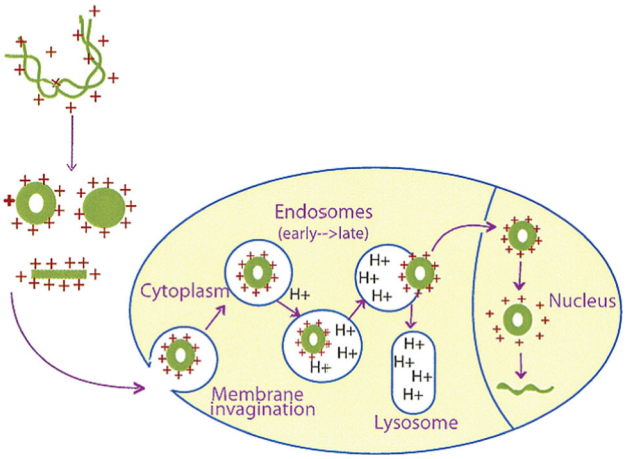

Fig. 4 Schematic representation of DNA uptake by mammalian cells. DNA is compacted in the presence of polycations into ordered structures such as toroids, rods, and spheroids. These particles interact with the anionic proteoglycans at the cell surface and are transported by endocytosis. The cationic agents accumulate in the acidic vesicles and inhibit the degradation of DNA by lysosomal enzymes. They also sustain a proton influx, which destabilizes the endosome, and release DNA. The DNA then is translocated to the nucleus either through the nuclear pore or with the aid of nuclear localization signals, and decondenses after separation from the cationic delivery vehicle (Vijayanathan et al. 2002; reproduced by permission of the publisher, American Chemical Society)

En route to the nucleus, cytosol presents multiple barriers before DNA can get access to the transcriptional machinery. Several methods, including the use of fusogenic peptides and chloroquine were used to facilitate endosomal release. However, polyamine-based vectors possessing titratable amino/imino groups, such as PEI, aid endosomal escape of DNA by their "proton sponge potential" wherein the amine groups with low pKa buffer the endosomal vesicle, leading to endosomal swelling and lysing, thereby releasing DNA into the cytoplasm (Kichler et al. 2001; Boussif et al. 1995). Polymeric polyamines such as polyamidoamines that undergo a pH-dependent conformational change can also afford endosomal escape in acidic environment of the endosome (Griffiths et al. 2004). Released DNA can traffic to the nucleus by passive diffusion through the nuclear pore or through the formation of nuclear pore complex or during nuclear envelope disassembly during cell division (Nigg 1997).

Recent studies unveil the important role of shape and size in addition to surface charge of the nanoparticles in regulating cellular uptake and transport. While conventional science limits the upper size to be in the nanometer range, particles with the size range of 1–3 μm were shown to be easily internalized by Hela cells (Gratton et al. 2008). Smaller nanoparticles (10–15 nm) can be easily removed by the reticuloendothelial cells (RES). Results from different laboratories using multiple cell lines and different carriers suggest that the optimal size of DNA nanoparticles for gene transfection is between 70 and 90 nm. Spherical particles within the size range of 100–200 nm exhibit prolonged circulation since their size prevents uptake by the liver (Champion et al. 2007). Transfection efficiency varied with the shape of nanoparticles, with worm-shaped particles giving a remarkable increase in gene expression compared to other shapes (Jiang et al. 2013). Filamentous micelles in the size range of 18 μm have a longer circulation time of 5 days, much higher than liposomes (Geng et al. 2007), indicating that the shape of the nanoparticle is as important as its size in controlling cellular internalization. Research on DNA nanoparticles resulted in the synthesis of several agents that could alleviate the negative attributes of conventional DNA condensing agents. Several polyamine-based degradable vectors and molecules that can exhibit a discontinuous state in their physical properties in response to internal or external stimuli, such as ionic strength, pH and temperature have been shown to improve the efficiency of non-viral vectors. For selective use in specific applications, targeting moieties have been incorporated to the vector system. The field of smart delivery agents is constantly expanding and has the potential to revolutionize the diagnosis and treatment of several diseases.

