J Membrane Biol (2011) 241:109–116
DOI 10.1007/s00232-011-9367-5

ORIGINAL ARTICLES

The Mechanism of a Nuclear Pore Assembly: A Molecular Biophysics View

Vasily V. Kuvichkin

Received: 23 March 2011 / Accepted: 22 April 2011 / Published online: 16 June 2011
© Springer Science+Business Media, LLC 2011

Abstract The basic problem of nuclear pore assembly is the big perinuclear space that must be overcome for nuclear membrane fusion and pore creation. Our investigations of ternary complexes: DNA–PC liposomes–Mg^{2+}, and modern conceptions of nuclear pore structure allowed us to introduce a new mechanism of nuclear pore assembly. DNA-induced fusion of liposomes (membrane vesicles) with a single-lipid bilayer or two closely located nuclear membranes is considered. After such fusion on the lipid bilayer surface, traces of a complex of ssDNA with lipids were revealed. At fusion of two identical small liposomes (membrane vesicles) <100 nm in diameter, a "big" liposome (vesicle) with ssDNA on the vesicle equator is formed. ssDNA occurrence on liposome surface gives a biphasic character to the fusion kinetics. The "big" membrane vesicle surrounded by ssDNA is the base of nuclear pore assembly. Its contact with the nuclear envelope leads to fast fusion of half of the vesicles with one nuclear membrane; then ensues a fusion delay when ssDNA reaches the membrane. The next step is to turn inside out the second vesicle half and its fusion to other nuclear membrane. A hole is formed between the two membranes, and nucleoporins begin pore complex assembly around the ssDNA. The surface tension of vesicles and nuclear membranes along with the kinetic energy of a liquid inside a vesicle play the main roles in this process. Special cases of nuclear pore formation are considered: pore formation on both nuclear envelope sides, the difference of pores formed in various cell-cycle phases and linear nuclear pore clusters.

V. V. Kuvichkin (✉)
Department of Mechanisms Reception, Institute of Cell Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow region, Russia
e-mail: vvkuvichkin@gmail.com

Keywords DNA · Phosphatidylcholine liposome · Fusion · Membrane vesicles · Nuclear pore assembly · Surface tension · Cryo-TEM · *Xenopus* egg extract

Introduction

According to modern notions, a nuclear pore represents a complex molecular machine containing 30–50 proteins—nucleoporins located at the surface of the hole connecting the outer and inner nuclear membranes of a nucleus (Hetzer 2010). Some authors believe that the hole is the result of interaction between nucleoporins and the nuclear envelope, although no evidence of this process, in vitro or by PC simulation, is available.

Many years ago DNA or DNA–RNA triplexes were assumed to be able to cause fusion of membrane vesicles consisting of zwitterionic lipids in the presence of a series of bivalent cations (Plum et al. 1995; Kuvichkin 2002).

Fusion of two nuclear membranes located a considerable distance from each other is a great difficulty in explaining nuclear pore assembly. As more than half of nuclear membranes are composed of zwitterionic lipids, a model of nuclear pore assembly by DNA-induced fusion of membrane vesicles, from which the nuclear envelope is formed in a cell, has been proposed (Kuvichkin 2002).

Although our biophysical and electron-microscopic data in vitro (Kuvichkin 2009) and in *Xenopus* egg extract (Kuvichkin and Uteshev 2003) support this hypothesis, the absence of nucleoporins in the model prevents its recognition by scientific society. It should be noted that no statement has been made that nucleoporins are not involved in nuclear pore assembly, but they promote fusion of membrane vesicles, which form the nuclear pore in a cell. Undoubtedly, without DNA, neither the process of

membrane vesicle transformation in the nuclear envelope nor the structure of a nuclear pore is seen.

The fusion of two membranes is complex, and liposome DNA-induced fusion is also studied insufficiently. Therefore, we think it is necessary to explain our position on this question, taking into account modern findings about a role of DNA and nucleoporins in nuclear pore assembly. Thus, we report the results of in vitro studies with DNA and PC liposomes as well as egg extract from *Xenopus laevis*, which more closely approximate a cell and the modern conception of nuclear pore assembly and structure.

It is known that a nuclear envelope is formed from membrane vesicles of at least two types (Vigers and Lohka 1991; Collas and Poccia 1996; Salpingidou et al. 2008). A pore-forming type binds with chromatin and can fuse with nuclear envelope fragments. The vesicle diameter of this type is about 70 nm. Another type of vesicle serves to increase the nuclear envelope surface through fusion with it, where no pore is formed. According to our model (Fig. 1a–c), the first type, at interaction with chromatin DNA, forms "string-of-pearl" structures, which further fuse with a nuclear envelope.

