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Isolation of Chromatin DNA Tightly Bound to the Nuclear Envelope of HeLa Cells

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Abstract Recent discovery of the role of nuclear pores in transcription, predicted by our early DNA-membrane complex (DMC) model, makes membrane-bound DNA (MBD) isolation from the cell nucleus and analysis of the MBD actual. The method of MBD isolation proposed by us retains DMC integrity during isolation. We used HeLa cells for DMC extraction. Changing the ionic composition of the isolation medium and replacing DNase I, used commonly for chromatin destruction, with a set of restriction enzymes allowed us to isolate the MBD. Treatment of a nuclear membrane with proteinase K and ultrasound has been used to increase the yield of MBD. Electron microscopic analysis of the purified fraction of isolated DMC supports our previous model of nuclear envelope lipid–chromatin interaction in the nuclear pore assembly.

Keywords Lipid–protein interaction · Membrane assembly · Membrane biophysics · Membrane fusion

Introduction

The question about chromatin DNA binding with the nuclear envelope (NE) has been discussed for more than 40 years (Moyer 1980). Comings (1968) suggested that DNA attaches to the nuclear membrane at the site of pore complexes. Later investigations confirmed that DNA binds with a nuclear pore (Riley and Keller 1978; Arlucea et al. 1998; Ishii et al. 2002) and that nuclear pores participate in transcriptional activity (Akhtar and Gasser 2007; Capelson et al. 2010; Van de Vosse et al. 2011; Mendjan et al. 2006). Nevertheless, many details of DNA interaction with the NE remain unclear.

In the past 40 years, attempts to isolate the DNA-membrane complex (DMC) from eukaryotic cells have been undertaken. The DMC fraction obtained contained all nascent DNA. As a result, it was concluded that DNA replication initiation also occurs at sites of DNA contact with a membrane (Crabb et al. 1980; Leno 1992; Infante et al. 1976; Sinha and Mizuno 1977; Kaufman et al. 1983).

However, many methods used for DMC isolation had an essential fault, namely, the possibility of artifact attachment of total-genome DNA to the DMC. Isolation from the cells of the nuclear matrix fraction (NM) has shifted the attention of researchers to a search for points of contact of chromatin DNA with NM. Later, the existence of NM in the nucleus was questioned (Cook 1988).

Several authors continued to try to isolate DMC; but no differences between DNA from this fraction and total DNA were revealed, or these differences were insignificant (Dvorkin et al. 1977; Prusov et al. 1980, 1982). Shabarina et al. (2006) found a unique DNA sequence in DMC, but in our opinion the method used for the isolation of this sequence is not applicable to membrane-bound DNA (MBD) isolation.

We have made one other attempt to isolate DMC from HeLa cells based on current data on structure and the possible functions of DNA–lipid interactions (Manzoli et al. 1974; Shabarshina et al. 1979; Sukhorukov et al. 1980; Kuvichkin and Sukhomudrenko 1987; Kuvichhkin 2002, 1983, 2010, 2011; Kuvichkin et al. 1999).

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According to the biophysical data available, the DMC model (a nuclear pore) has been proposed based on direct interactions between DNA and zwitterionic lipids in the presence of bivalent metal cations (Kuvichhkin 2002, 2009, 2010, 2011).

Four Principles of DMC Isolation

It should be noted that factors that destroy ternary complexes: DNA–phosphatidylcholine liposomes–Mg^{2+} (Kuvichkin and Sukhomudrenko 1987) also influence the stability and structure of the NE. Agutter (1972) showed the necessity of a small percent of DNA to the structural integrity of the NE. The presence of a minimal amount of DNA in a nuclear membrane protects the NE from disintegration into membrane vesicles, maintaining a constant number of nuclear pores (Agutter 1972).

The main conditions necessary for NE isolation, at which DNA–lipid interactions and "membrane" DNA are kept, have been suggested as follows:

1. The absence of large concentrations of univalent cations (K^+ ≤0.1 M, Na^+ ≤0.2 M)
2. The presence of at least insignificant concentrations of bivalent metal cations (≥1 mM Ca^{2+}, Mg^{2+}) and the complete lack of chelating agents (EDTA, etc.)
3. The absence of enzymes able to destroy single-stranded DNA
4. The absence of ionic or non-ionic detergents that are capable of destroying the lipid bilayer; accordingly, DNA–lipid interactions are also desirable