Enzyme hosting nanoparticles

Polysaccharide-based nanoparticles are useful tools in drug and protein delivery for numerous therapeutic purposes (Nakai et al. 2012). Linear polysaccharides like hyaluronic acid (HA) have been widely investigated for pharmaceutical and biomedical applications. HA is a well-known biocompatible, polyanionic, non-sulfated glycosaminoglycan present in the extracellular matrix and is involved in several biological functions. HA can be easily derivatized with small molecules to modify their physico-chemical properties, and in particular, their solubility and gelling ability (Coviello et al. 2007). By choosing an appropriate hydrophobic moiety and a suitable derivatization degree, polysaccharide chains can spontaneously form self-assembled nanohydrogels (NHs) (Nakai et al. 2012; Akagi et al. 2007; Akiyoshi et al. 1993, 1998; Akiyoshi and Sunamoto 1996). Their excellent biocompatibility and high water content allows excellent diffusion rate that can be exploited for enzyme immobilization for controlled drug delivery systems. (D'Urso et al. 1995; Jean-François et al. 1997). Agostinelli and colleagues used the polyamine degrading enzyme, bovine serum amino oxidase (BSAO), as a new strategy to degrade and deplete polyamines in cancer cells (Averill-Bates et al. 1993; Agostinelli et al. 1994, 1996, 2004, 2009, 2010b). BSAO catalyzes the oxidative deamination of primary amines, leading to the formation of the corresponding aldehyde (or acrolein from spermine) and hydrogen peroxide, which are toxic for tumor cells. BSAO immobilization procedure allowed optimization of the enzyme hydrogel bioreactor properties, such as its plasmatic half-life or its targetability under injectable form, in the perspective of obtaining an enzymotherapeutic device for cancer treatment. Moreover, these BSAO immobilized nanoparticles exhibited excellent stability with no diminution of enzymatic activity upon freeze-thawing and freeze-drying processes (Montanari et al. 2013 in press).

Due to their good biocompatibility and adequate functional groups for chemical fixation, magnetic nanoparticles modified on the surface by various recognition polymers can be used to immobilize specific biomolecules (Taylor et al. 2000). A new method has been developed to synthesize superparamagnetic nanoparticles constituted of stoichiometric maghemite (γ-Fe2O3) with a dimension of approximately 10 nm. These nanoparticles are stable in water and present a high average magnetic moment and can be easily derivatized to immobilize specific organic molecules in solution (Magro et al. 2010, 2011). We reported a unique synthetic route of maghemite nanoparticles exhibiting excellent colloidal behavior without any additional organic or inorganic modification of their surface (Sinigaglia et al. 2012). Consequently, nanoparticles immobilized

of rhodamine B isothiocyanate, which acts as a fluorescent label and a magnetically controllable rhodamine-based fluorescent nanocomposite, have been synthesized. The rhodamine structure allows immobilization of BSAO and retains its catalytic activity toward oxidation of polyamines. The magnetically drivable fluorescent nanocatalyst can be used as a nanodevice with the ability to selectively induce tumour cell death by the in situ production of hydrogen peroxide and aldehydes (Sinigaglia et al. 2012). The versatility of nanogels both in drug/enzyme encapsulation and its adaptability to be tailor made by incorporating sensors and functionalities to respond to external stimuli provide immense potential for biomedical applications.

Future outlook

Significant progress has been achieved in the field of synthetic gene delivery vectors and several key parameters have been identified to improve successful gene delivery applications. Insufficient knowledge of the biophysical characteristics of nanoparticles and their interaction with various cellular organelles impedes the development of non-viral delivery vectors. In addition, conflicting properties, such as high stability versus efficient release of DNA and biocompatibility versus endosome disrupting, also limit progress in this area. Nanoparticle characteristics have emerged as key determinants in gene delivery and hence knowledge on the interaction of nanoparticles with cellular targets remains to be fundamental to advance this area of research. Designing multifunctional smart delivery systems that possess adaptability to respond to changes in the environment is the most desirable improvement to advance the field of synthetic delivery systems. Biodegradable nanohydrogels with exceptional drug/enzyme loading capacities and large surface area can function as templates for multifunctional conjugation and have great promise in improving current treatments and diagnostic modalities. Current research on polyamine-based systems augurs the future development of multifunctional next generation smart delivery systems as theranostic agents.