String-of-pearl structures are observed in cells in anaphase when the new nuclear envelope is only beginning to be formed (Zhanga et al. 1998; Kiseleva et al. 2001, 2004). Although the authors who have revealed such structures think that fibrils—bridges between vesicles—are keratin (Zhanga et al. 1998) or actin-like proteins (Kiseleva et al. 2004), it is impossible to exclude the presence of DNA in fibrils. It is known that the proteins mentioned and vimentin bind to DNA, providing a shield against DNase I (Zahm et al. 2001; Chelobanov et al. 2003). DNA at the resolution of an electronic microscope used in the cited

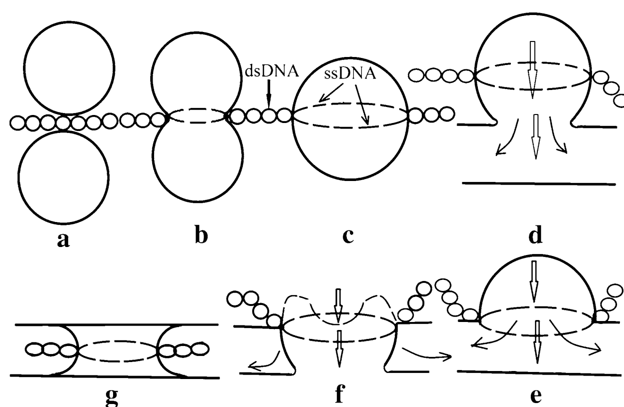

Fig. 1 **a–c** "Big" vesicle formation after DNA-induced fusion of small unilamellar vesicles. **d** Fusion of a "big" liposome with the first nuclear membrane. **e** Fusion of a half-vesicle with the nuclear membrane, with a delay in further fusion. **f** Turning inside out of half of a "big" vesicle and its fusion with a second nuclear membrane. **g** Full fusion of a vesicle with two nuclear membranes, hole formation and the beginning of nuclear pore assembly by nucleoporin binding to ssDNA

studies can be invisible. In early work where so-called nuclear ghosts were investigated (Riley and Keller 1978), it has been shown that annuli of nuclear pores relate to two types (thick and thin) of fibrils. When thick fibrils are destroyed by proteinases, thin ones are in turn digested by DNase I (Riley and Keller 1978).

Materials and Methods

Chemicals

Calf thymus DNA and magnesium chloride were obtained from Sigma-Aldrich (St. Louis, MO). Egg yolk 3-*sn*-phosphatidylcholine, $MgCl_2$, $BaCl_2$, terbium (III) acetate and NaCl were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). HEPES (2-[4-2-hydroxyethyl]-1-perazinylethenesulfonic acid) was from Dojindo (Kumamoto, Japan). DOPC and DOPE were from Avanti Polar Lipids (Alabaster, AL). Pyrene-PC was from Invitrogen (Carlsbad, CA). The DNA solution and the liposomes were prepared in 0.01 M HEPES buffer, pH 7.2. DNA segments 400 bp long were prepared by ultrasonic treatment of DNA solution for 20 min in an ultrasonic bath (Uhrikova et al. 2005). DOPC liposomes were prepared by the extrusion method using 400 and 50 nm pore polycarbonate membranes (Frisken et al. 2000). The appropriate amounts of lipids (e.g., 100% DOPC and 95% DOPC/5% pyrene-PC) in chloroform were mixed and dried under a stream of N_2 gas, after which the solvent was completely removed by placing the sample in a vacuum desiccator connected to a rotary vacuum pump for more than 12 h. Then 1 ml of 10 mM HEPES (pH 7.2) was added to the dry lipid film, and the suspension was vortexed 5 minutes at room temperature. The resulting solution was extruded ten times through a 400 nm pore membrane, and then a 50 nm pore membrane using a LF-1 LiposoFast apparatus. The turbidity of the liposome suspension was measured by a spectrophotometer U-3300; Hitachi (Tokyo, Japan).

Lipid Mixing Induced by Interaction of DNA with DOPC Liposomes in the Presence of Mg^{2+}

For the lipid-mixing experiment, we first mixed 95% DOPC/5% pyrene-PC liposomes with DOPC liposomes, where the molar ratio (or the number ratio) of 95% DOPC/5% pyrene-PC liposomes to DOPC liposomes was 3 to 1, then mixed the suspension with various concentrations of Mg^{2+} solution and finally mixed it with various concentrations of DNA solution. The final total lipid concentration was 1.0 mM. We incubated these suspensions for 1 h at room temperature. Then, we diluted the suspensions 8.5

times with 10 mM HEPES (pH 7.2) and measured the fluorescence intensities of these suspensions.

A Hitachi F3000 spectrofluorimeter (Hitachi Ltd., Tokyo, Japan) was used for fluorescence measurements. Fluorescence intensities of samples were measured at room temperature. The excitation wavelength of pyrene-PC was 347 nm, and the emission wavelength was 376 nm for monomer fluorescence and 481 nm for excimer fluorescence. The excitation and emission bandpasses were 3 and 3 nm, respectively. The ratio of excimer to monomer fluorescence intensities (*E*/*M*) of pyrene-PC was calculated. The concentration of total phospholipids in the samples for fluorescence measurements was 120 μM.

Zernike Phase-Contrast Electron Microscopy

Phase Plate Preparation

The phase plate was prepared from amorphous carbon films. The films were deposited by vacuum evaporation by means of vacuum evaporator JEE-400 (JEOL, Tokyo, Japan) on a freshly cleaved mica surface. For observation at 300-kV acceleration voltage, the film thickness corresponding to the *p*/2-phase plate was approximately 32 nm. At that thickness, the transparency of 300-kV acceleration electrons was estimated to be 70% (Danev and Nagayama 2001).

