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DNA–lipid interactions in vitro and in vivo

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Abstract

The data on lipid–nucleic interactions and their role in vitro and in vivo are presented. The results of study of DNA–lipid complexes in absence and in presence of divalent metal cations (triple complexes) are discussed. The triple complexes represent the generation of cellular structures such as pore complexes of eucaryotes and ''Bayer's junctions'' of procaryotes. The participation of triple complexes in the formation of structure of bacterial and eucaryotic nucleoid and nuclear matrix is analysed. A model of formation of triple complexes and cellular structures and their role in DNA–lipid interactions are discussed.

Keywords: DNA; DNA–membrane complexes; Cations; Triple complexes

1. Introduction

Understanding as an essence of life, a mode of existence of protein bodies was transformed to a chain DNA–RNA–protein, thanks to the discovery of double helix structure of DNA, its replication and transcription modes by Watson and Crick. This chain reflects the main paradigm of molecular biology, which is practically unchanged to date. At the same time, the coexistence of three main components in a cell, namely proteins, nucleic acids and lipids, assumes potentiality of existence of three main interactions between them: protein–nucleic acids, lipid–proteins and lipid–nucleic acids interactions. In spite of the evidence on existence of these types of interactions, the DNA–lipid interactions were almost completely ignored by most of the biologists to date. It is therefore necessary to discuss some aspects of DNA–lipid interactions and their role in the functioning of cells and formation of a number of cellular structures.

2. DNA–lipid complexes in vitro

An idea about the possible involvement of bacterial membranes in cellular genome functioning has appeared for the first time in 1963. Observation of attachment of DNA to a bacterial membrane by Jacob et al. [1] by means of electron microscopy lead to the assumption that DNA–membrane contacts (complexes) can participate in the segregation of chromosomes between daughter cells. In this work, the role of lipids in the attachment of DNA to a membrane was not mentioned. However, data on electron microscopy that provided evidence on numerous DNA–membrane contacts (further DMC) in vivo [2], as well as the fact that the membranes are composed of lipids by 40–60%, have forced some scientists toward research, focused on the study of the DNA–lipid interactions on various model systems.

Manzoli et al. [3,4] reported considerable changes of parameters of DNA melting as a consequence of interaction of DNA with various lipids dissolved in 70% ethanol solution. The ethanol was used for better solubility of lipids. Lipids are only weakly soluble in water, but form micelles or liposomes. These particles, however, cause intensive light scattering, which represent major limit for application of light spectroscopy methods. Therefore, the results of the study of DNA–liposome complexes by spectroscopy methods were ambiguous. Manzoli et al. showed both stabilizing and denaturing effect of various lipids on DNA. Experiments with DNA–liposomes complexes revealed a depletion of DNA melting peak when DNA molecules were inside the liposomes [5]. However, this observation has not been confirmed by other authors [6].

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IR spectroscopic analysis of DNA–lipid complexes (total fraction of lipids of a rat liver) showed existence of DNA denaturation at the presence of these lipids [7]. It is, however, possible, that denaturation of DNA could be caused by the procedure of drying–rehydration. This procedure is necessary for preparation of DNA–lipid complexes for IR-spectroscopy.

The data of calorimetric study of the complexes: polyA-polyU-phosphatidylcholine liposomes in absence of divalent cations have shown minor changes of profile in the melting of lipid and polynucleotide [8]. Thus, despite the existence of the interactions of DNA with liposomes composed of zwitterionic or negatively charged phospholipids, these interactions do not cause significant changes in the structure of complexes or their components, except in some special methods of preparation of complexes (using organic solvents, drying–rehydration, placement of DNA inside liposomes, etc.).

Almost simultaneous with the start of the research of DNA–lipid interactions in vitro, Budker et al. [9] have revealed the existence of specific interactions between zwitterionic phospholipids and polynucleotides at the presence of divalent metal cations (Me^{2+}). These complexes, according to our opinion, play an important role in a cell.

2.1. Role of divalent cations in the interaction of DNA with phospholipids

During the study of DNA–phosphatidylcholine liposomes–Mg^{2+} complexes, Budker et al. [9] have detected that DNA can interact with liposomes composed of zwitterionic liposomes by means of a bridge from divalent cations. In further works, an extensive study of triple complexes has been performed [8,10–13].