Having analyzed methods for isolating MBD and the NE from the cell nucleus (Prusov et al. 1980, 1982; Shabarina et al. 2006; Matunis 2006), we came to the conclusion that in almost all techniques most of the conditions mentioned above were not taken into consideration. DNase I was used as the basic enzyme for destruction of chromatin DNA. DNase I is known to destroy double-stranded DNA as well as single-stranded DNA, resulting in detachment of MBD from the NE. In many techniques, EDTA, high NaCl and KCl concentrations and detergents were also used. None of these researchers discussed the maintenance of DNA–lipid interactions during the isolation procedure. As a result, the DNA had already detached from the membrane at the stage of NE isolation, and the nuclear membrane was composed only of proteins of lamina and intramembrane proteins. Nucleoporins, linked to each other and to nuclear membranes by protein–protein and protein–lipid interactions, likely retain the shape of DNA-free nuclear pores. According to our DMC model (Kuvichkin 1983), DNA is an important structural element of the nuclear pore and participates in both transcription and replication. We assume that the DMC structure is more complex and includes DNA–RNA hybrids and single-stranded DNA arising at the unwinding of the triple-stranded DNA–RNA hybrid (Fig. 1) (Kuvichhkin 2002, 2010, 2011).

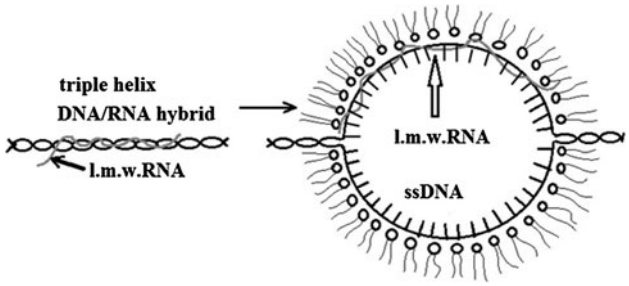

Fig. 1 Model DMC (nuclear pore complex). DNA–RNA hybrids located on chromatin fuse two membrane vesicles and form nuclear pores at the intersection of fused vesicles with the NE. The triple-stranded hybrid of DNA—l.m.w. RNA untwists at DMC formation up to hybrid DNA–RNA and single-stranded DNA (Kuvichkin 2010)

With the aim of isolating MBD, the new isolation method must be in agreement with the four principles stated above. It is rather easy to lower the concentration of univalent cations, add a little Mg^{2+} and not use EDTA and detergents. What can be substituted for DNase I, if all known DNases that are able to destroy double-stranded DNA digest single-stranded DNA in a nonspecific manner too? Therefore, we had to use a specific enzyme in this work—to be more precise, a set of four restriction enzymes, typically used in genetic engineering. Four enzymes digested double-stranded DNA as effectively as DNase I did and did not disturb single-stranded DNA. As a result, we first isolated the fragments of the MBD-containing NE from the nucleus of HeLa cells and then separately isolated the MBD.

Methods and Results

Reagents

Calf thymus DNA, deoxyribonuclease I from bovine pancreas, ribonuclease H (RNase H) from *Escherichia coli* H 560 pol A1, proteinase K from *Tritirachium album*, protease inhibitor cocktail, HEPES, sucrose (SigmaUltra), Triton X-100, DL-dithiothreitol, chloroform and magnesium chloride hexahydrate were from Sigma-Aldrich (St. Louis, MO). Restriction enzymes *Bam*HI, *Nco*I, *Nhe*I and *Pst*I were from New England Biolabs (Hitchin, UK).

Equipment

We used the 5402 Eppendorf (Hamburg, Germany) Vacufuge Concentrator and the Thermo Scientific (Waltham, MA) Sorvall ST 40. Equipment for agarose gel electrophoresis included a Midi horizontal electrophoresis unit

(Sigma-Aldrich, St. Louis, MO), the Micro-Volume UV-Vis Spectrophotometer NanoDrop 2000 (NanoDrop, Wilmington, DE) and ultrasonic disintegrator Dailymag Magnetic Technology (Ningbo, Zhejiang, China). We also used the CKX31 compact inverted microscope (Olympus, Tokyo, Japan), the FEI (Hillsboro, OR) Tecnai T12 and the JEOL (Tokyo, Japan) JEM 1220 transmission electron microscopes as well as the JEOL JSM-5200 scanning electron microscope (JEOL, Tokyo, Japan).

MBD Isolation

The isolation of MBD consists of three stages: (1) isolation of nuclei from HeLa cells, (2) isolation of DMC from nuclei and (3) isolation of MBD from DMC. Nuclei were isolated from HeLa cells by the technique developed by the Collas lab (Department of Biochemistry, Institute of Basic Medical Sciences, University of Oslo, Norway; http://www.collaslab.com/UserFiles/File/Isolation%20of%20somatic%20cell%20nuclei.pdf). The isolation procedure was controlled by means of an inverted microscope for cells in culture. Isolated nuclei were frozen in the presence of 50 % glycerol and stored at −80 °C. The DMC was isolated according to a considerably modified version of the procedure of NE isolation using animal cells. First, DNase I was replaced by a set of four restriction enzymes. This set may include different restriction enzymes, provided that each of the enzymes works well in the buffer used for isolation, maintaining at least 75 % of its maximal activity. The buffer for restriction enzymes (NEB 2) also was selected to conform with the four principles set forth above. Thus, the procedure for NE isolation from HeLa cells was as follows.