After preparation, the films were floated on the water's surface, then transferred to a molybdenum aperture with several holes 50 μm in diameter, which resulted in a cutoff frequency for special resolution of 0.5 nm. A hole approximately 0.5 μm in diameter in the center of the carbon film was used by a focused ion beam machine (JEOL JFIB-2000) (Schmutz et al. 1999).

300 kV TEM Observation

The experiments were carried out on a JEOL JEM-3100FFC electron microscope operated at 300-kV acceleration voltages with or without the Hilbert differential contrast phase plate. The microscope was equipped with a field-emission gun and omega-type energy filter. Objective lens parameters were as follows: spherical aberration coefficient 5 nm, chromatic aberration coefficient 4.7 nm. All observations were performed with a nominal magnification of 940,000, a final magnification with a CCD camera of 960,000 and an electron dose of 200 e^{-}/nm^2 in zero-loss filtering mode. The energy window width was set at 10 eV. A special heated aperture holder was used to support the phase plates. To avoid contamination, the phase plates were kept at approximately 200°C at all times. All images were recorded with a Gatan (Pleasanton, CA) Mega Scan 795 2 K 9 2 K CCD camera (Danev and Nagayama 2001).

Results and Discussion

We believe that the first stage of nuclear pore formation is an interaction of two membrane vesicles with chromatin DNA, located between vesicles (Fig. 1a). As a result of DNA-induced fusion, approximately equal two vesicles form one "big" vesicle (its diameter being 1.26 times more than the diameter of the initial vesicles). Simultaneous DNA unwinding in a zone of vesicle fusion (Fig. 1b) results in the appearance of single-stranded DNA (ssDNA) belting of the "big" vesicle along the equator (Fig. 1c, the ssDNA chain is shown as a dashed line).

The second stage includes interaction of the structure represented in Fig. 1c with a nuclear envelope. We will consider it in more detail using the scheme given below.

The "big" vesicle, belted with ssDNA, fuses with an inner nuclear membrane. In the course of fusion, various proteins—fusogenes, including nucleoporins—are most likely involved, but apparently the ability of the nuclear membrane to fuse is determined by the lipid composition of the vesicles.

Fusion of vesicles with a nuclear membrane follows a standard mechanism, either involving a hemifusion stage or not. Fusion occurs quickly (several microseconds), similar to liposome fusion (Chizmadzhev et al. 2000). If the liposome is fused with a flat membrane or with a liposome of greater diameter, the result is a structure such as that represented in Fig. 7 in Kuvichkin et al. (2009).

Traces of such fusion—ssDNA in lipid surroundings—at the surface of the membrane or other liposomes are visible. Thus, the lipid bilayer structure close to ssDNA will be essentially changed. Such structures are revealed by a cryo-TEM method on the surface of the big liposomes, with which many liposomes of smaller diameter (Fig. 2a) are fused. Figure 2b demonstrates double-stranded DNA (bold short line) and ssDNA (dotted line). Staining ternary complexes (PC liposomes DNA Mg^{2+}) by Ba^{2+} ions, dsDNA and ssDNA are clearly seen (Fig. 2c). The Tb^{3+} cation, staining mainly ssDNA, also reveals sites of DNA unwinding on the liposome surface (Fig. 2d). Ring structures are visible on PC liposomes added in *X. laevis* egg extract upon staining with uranyl acetate (Fig. 2e), but DNA and lipids are rather poorly stained by this dye. An explanation of this effect can be that the proteins in egg extract bind to ssDNA, making these sites visible upon uranyl acetate staining. Openings of the double helix in DNA are also found by a freeze-fracture method (Kuvichkin 2009).

Similar ring structures, but more close to nuclear pores, are observed in *X. laevis* egg extract with a high content of membrane vesicles (Fig. 2f). In this case, as shown earlier (Kuvichkin and Uteshev 2003), fragments of a nuclear envelope with pore-like structures or annulate lamellae are formed.