The brief summary of the results of the study of triple complexes is listed below:

(1) DNA forms complexes with three main lipids: phosphatidylcholines (PC), phosphatidylethanolamines (PE) and sphingomyelines (SM), the addition of other lipids increases or decreases these interactions [8];
(2) Ability of divalent cations to form complexes with DNA and PC correlates with a degree of binding of these cations with PC [8];
(3) The liposomes fuse partially or completely with presence of Me^{2+}, and DNA, i.e. DNA plays a role of fusogene [12];
(4) DNA is partially unwounded in triple complexes [8,9];
(5) DNA molecules in triple complexes become restrictedly accessible to action of DNAse I, but are more accessible to digestion of S1-endonuclease [9];
(6) By means of ^{31}P-NMR technique, a strong change of signal connected with phosphate groups of PC was shown, an evidence on the formation of triple complexes between phospholipids, DNA and Mg^{2+} [10];
(7) A cooperative character of binding of Mn^{2+} in the complex DNA–PC was shown [12];
(8) In addition to the double-stranded DNA, a three-stranded polynucleotide polyA * 2polyU forms triple complexes as revealed by microcalorimetry [13].

Binding energy between phospholipid and DNA molecules by means of bridges of Mg^{2+} is comparable with that of hydrogen bounds between the basis of DNA (~ 2 to 3 kcal/mol per one base pair).

The triple complexes and DNA–membrane complexes were studied simultaneously but independently from each other, that did not promote development of these both directions.

2.2. Interaction of DNA with cationic liposomes

The study of the mechanisms of interaction of aliphatic amines with DNA, first interested some physico-chemists, has become explosive after successful application of DNA–cationic liposomes complexes in gene therapy [14]. Such complexes are related to triple complexes: DNA–liposomes from zwitterionic phospholipides–Mg^{2+}. According to our opinion, triple complexes represent a simplified model of DMC, and complexes of aliphatic amines with DNA could be considered as a model of triple complexes. In contrast with labile triple complexes, where the bridge between DNA and phospholipid formed by Mg^{2+} represents a certain analog of a joint connection of molecular skeleton, the direct bounds between amines and phosphates reveal lower degree of freedom. A strong interaction between DNA molecules and aliphatic amines also allowed the study of these interactions easier by various techniques. Nevertheless, the complexes of cationic liposomes and DNA reveal many properties that are similar to the triple complexes.

It has been shown that aggregation and fusion of cationic liposomes take place in the presence of DNA [15–17]. The changes of DNA structure were observed at certain methods of preparation of complexes. Cationic liposomes composed of 100% DOTAP bor DOTAP/DOPE (1/1) liposomes, induced instantaneous transition of the plasmid DNA from the B- toward a partial C-type conformation as shown by circular dichroism (CD) spectroscopy [18]. The interaction of DNA with aliphatic amines also induces changes in phase state of lipid bilayer. For a wide range of lipid compositions, the phase evolution is characterized by lipid to DNA charge ratio [19]. These complexes of type “spaghetti” and lamellar structures [19] have been found also in triple complexes and invaginations of liposomes similar to endocytosis has been observed at contacts of DNA with large liposomes or cells [20]. The possibility of formation of various three-dimensional structures from neutral unilamellar liposomes in triple complexes is, according to our opinion, higher in comparison with positively charged liposomes. It is due to the fact that Mg^{2+} bridges are easily disturbed and

rebounded at a complex formation, which, obviously, does not hold for DNA–cationic lipid complexes. Therefore, the study of triple complexes is more valuable for understanding the DMC formation in a cell.

Complexes of DNA and lipid monolayers composed of cationic lipids (Langmuir–Blodgett films) unlikely form the triple complexes [21,22] and are far from cell-like DMC. However, these complexes are probably good models of the so-called tightly DNA-bounded lipids [23]. In order to understand the nature and possible functions of intranuclear lipids, we shall consider how these lipids can be linked to DNA–membrane complexes.

2.3. Lipids of chromatin and nuclear matrix and their involvement in function of cell nucleus

In addition to DNA, RNA and proteins, a minor amount of lipids are present in isolated nucleus. The composition of lipids in chromatin is different from that of nuclear envelope. This difference cannot be explained by contamination of the chromatin lipids by components of nuclear membrane during the isolation procedure [24]. The kinetics of lipid accumulation in a chromatin differs from that in the whole nucleus. This allows us to assume the existence of space separation of chromatin lipids and other lipids present in the nucleus [25]. The changes of lipid composition of a chromatin at partial haepatectomy [26] and at cell treatment by hormones [27] have been shown.

With an organism ageing, the composition of lipids in a chromatin of rat haepatocytes undergoes changes in such a way that it does not correlate with changes of lipid composition in nuclear membrane [28]. The phospholipids in nucleus of haepatocytes are localized close to RNA in a fraction of euchromatin. This may be an evidence of their participation in DNA and RNA synthesis [28,29].