Preparation of NEs from Somatic Nuclei

This protocol was adapted from the procedure developed by Dwyer and Blobel (1976). This procedure entails only one digestion step. "Classical" protocols include two digestion steps.

Working Solutions

Prepare the following solutions before starting isolation or washing nuclei. Alternatively, thaw these solutions outside of the freezer. All solutions should contain 1 mM PMSF (10 µl/ml solution) and 1 mM DTT (1 µl/ml solution), added to an aliquot just prior to use. Solutions should be kept on ice at all times.

1. TKM buffer (pH 7.9) at 25 °C
2. Tris–HCl (pH 7.5, 2.5 ml of 1 M stock), KCl 25 mM (0.4 ml of 3 M stock), $MgCl_2$ 5 mM (0.25 ml of 1 M stock), H_2O 47 ml

Table 1 Comparison of two types of digestion solutions

Collas lab solution (50 ml)	NeB buf2
10 % sucrose, 7.8 ml of 2 M stock	50 mM NaCl
20 mM Tris (pH 7.5), 1 ml 1 M stock Tris–HCl (pH 7.5)	10 mM Tris–HCl
H_2O 41.2 ml	10 mM $MgCl_2$
1 mM DTT, PMSF	1 mM DTT
pH 7.5	pH 7.9 at 25 °C

3. $MgCl_2$ solution: 1 mM (10 µl of 1 M $MgCl_2$ stock into 10 ml H_2O)
4. Digestion solution (50 ml): NeB buffer 2 (right row in Table 1) instead of the Collas lab digestion solution (left row)
5. Restriction enzyme mixture prepared by mixing 10 µl of each enzyme in the next step
6. *Bam*HI, *Eco*RI, *Nco*I and *Nhe*I, keeping 100 % enzyme activity, and *Pst*I, 75 %, in NEB 2 buffer
7. Sucrose cushion (50 ml): 25 % sucrose, 19.5 ml of 2 M stock; 20 mM Tris (pH 7.5), 1 ml of 1 M stock; H_2O 29.5 ml

Procedure

- Start with 1–2 ml of packed purified nuclei.
- Wash nuclei in 5 ml of TKM buffer at 1,000 rpm (200×*g*), 10 min, 4 °C.
- Decant supernatant.
- While vortexing, add 4 ml of 1 mM $MgCl_2$ solution. It is important to vortex or else nuclei cannot be resuspended.
- Add 12 ml of digestion solution (NEB 2) and mix by inversion.
- Add 10 µl of each of restriction enzyme and mix well by inversion at room temperature.
- Incubate for 20 min at room temperature, then make 50 strokes with a glass Dounce pestle B in a sterile environment. The nuclear suspension becomes very viscous at first but clarifies over time as the DNA is digested. Repeat these steps two to three times, if necessary, until the solution turns clear. After this step, cool samples on ice and work subsequently at +4 °C.
- Transfer the solution into two 10-ml clean plastic centrifuge tubes (2 × 6 ml).
- Underlay solution with a 3-ml sucrose cushion.
- Centrifuge at 10,000×*g* in a Sorvall or Beckman centrifuge for 15 min. The NE pellet will be concentrated at the bottom of the tube.
- Remove the supernatant, wash the walls of the tube and remove all supernatant.
- Resuspend the NE pellet in 500 µl of TKM buffer.
- Keep on ice until use or freeze in TKM buffer.

For MBD isolation from the NE, it is possible to use standard techniques, but we conducted several experiments to increase the yield of DNA and DNA purity on the basis of our DMC model and interphase chromosome structure.

Model of Interphase Chromatin Organization in the Nucleus

To formulate a research program and understand the results, it is necessary to choose one of two possible ways to characterize DNA interaction with the nuclear membrane: the standard mode, in which the interaction occurs by means of proteins, or the mode developed by this study's author, in which DNA directly interacts with membrane lipids as the DNA simultaneously unwinds. The latter does not exclude but assumes that MBD interacts with nucleoporins. Since we work with cells but not with DNA in vitro, we suppose that not double-stranded DNA but rather the triple-stranded hybrid of DNA and low-molecular weight RNA interacts with the nuclear membrane (Fig. 1).

The temperature of this transition is considerably lower than the temperature of DNA melting, which results in the preferential attachment of triple-stranded hybrids to the NE. Acceptance of this precondition allows us to formulate a research program and to explain the results obtained. We would propose the real arrangement of chromatin DNA in an interphase nucleus as represented in Fig. 2.