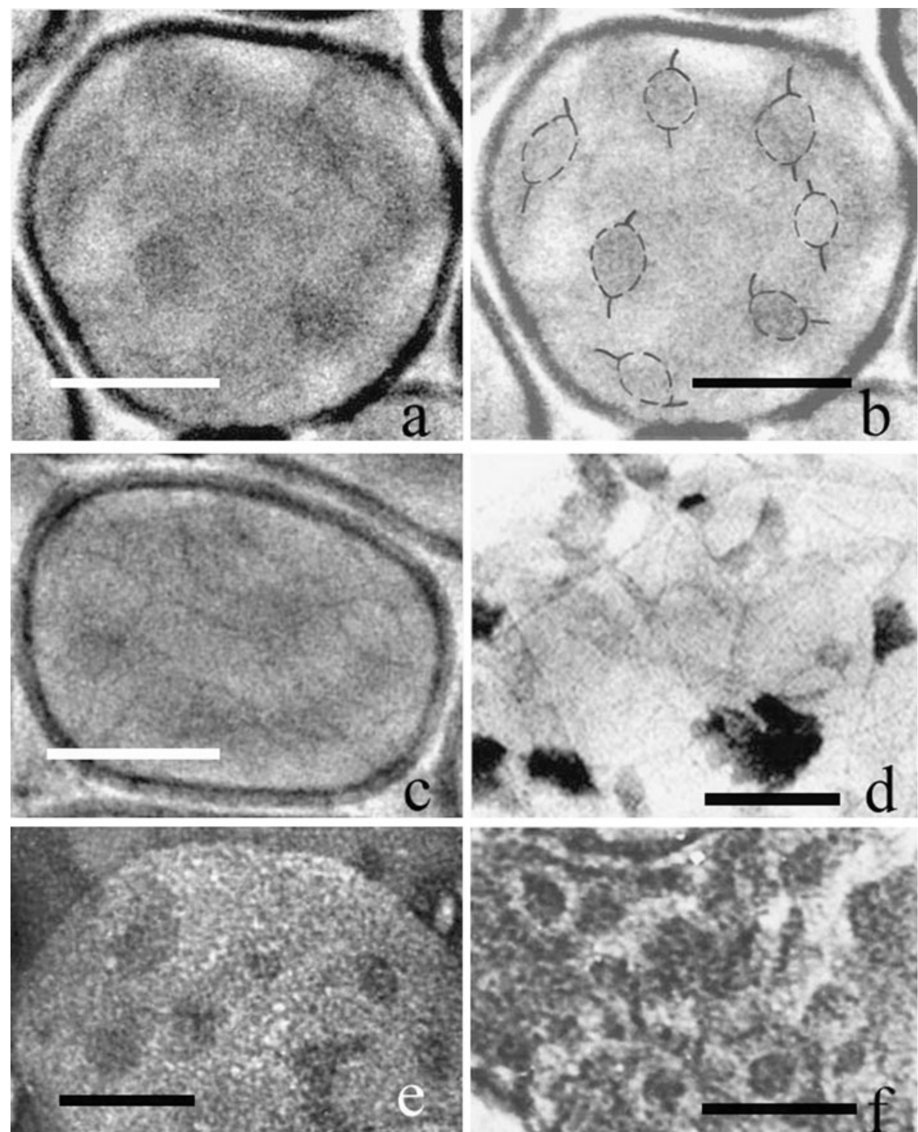

Fig. 2 Areas of DNA double helix opening revealed by various methods: **a** cryo-TEM; **b** elucidation of **a**; **c** $BaCl_2$ staining ternary complexes, DNA–PC liposome Mg^{2+} (cryo-TEM); **d** Tb^{3+} staining of ternary complexes with Mg^{2+}; **e** uranyl acetate staining of PC liposomes added to *X. laevis* egg extract; **f** annulate lamellae in preparation of **e** after addition of *Xenopus* egg membrane vesicles. *Bar* 50 nm, PC liposome size 100 nm, $[Mg^{2+}] = [Ba^{2+}]$ 5 mM, $[Tb^{3+}]$ 10 mM, DNA 20 µg/ml, PC liposome 1.0 mg/ml

It should be noted that a nuclear envelope consists of two membranes, outer and inner, separated by a perinuclear space of 20–40 nm. Just this small distance turned out to be an impassible barrier for experimental and computer modeling of nuclear pore assembly. The hypothesis stated here, according to the author's opinion, explains how the channel between the outer and inner nuclear membranes is formed.

The Mechanism of a Nuclear Pore Assembly

So, when the "big" vesicle, surrounded by ssDNA, is fused by half with an inner nuclear membrane, ss DNA reaches a fusion zone (Fig. 1e). It considerably reduces fusion rate of the rest vesicle with a membrane owing to the changed structure of lipid bilayer near ssDNA. The evidence of it is data on kinetics of DNA induced fusion of PC liposomes in the presence of Mg^{2+}.

Fusion of DOPC-liposomes, 50 nm in diameter, observed by the lipid mixing method occured within 3 hours has two distinct phases (Fig. 3). The first fast phase reaches a plateau approximately for 15 minutes. The second phase lasts 180–200 minutes, i.e., fusion rate after the first fast stage slows down by an order. It is explained by fast liposomes fusion at the first stage, when fast lipid exchange between liposomes takes place (Fig. 1b) until DNA unwinding and ssDNA rim formation (Fig. 1c). After that the lipid exchange between fusing (hemi-fused) liposomes decreases as a result of the altered structure of lipid bilayer close to ssDNA at liposome equator. Similar two fusion phases can be observed during fusion of the "big" vesicle with the flat membrane.

