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REVIEW

Cell Transfection by DNA-Lipid Complexes – Lipoplexes

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Abstract—Nonviral vectors such as complexes of plasmid DNA with cationic lipids known as lipoplexes are considered as an attractive alternative to virus-based delivery systems. Unlike viruses, lipoplexes do not suffer from immunological and mutational hazards, though the efficiency of lipoplexes is often not sufficient for therapeutic purposes and require higher level of transfection than achieved until now. A number of critical steps responsible for transfection efficiency are discussed here. They include processes of lipoplexes formation, interaction with cell surface, their internalization into cell, and DNA release and delivery into the nucleus. All these processes should be thoroughly studied to be able to enhance the transfection efficacy.

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Key words: liposomes, cationic lipids, transfection, nonviral vectors, lipoplexes

The idea of treating disease on the genetic level, known as gene therapy, was first suggested more than 35 years ago [1]. At the beginning the idea looked quite abstract, but progress in molecular biology, gene engineering, and cell biology transformed gene therapy into a subject of intensive research and numerous clinical trials. In 2004 China approved for clinical use the first in the world licensed gene therapy anticancer drug called Gendicine. Gendicine is a recombinant adenovirus that delivers gene *p53* [2-5], which belongs to a group of the most effective tumor suppressors [6]. Unfortunately, the clinical trials and subsequent investigation of this drug was rather restricted [7]. In addition, at present the new drug Advexin based on recombinant adenovirus that delivering gene *p53* is in extensive clinical trials (phases 1-3) in the USA [8]. However, tragic events related to application of viral vectors [9] sharply decreased the number of clinical trials at 2008. It was found also that viral vectors can initiate mutagenesis and cause carcinogenic transformations [10-14]. Artificial containers based on complexes of polynucleotides with polymers (polyplexes) or lipids (lipoplexes) could become more attractive than viral vectors if the artificial compounds could achieve a level of expression comparable to viruses. The lipo- and polyplexes are less expensive and considerable more simple to produce. Their handling is safer and simpler and does not require highly trained personal. In contrast to viruses, the size of delivered DNA with artificial containers is not highly restricted [15-17].

The application of cationic lipids for transfection and the term lipofection were introduced more than 20 years ago [18, 19]. Though viral vectors are still the most extensively studied in biomedicine [20-22], the application of nonviral vectors have been constantly increasing, and clinical trials of liposomal compounds take fifth place after some viruses. Up to 1500 clinical trials were approved from 1989 to September 2008, and 105 of these involved lipofection. In 2008 two liposomal drugs for malignant melanoma were in stage III clinical trial (www.wiley.co.uk/genmed/clinical).

A number of new sorts of genetic materials that could be preferably delivered with nonviral containers have been developed in recent years. They include very large molecules like artificial chromosomes [23], or very small molecules like antisense oligonucleotides and short interfering RNA (siRNAs) [24, 25] designed to selectively switch-off or silence selected genes. It seems that the opposite approach suggesting selective repair and activation of desirable genes, which supposes the delivery of multi-compound architectures containing zinc-finger nucleases and a correct copy of the gene [26, 27] might also be better achieved with nonviral vectors.

A number of sophisticated molecular containers able to circulate for a long time in the blood stream and target the desired cells have been suggested during the last decade [28-30], while the number of delivered gene copies and the level of gene expression still remains lower than required to achieve reasonable therapeutic effect. To

explain difficulties in achievement of the necessary level of gene expression, the concept of cellular barriers was developed. The existence of barriers for DNA delivery was known from studies of viral infection [31, 32]. Barriers for lipofection were discovered in early studies of lipoplexes [18]. Later this concept was developed in a number of laboratories [29, 33-39]. According to the concept, the plasma membrane is the first barrier to lipoplex entry into the cytoplasm. Then the lipoplex should be delivered into the perinuclear area, and finally DNA should be released from the lipid surrounding and enter the nucleus. Numerous obstacles including lytic enzymes and molecular crowding hamper the transportation and expression of DNA. Here we analyze the recent efforts to overcome obstacles for successful delivery of genetic information to the nucleus.

LIPIDS USED FOR TRANSFECTION

Cationic lipids suggested in 1987 became a popular and useful means of nonviral cell transfection. The first cationic lipid DOTMA (Fig. 1) is still used in numerous studies. The first commercial compound Lipofectin composed of DOTMA and neutral lipid DOPE remains to be a gold standard used for testing the effectiveness of transfection reagents, although the use of Lipofectin is now restricted because of its toxicity [40]. The toxicity of biodegradable compounds chemically resembling natural molecules is usually lower. Thus, low toxicity cationic derivatives of diacylglycerol like DOTAP and DODAP found wide application. The low toxicity cationic compounds DDAB [41-43] and CTAB [44-46] are also quite popular in designing of nanoparticles and nanoliposomes for transfection. Synthesis of the cationic derivative of phosphatidylcholine (EDOPC) is the culmination of attempts to develop biodegradable cationic lipids chemically resembling natural phospholipids [47, 48]. Significant progress was achieved also with cationic derivatives of cholesterol. The most often used DC-cholesterol and CTAP are distinguished by low toxicity and high transfection activity [49-51]. The transfection activity of some compounds can correlate with the number of cationic charges in the molecule. Thus, lipids with polyamine moieties can be rather active in transfection. The most often used polyamine spermine with four positive charges constitutes well known cationic lipids DOSPER, DOSPA, and DOGS. High activity was expressed with lipid dendrimer MVLBG2 bearing 16 cationic charges [52].