Here, DNA is attached to nuclear pores not as a double helix but in the form of hybrid DNA–RNA and single-stranded DNA located on the periphery of a nuclear pore annulus. As can be seen, several nuclear pores close located on one DNA chain form linear clusters (the long black arrows).

Nucleases as a Tool for Study of MBD and Interphase Chromatin Structure in the Nucleus

Based on this structure of the cellular nucleus and with a set of restriction enzymes instead of DNase I during DMC isolation, it is possible to isolate a real MBD and "tails" of genome DNA not removed by restriction enzymes. After isolation of a fraction of the NE with DMC, the following nucleases were added to equal volumes of the fraction: S1-endonuclease, RNase H, RNase H + nuclease S1 and DNase I. After reaction with the enzymes for 20 min (in the buffer recommended by the nuclease manufacturer), MBD was isolated from each fraction and then analyzed by horizontal agarose electrophoresis (Fig. 3).

Figure 3 shows that the molecular weight of DNA isolated from DMC is abnormally large, about 12 kDa, instead of the expected 500–1,000 Da. We can assume that the rest of the genome DNA, which is present at the ends of "membrane" DNA after genome treatment by restriction enzymes, is too long. Nevertheless, the nuclease action on "membrane" DNA needs to be explained. S1 nuclease (lane 2) and DNase I (lane 5) destroy MBD completely. RNase H (lane 3) also strongly disturbs MBD. At the same time that DMC is treated with RNase H and nuclease S1 sequentially, a portion of "membrane" DNA remains undamaged. DNase I was observed by completely removing DNA from the nuclear membrane. Therefore, when techniques for NE isolation are used for DNase I treatment, nuclear membranes can be isolated but without MBD. The fact that the effect of nuclease S1 is similar shows that in the area of DNA attachment to the NE, sites where the double helix opens exist. These sites keep DNA in contact with the nuclear membrane.

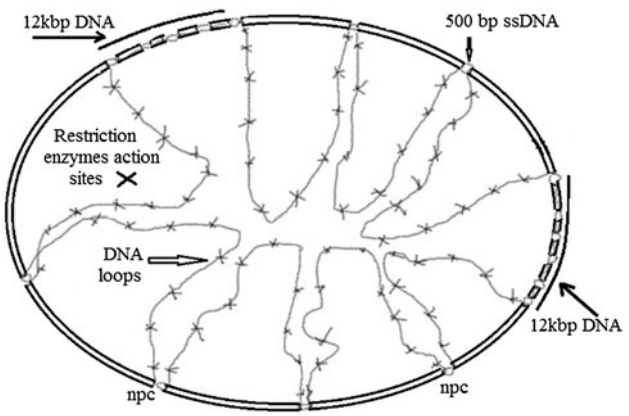

Fig. 2 DNA structure in an interphase nucleus. DNA is attached to the nuclear pores, forming big intranuclear loops of DNA (*white arrow*). In addition to single pores (*npc*) there exist pore clusters 12 kbp in length (*long black arrows*). *Crosses* indicate where genome DNA is cut by restriction enzyme

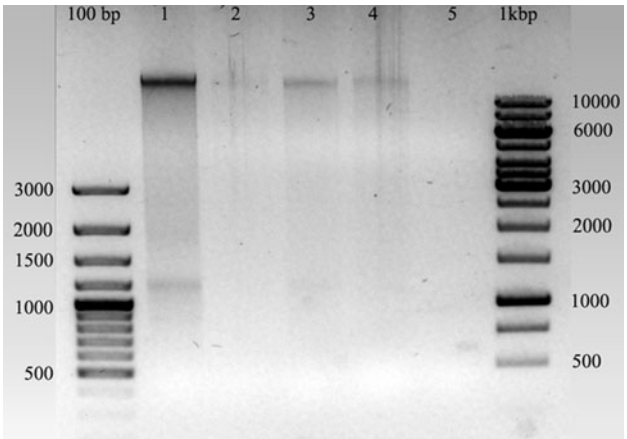

Fig. 3 Electrophoresis of MBD after treatment of NE by nucleases: *lane 1* NE without nucleases, *lane 2* NE treated by nuclease S1, *lane 3* NE after treatment with RNase H, *lane 4* NE treated RNase H + nuclease S1, *lane 5* NE treatment with DNase I

The ability of RNase H to destroy DMC is observed due to the presence of the DNA–RNA hybrid in the area where DNA is attached to the nuclear membrane. Destruction of this hybrid can lead to recovery of the DNA double helix and its detachment from a membrane (Fig. 1). Partial recovery of the DNA double helix with the action of RNase H in combination with nuclease S1 may result in a weakened destructive effect of nuclease S1 on DMC (lane 5).