The relation between lipids of chromatin and lipids of nuclear matrix is not clear yet [30]. Namely, the phospholipids in nuclear matrix directly or indirectly link DNA with matrix proteins [30]. Evidence that removal of sphingomyelin from a nuclear matrix by means of sphingomyelinase results in displacement of all DNA from matrix [31] may indicate an existence of direct DNA–sphingomyelin interaction. Evidence that the sphingomyelin forms the strongest triple complexes in vitro [8] only confirms this suggestion. At the same time, approximately 50% of a sphingomyelin ware removed during treatment of rat liver nucleus by RNAase [29]. It can be suggested that lipids help keep DNA and RNA in a nuclear matrix.

Manzoli et al. investigated the influence of phospholipids on both the structure and matrix function of chromatin of an isolated cell nucleus. They found that the phosphatidylserine vesicles have removed the histone H1 from chromatin. This effect is probably the main reason of decondensation of chromatin [32]. In a similar manner, the anionic lipids induce chromatin decondensation and increase its transcription activity [4]. In contrast, zwitterionic phospholipids reveal an opposite effect [33]. The treatment of nuclear matrix by phospholipases results in a release of newly synthesized DNA from matrix [30]. This evidence coincides with the data reported by Alesenko et al. [31].

In addition to the lipids of chromatin and nuclear matrix, another strongly DNA-bounded lipid is present in the nucleus. These lipids have specific composition in different phases of cell cycle and depending on superhelicity of DNA, at carcinogenesis [34]. According to Struchkov and Strazevskaya [35], these lipids participate in the regulation of DNA transcription. They also suggested that these lipids play an important role in the organization of DNA in the nucleus.

We suggest that DNA–lipid complexes reported in paper [35] can be formed in a cell in the presence of divalent cations, i.e. like complexes of DNA with synthetic cationic lipids or the complexes of DNA with sphingosine. In addition, the appearance of these complexes during isolation of chromatin cannot be excluded. The complexes between DNA and separate lipids could appear in a nonpolar environment, e.g. when detergents and phenol are used at chromatin isolation. It is obvious that the composition of lipids interacting with DNA will be different in the presence of TritonX-100, which is used at isolation of nuclear matrix, or phenol, used at the procedure of determination of chromatin lipids [35]. Moreover, the composition of these lipids will differ from lipids of a nuclear membrane. From the data mentioned above, it follows that at least three types of localization of lipids inside a nucleus reported by different authors could have an artifact origin. There exist only a hypothesis on how these lipids interact with DNA and what their functions are in a cell.

In addition to Capitani et al. [36], Alesenko and Burlakova [37] also studied influence of lipids on enzymes of template synthesis. For review on influence of lipids on activity of the enzymes controlling replication and transcription, see Ref. [35]. It has been shown that lipids affect an isolated DNA and RNA polymerases as well as isolated nuclei, when liposomes are added in corresponding solutions [36]. For example, phosphatidylserine increased both DNA-, and RNA-polymerase activity, and in contrast, zwitterionic lipids reduced it. At the same time, phosphatidylcholine vesicles at the presence of Mg^{2+} ions stimulate the activity of a DNA polymerase alpha [38] and DNA polymerase of *Escherichia coli* [39].

The mentioned data represent convincing proof of involvement of phospholipids in a gene statement at a level of a regulation of activity of enzymes of template synthesis.

3. DNA–membrane complexes in vivo

As we mentioned above, the first data on DNA binding to a membrane were obtained by electron microscopy [2]. Results of study of DMC were reviewed in a number of works [40–43]. Bayer [44] has shown that in bacteria there exist so-called zones of adhesion between a bacterial wall and

cytoplasmic membrane, to which DNA is attached. There exist from 100 to 400 of such contacts in a cell. It has been assumed that the site of DNA attachment to a nuclear membrane of eucaryotes are the pores [13,45]. Near to the nuclear pores the chromatin is in a diffuse state, and therefore, is transcriptionally active [25,40].

In 1970–1980, an attempt was made to isolate a fraction of DMC and to analyze its biochemical composition. For this purpose, the cells have been gently lysed in presence of Mg-sarcosylate and so-called "M-band" fraction has been obtained. This fraction contained DNA, enriched by moderate repeats, newly synthesized DNA, RNA and proteins [45–47]. There is no information on lipid composition of "M-band" fraction. However, there is evidence on changes of lipid composition of nuclear envelope in cells, with increased level of transcription and the replication. This fact may suggest on important role of lipids in template synthesis of DNA and RNA [31].

The involvement of DMC in an initiation of replication and transcription and in a regulation of gene statement has been suggested. Just like these functions have been assigned for the existence of moderate repeats in DNA [40,41]. Further research as well as improvement of the methods of isolation of DMC fractions has not been continued, mostly due to a general fascination in study of nuclear matrix [48]. In connection with a present topic, it is necessary to stress a problem of structure of bacterial and eucaryotic nucleoid, which are directly related both to DMC and to the nuclear matrix.