Some common features are present in the most successfully used cationic lipids [16, 53-57]. As follows from the presented examples, the cationic charge is determined by amino groups of quaternary and tertiary amines. The quaternary amines generally express higher activity, though in cationic cholesterols the tertiary amines are often more active. A number of carbon atoms can serve as a spacer between the charged and nonpolar parts of the molecule. The length of spacer is important and can influence activity. The length of the nonpolar hydrocarbon moiety is also very important. Chains shorter than 14 carbon atoms can destabilize the lipid bilayer and express toxicity for cells. Very long and saturated chains can have undesirable high temperature of melting. Oleic acid (C18:1, *cis* 9) or fatty alcohols with a similar structure are liquid above 0°C and are the most widely used.

STRUCTURE AND PROPERTIES OF DNA-LIPID COMPLEXES – LIPOPLEXES

DNA initiates drastic structural changes in cationic liposomes. It is supposed that electrostatic forces are responsible for the interaction of DNA with the surface of the lipid bilayer [58], liposomes aggregation, and collapse [59]. The resulting structure depends on many factors. The DNA/lipid ratio or the size of liposomes used for lipoplex preparation can influence the structure of lipoplexes. For example DNA excess can prevent fusion of small cationic liposomes, but it initiates membrane formation and formation of gigantic multilamellar lipoplexes when it interacts with large monolamellar cationic liposomes (Fig. 2, a and b). Fusion of small liposomes and formation of multilamellar complexes was found when an excess of lipid was added to DNA (Fig. 2c). A number of laboratories have described the influence of lipid composition, net charge, and preparation protocol on the structure and properties of lipoplexes [60-62]. It is remarkable that DNA molecules produced clusters of parallel and periodically arranged threads when adsorbed on the surface of phospholipid bilayer or trapped between bilayer surfaces (Fig. 2d). Under the electron microscope, the clusters looked like fingerprints [63-65]. Small angle X-ray scattering (SAXS) also confirmed the existence of periodic DNA clusters located between lipid bilayers [66-70]. It is believed that DNA packaged in lipoplexes is comparable to the pseudocrystalline DNA structure of viruses [71, 72].

Formation of vesicular stacks glued together by DNA molecules is the simplest example of lipoplex structure (Fig. 3b). The most abundant multilamellar structures (Fig. 3, c and d) can be produced after considerable reorganization of the lipid bilayer [62, 64, 71, 73, 74] vesicle disruption and formation of specific membrane fusion intermediates [75, 76], DNA entrance into vesicles, and covering of both sides of the lipid bilayer [77]. Besides multilamellar structures, lipids can produce bilayer tubes [78] with DNA inside (Fig. 3e). This structure called spaghetti [79] can coexist with multilamellar lipoplexes or protrude from lipoplexes, resembling map pins [80]. DNA could be also located inside of inverted tubes (Fig. 3f) of hexagonal H_{II} phase of lipid [67, 68, 70, 81].