Thus, it is possible to conclude that double-stranded DNA or the DNA–RNA hybrid easily detaches from DMC. Apparently, double-stranded DNA after replication also detaches from nuclear pores, although pores keep their shapes because of interaction between nucleoporins. Pore complexes lose any matrix activity and keep only their transport functions. Pore complexes without DNA are most likely unstable and quickly disassembled. This assumption is supported by their full disappearance from the NE in the beginning of mitosis (Chatel and Fahrenkrog 2011).

MBD and Linear Pore Clusters

The above-mentioned data on the structure and sensitivity of DMC to nucleases give us the basis to consider our DMC model to be true (Figs. 1, 2), taking into account the following remarks. We assume that usually DNA is attached to a membrane at one site (single nuclear pore), with the formation of big loops of DNA between these pores. In rare instances linear pore clusters exist (Fig. 2, long black arrows). These clusters are formed when pores are too closely located to each other along one DNA thread. In this case, the DNA segment that connects these pores is so short that it is located in the perinuclear space (Fig. 2).

The occurrence of such pore clusters can be explained by the presence of highly repeated sequences in DNA that have enhanced affinity to the membrane vesicles from which the NE is formed. The DNA-induced fusion membrane of these vesicles leads to formation of "string of pearls"—type structures on a DNA thread (Shaulov and Harel 2012) and then nuclear pore clusters, according to the model described earlier (Kuvichkin 2011). Electron microscopic data about the existence of linear pore clusters at the NE are available (Fiserova et al. 2009).

Provided that the size of the DNA site connected with one pore is 400–500 bp and the length of DNA between pores in the cluster is the same, to obtain a DNA cluster size around 10 kbp, the average number of pores in the cluster should be equal to 10, as observed in previous experiments (Fiserova et al. 2009).

Our technique is also used while isolating "membrane" DNA from a single pore, but it is accompanied by big losses. If the fragment of the membrane connected with a pore is insignificant, because of insignificant density, it can be lost at the last stage of isolation through a sucrose gradient. Therefore, the band of 10 kbp observed in our experiments is connected with a large fragment of an NE and belongs to nuclear pore clusters where interpore DNA sites are located in the perinuclear space. Our task is to find a way of isolating the "membrane" or "pore" DNA from these clusters. For this purpose, we studied how to increase the yield of cluster DNA by treatment of DMC with various enzymes and ultrasound for MBD isolation.

Furthermore, we compared the isolation of cluster and "membrane" DNA, in which phenol and a mix of chloroform-isoamyl alcohol (24:1) were used as extracting substances at the last stage of isolation. The DMC fraction was exposed to ultrasound or treated with proteinase K or a combination of these two factors (Fig. 4).

As seen in Fig. 4, phenol or chloroform cannot extract DNA from membrane fractions (lanes 1, 2). Ultrasound also fails to detach DNA from DMC (lanes 5, 6). However, proteinase K and subsequent treatment with phenol or chloroform greatly increased the yield of cluster DNA (lanes 7, 8). Proteinase K action and the subsequent ultrasound led to the appearance of a fraction of MBD 500 bp in length. This was most likely a consequence of destruction of the cluster DNA band (10–12 kbp), the residues of which are visible in Fig. 4 (lanes 3, 4).

Electron Microscopic View of the NE with DMC

We tried to observe DMC isolated with our method by electronic microscopy (a method of negative contrast). In Fig. 5, it is possible to see nuclear pores in the DMC fraction, which still have a hole; however, annuli and edges of

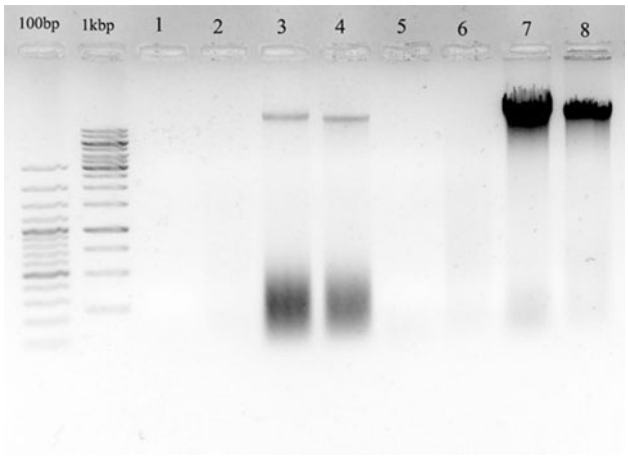

Fig. 4 Effect of NE treatment with chloroform and phenol. *Lanes 1* extraction by chloroform, *2* extraction by phenol, *3* proteinase K + ultrasound 5 min (extraction chloroform), *4* same as lane 3 with phenol extraction, *5* ultrasound 5 min with chloroform extraction, *6* same as lane 5 with phenol extraction, *7* proteinase K with chloroform extraction, *8* same as lane 7 with phenol extraction. The 100-bp and 1-kbp DNA ladders are the same as in Fig. 3