3.1. DMC and nucleoid of bacteria and eucaryotes

The problem of packaging of giant DNA (1–2 cm in length) in bacteria, whose size does not exceed several microns, is comparable with complexity of packaging DNA in eucaryotic chromosomes. After a mild lysozyme-detergent lysis of bacteria, the structure called as a nucleoid ("chromosome") of bacteria was detected by electron microscope. The nucleoid represents closed loops of DNA, outgoing from common center. There exist about 100 such closed loops. The amount of loops is influenced by a number of factors [49]. Under the action of RNAase H, pronase, S1-endonuclease and other factors, these closed loops are split off from a center and the nucleoid undergoes relaxation. This fact is an evidence of the existence of open structures in DNA [50].

As soon as the nucleoids have been prepared from eucaryotic nucleus [51], the assumption was made that inside the bacteria there exists same compact structure as in chromosomes of eucaryotes. However, electron microscopy of bacteria did not reveal any structure similar to chromosomes-like structures and no central core nucleoid containing outer and inner membrane of bacteria, as in "membrane bound" nucleoid [49].

We developed a model on the formation of bacterial nucleoid with participation of DNA–membrane complexes, and a model of the DNA–membrane contacts [52,53]. The essence of this model is in the following. Both in a native bacterial cell, and in an interphase nucleus of eucaryotes, DNA is attached to a membrane in many points. This arrangement is the basis of further structure of nucleoid. This structure can arise from the treatment of a nucleus or bacterial cell by detergents and the removal of a large part of the membrane preserving some part of DMC. As a result of this process, a small membrane island with closed loops of attached DNA appears. This structure, i.e. nucleoid, is observed in electron microscope; however, it does not exist in a native cell (Fig. 1).

According to our opinion, the similarity of a nucleoid of bacteria and eucaryotes consists, in fact that DNA in an interphase nucleus and dividing cells of bacteria is attached to a membrane in a number of sites. This condition is necessary for normal transcription and replication. In addition, the structure of DMC and the method of its formation in bacteria and eucaryotes are according to our similar model. According

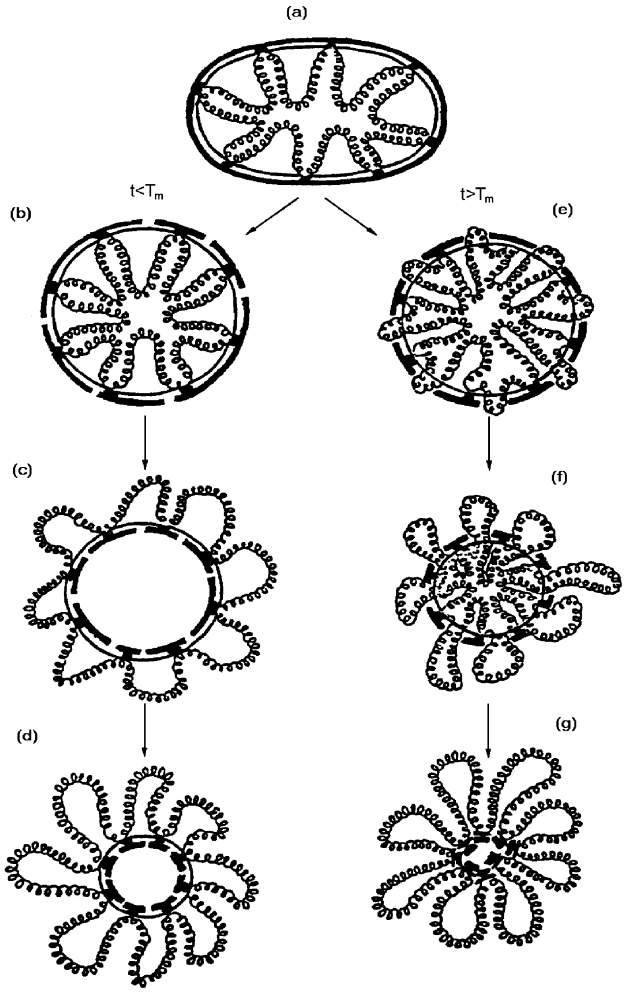

Fig. 1. The model of formation of bacterial and eucaryotic nucleoid: (a) native state of bacterial cell (nucleus of eucaryotes) with many DMC; (b)–(d) the formation of membrane-bound nucleoid of bacteria; (e)–(g) the formation of membrane-free nucleoid of bacteria (eucaryotic nucleoid). T_m—the temperature of phase transition of a membrane (see Ref. [35] for details).

to this model, DNA is attached to a membrane at the so-called regulatory sites (promotors, sites of initiation of replication, which according to reassociation kinetics belong to the moderate repeats). We suggest that at DNA–membrane, binding sites are also localized low molecular RNA (l.m.w. RNA). These RNA form a triple helix with DNA, which due to its interaction with a membrane lipids unwind already at a room temperature and forms a classical R-loop: DNA–RNA hybrid and a single-stranded DNA [40,41]. In contrast to a double helix DNA, the three-stranded hybrids unwind at lower temperature, and thus could be easily attached to a membrane.