This delay of the lipid exchange resulted in the delay of vesicle fusion with the nuclear membrane, in our opinion, is a crucial factor at pore formation. Sharp delay of fusion

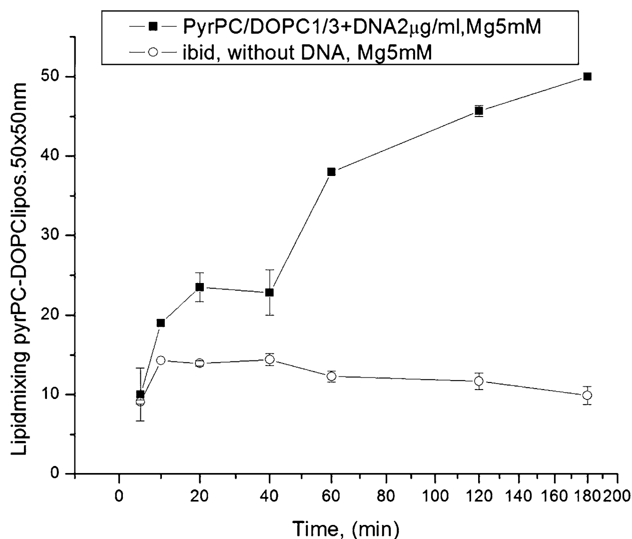

Fig. 3 Time course of lipid mixing of DOPC liposomes (50 nm diameter) studied with Pyrene-PC. DNA 2 μg/ml, $MgCl_2$ 5 mM, DOPC 1 mg/ml, Pyrene-PC 5 mol%

is the reason that the water which is inside the vesicle, will continue to flow into perinuclear space, carrying along unfused half of the vesicle (Fig. 2d). Moreover the water which is outside vesicle, carried along by the vesicle surface at sharp delay of surface movement will press on the unfused part of the vesicle and promote turn the rest half of the vesicle inside out. Taking into account a high speed of the first stage of fusion, water kinetic energy converted from energy of a surface tension of the fused part of the vesicle should be sufficient to turn the unfused part of the vesicle inside out and to contact it with an outer nuclear membrane. Suppose a surface tension of the nuclear membrane is equal to 1 dyn/s, and the vesicle radius is 30 nm, then the rate of the issue of water from the vesicle in perinucleare space is around 3×10^{-6} m/s. It means that the vesicle after extraversion will reach an outer nuclear membrane for $\sim$10 ms and fuse with it (Fig. 2f). Originally during fusion of the rest half of membrane vesicle with an outer nuclear membrane a small hole in diameter of 10–20 nanometers is formed in a fusion zone (Fig. 1e), according to physics of liposomes fusion process (Chizmadzhev et al. 2000). Such small holes (dimple as some authors report) are shown on the cytoplasm side of a nuclear membrane at the initial stage of a nuclear pore assembly (Kiseleva et al. 2001). Since no restrictions in the form of ssDNA exist for fusion of the inverted part of the vesicle with an outer membrane, ssDNA moves from an inner nuclear membrane to an outer one together with movement of lipids or locates in the middle of pore annulus (Fig. 1g). Here nucleoporins bind to ssDNA and with each other, forming native structure of a nuclear pore.

As the process described is purely physical, the possibility of turning a rubber cylindrical membrane inside out at its tension in water is demonstrated.

Hydrodynamic Model of Molecular Biological Processes

The device photo and graphic illustrations are represented in Fig. 4a. The device is placed in the box filled with tap water (partially represented in Figure). A cylinder used is from a plastic syringe. A rubber fingerstall used as a membrane has been covered on a plastic ring, 5 cm in diameter and 0.5 cm in depth . A truncated end of a syringe has been tightly pressed to a rubber surface so that when withdrawing the hub, the fingerstall surface is stretched along the cylinder (Fig. 4b). The hub is then removed, the surface of the fingerstall begins to contract under the action of rubber tension, pushing out water. When rubber surface reaches its initial level (Fig. 4c), the fingerstall continues to move toward outside a syringe (Fig. 4d). This is the result of an action of water kinetic energy converted from surface energy of rubber in a syringe. In our opinion, this system is an adequate approach to a situation with a membrane vesicle where the rubber tension is analog of vesicle surface tension.

In our reasoning, we easily pass from consideration of a process with liposomes to a situation with a nuclear membrane because, according to recent data, the surface tensions of a liposome lipid bilayer and a nuclear membrane are similar (Dahl et al. 2004; Chizmadzhev et al. 2000). Also, the diameter of liposomes used in our study is 50 nm (Kuvichkin et al. 2009), and the liposomes used by a number of authors for PC simulation of liposome fusion with lipid bilayer are of similar size (Chizmadzhev et al. 2000). Though intermembrane proteins—fusogenes—can modulate fusion processes, there are data that their influence is minimal (Ramos et al. 2009). Apparently, the basic role in the fusion course belongs to a surface tension of the lipid bilayer of nuclear membrane and membrane vesicles.

Why in the majority of preparations of pore complexes is ssDNA not seen? The answer is very simple: If it is difficult to reveal ssDNA on the liposome surface by cryo-TEM, then it is actually impossible to observe it in the surroundings of proteins and lipids in the structure of a nuclear pore. The only way to detect it is the use of any known method of electron microscopy and staining with rare-earth element (Tb^{3+}, La^{3+}) cations. The literature provides data that nuclear pores are well stained by lanthanum (Shaklai and Tavassoli 1982), which, like Tb^{3+}, has high affinity to ssDNA.