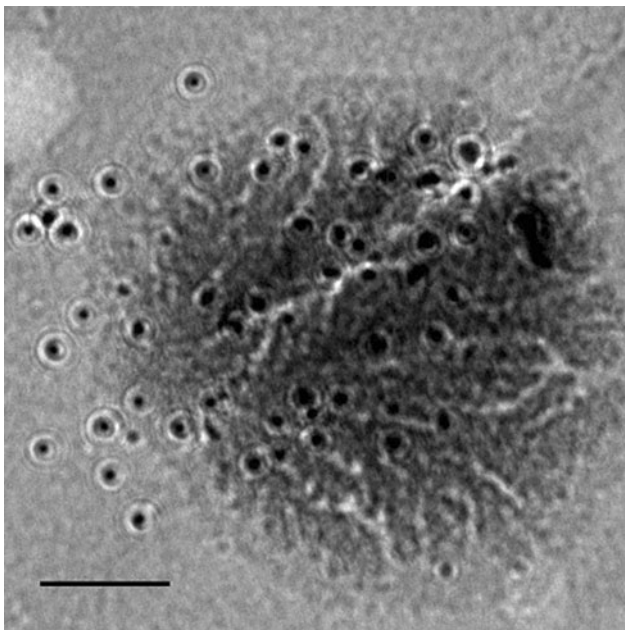

Fig. 5 Nuclear pore structure after NE isolation with restriction enzymes and 30 min proteinase K treatment. *Bar* = 50 nm

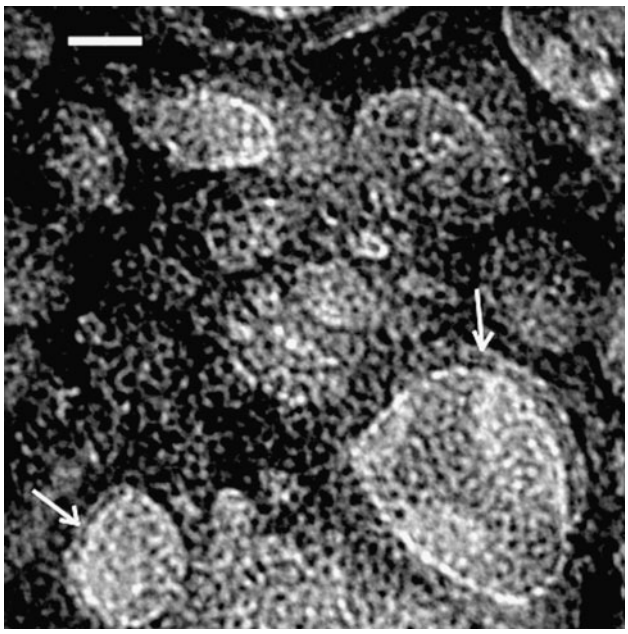

Fig. 6 NE fragments isolated with restriction nuclease enzymes and proteinase K (*arrows* show the double membrane of NE). *Bar* = 50 nm

pores are smooth. Octagonal symmetry and other structural peculiarities of pore complexes were not observed. Apparently, this is the result of proteinase K action. Annuli of pores are connected to each other by the threads of DNA that branch from the annuli in opposite directions.

The elements of a double nuclear membrane and MBD connected with it are more clearly shown in Fig. 6. In Fig. 6, we can see the inner and outer nuclear membranes (white arrows). The density of a nuclear pore on fragments of the NE is very high, which confirms that DMC remains in the course of isolation of the nuclear membranes by our method. At the same time, several areas were observed where the NE split into two membranes. The increased fragment of the NE with sharply visible MBD is shown in Fig. 7.

Based on the thickness of fibrils in Figs. 6, 7, the DNA is presented in a complex with lipids and proteins partially destroyed by proteinase K. Numerous zones of the DNA–RNA hybrids opening (R-loops) in the area of nuclear pores are observed (arrows).

Scanning electron microscopic data showed high packaging of nuclear pores in our samples of NE prepared after ultrasonic and proteinase K treatment of NE (Fig. 8).