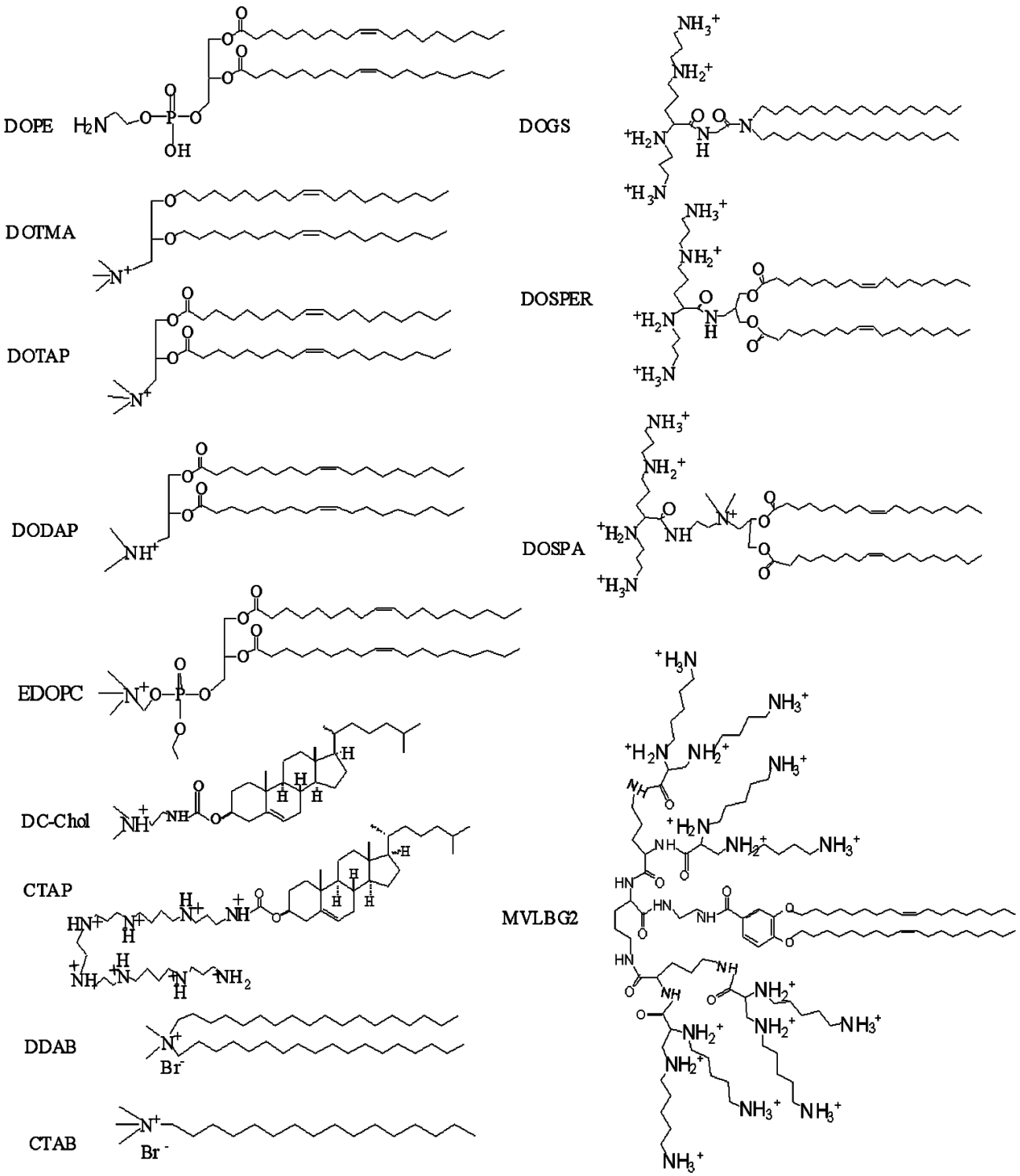

Fig. 1. Cationic lipids used for cell transfection and gene therapy. DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine; DOTMA, *N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride; DOTAP, 1,2-dioleoyloxy-3-(trimethylammonium)propane; DODAP, 1,2-dioleoyloxy-3-(dimethylammonium)propane; EDOPC, ethyldioleoyloxyphosphatidylcholine; DC-Chol, DC-cholesterol or 3β-[N-(N′,N′-dimethylaminoethyl)carbomoyl]cholesterol; CTAP, N-15-cholesteryloxycarbonyl-3,7,12-triazapentadecane-1,15-diamine; CTAB, cetyltrimethylammonium bromide; DDAB, dioctadecyldimethylammonium bromide; DOGS, dioctadecylamidoglycylspermine (transfectam); DOSPER, 1,3-dioleoyloxy-2-(6-spermylcarbonylamino)propane; DOSPA, *N*-[1-(2,3-dioleoyloxy)propyl]-*N*-[2-(6-spermylcarbonylamino)ethyl]-*N*,*N*-dimethylammonium [52, 56]; MVLBG2, lipid dendrimer carrying 16 positive charges.

