



DNA-Lipids Interactions in Nuclear Pore Assembly and Hormone Actions

Kuvichkin VV*

Department of Reception Mechanisms, Institute of Cell Biophysics, Russian Academy of Sciences, Russia

Abstract

In a modern science there are ideas which were born earlier, than there was a necessary tool for their proof and development. Finally such ideas the oblivion waits, but it does not mean, that they are incorrect, simply scientific community has not ripened to their necessity, that result in scientific progress braking. The author shows on an example of one such idea: DNA-lipids interactions and DNA-membrane complexes. Having analyzed role of DNA-lipids complexes *in vitro* and *in vivo* we have proposed model of a nuclear pore which based on our understanding method such complexes assembly including the unwinding three-stranded DNA/RNA hybrid during pre-pore formation with ssDNA and double stranded DNA/RNA hybrid production. Interaction of nucleoporins with these polynucleotides in pore complex annulus leads to native structure of nuclear pore. The author propose new model of the first stages of a nuclear pore formation with participation three-stranded DNA/RNA hybrids and membrane vesicles without any proteins.

Keywords

DNA-lipids interaction, Nuclear pore, Vesicles, Hormones

Abbreviations

DMC: DNA-Membrane Complex; ssDNA: Single-stranded DNA; LMWRNA: Low Molecular Weight RNA

Introduction

There are a lot of ways to regulate genome activity at the level of transcription and most of them are related to DNA-protein interactions, where either transcription enzymes (RNA polymerase) or peptidic or protein hormones or huge other protein regulators of gene activity. The problem of structure and the role of DNA-lipid interactions, the existence of which was proven by studies conducted over the past 40 years are more complex as seemed to many. The biophysical and biochemical methods to study of DNA interaction with membranes, mainly with artificial ones (liposomes, Langmuir-Blodgett layers, etc.) used.

It is clear that the regulatory effect of some lipids on transcription processes is associated with the formation of DNA complexes with a membrane proteins or lipid bilayer. It is possible that some hormones that are hydrophobic in nature also affect DMC, changing the transcriptional properties of ssDNA (Figure 1).

Analyzing the properties of DNA-lipid complexes *in vitro*, the author proposed a model for the formation of

such complexes in a cell and their possible participation in the formation of nuclear pores. It has been discovered recently that chromatin areas have an increased transcriptional activity near nuclear pores [1]. We believe that our model will well explain these regulatory functions of nuclear pores at the level of transcription. Our model also explains the structure of "transcription factories", which, in our opinion, are precursors of nuclear pores and also are a regulator of transcription in the nucleoplasm (Figure 1). Undoubtedly hormones should interact with both nuclear pores and with "transcription factories", which is currently shown by many authors, but the structure of such complexes is not clear [2]. Be-

***Corresponding author:** Kuvichkin VV, Department of Reception Mechanisms, Institute of Cell Biophysics, Russian Academy of Sciences, 142290, Pushchino, Moscow Region, Russia, Tel: +79166405267, E-mail: vvkuvichkin@gmail.com

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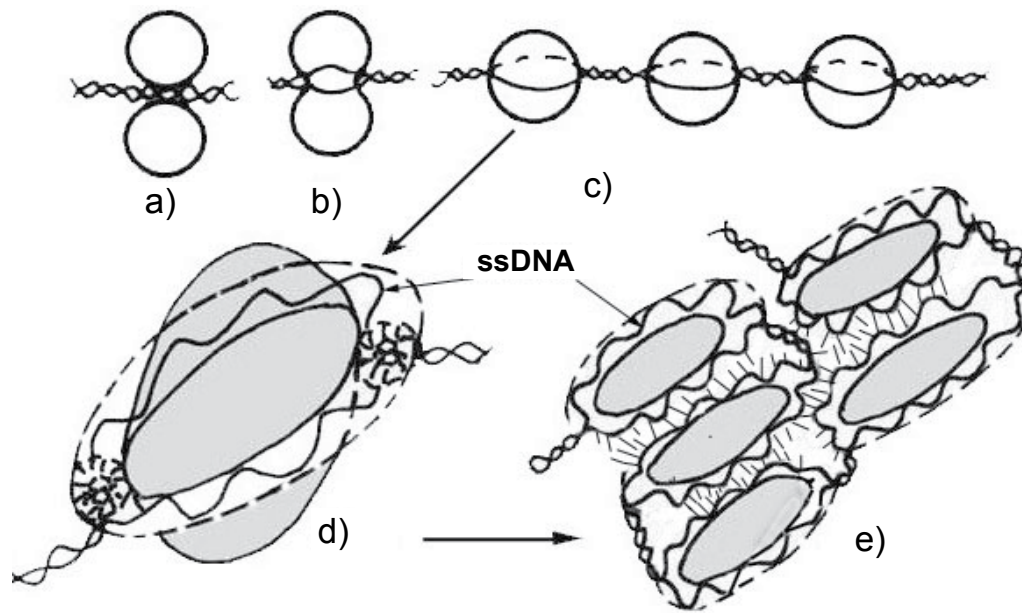