Acknowledgments This work was partially supported by Italian MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca), by Istituto Superiore di Sanità "Project Italy-USA", by funds MIUR-PRIN (Cofin) and by Istituto Pasteur -Fondazione Cenci Bolognetti (EA), and by a grant from the Foundation of the University of Medicine and Dentistry of New Jersey (PC28-11).

Conflict of interest The authors declare that they have no conflict of interest.

References

Agostinelli E (2012) Role of polyamines, their analogs and transglutaminases in biological and clinical perspectives. Amino Acids 42:397–409

Agostinelli E, Przybytkowski E, Mondovi B, Averill-Bates DA (1994) Heat enhancement of cytotoxicity induced by oxidation products of spermine in Chinese hamster ovary cells. Biochem Pharmacol 48:1181–1186

Agostinelli E, Przybytkowski E, Averill-Bates DA (1996) Glucose, glutathione, and cellular response to spermine oxidation products. Free Rad Biol Med 20:649–656

Agostinelli E, Arancia G, Dalla Vedova L, Belli F, Marra M, Salvi M, Toninello A (2004) The biological functions of polyamine oxidation products by amine oxidases: perspectives of clinical applications. Amino Acids 27:347–358

Agostinelli E, Condello M, Molinari A, Tempera G, Viceconte N, Arancia G (2009) Cytotoxicity of spermine oxidation products to multidrug resistant melanoma M14 ADR2 cells: sensitization by the MDL 72527 lysosomotropic compound. Intern J Oncol 35:485–498

Agostinelli E, Marques MP, Calheiros R, Gl FP, Tempera G, Viceconte N, Battaglia V, Grancara S, Toninello A (2010a) Polyamines: fundamental characters in chemistry and biology. Amino Acids 38:393–403

Agostinelli E, Tempera G, Viceconte N, Saccoccio S, Battaglia V, Grancara S, Toninello A, Stevanato R (2010b) Potential anticancer application of polyamine oxidation products formed by amine oxidase: a new therapeutic approach. Amino Acids 38:353–368

Akagi T, Baba M, Akashi M (2007) Preparation of nanoparticles by the self-organization of polymers consisting of hydrophobic and hydrophilic segments: potential applications. Polymer 48:6729–6747

Akiyoshi K, Sunamoto J (1996) Supramolecular assembly of hydrophobized polysaccharides. Supramol Sci 3:157–163

Akiyoshi K, Deguchi S, Moriguchi N, Yamaguchi S, Sunamoto J (1993) Self-aggregates of hydrophobized polysaccharides in water. Formation and characteristics of nanoparticles. Macromolecules 26:3062–3068

Akiyoshi K, Kobayashi S, Shichibe S, Mix D, Baudys M, Kim SV, Sunamoto J (1998) Self-assembled hydrogel nanoparticle of cholesterol-bearing pullulan as a carrier of protein drugs: complexation and stabilization of insulin. J Control Release 54:313–320

Allison SA, Herr JC, Schurr JM (1981) Structure of viral φ29 DNA condensed by simple triamines: light scattering and electron microscopic studies. Biopolymers 20:469–488

Averill-Bates DA, Agostinelli E, Przybytkowski E, Mateescu MA, Mondovi B (1993) Cytotoxicity and kinetic analysis of purified bovine serum amine oxidase in the presence of spermine in Chinese hamster ovary cells. Arch Biochem Biophys 300:75–79

Baase WA, Staskus PW, Allison SA (1984) Precollapse of T7 DNA by spermidine at low ionic strength: a linear dichroism and intrinsic viscosity study. Biopolymers 23:2835–2851

Beauchemin R, N'soukpoé-Kossi CN, Thomas TJ, Thomas T, Carpentier R, Tajmir-Riahi HA (2007) Polyamine analogues bind human serum albumin. Biomacromolecules 8:3177–3183

Beck A, Vijayanathan V, Thomas T, Thomas TJ (2013) Ionic microenvironmental effects on triplex DNA stabilization: cationic counterion effects on poly(dT)·poly(dA)·poly(dT). Biochimie 95:1310–1318

Becker M, Misselwitz R, Damaschun H, Damaschun G, Zirwer D (1979) Spermine-DNA complexes build up metastable structures. Small-angle X-ray scattering and circular dichroism studies. Nucleic Acids Res 7:1297–1309

Behr JP (2012) Synthetic gene transfer vectors II: back to the future. Acc Chem Res 45:980–984