Some Special Cases

As the mechanism of nuclear pore assembly is still unclear and there are many variants in its elucidation, based on our

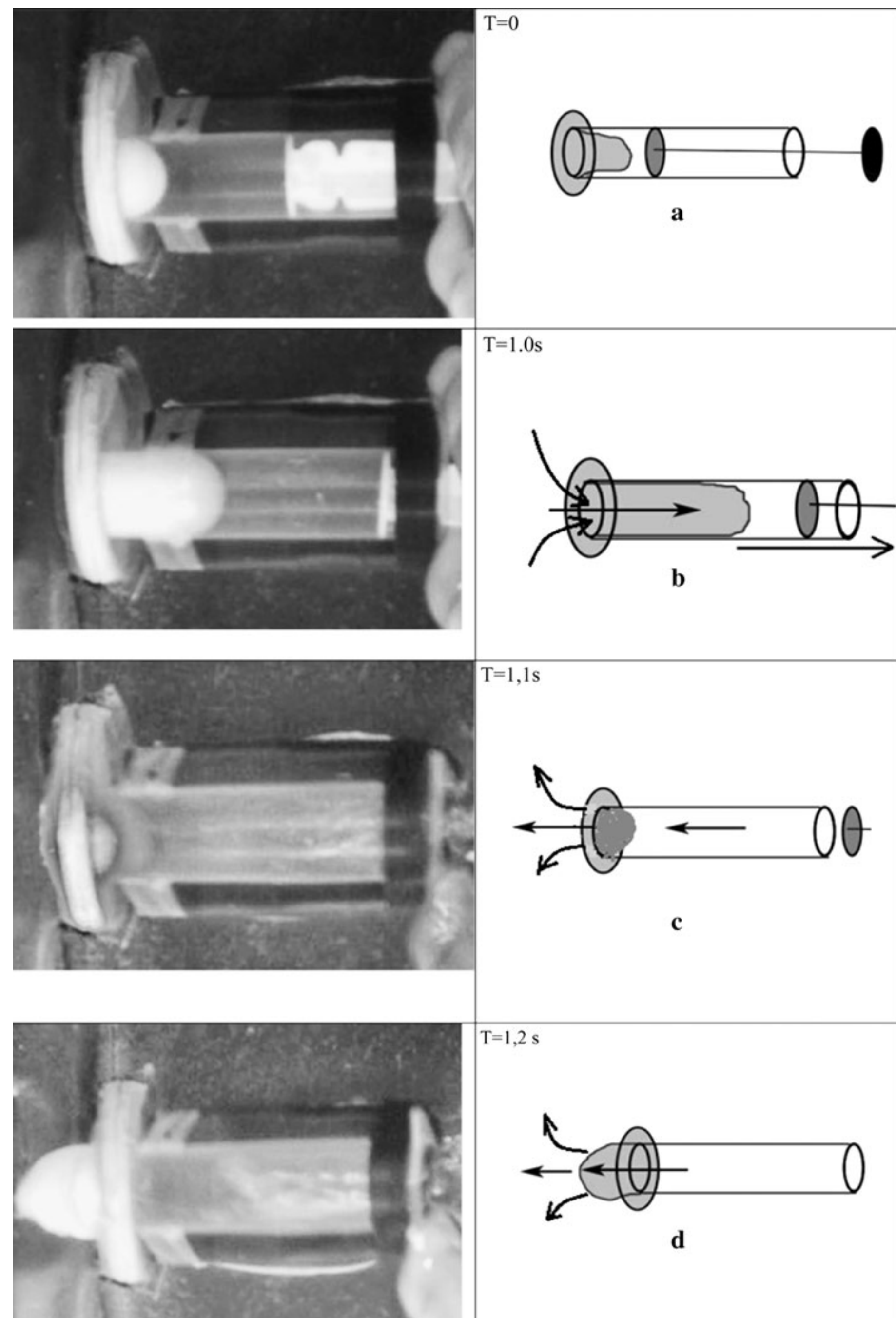

Fig. 4 Hydrodynamic model of membrane vesicle fusion with nuclear envelope: **a** zero position of rubber membrane; **b** maximum of membrane tension and beginning of rubber contraction; **c** fast passing by membrane of zero position; **d** turning inside out of membrane and its protrusion from syringe (arrowheads show the water stream moving)

model we will try to explain some well-known facts from the biology of a cell nucleus.

Linear Clusters of Nuclear Pores

According to our model, when more than one vesicle is closely located along one DNA strand forming a string-of-pearl structure, they are fused with a nuclear membrane. At fusion of this structure with a nuclear membrane, nuclear pore clusters arranged in a line are formed. In this case, DNA between nuclear pores is located in the perinuclear space (Fig. 1g). The fact that DNA crosses the lipid bilayer should not confuse us since in crossing places DNA can strongly change its structure, forming spaghetti-like complexes or similar structures with lipids, as in DNA–cationic amphiphile complexes (Liang et al. 2005; Hohner et al. 2006). In many samples obtained by freeze-fracture EM methods, where the outer nuclear membrane is absent, on

the surface of an inner nuclear membrane fibrils binding a pore annulus (Monneron et al. 1972) are visible. The linear arrangement of pore clusters is often observed in nuclei of various types of cells (Fiserova et al. 2009). Since the pore is a site of transcription initiation, in such DNA area transcription promoter-gene clusters are assumed to be present. Sequencing DNA (pore DNA), isolated from pore complexes in our study (unpublished data), could help us in the above-mentioned tasks.