Figure 1: TC formation (a-c) and their aggregation (d,e); a) DNA situated between two membrane vesicles; b) Partial fusion of vesicles caused by DNA; c) Full fusion vesicles and threads of chromatin formation binding with vesicles belted by single-stranded DNA in complex with lipids (possible candidate for “strings-of pearls” structures, pre-pores [19]; d,e) Aggregates of pre-pores in the nucleoplasm-possible candidates in “transcription factories”).

fore explaining the mechanism of genome regulation through DMC, we should briefly state the data available today on the structure and functions of Ternary Complex (TC): DNA-zwitterionic liposomes-Mg⁺⁺, nuclear pores and transcription factories. Then we try to introduce into their structure hormones and get a complete picture of the regulation by hormones cells activity.

DNA-Lipids Interactions *In Vitro*

Twenty years ago my attempts to have a talk about DNA-lipid interactions were usually accompanied with the question: “Whether such interaction exist really?”

As it is known in 1972 an Italian scientist Manzoli and his colleagues investigated DNA-lipid interactions *in vitro* and showed that this interaction was present. Depending on the type of lipid, DNA-lipid interaction is able to stabilize or destabilize DNA double helix [3,4]. A big progress in study of lipid-nucleic acid interactions has been made by Budker who described the formation of TC in 1978 [5-10]. Some features of DNA-lipid interactions are highlighted in our works [11-20].

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

DNA forms complexes with three main lipids: Phosphatidylcholine (PC); Phosphatidylethanolamine (PE) and Sphingomyelin (SM), the addition of other lipids increases or decreases these interactions force [12], Ability of divalent cations to form complexes with DNA and PC correlates with a degree of binding of these cations with PC [13], A cooperative character of binding of Mn²⁺

in the complex DNA-PC was shown [14], DNA is partially unwound in TC [5,15], DNA molecules in TC become restrictedly accessible to action of DNase I, but are more accessible to digestion of S1-endonuclease [5], In addition to the double-stranded DNA, a three-stranded polynucleotide polyA²polyU forms TC as revealed by microcalorimetry [16].

Double-stranded polynucleotides are able to absorb on the surface of-zwitterionic liposomes in the presence of divalent cations and also to induce liposome fusion (hemi-fusion). DNA-induced fusion or aggregation of liposomes depends on the liposomes size [17-20].

DNA-Membrane Complexes *In Vivo*

Observations of TC using an optical and an electron microscope revealed a huge diversity of structures formed by liposomes in TC. They are giant flat surfaces, sites of DNA unwinding on the surface of a lipid bilayer, bilayer fusion and hemi-fusion, etc. [21,22]. This promotes further search in the cell for similar structures which could be formed as a result of DNA-lipid interactions. Since chromatin DNA is positioned in the nucleus surrounded by nuclear envelope composed of lipids, 50%, then the desired structures (nuclear pores? pore complex lamina?) should be found at the periphery of chromatin which contacts with internal nuclear membrane. Therefore, we should consider the issue on DMC which was touched upon at the same time as DNA-lipid interactions *in vitro* study [22-26].

In 1970-1980, an attempt was made to isolate a frac-

tion of DMC and to analyze its biochemical composition. For this purpose, the cells have been gently lysed in presence of Mg^{2+} sarcosylate and so-called “M-band” fraction has been obtained. This fraction contained DNA, enriched by moderate repeats, newly synthesized DNA, RNA and proteins [24-26]. There was 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 [26].