Blagbrough IS, Geall AJ, Neal AP (2003) Polyamines and novel polyamine conjugates interact with DNA in ways that can be exploited for non-viral gene therapy. Biochem Soc Trans 31:397–406

Blessing T, Remy JS, Behr JP (1998) Monomolecular collapse of plasmid DNA into stable virus-like particles. Proc Natl Acad Sci USA 95:1427–1431

Bloomfield VA (1996) DNA condensation. Curr Opin Struct Biol 6:334–341

Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP (1995) A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci USA 92:7297–7301

Byk G, Dubertret C, Escriou V, Frederic M, Jaslin G, Rangara R, Pitard B, Crouzet J, Wils P, Schwartz B, Scherman D (1998) Synthesis, activity and structure-activity relationship studies of novel cationic lipids for DNA transfer. J Med Chem 41:224–235

Champion JA, Katare YK, Mitragotri S (2007) Particle shape: a new design parameter for micro- and nanoscale drug delivery carriers. J Control Release 121:3–9

Chen N, Murata S, Yoshikawa K (2005) Dramatic change in the tertiary structure of giant DNA without distortion of the secondary structure caused by pteridine-polyamine conjugates. Chemistry 11:4835–4840

Chen AM, Taratula O, Wei D, Yen HI, Thomas T, Thomas TJ, Minko T, He H (2010) Labile catalytic packaging of DNA/siRNA: control of gold nanoparticles "out" of DNA/siRNA complexes. ACS Nano 4:3679–3688

Conwell CC, Vilfan ID, Hud NV (2003) Controlling the size of nanoscale toroidal DNA condensates with static curvature and ionic strength. Proc Natl Acad Sci USA 100:9296–9301

Coviello T, Matricardi P, Marianecci C, Alhaique F (2007) Polysaccharide hydrogels for modified release formulations. J Control Release 119:5–24

D'Urso EM, Jean-Francois J, Doillon CJ, Fortier G (1995) Poly(ethylene glycol)-serum albumin hydrogel as matrix for enzyme immobilization: biomedical applications. Artif Cell Blood Substit Immobil Biotechnol 23:587–595

Derouchey J, Hoover B, Rau DC (2013) A comparison of DNA compaction by arginine and lysine peptides: a physical basis for arginine rich protamines. Biochemistry 52:3000–3009

Díaz-Moscoso A, Vercauteren D, Rejman J, Benito JM, Ortiz Mellet C, De Smedt SC, Fernández JM (2010) Insights in cellular uptake mechanisms of pDNA-polycationic amphiphilic cyclodextrin nanoparticles (CDplexes). J Control Release 143: 318–325

Dubeau S, Bourassa P, Thomas TJ, Tajmir-Riahi HA (2010) Biogenic and synthetic polyamines bind bovine serum albumin. Biomacromolecules 11:1507–1515

Dunlap D, Maggi A, Sori MR, Monaco L (1997) Nanoscopic structure of DNA condensed for gene delivery. Nucleic Acids Res 25:3095–3101

Eickbush TH, Moudrianakis EN (1978) The compaction of DNA helices into either continuous supercoils or folded-fiber rods and toroids. Cell 13:295–306

Essemine J, Hasni I, Carpentier R, Thomas TJ, Tajmir-Riahi HA (2011) Binding of biogenic and synthetic polyamines to β-lactoglobulin. Int J Biol Macromol 49:201–209

Fang Y, Hoh JH (1999) Cationic silanes stabilize intermediates in DNA condensation. FEBS Lett 459:173–176

Geall AJ, Eaton MA, Baker T, Catterall C, Blagbrough IS (1999) The regiochemical distribution of positive charges along cholesterol polyamine carbamates plays significant roles in modulating DNA binding affinity and lipofection. FEBS Lett 459:337–342

Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, Discher DE (2007) Shape effects of filaments versus spherical particles in flow and drug delivery. Nat Nanotechnol 2:249–255

Godbey WT, Wu KK, Mikos AG (1999) Size matters: molecular weight affects the efficiency of poly(ethyleneimine) as a gene delivery vehicle. J Biomed Mater Res 45:268–275

Grandinetti G, Reineke TM (2012) Exploring the mechanism of plasmid DNA nuclear internalization with polymer-based vehicles. Mol Pharm 9(8):2256–2267

Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, Napier ME, DeSimone JM (2008) The effect of particle design on cellular internalization pathways. Proc Natl Acad Sci USA 105:11613–11618

Griffiths PC, Paul A, Khayat Z, Wan KW, King SM, Grillo I, Schweins R, Ferruti P, Franchini J, Duncan R (2004) Understanding the mechanism of action of poly(amidoamine)s as endosomolytic polymers: correlation of physicochemical and biological properties. Biomacromolecules 5:1422–1427

Grosberg AY, Kuznetsov DV (1992) Quantitative theory of the globule-to-coil transition. 2. Density-density correlation in a globule and the hydrodynamic radius of a macromolecule. Macromolecules 25:1980–1990

Ha BY, Liu AJ (1998) Effect of non-pairwise-additive interactions on bundles of rod like polyelectrolytes. Phys Rev Lett 81:1011–1014

Hud NV, Downing KH (2001) Cryoelectron microscopy of λ phage DNA condensates in vitreous ice: the fine structure of DNA toroids. Proc Natl Acad Sci USA 98:14925–14930

Jean-François J, D'Urso EM, Fortier G (1997) Immobilization of L-asparaginase into a biocompatible poly(ethylene glycol)-albumin hydrogel: evaluation of performance in vivo. Biotechnol Appl Biochem 26:203–212

Jeong JH, Kim SW, Park TG (2007) Molecular design of functional polymers for gene therapy. Prog Polym Sci 32:1239–1274

Jiang X, Qu W, Pan D, Ren Y, Williford JM, Cui H, Luijten E, Mao HQ (2013) Plasmid-templated shape control of condensed DNA-block copolymer nanoparticles. Adv Mater 25:227–232

Kichler A, Leborgne C, Coeytaux E, Danos O (2001) Polyethylenimine-mediated gene delivery: a mechanistic study. J Gene Med 3:135–144

Kircheis R, Wightman L, Wagner E (2001) Design and gene delivery activity of modified polyethyleneimines. Adv Drug Deliv Rev 53:342–358

Korolev N, Lyubartsev A, Nordenskiold L (2010) Cation-induced polyelectrolyte–polyelectrolyte attraction in solutions of DNA and nucleosome core particles. Adv Colloid Interface Sci 158:32–47

Korolev N, Berezhnoy NV, Eom KD, Tam JP, Nordenskiod L (2012) A universal description for the experimental behavior of salt-(in)dependent oligocation-induced DNA condensation. Nucleic Acids Res 40:2808–2821

Li S, Huang L (2000) Nonviral gene therapy: promises and challenges. Gene Ther 7:31–34

Lin Z, Wang C, Feng X, Liu M, Li J, Bai C (1998) The observation of the local ordering characteristics of spermidine-condensed DNA: atomic force microscopy and polarizing microscopy studies. Nucleic Acids Res 26:3228–3234

Livolant F, Leforestier A (1996) Condensed phases of DNA: structures and phase transitions. Prog Polym Sci 21:1115–1164

Luo D, Saltzman WM (2000) Synthetic DNA delivery systems. Nat Biotechnol 18:33–37

Magro M, Valle G, Russo U, Nodari L, Vianello F (2010) Inventors University of Padua, Magro M,Valle G, Vianello F. Assignees. Maghemite nanoparticles and method for preparing thereof. EU Patent n. PCT/EP2010/060486

Magro M, Sinigaglia G, Nodari L, Tucek J, Polakova K, Zdeněk M, Cardillo S, Salviulo G, Russo U, Zboril R, Stevanato R, Vianello