The hypothesis on the role of R-loops in a regulation of gene statement was popular in 1970s, however, many authors follow it at present [54]. We recently showed that three-stranded nucleic acids could form complexes with PC-liposomes, which results from the unwinding at a room temperature [13]. It is known that a low molecular weight RNA (l.m.w. RNA) is able to bind with chromatin and could form three-stranded helix. The role of l.m.w. RNA in a regulation of transcription was earlier reported in Ref. [55].

The data on relaxation of the nucleoid structure induced by RNAse enzyme, which destroys the DNA–RNA hybrids, confirm the opinion on participation of triple helix structures on DNA attachment to a membrane [49]. An opinion exists that nuclear pores and "Bayer's junction" are the sites of attachment of DNA to a membrane. The DNA sites between pores and "Bayer's junction" are, according to our model, replicons. However, number of loops in a nucleoid is less than the number of replicons owing to particular destruction of DMC by detergents during the isolation of nucleoids.

3.2. Nuclear pores and lipid nucleic acids interactions

It is known that at the end of a prophase, the nuclear pores are formed simultaneously with assembly of new nuclear envelope. The membrane vesicles that appeared due to breakdown of old nuclear envelope and sarcoplasmic retikulum are involved in this process [56]. Apparently, the assembly of nuclear pores in an interphase is realised by another way [53].

We suggested following multistage way of formation of interphase pore (Fig. 2): Interaction of chromatin DNA, which is in form of nucleosomes, with internal nuclear membrane resulting in an invagination of a membrane in the direction of cytoplasm. This process has some peculiarities of endocytosis and is commonly observed in gene therapy during lipofection. As a result, a contact with outer nuclear membrane arises (Fig. 2a).

The histones should not prevent the interaction of DNA with a membrane, because as it is known, they interact both with negatively charged [58], and with neutral phospholipids [59]. Moreover, recent studies on DNA transfer into the cells showed, that histones promote penetration of DNA through a membrane [60]. Thus, it might be possible that DNA (chromatin) is capable to penetrate into lipid bilayers