Formation of Pores on Two Sides of a Nuclear Envelope

Fusion of “big” vesicles, belted by ssDNA, is possible with both inner and outer nuclear membranes as a consequence of “big” vesicle symmetry. The result will be identical with any way of vesicle fusion, but the kinetics of pore assembly may differ. The existing data that nuclear pore assembly occurs on both sides of a nuclear envelope (D’Angelo et al. 2006) support our hypothesis. Fusion of “big” vesicles with a nuclear membrane and formation of a pore complex hole occur quickly. The time required for “big” vesicle fusion is comparable to that necessary for liposome fusion (some microseconds). Binding of nucleoporins to ssDNA and formation of a mature nuclear pore last several minutes (Dultz et al. 2008).

“Anaphase” and “Interphase” Nuclear Pore

Some “big” vesicles, bound earlier to chromatin, are unable to fuse with an inner nuclear membrane because of weak decondensation of this chromatin fraction. As a result of DNA replication in S phase, decondensation of the same chromatin areas increases and vesicles bound to this chromatin fraction will be able to reach the nuclear membrane and create additional “interphase” pores. This can explain the second peak of nuclear pore formation in S phase (D’Angelo and Hetzer 2008). These pores may differ from “anaphase” pores in nucleoporin composition and in the dynamics of pore complex assembly. The reason could be depletion of nucleoporins stock in the nucleus or the use of nucleoporins in the construction of intranuclear annulate lamellae.

Intranuclear Annulate Lamellae

Some other “big” vesicles (Fig. 2c), bound to certain chromatin fractions, are unable to reach a nuclear envelope at any time of the cell cycle. But since they are bound to promoter DNA regions and belted by ssDNA, this can provide enhanced transcription activity of the chromatin sites having no contact with the nuclear envelope. Some nucleoporins can interact with ssDNA of such vesicles. A regulatory role in transcription is attributed to these nucleoporins (Mendjan et al. 2006; Capelson et al. 2010); nevertheless, their location inside the nucleus is determined by their affinity to ssDNA. If the fact that chromosomes contain very many lipids is taken into account (Albi and Magni 2004; Albi and Villani 2009; Struchkov et al. 2002), formation of intranuclear annulate lamellae on the surface of chromosomes at the interaction of “big” vesicles with chromosomal lipid clusters is possible.

Thus, we showed how the area where two nuclear membranes are fused can be formed and a pore can be created. The energy of the surface tension of nuclear membranes plays a crucial role in this process. The presence of an ssDNA rim around fusing vesicles is very important in the fusion process. It defines its contact with a nuclear membrane and fusion to it, leading to complete formation channel between two membranes. The average diameter of membrane vesicles is 70 nm; during fusion, they form “big” vesicles, 100 nm in diameter. Half of this diameter exceeds the distance between the outer and inner nuclear membranes. Even smaller vesicles can make it by invagination of parts of a nuclear membrane in perinuclear space at the expense of the same kinetic energy of water.

References

Albi E, Magni VMP (2004) The role of intranuclear lipids. Biol Cell 96:657–667

Albi E, Villani M (2009) Nuclear lipid microdomains regulate cell function. Commun Integr Biol 2:23–24

Capelson M, Liang Y, Schulte R, Mair W, Wagner U, Hetzer MW (2010) Chromatin-bound nuclear pore components regulate gene expression in higher eukaryotes. Cell 140:372–383

Chelobanov BP, Laktionov PP, Kharkova MV, Rykova EY, Pyshnyi DV, Pyshnaya IA, Marcus K, Meyer HE, Vlassov VV (2003) Interaction of keratin K1 with nucleic acids on the cell surface. Biochemistry (Moscow) 68:1239–1246

Chizmadzhev YA, Kuzmin PI, Kumenko DA, Zimmerberg J, Cohen FS (2000) Dynamics of fusion pores connecting membranes of different tensions. Biophys J 78:2241–2256

Collas P, Poccia D (1996) Distinct egg membrane vesicles differing in binding and fusion properties contribute to sea urchin male pronuclear envelopes formed in vitro. J Cell Sci 109:1275–1283

D’Angelo MA, Hetzer MW (2008) Structure, dynamics and function of nuclear pore complexes. Trends Cell Biol 18:456–466

D’Angelo MA, Anderson DJ, Richard E, Hetzer MW (2006) Nuclear pores form de novo from both sides of the nuclear envelope. Science 312:440–443

Dahl KN, Kahn SM, Wilson KL, Discher DE (2004) The nuclear envelope lamina network has elasticity and a compressibility limit suggestive of a molecular shock absorber. J Cell Sci 117:4779–4786

Danev R, Nagayama K (2001) Transmission electron microscopy with Zernike phase plate. Ultramicroscopy 88:243–252

Dultz E, Zanin E, Wurzenberger C, Braun M, Rabut G, Sironi L, Ellenberg J (2008) Systematic kinetic analysis of mitotic dis- and reassembly of the nuclear pore in living cells. J Cell Biol 180:857–865