F (2011) Novel surface active maghemite nanoparticles: synthesis and characterization. Acta Biomater 8:2068–2076
Mann A, Thakur G, Shukla V, Singh AK, Khanduri R, Naik R, Jiang Y, Kalra N, Dwarakanath BS, Langel U, Ganguli M (2011) Differences in DNA condensation and release by lysine and arginine homopeptides govern their DNA delivery efficiencies. Mol Pharm 8:1729–1741
Manning GS (1978) The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. Q Rev Biophys 11:179–246
McGregor C, Perrin C, Monck M, Camilleri P, Kirby AJ (2001) Rational approaches to the design of cationic gemini surfactants for gene delivery. J Am Chem Soc 123:6215–6220
McKenzie DL, Collard WT, Rice KG (1999) Comparative gene transfer efficiency of low molecular weight polylysine DNA-condensing peptides. J Pept Res 54:311–318
Mintzer M, Simanek EE (2009) Non viral vectors for gene delivery. Chem Rev 109:259–302
Montanari E, Capece S, Di Meo C, Meringolo M, Coviello T, Agostinelli E, Matricardi P (2013) Hyaluronic acid nanohydrogels as a useful tool for BSAO immobilization in the treatment of melanoma cancer cells. Macromol Biosci. doi:10.1002/mabi.201300114
N'soukpoé-Kossi CN, Ouameur AA, Thomas T, Thomas TJ, Tajmir-Riahi HA (2009) Interaction of tRNA with antitumor polyamine analogues. Biochem Cell Biol 87:621–630
Nakai T, Hirakura T, Sakurai Y, Shimoboji T, Ishigai M, Akiyoshi K (2012) Injectable hydrogel for sustained protein release by salt-induced association of hyaluronic acid nanogel. Macromol Biosci 12:475–483
Nayvelt I, Thomas T, Thomas TJ (2007) Mechanistic differences in DNA nanoparticle formation in the presence of oligolysines and poly-L-lysine. Biomacromolecules 8:477–484
Nayvelt I, Hyvönen MT, Alhonen L, Pandya I, Thomas T, Khomutov AR, Vepsäläinen J, Patel R, Keinänen TA, Thomas TJ (2010) DNA condensation by chiral alpha-methylated polyamine analogues and protection of cellular DNA from oxidative damage. Biomacromolecules 11:97–105
Nigg EA (1997) Nucleocytoplasmic transport: signals, mechanisms and regulation. Nature 386:779–787
N'soukpoé-Kossi CN, Ouameur AA, Thomas T, Shirahata A, Thomas TJ, Tajmir-Riahi HADNA (2008) Interaction with antitumor polyamine analogues: a comparison with biogenic polyamines. Biomacromolecules 9:2712–2718
Oosawa F (1971) Polyelectrolytes. Marcel Dekker, New York
Ouameur AA, Bourassa P, Tajmir-Riahi HA (2010) Probing tRNA interaction with biogenic polyamines. RNA 16:1968–1979
Park TG, Jeong JH, Kim SW (2006) Current status of polymeric gene delivery systems. Adv Drug Deliv Rev 58:467–486
Pelta J, Livolant F, Sikorav JL (1996) DNA aggregation induced by polyamines and cobalthexamine. J Biol Chem 271:5656–5662
Petros RA, DeSimone JM (2010) Strategies in the design of nanoparticles for therapeutic applications. Nat Rev Drug Discov 9:615–627
Pettit MW, Griffiths P, Ferruti P, Richardson SC (2011) Poly(amidoamine) polymers: soluble linear amphiphilic drug-delivery systems for genes, proteins and oligonucleotides. Ther Deliv 2:717–907
Rau DC, Parsegian VA (1992) Direct measurement of the intermolecular forces between counterion-condensed DNA double helices: evidence for long range attractive hydration forces. Biophys J 61:246–259
Record MT Jr, Anderson CF, Lohman TM (1978) Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. Q Rev Biophys 11:103–178
Rouzina I, Bloomfield VA (1997) Competitive electrostatic binding of charged ligands to polyelectrolytes: practical approach using the non-linear Poisson-Boltzmann equation. Biophys Chem 64:3139–3155
Rowat E, Williams RJ (1992) The binding of polyamines and magnesium to DNA. J Inorg Biochem 46:87–92
Sala-Rabanal M, Li DC, Dake GR, Kurata HT, Inyushin M, Skatchkov SN, Nichols CG (2013) Polymine transport by the polyspecific organic transporters OCT1, OCT2 and OCT3. Mol Pharm 10:1450–1458