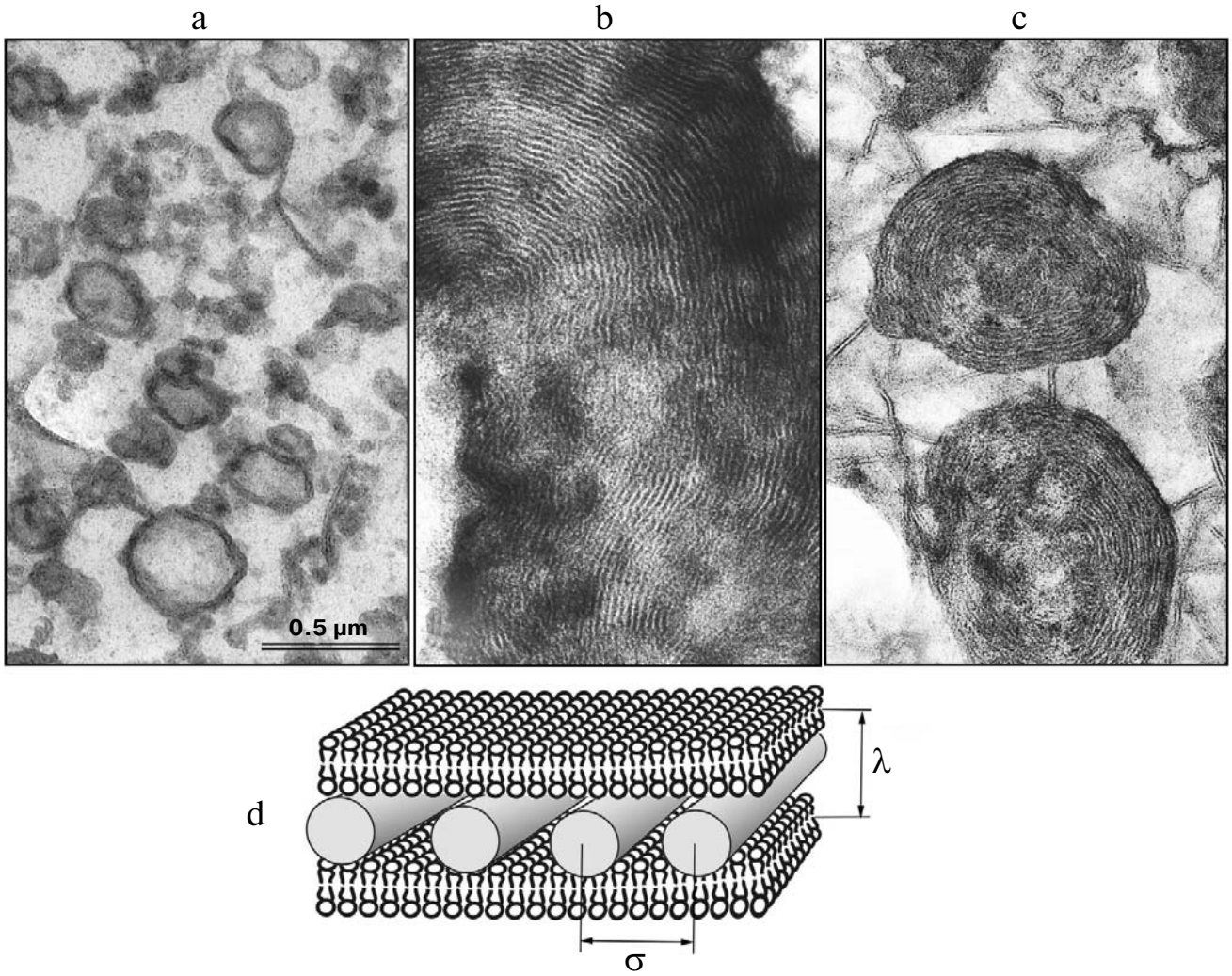

Fig. 2. Structure of some lipoplexes. Thin-section electron microscopy of lipoplexes (a-c) prepared as described earlier [48]. a) Small monolamellar complexes prepared by mixing of small monolamellar vesicles of cationic phosphatidylcholine with an excess of herring sperm DNA (DNA/lipid = 2 : 1); b) gigantic multilamellar lipoplexes prepared as the previous sample, but with large monolamellar liposomes; c) multilamellar lipoplexes prepared as sample (b) but with lipid excess (DNA/lipid = 1 : 2); d) schematic picture of parallel DNA threads located between lipid bilayers in multilamellar lipoplexes. It is known that the repeat distance between DNA threads (σ) can range 2.5-6 nm depending of net charge of lipoplexes. The repeat distance between layers (λ) is approximate 2.4 nm, which is sufficient for intercalation of DNA threads between membranes [66].

The physical properties of lipid can change on lipoplex formation. Thus, the temperature of lipid melting can decrease or increase in different lipoplexes [82-84]. The stability and temperature of DNA denaturation can also be considerable increased up to 105-115°C as a result of interaction with multilamellar surfaces of cationic lipid [85, 86]. Conformational changes in the DNA chain designated as Ψ^- DNA were also revealed by circular dichroism [87]. The presence of this form of DNA depends on the charge ratio and the lipid structure. It should be noted, that a correlation between the transfection activity and appearance of Ψ^- DNA has not been found.

The physical properties of lipoplexes determined mostly by DNA and lipid packaging can influence transfection effectiveness. It is supposed that the net charge and charge density could be responsible for the correlation between lipoplexes stability and transfection activity [88]. In some experiments the transfection activity of hexagonal H_{II} phase was higher than that of bilayer forming lipoplexes [52, 67, 69, 70, 72, 89-91]. However, this point of view was not supported in some other studies [87, 92, 93]. Considerable improvement in transfection could be achieved also in lipid mixtures with different length of hydrocarbon chains or different polar heads [94-96]. As recently demonstrated, the transfection effectiveness cor-

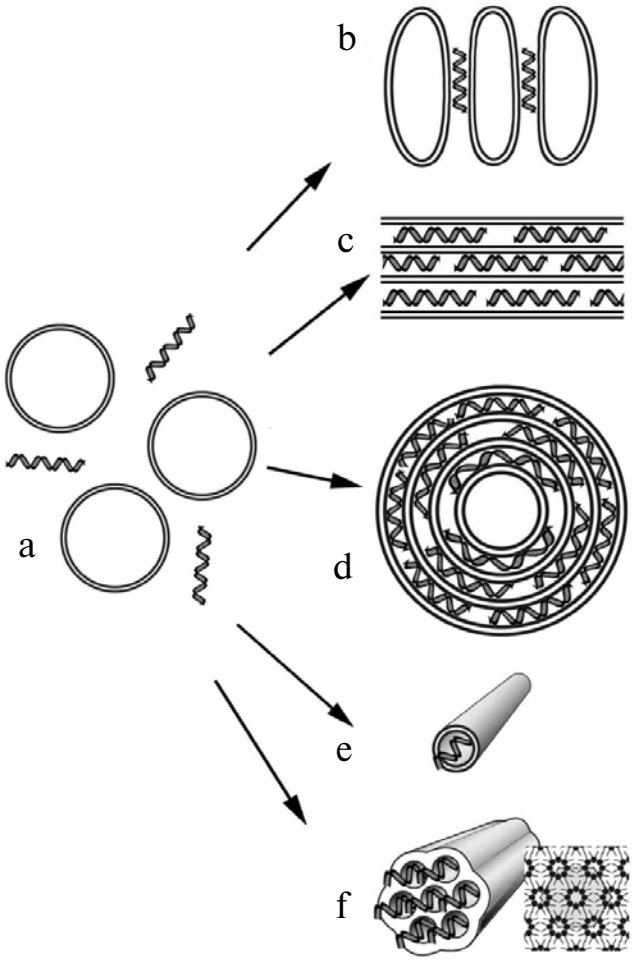

Fig. 3. DNA molecules after adsorption on the surface of cationic liposomes (a) can function as a molecular glue and initiate formation of vesicle stacks (b) or initiate drastic transformation of lipid to produce different structures including stacks of flat bilayers (c), multilamellar vesicles (d), bilayer tubes (e), or bunches of inverted lipid tubes of hexagonal H_{II} phase (f). The inset shows the lipid arrangement on a transverse section of inverted hexagonal H_{II} tubes.