Fiserova J, Kiseleva E, Goldberg MW (2009) The nuclear envelope and nuclear pore complex structure and organization in tobacco BY-2 cells. Plant J 59:243–255

Frisken BJ, Asman C, Patty PJ (2000) Studies of vesicle extrusion. Langmuir 16:928–933

Hetzer MW (2010) The nuclear envelope. Cold Spring Harb Perspect Biol 2:a000539

Hohner A, Bayer J, Radler JO (2006) Wormlike lipid/DNA micelles in a non-polar solvent. Eur Phys J E Soft Mater 21:41–48

Kiseleva E, Rutherford S, Cotter LM, Allen TD, Goldberg MW (2001) Steps of nuclear pore complex disassembly and reassembly during mitosis in early *Drosophila* embryos. J Cell Sci 114:3607–3618

Kiseleva E, Drummond SP, Goldberg MW, Rutherford SA, Allen TD, Wilson KL (2004) Actin- and protein-4.1-containing filaments link nuclear pore complexes to subnuclear organelles in *Xenopus* oocyte nuclei. J Cell Sci 117:2481–2490

Kuvichkin VV (2002) Lipid–nucleic acids interactions in vitro and in vivo. Bioelectrochemistry 58:3–12

Kuvichkin VV (2009) Investigation of ternary complexes: DNA–phosphatidylcholine liposomes–Mg^{2+} by freeze-fracture method and their role in the formation of some cell structures. J Membr Biol 231:29–34

Kuvichkin VV, Uteshev VK (2003) The role of lipid–nucleic acids interactions in nuclear envelope and pore complex assembly. Biophysics 48:236–239

Kuvichkin VV, Danev R, Shigematsu H, Nagayama K (2009) DNA-induced aggregation and fusion of phosphatidylcholine liposomes in the presence of multivalent cations observed by the cryo-TEM technique. J Membr Biol 227:95–103

Liang H, Harries D, Wong GCL (2005) Polymorphism of DNA-anionic liposome complexes reveals hierarchy of ion-mediated interactions. Proc Natl Acad Sci USA 102:11173–11178

Mendjan S, Taipale M, Kind J, Holz H, Gebhardt P, Schelder M, Vermeulen M, Buscaino A, Duncan K, Mueller J, Wilm M, Stunnenberg HG, Saumweber H, Akhtar A (2006) Nuclear pore components are involved in the transcriptional regulation of dosage compensation in *Drosophila*. Mol Cell 21: 811–823

Monneron A, Blobel G, Palade GE (1972) Fractionation of the nucleus by divalent cations. Isolation of nuclear membranes. J Cell Biol 55:104–125

Plum GE, Pilch DS, Singleton SF, Breslauer KJ (1995) Nucleic acid hybridization: triplex stability and energetic. Annu Rev Biophys Biomol Struct 24:319–350

Ramos C, Rafikova ER, Melikov K, Chernomordik LV (2009) Transmembrane proteins are not required for early stages of nuclear envelope assembly. J Biol Chem 284:29847–29859

Riley DE, Keller JM (1978) The ultrastructure of non-membranous nuclear ghosts. J Cell Sci 32:249–268

Salpingidou G, Rzepecki R, Kiseleva E, Lyon C, Lane B, Fusiek K, Golebiewska A, Drummond S, Allen T, Ellis JA, Smythe C, Goldberg MW, Hutchison CJ (2008) NEP-A and NEP-B both contribute to nuclear pore formation in *Xenopus* eggs and oocytes. J Cell Sci 121:706–716

Schmutz M, Durand D, Debin A, Palvadeau Y, Etienne A, Thierry AR (1999) DNA packing in stable lipid complexes designed for gene transfer imitates DNA compaction in bacteriophage. Proc Natl Acad Sci USA 96:1293–1298

Shaklai M, Tavassoli M (1982) Preferential localization of lanthanum to nuclear pore complexes. J Ultrastruct Res 81:139–144

Struchkov VA, Strazhevskaya NB, Zhdanov RI (2002) DNA-bound lipids of normal and tumor cells: retrospective and outlooks for functional genomics. Bioelectrochemistry 58:23–30

Uhrikova D, Hanulova M, Funari SS, Khusainova RS, Serxen F, Balgavy P (2005) The structure of DNA–DOPC aggregates formed in presence of calcium and magnesium ions: a small angle synchrotron X-ray diffraction study. Biochim Biophys Acta 1713:15–28

Vigers GPA, Lohka MJA (1991) Distinct vesicle population targets membranes and pore complexes to the nuclear envelope in *Xenopus* eggs. J Cell Biol 112:545–556

Zahm JM, Debordeaux C, Maurer C, Hubert D, Dusser D, Bonnet N, Lazarus RA, Puchelle E (2001) Improved activity of an actin-resistant DNase I variant on the cystic fibrosis airway secretions. Am J Respir Crit Care Med 163:1153–1157

Zhanga B, Chena Y, Hana Z, Risb H, Zhaia Z (1998) The role of keratin laments during nuclear envelope reassembly in *Xenopus* egg extracts. FEBS Lett 428:52–56