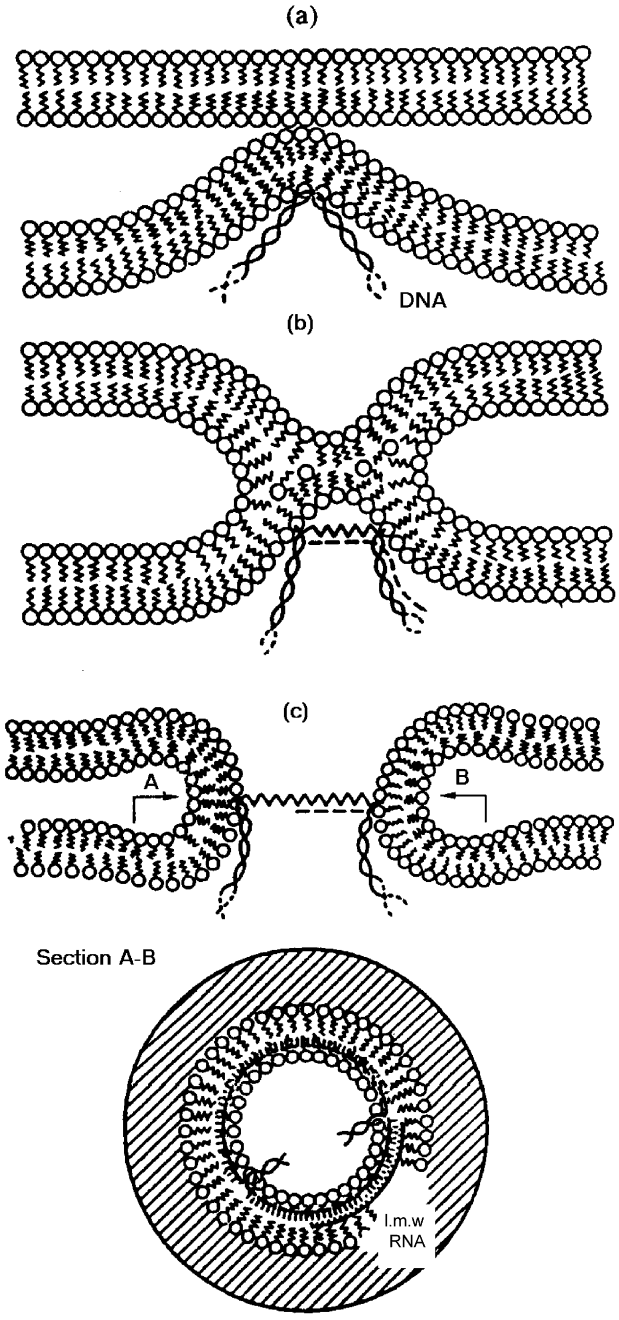

Fig. 2. Scheme of the interphase of DMC formation: (a) contact of DNA (chromatin with inner membrane and their invagination by endocytosis type); (b) partial fusing of two membranes, unwind of R-loop, the formation of pore complexes or Bayer's junction of procaryotes; (c) break of membrane diaphragm and DNA unwinding for the size more than R-loop under action of surface tension.

where, in lipid environment, the triple helix unwinds with formation of R-loop (Fig. 2b). This process is accompanied by a fusion of two membranes and formation of a nuclear pore (Fig. 2c) [53].

Despite that we will not discuss the role of nuclear or membrane proteins in formation of nuclear pores, this process should not be completely eliminated. We suppose that there

are no, other than RNA, molecules that would be able to recognize DNA regulatory sites and promote their attachment to a membrane.

For low molecular weight RNA (200 nucleotides that corresponds to the length of l.m.w. RNA U1 and U2 types and at a surface tension of nuclear membrane, which is in a range of 0.02–5.0 mN/m) corresponding to a surface tension of the majority of biological membranes, the calculated diameter of pores (616 nm for U1 RNA and 722 nm for U2 RNA) is consistent with a diameter of nuclear pores observed by electron microscopy (600–800 nm) [41,11,53]. The process of formation of nuclear pores in late anaphase from membrane vesicles at the moment of a chromatin decondensation [11,56] is presented schematically in Fig. 3. This process consists of several stages:

- the fusion of two vesicles when DNA (or DNA–RNA hybrid) is localized between vesicles. The DNA plays a role fusogene;
- uwinding of R-loop on equator of the large vesicle which appeared from two fused vesicles;
- fusion of eight vesicles with the large vesicle, which is encircled on equator with single stranded DNA and DNA–RNA hybrids, formation of prepore;
- fusion of remaining vesicles with a prepore membrane on its perimeter with formation of fragments of a nuclear envelope with pores;
- fusion of fragments of the nucleus envelope and vesicles in a native nuclear envelope.

From the proposed model, it follows that there are two various ways of fusion of vesicles: first is induced by fusogene DNA, second consisting in a fusion of vesicles with prepore and among them is induced by other factors, different from DNA, and leads to formation of a nuclear envelope.

The scheme presented above is supported by data, which evidence that the pores and the membrane of nuclear enve-