relates with the ability of lipid mixtures to undergo the gel–liquid crystal phase transition at physiological temperatures [97] or to produce nonbilayer polymorphic structures that correlate with lipoplexes fusion with anionic membranes [98-102]. This subject will be discussed later.

INTERACTION OF LIPOPLEXES WITH THE CELL SURFACE AND THEIR INTERNALIZATION INTO THE CYTOPLASM

It is supposed that electrostatic interaction between cationic lipoplexes and negatively charged cell surface mediates interaction of cationic lipids with the cell. The negative charge of the cell surface contributed mostly by proteoglycan sulfates can be involved in the interaction [103]. Besides, sialic acids can also contribute to negative charge of the cell surface and mediate adsorption of particles bearing cationic sites [104]. Then, the plasma membrane contains some amount of negatively charged lipids that might influence the interaction of liposomes with the cell surface [105].

Though it is supposed that the net positive charge of lipoplexes is necessary for interaction with the cell surface, the relationships between transfection effectiveness and charge of lipoplexes is not simple. Depending on the DNA/lipid ratio, a considerable portion of the lipid positive charge is neutralized by the negative charge of the polynucleotide that results in decrease or even in inversion of the zeta potential of lipoplexes [106] that is experimentally revealed from electrophoretic motility of particles. To achieve the highest level of transfection, zeta potential should be slightly positive, though the transfection could be high even at slightly negative values of zeta potential [107, 108].

The size of lipoplexes can influence the way of their internalization and effectiveness of transfection [109, 110]. There is a correlation between lipoplex size and zeta potential. The size of lipoplexes increases at low zeta potential because electrostatic repulsion forces dissipate and cannot prevent particles aggregation and fusion. The optimal sizes of lipoplexes can vary in different studies. For lipoplexes containing cationic cholesterol, the optimal size ranges from 0.4 to 1.4 μ [111, 112]. Similar sizes were optimal for lipoplexes containing DOTAP [113, 114].

In most cases, after adsorption on the ell surface lipoplexes enter cells through endocytotic pathways [19, 39, 115-119]. As a result of plasma membrane invagination, lipoplexes appear inside endosomes [39, 115-123] and are transported along microtubules to the perinuclear area [124, 125].

It is known that the pathways of internalization of different particles can determine further routes of their transportation and localization in the cytoplasm, delivery to lysosomes, and subsequently the probability of their enzymatic degradation, or vice-versa DNA release into the cytoplasm and delivery into the nucleus (Fig. 4). The pathways through clathrin-mediated endocytosis, macropinocytosis, or phagocytosis lead to lysosomes, while the pathways through caveolin-mediated endocytosis allows escape from lysosomal delivery [125-127]. Though, as demonstrated in a number of laboratories, clathrin-mediated endocytosis is responsible for internalization of lipoplexes [116, 127-132], it seems very attractive to find possibilities of DNA release before it is delivered to the lysosomes or to change the direction of lipoplexes to the paths avoiding lysosomes [29, 133].

A number of approaches to prevent lysosomal delivery of lipoplexes have been developed. For example, it

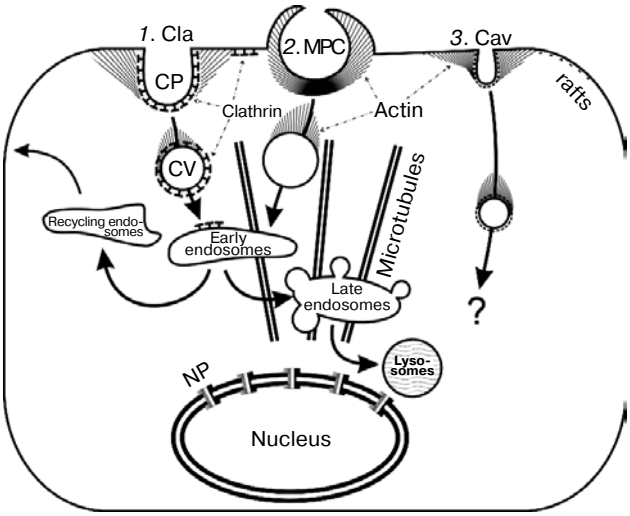

Fig. 4. Simplified scheme of three main endocytotic pathways. *1*) Clathrin-mediated endocytosis (Cla) begins from plasma membrane invagination in clathrin-coated pits (CP) and subsequent isolation of clathrin-coated vesicles (CV). It was found that fibrillar actin is also involved in this process. *2*) Macropinocytosis (MPC), or phagocytosis, is characterized by formation of plasma membrane protrusions driven by actin fibers. The endocytotic vesicles of Cla and MPC deliver cargo to early endosomes, but then the path bifurcates. The cargo could be excreted from the cytoplasm through the compartment of recycling endosomes, or it can be transferred to late endosomes and then to lysosomes in the perinuclear space. This movement towards the nucleus is guided by microtubules. *3*) Caveolin-mediated endocytosis (Cav) represents the alternative route of internalization of extracellular material. Endosomes are produced from specialized caveolin-containing structures of plasma membrane called membrane rafts. Though this kind of endocytosis is less studied, it is supposed that the internalized material is not delivered to lysosomes. NP, nuclear pore.