Saminathan M, Antony T, Shirahata A, Sigal LH, Thomas T, Thomas TJ (1999) Ionic and structural specificity effects of natural and synthetic polyamines on the aggregation and resolubilization of single-, double-, and triple-stranded DNA. Biochemistry 38:3821–3830
Saminathan M, Thomas T, Shirahata A, Pillai CKS, Thomas TJ (2002) Polyamine structural effects on the induction and stabilization of liquid crystalline DNA: potential applications to DNA packaging, gene therapy and polyamine therapeutics. Nucleic Acids Res 30:3722–3731
Schaffer DV, Lauffenburger DA (2000) Targeted synthetic gene delivery vectors. Curr Opin Mol Ther 2:155–161
Schellman JA, Parthasarathy N (1984) X-ray diffraction studies on cation-collapsed DNA. J Mol Biol 175:313–329
Sinigaglia G, Magro M, Miotto G, Cardillo S, Agostinelli E, Zboril R, Bidollari E, Vianello F (2012) Catalytically active bovine serum amine oxidase bound to fluorescent and magnetically drivable nanoparticles. Int J Nanomed 7:2249–2259
Taylor JI, Hurst CD, Davies MJ, Sachsinger N, Bruce IJ (2000) Application of magnetite and silica-magnetite composites to the isolation of genomic DNA. J Chromatogr A 890:159–166
Thomas TJ, Bloomfield VA (1983) Collapse of DNA caused by trivalent cations: pH and ionic specificity effects. Biopolymers 22:1097–1106
Thomas TJ, Bloomfield VA (1984) Ionic and structural effects on the thermal helix-coil transition of DNA complexed with natural and synthetic polyamines. Biopolymers 23:1295–1306
Thomas TJ, Bloomfield VA (1985) Quasielastic laser light scattering and electron microscopy studies of the conformational transitions and condensation of poly(dA-dT)·poly(dA-dT). Biopolymers 24:2185–2194
Thomas TJ, Thomas T (1993) Selectivity of polyamines in triplex DNA stabilization. Biochemistry 32:14068–14074
Thomas T, Thomas TJ (2001) Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell Mol Life Sci 58:244–258
Thomas RM, Thomas T, Wada M, Sigal LH, Shirahata A, Thomas TJ (1999) Facilitation of the cellular uptake of a triplex-forming oligonucleotide by novel polyamine analogues: structure–activity relationships. Biochemistry 38:13328–13337
Todd BA, Parsegian VA, Shirahata A, Thomas TJ, Rau DC (2008) Attractive forces between cation condensed DNA double helices. Biophys J 94:4775–4782
Vijayanathan V, Thomas T, Shirahata A, Thomas TJ (2001) DNA condensation by polyamines: a laser light scattering study of structural effects. Biochemistry 40:13644–13651
Vijayanathan V, Thomas T, Thomas TJ (2002) DNA nanoparticles and development of DNA delivery vehicles for gene therapy. Biochemistry 41:14085–14094
Vijayanathan V, Thomas T, Antony T, Shirahata A, Thomas TJ (2004) Formation of DNA nanoparticles in the presence of novel polyamine analogues: a laser light scattering and atomic force microscopic study. Nucleic Acids Res 32:127–134
Vijayanathan V, Lyall J, Thomas T, Shirahata A, Thomas TJ (2005) Ionic, structural, and temperature effects on DNA nanoparticles formed by natural and synthetic polyamines. Biomacromolecules 6:1097–1103

Vilar G, Tulla-Puche J, Albericio F (2012) Polymers and drug delivery systems. Curr Drug Deliv 9:367–394

Wadhwa MS, Collard WT, Adami RC, McKenzie DL, Rice KG (1997) Peptide-mediated gene delivery: influence of peptide structure on gene expression. Bioconjug Chem 8:81–88

Widom J, Baldwin RL (1980) Cation-induced toroidal condensation of DNA. Studies with $Co(NH_3)_6$. J Mol Biol 144:431–453

Wilson RW, Bloomfield VA (1979) Counterion-induced condensation of deoxyribonucleic acid. A light scattering study. Biochemistry 18:2192–2196

Yan H, Li Z, Guo ZF, Lu ZL, Wang F, Wu LZ (2012) Effective and reversible DNA condensation induced by bifunctional molecules containing macrocyclic polyamines and naphthyl moieties. Bioorg Med Chem 20:801–808

Yoshikawa Y, Umezawa N, Imamura Y, Kanbe T, Kato N, Yoshikawa K, Imanaka T, Higuchi T (2013) Effective chiral discrimination of tetravalent polyamines on the compaction of single DNA molecules. Angew Chem Int Ed Engl 52:3712–3716