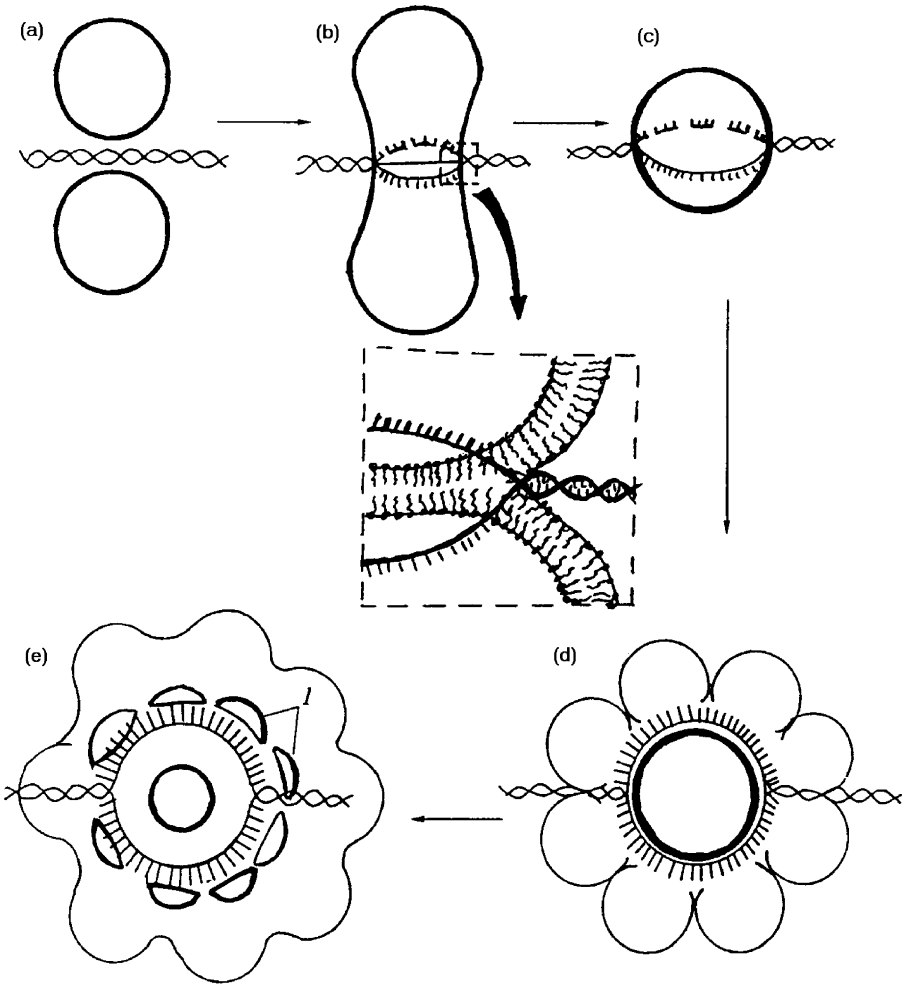

Fig. 3. The model of nuclear pores assembly in late anaphase: (a) DNA is located between two membrane vesicles; (b) partial fusion of two membrane vesicles under action of DNA as fusogene, the R-loop formation; (c) full vesicles fusion, prepore formation; (d) fusion of eight vesicles with prepore under action of another's fusogenes (not DNA); (e) the formation of functionally active pore complexes with the patches of nuclear envelope. The role of many proteins in formation of pore complexes was not shown. The number 1 in scheme is the hole in nuclear envelope forming true "diffused diameter" of nuclear pore [38].

lope are formed from different populations of membrane vesicles [57]. The structure and the way of formation of pores have been scopes of a number of papers; however, the role of lipids and DNA in this process was not discussed, i.e. the existence of direct DNA–lipid interactions is ignored. However, there are many data supporting our model. Thus, it is obvious that the existence of membrane diaphragm almost completely overlaps the pore of the channel. The electron microscopy data obtained by freeze-etching method, which in a most degree preserve the native state of nuclear envelope, evidence on the existence of such a diaphragm in nucleus pores at the samples of nucleus envelope. Considering the above-mentioned experimental data, it seems that our model is preferable in comparison, with suggestion on open and close nucleus pores presented by other authors [61].

According to our model, the DNA unwinds in areas of nuclear pore. Therefore, the filaments connecting the pores are visible on the fraction of a nuclear envelope, after various treatments that expose the pore complexes. The treatment of nuclear envelope by 1 M NaCl, trypsin and RNAase does not result in disappearance of these filaments [62]. It is clear that the treatment of the nuclear envelope by DNAase would result in disruption of the filaments; however, this peculiarity has not been studied in the mentioned work. The nuclear pores linked by DNA filaments DNA seems to be the most stable structures of a nucleus. They are stable after the treatment by DNAase-I, RNAase, 1.5 M NaCl, but can be destroyed by 25 mM NaOH [63]. This peculiarity confirms our model of nuclear pores based on the presence of hybrid DNA–l.m.w. RNA, which as it is known is destroyed by alkali.

The necessity of divalent cations for formation of pore complexes was proved in experiments with chelators that bind Ca^{2+} and Mg^{2+}. Addition of chelators results in the diminishing of pore complexes [59]. The data on possibility of formation of a nuclear envelope with pores in an extract *Xenopus laevis* in absence of DNA [64] are on a first view against our model. However, presence of a number of double-stranded RNA in plasm [65] as well as presence of not chromosomal DNA, which is present in cytoplasm of many cells [65] and which can initiate the formation of pore complexes, makes this argument inconsistent. The evidence of our opinion is the last data about connection of chromatin with nuclear envelope [66].

3.3. Nuclear matrix and lamina

The data presented above give a new view on the role of such structures as nuclear lamina and nuclear matrix in nuclear pores assembly and on their function in gene statement. The procedure of preparation of a nuclear matrix includes application of detergents that are also used for isolation of nucleoid. It also includes DNAase that destroys total DNA. During this procedure, the DNA linked with a membrane (DMC) is not destroyed and is transformed to a nuclear matrix structure together with residuals of lipids and proteins of a nuclear envelope. The resulting complex is far from structures existing in native cells. As a result, the proteins, lipids and the residuals DNA of nucleotide core (center of a nucleoid) should be identical to the proteins and DNA of a nuclear matrix. Moreover, according to our opinion, the condensation of eucaryotic chromosome starts on a surface of a nuclear envelope and the looped chromosome structure completely reproduce DNA loops between DMC and loops of nucleoids. This leads to similarity of proteins of nuclear matrix with so-called ‘‘scaffold’’ chromosomes.