was found that dysfunction of microtubules can improve transfection. A positive effect could be achieved both by disaggregation or stabilization of microtubules because in both cases the lysosomal delivery of lipoplexes is hampered [124, 134]. As demonstrated by confocal microscopy, after disaggregation of microtubules the lipoplexes were not transported to the perinuclear region and remain in the periphery of the cytoplasm, where they can release DNA into the cytoplasm. However, the DNA release on the periphery of the cytoplasm can considerably increase the time of DNA delivery to the nucleus and elevate the risk of DNA degradation by cytoplasmic enzymes. Slow diffusion of large molecules through the cytoplasm can be explained by the molecular crowding effect [135, 136]. For DNA molecules larger than 2000 bp, the diffusion rates in cytoplasm is 100 times lower than that in water [137].

As mentioned above, normally lipoplexes are delivered to the lysosomes and gathered in the perinuclear area. In this case fast DNA release into the cytoplasm is one of the most important factors responsible for transfection effectiveness [138, 139]. Lysosomotropic compounds, like chloroquine and its derivatives, or polyvinylpyrrolidone that prevents pH decrease and activation of lytic enzymes, can be used to protect DNA and increase transfection [140-142]. Compounds able to increase the osmotic pressure inside lysosomes, like sucrose [141], or some polycationic compounds functioning as a proton sponge [143, 144], can initiate lysosome swelling and disruption that initiates DNA release in cytoplasm before it can be lysed by enzymes. An alternative smart solution of the problem was development of tunable liposomes that are able to release DNA when pH decreases [145, 146]. It was demonstrated that tunable liposomes release DNA because of the bilayer–hexagonal H_{II} phase transition and activation of membrane fusion processes at low pH.

DISINTEGRATION OF LIPOPLEXES BY ANIONIC LIPIDS AND DNA RELEASE

DNA must be released from the lipid surrounding to perform its biological functions. The release could be facilitated by cationic lipid charge neutralization by anionic cellular lipids. This phenomenon was initially demonstrated in model experiments with anionic liposomes [147-149]. It was found that DNA can be released in the case of complete neutralization of the positive charge. Lipid composition of both lipoplexes and cellular membranes can influence the phase behavior of lipid mixtures and can be responsible for DNA release [102].

The analyses of lipid molecule shape suggest a simple explanation for phase behavior of cationic and anionic lipid mixtures. The idea of correlation between lipid molecule shape and phase behavior of lipid was suggested more than 30 years ago [150-152]. Together with the theory of lipid layer intrinsic curvature [153, 154], this idea became a theoretical background for lipid structural polymorphism and the mechanism of transition from lamellar to nonlamellar cubic and hexagonal phases of lipid [155, 156]. It is supposed that lipid with cylindrical shape of molecules can produce flat or slightly curved bilayer structures present in the majority of biological membranes and large liposomes. The intrinsic curvature of these membranes is low, but not all lipid molecules are able to produce flat structures. If the polar head is very big compare to the nonpolar moiety, the shape of lipid molecule is conical. If the polar head is relatively small the molecule has the shape of a reversed cone. The conical or reversed conical molecules cannot arrange in a flat bilayer, and they produce various nonlamellar structures with high intrinsic curvature [157].

Repulsive forces appearing between like charges increase the distance between the polar heads of lipid molecules, and this factor should be considered when we determine the shape of lipid molecules and the intrinsic

curvature of the lipid layer (Fig. 5a). In a mixture of cationic and anionic lipids, the repulsive forces between polar heads are replaced by attractive forces. This can be regarded as a change in the lipid molecule shape and corresponding change in the intrinsic curvature of the lipid layer (Fig. 5b). The decrease in the polar lipid surface and increase in the intrinsic curvature favor lipid polymorphism and appearance of cubic or hexagonal H_{II} phases. Thus, if for example an anionic or cationic lipid separately produces large bilayer vesicles, after lipid mixing nonlamellar structures with high intrinsic curvature can appear [89, 94, 158, 159].

DNA release from lipoplexes can depend from the lipoplex topology. Topologically closed structures, like multilayer vesicles for example, prevent DNA release (Fig. 6b) even if the DNA–lipid interaction is abolished by complete neutralization of cationic charge. For DNA release into the surrounding space the bilayer barrier should be disintegrated. In case of multilamellar structure of lipoplexes the disintegration can begin from formation of intermembrane contacts, membrane fusion, and formation of intermembrane stalks, which represents the beginning of polymorphic phase transition to nonlamellar structures [160-164]. DNA release correlates with the intrinsic curvature of lipid mixtures, and the best result should be expected in lipid mixtures where the bilayer structure disintegrates completely (Fig. 6, c-e). A correlation between membrane fusion and transfection efficiency has been demonstrated in a number laboratories [165-167]. As recently demonstrated for lipid mixtures, there is a correlation between the layer curvature, the intensity of fusion between cationic and anionic liposomes, the intensity of DNA release, and transfection effectiveness [98, 99, 102].