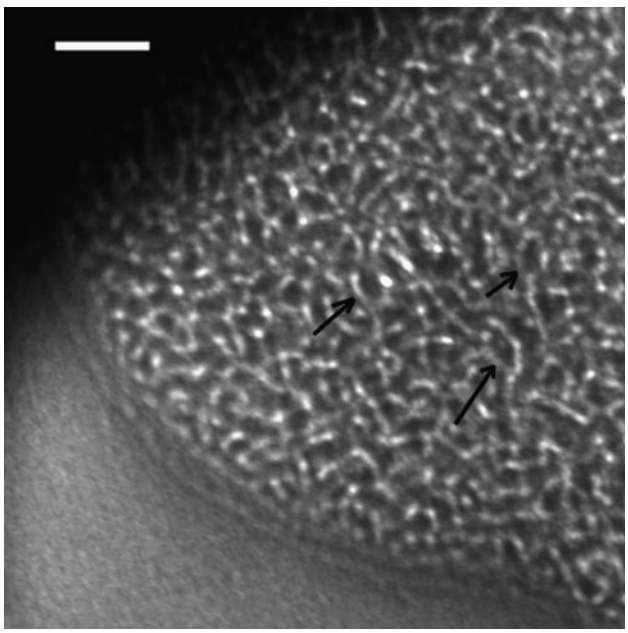

Fig. 7 NE fragment with two nuclear membranes (*arrows*) and MBD. *Bar* = 50 nm

MBD Extraction

Thus, the final stage of MBD isolation, taking into account the investigations performed, is as follows. The DNA extraction protocol utilized the fresh, native NE containing membrane DNA. Preparation was started with 50–500 µl of an NE sample.

We added 1–10 µl of proteinase K (10 mg/ml) to 50–500 µl of NE, held for 30 min on ice, then sonicated for 5 min at 0 °C. DNA was extracted with an equal volume of chloroform/isoamyl alcohol (Sigma, 24:1) and centrifuged for 15 min at 12,800×*g* (eppendorf centrifuge) at 4 °C. The aqueous phase was transferred into a new eppendorf

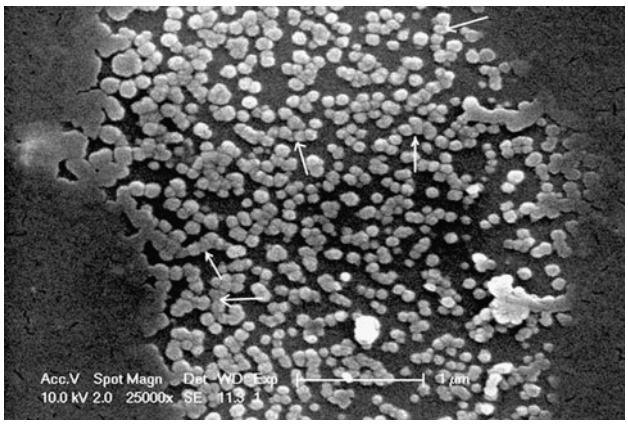

Fig. 8 Scanning electron microscopic image of NE fragments isolated with restriction nuclease enzymes and proteinase K (*arrows* show probable nuclear pore clusters). *Bar* = 1 µm

tube and extracted repeatedly with chloroform/isoamyl alcohol (24:1).

The clear supernatant was concentrated to two times less volume in a vacuum concentrator. During this procedure, most of the chloroform-isoamyl alcohol removed from DNA samples. DNA concentrations were measured by UV spectroscopy (NanoDrop), and their concentrations were 20–30 ng/µl (optimal for agarose electrophoresis).

Conclusion

A variety of factors—DNase I, high ionic force, the presence of EDTA, detergents, etc.—can destroy a complex of DNA with lipids. Based on our DMC model (Fig. 1), we selected the conditions that protect MBD and the DNA complex with membrane lipids from destruction in the course of isolation.

The isolation techniques of nucleoid (Risley et al. 1986) and nuclear matrix (Nickerson 2001) should be reconsidered because none of the authors who described such techniques for the NE (Agutter 1972; Rout and Blobel 1993; Matunis 2006) or MBD (Prusov et al. 1980, 1982; Shabarina et al. 2006) took lipid–nucleic acid interactions into account.

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References

Agutter PS (1972) The isolation of the envelopes of rat liver nuclei. Biochim Biophys Acta 255:397–401

Akhtar A, Gasser SM (2007) The nuclear envelope and transcriptional control. Nat Rev Genet 8:507–517

Arlucea J, Andrade R, Alonso R, Arachaga J (1998) The nuclear basket of the nuclear pore complex is part of a higher-order filamentous network that is related to chromatin. J Struct Biol 124:51–58

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

Chatel G, Fahrenkrog B (2011) Nucleoporins: leaving the nuclear pore complex for a successful mitosis. Cell Signal 23:1555–1562

Comings D (1968) The rationale for an ordered arrangement of chromatin in the interphase nucleus. Am J Hum Genet 20:440–460