Some authors assume that the nuclear matrix can be artificial structure [67]. The fraction of nuclear matrix obtained by biochemists has structure which is far from native structure of the matrix in cell nucleus because both proteins of a nuclear envelope, as well as the DNA fragments (regulatory sites, newly synthesize DNA and RNA) passing into a nuclear matrix as a result of the removal of a nuclear envelope by detergents [68]. Therefore, all functions, namely attachment of DNA loops, regulatory sites of DNA, newly synthesized DNA and RNA, enzymes of matrix synthesis, etc., assigned earlier to DMC, refer to a nuclear matrix [48]. According to our opinion, there is only one advantage of this structure, which consists in the fact that isolation of nuclear matrix by means of biochemical methods is easier than isolation of DNA–membrane complexes. In addition to the proteins, lipids are also involved in a nuclear matrix [33,69]. It has been shown in vitro experiments that these lipids can form complexes with DNA in the presence of detergents [68,70]. This is also a way of allowing transfer of some lipids of nuclear envelope into the nuclear matrix [68].

The composition of lipids of a nuclear matrix will be obviously different from lipids of a nuclear envelope, because not all lipids will strongly contact with DNA at these conditions. At the same time, the part of intranuclear proteins can aggregate with nuclear membrane protein residues, forming, as a result, the structure of a classical nuclear matrix. Recent electrophoretic analysis of nuclear matrix proteins has shown that the majority of its proteins belong to heterogenous nuclear ribonucleorproteins (hnRNP) [71]. The data [72] that the protein composition of nuclear matrix changes depending on presence and concentration of Mg^{2+} ions confirm, according to our opinion, the artificial character of matrix.

The above-mentioned suggestion is also confirmed by data evidencing on the removal of all DNA from the matrix after its treatment by sphingomyelinase [31]. According to our data, the sphingomyelin forms the strongest bound with DNA with the presence of divalent metal cations [8]. The detachment of all DNA from a nuclear matrix by action of sphingomyelinase indicates that lipids and not proteins provide link of DNA to the matrix and consequently also with nuclear envelope, from which DNA moves to a nuclear matrix.

Concerning nuclear lamina (‘‘net’’ of proteins, connected with internal nuclear membrane), it can be assumed that this

structure appears due to accumulation of appropriate proteins near the nuclear envelope around decondensed chromatin. Data on linkage of lamina proteins with DNA in a prophase of cell cycle and their subsequent accumulation between DNA and internal nuclear membrane after release from this link [73] are not in contrast with previous opinion. We suppose that lamina represent simply an aggregation of proteins that are exported to the cell nucleus through pores or detached from a chromatin after its complexation with a membrane, or during of DNA transcription and replication. On the other hand, the lamina proteins of types A, B and C are detected in the nuclear envelope [74] and they are probably moved from this place to a near membrane area. This process is initiated by protein release from protein–DNA complexes. Additionally, DNA sites are detached from the complex with a membrane in the S-phase, and are permanently substituted by a newly formed DNA–membrane complexes.

The monoclonal antibodies against lamina proteins of *Drosophila melanogaster* specifically interact with a fraction of a nuclear envelope in situ [75]. Recent studies show that the proteins of lamina are very motile in cell cycle both in respect to their structure and quantity as well as with respect to subcellular localization [76]. Thus, classification of lamina proteins as a special cell structure and relation of their function with a nuclear skeleton is not justified. We suppose this is the function of chromatin. Nevertheless, the large number of works is devoted to the study of the role of lamina proteins, lamina in pore complex formation. Due to evident role of some nuclear proteins in assembly of a nuclear envelope, it is impossible to ignore these works, as it is impossible to ignore a role of direct phospholipid–nucleic acids interactions in this process. With respect to our model, these proteins can promote fusion of membrane vesicles, but can also block this process by means of decreased interaction between lipids and DNA in a defined phase of cell cycle.

Without any doubt, the double-nuclear membrane represent basis of the nuclear structure. The DNA is attached to this membrane at sites of nuclear pores. The study of structure, formation and destruction of DMC in a cell as well as study of involvement of lipids and proteins of cell envelope into replication, transcription and in formation of chromosomes is possible with the existing methods. At the same time, it is necessary to understand the contribution of DMC to formation of nuclear matrix, nucleoid and lipids of a chromatin by means of the methods utilizing destruction of cell nucleus. We suppose that investigation of DMC is the basic way to study the assembly and function of a cell nucleus and statement of cell genome. Without this knowledge, the gene sequence will only be dead letters in the great book of life.

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