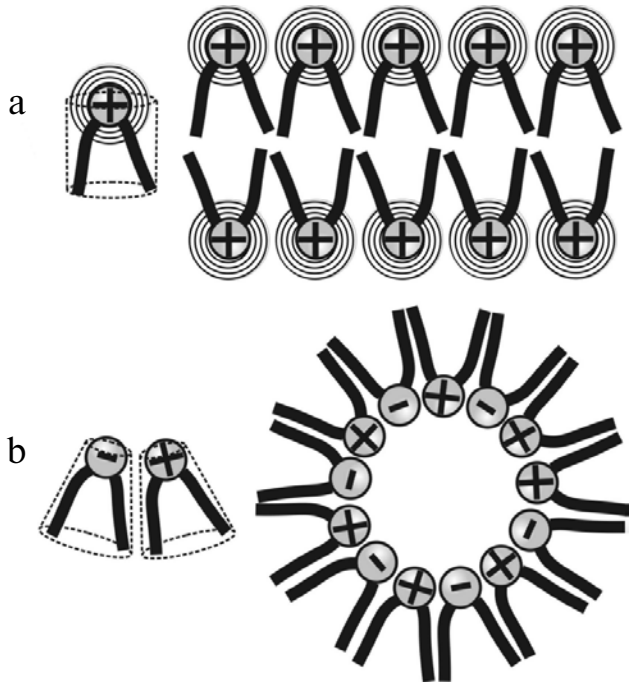

Fig. 5. Correlation between lipid net charge, molecular shape, and produced structures. a) With excess cationic or anionic (not shown) charge, the shape of lipid molecules approximates a cylinder and flat bilayer structures are produced. b) In a mixture of anionic and cationic lipids the repulsive forces between polar heads are replaced by attractive forces, while molecular shape approximates cones. The cone-shaped molecules tend to produce inverted tubular or spherical micelles belonging to hexagonal H_{II} or cubic phases.

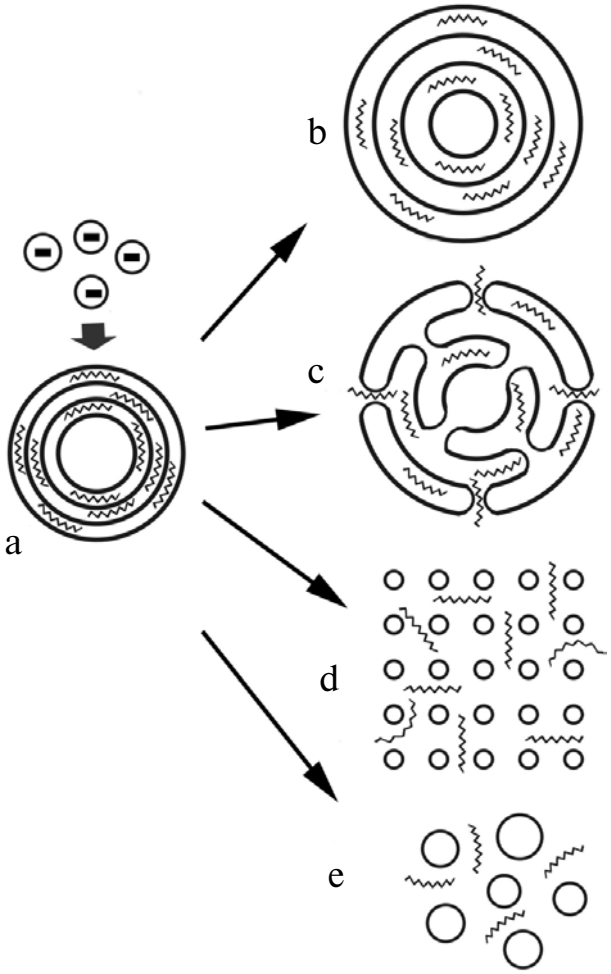

Fig. 6. Interaction of lipoplexes with anionic liposomes and lipid exchange with them (a) leads to cationic charge neutralization and DNA desorption from the bilayer surface. However, DNA cannot be released from topologically closed multilamellar structures (b). Drastic structural changes leading to appearance of topologically open sponge (c), cubic (d), or micellar (e) structures are necessary to allow DNA release from lipoplexes.

The exchange of lipid between lipoplexes and cellular membranes begins after adsorption of the lipoplexes on the surface of the plasma membrane. The influence of lipid exchange on the endocytosis of lipoplexes has been demonstrated in many laboratories [18, 130, 168-170]. We suppose that lipid exchange can influence processes of membrane invagination and endosome isolation and

thus can influence the choice of endocytotic pathway and direction of endosome transportation. As mentioned earlier, lipoplexes usually enter cells through clathrin-mediated endocytosis (Fig. 4) and accordingly can be delivered to lysosomes, where degradation of plasmid DNA is possible [116, 127, 129-131, 171]. On the contrary, if the exchange of material with plasma membrane is not possible, for example for endocytosis of solid spheres of cationic polymers, the caveolin-mediated endocytosis prevails [110, 126, 127]. However, not all lipoplexes can exchange lipid with plasma membrane [91, 115, 131]. Lipid exchange was not demonstrated for lipoplexes with large polymeric polar heads and numerous cationic charges resembling cationic polymers [91, 172, 173]. It seems very attractive to be able to control the endocytotic pathways by varying the composition of lipoplexes.