Cook R (1988) The nucleoskeleton: artifact, passive framework or active site? J Cell Sci 90(Pt 1):1–6

Crabb WD, Firshein W, Infante AA (1980) A potential DNA replication complex isolated from sea urchin embryos by renografin gradient centrifugation. Biochim Biophys Acta 609:456–463

Dvorkin VM, Prusov AN, Fais D, Kir'ianov GI, Vaniushin BF (1977) DNA from chromatin-nuclear membrane complex: reassociation kinetics at different stages of the cell cycle (in Russian). Biokhimiia 42:74–78

Dwyer N, Blobel G (1976) A modified procedure for the isolation of a pore complex-lamina fraction from rat liver nuclei. J Cell Biol 70:581–591

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

Infante AA, Firshein W, Hobart P, Murray L (1976) A nuclear membrane-associated DNA complex in cultured mammalian cells capable of synthesizing DNA in vitro. Biochemistry 15:4810–4817

Ishii K, Arib G, Lin C, Van Houwe G, Laemmli UK (2002) Chromatin boundaries in budding yeast: the nuclear pore connection. Cell 109:551–556

Kaufman DG, Cordeiro-Stone M, Rude TH, Nelson KG, Kaufmann WK (1983) Fractionation and characterization of DNA at sites of replication from rat liver nuclei. Exp Cell Res 146:339–347

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

Kuvichkin VV (1983) Theoretical model of DNA-membrane contacts. Biofizika 28:771–775

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. Membrane Biol 231:29–34

Kuvichkin VV (2010) Lipid–nucleic acids interactions as base for organization and expression of cellular genome. Int J Quant Chem 110:120–126

Kuvichkin VV (2011) The mechanism of a nuclear pore assembly: a molecular biophysics view. J Membr Biol 241:109–116

Kuvichkin VV, Sukhomudrenko AG (1987) Interaction of natural and synthetic polynucleotides with liposomes in the presence of divalent cations (in Russian). Biofizika 32:628–633

Kuvichkin VV, Kuznetsova SM, Emeljanenko VI, Zhdanov RI, Petrov AI (1999) Calorimetric study of the complexes: polyA*polyU-phosphatidylcholine liposomes-Mg^{2+}. Biofizika 44:430–435

Leno GH (1992) DNA replication by the nuclear envelope. Semin Cell Biol 3:237–243

Manzoli FA, Muchmore JH, Capitani S, Bonora B, Bartoli S (1974) Lipid–DNA interactions: II. Phospholipids, cholesterol, glycerophosphorylcholine, sphingosine and fatty acids. Biochim Biophys Acta 340:1–15

Matunis MJ (2006) Isolation and fractionation of rat liver nuclear envelopes and nuclear pore complexes. Methods 39:277

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

Moyer MP (1980) The association of DNA and RNA with membranes. Int Rev Cytol 61:1–61

Nickerson JA (2001) Experimental observations of a nuclear matrix. J Cell Sci 114:463–474

Prusov AN, Fais D, Polyakov VY, Vanyushin BF (1980) Chromatin–nuclear envelope complex from rat liver: isolation and purification. Cell Biol Int Rep 4:391–398

Prusov AN, Fais D, Poliakov VIu, Vaniushin BF (1982) Properties of rat liver chromatin bound to the nuclear membrane. Biokhimiia 47:502–510

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

Risley MS, Einheber S, Bumcrot DA (1986) Changes in DNA topology during spermatogenesis. Chromosoma (Berl) 94:217–227

Rout MP, Blobel G (1993) Isolation of the yeast nuclear pore complex. J Cell Biol 123:771–783

Shabarina AN, Prilepa EI, Glazkov MV (2006) Unusual nucleotide sequence of a DNA fragment isolated from nuclear envelopes of mouse hepatocytes. Genetika 42:879–886

Shabarshina LI, Sukhorukov BI, Kuvichkin VV (1979) Infrared spectroscopic study of DNA–lipid interactions. DNA compacting on disperse particles. Biofizika 24:990–994

Shaulov L, Harel A (2012) Improved visualization of vertebrate nuclear pore complexes by field emission scanning electron microscopy. Structure 20:407–413

Sinha AA, Mizuno NS (1977) DNA synthesis associated with a DNA-nuclear-membrane complex from rat liver. Cell Tissue Res 183:191–201

Sukhorukov BI, Kuvichkin VV, Shabarchina LI (1980) Structure and function of DNA-membrane contact in cells. Biofizika 25:270–275

Van de Vosse DW, Wan Y, Wozniak RW, Aitchison JD (2011) Role of the nuclear envelope in genome organization and gene expression. Wiley Interdiscip Rev Syst Biol Med 3:147–166