After internalization into endosomes, lipoplexes exchange lipids mostly through interaction and fusion with the surrounded endosomal membrane [112, 174]. Cellular membranes usually contain 10-20% negatively charged lipids including phosphatidylserine and phosphatidylinositol [175]. The amount of anionic lipid present in the endosomal membrane is not sufficient to neutralize numerous bilayers of lipoplexes containing 50-100% cationic lipids. Endosomes containing lipoplexes interact with various cytoplasmic membranes and exchange lipids with them until complete neutralization of cationic lipid charge, which is necessary for DNA release. Numerous contacts between endosome and membranes of mitochondria, endoplasmic reticulum, and nucleus could be observed under the electron microscope. After disintegration of lipoplexes confocal microscopy reveals a broad distribution of cationic lipids and DNA in the cytoplasm, while a minor part of the DNA and lipid enters the nucleus [99].

ROUTES OF DNA PENETRATION INTO THE NUCLEUS

The above-described strategies of DNA delivery into the cytoplasm are not sufficient to achieve high level of transfection because the entrance of DNA into the nucleus is the main limiting factor. Small polynucleotides like antisense DNA could be accumulated in the nucleus during a few dozens of minutes. On the contrary, plasmid DNA enters the nucleus very slowly. Plasmid DNA could appear in the nucleoplasm usually 12-24 h after treatment of the cell [117, 176]. Transfection is higher in dividing cells and correlates with mitosis, when the nuclear envelope disintegrates [177-180]. However, it was demonstrated that even in dividing cells DNA can enter the nucleus not only during the mitosis, but also in the intervals between cell division [181]. Though the transfection level of dividing cells is usually higher, non-dividing cells, like neurons for example, are also susceptible for transfection [182], which indicates DNA passage through the intact nuclear envelope [183].

Moreover, the supposed entrance of lipoplex into the nucleoplasm during mitosis will hardly increase the level of DNA expression because this parameter was very low even after direct injection of lipoplexes into the nucleus. On the contrary, the level of DNA expression after injection of naked DNA into the nucleus was 100-1000-fold higher than the injection into the cytoplasm [184]. It is supposed that normally in cytoplasm DNA should be released from lipoplexes, and after that naked DNA should enter the nucleus. During transportation through the cytoplasm, DNA is enzymatically degraded and less than 1% remains intact and expressed [185, 186].

Nuclear pores seem the most probable gate for DNA penetration into the nucleus. Each nucleus can have a few thousand pores [187] constituted of nuclear pore complex (NPC) proteins [188, 189]. As revealed by electron microscopy, the pore diameter is about 40-45 nm while its length is about 90 nm [190]. Accordingly, macromolecules with diameter as large as 39 nm [191] and DNA of about 300 bp can penetrate through the pore [192], while the size of plasmid DNA is usually larger than 5000 bp. The compaction of DNA with cationic substances like lipids, polymers, or cationic polypeptides seems a smart solution of the problem. Thus, plasmid DNA can be compacted in pseudocrystalline hexagonally [52, 69] or toroidal ordered structures [193, 194]. Molecule of 5500 bp can be organized in a sphere with diameter of 34 nm, which is sufficiently small to pass through a nuclear pore [195].

It seems very attractive to combine cationic lipids with other compounds that can facilitate DNA delivery into the nucleus. A number of natural and synthetic cationic peptides of different origin are able to mediate the cytoplasmic and nuclear delivery of macromolecules and potentially could be utilized to enhance transfection [196-198]. Transfection can be improved by attachment of nuclear localization signal (NLS) peptides to a DNA molecule [187, 199-202]. It was recently found that NLS peptides are not only an effective tool for overcoming the barrier of the nuclear envelope, but also an intermediate of macromolecular transportation along microtubules from the cell periphery to the nucleus [203]. In recent years much attention has been given to the cationic domain of transactivator (Tat) protein of human immunodeficiency virus HIV-1 (residues 48-60) known as the TAT-peptide. It was found that TAT-peptide or its artificial derivatives can penetrate into cytoplasm through caveolin-dependent fluid phase endocytotic pathway and assist nuclear delivery of various macromolecules, including plasmid DNA [204, 205]. Then the cationic bee venom peptide melittin and its derivatives were also successfully utilized for nuclear delivery of peptide–DNA complexes [206-208].

Complexes of plasmid DNA with cationic lipids (lipoplexes) are not just ordinary vesicular containers with DNA molecules enclosed in the internal volume. Drastic structural reorganization of both polynucleotides and lipid accompanied lipoplex formation. The final structure and physical properties of lipoplexes are responsible for effectiveness of cell transfection. The processes responsible for effectiveness of transfection comprises the interaction of lipoplexes with the cell surface, their endocytosis, transportation to the perinuclear region, and DNA release and translocation into the nucleoplasm where the genetic information can be expressed. While studying the journey of a lipoplex to the nucleus, new details and complexities are being revealed. Though new smart and more effective transfection vehicles have been regularly suggested, the tiny and perfect virus particles are still leading in the competition between human intellect and nature.

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