The involvement of DMC in an initiation of replication and transcription and in a regulation of gene expression has been suggested [19,27,28].

Data on DNA-lipid interactions *in vitro* are beyond any doubt and they widen our knowledge in biophysics. DMC findings are thought to be an artifact since no appropriate techniques have been available to estimate their significance. In molecular biology the methods of PCR and DNA sequencing have not yet been developed at that period of time.

DNA-Membrane Complexes and Nuclear Pores

Now we may talk about interaction between DMC and nuclear pores. In 1968 Comings suggested that chromatin DNA is attached to the nuclear pores [21,22]. Modern interpretation of DNA attachment to nuclear pores through DNA-protein interactions appears to be unconvincing [29]. In the context of modern knowledge about the involvement of nuclear pores in transcription [30-32] this suggestion seems to be true since dense heterochromatin close to nuclear pores is diffuse, hence it is transcriptional active euchromatin. It is viewed in any electron microscopic images of the nucleus. Because of the absence of the biochemical techniques developed to date it was impossible for Comings to analyze a detailed DNA attachment to nuclear pores.

Biophysical study of TC showed that DNA in the course of TC formation is partially unwinding. Based on it the model of TC formation *in vitro* has been proposed and an attempt to explain the mechanism of nuclear pore formation with the involvement of DNA and membrane vesicles has been made [15,17,19,20]. In the course of study of TC the early model underwent changes and now it can be used to describe the formation of nuclear envelope from membrane vesicles and from endoplasmic reticulum too [33].

Simultaneously with our model of the formation of nuclear envelope and pore complexes with the involvement of lipids and chromatin DNA, the model of nucleoporin-based nuclear pores has been developed [34-36].

Although almost all nucleoporins are known, an attempt to build nuclear pore complex *in vitro* using only nucleoporins failed.

The use of our model provided us with the possibility to produce pore-like structures in lipid bilayer of liposomes which were added to the extract of *Xenopus laevis* eggs [37]. The annulate lamellae-like structures were also obtained. These structures are present in *Xenopus* eggs extract and very similar to nuclear pore complexes.

Are there irreconcilable contradictions between two approaches to study the formation of nuclear pores? Now we try to give an answer to the question concerning DMC. If DNA is bound to the pore complex, then why DNA is invisible in the area of nuclear pores in modern Electron Microscopy (EM) images of cellular nuclei? First, many scientists observed links between chromatin and nuclear pores [38,39]. Naked DNA is hardly visible even on the surface of liposome with the use of cryo-TEM (cryo-Transmission Electron Microscopy) high resolution method, but it is actually impossible to view it on the surface of nuclear membrane which contains proteins and lipids. At the same time cryo-TEM images are available where it is possible to view threads which extend from nuclear pore annuli inside nucleus.

It is difficult to select a dye for DNA for EM study since all dyes change DMC structure. Only staining with lanthanum and terbium which preferentially bind single-stranded DNA may make a situation clear. Staining with terbium of TC supports our model of TC where DNA double helix is unwinding in TC [18]. Nuclear pores are also stained with lanthanum [40,41].

From our view the combination of our model of pore complexes and alternative models provides us with the possibility to produce nuclear pores *in vitro* system: DNA-liposomes- Mg^{2+} with the addition of nucleoporins or extract of *Xenopus laevis* eggs [38]. The modern model of nuclear pore taking into account the role of DNA and lipids is represented in Figure 2.

As it is seen ssDNA and DNA/RNA hybrid located in pore annulus were attached to Figure 2B. The model obtained seems not complex and has a series of advantages over the previous model. First, the presence of three-stranded DNA/RNA hybrids in chromatin induces fusion of membrane vesicles from which pre-pores are formed (Figure 1C and Figure 1D). Unwinding of DNA-RNA hybrid and the formation of single stranded DNA and LMWRRNA-DNA hybrid in pore annulus make annulus the site of nucleoporin attachment (FG-nucleoporins). Then other nucleoporins join them forming intranuclear and cytoplasmic surface of the pore (Figure 2B).

Thus, chromatin is attached to the nuclear envelope

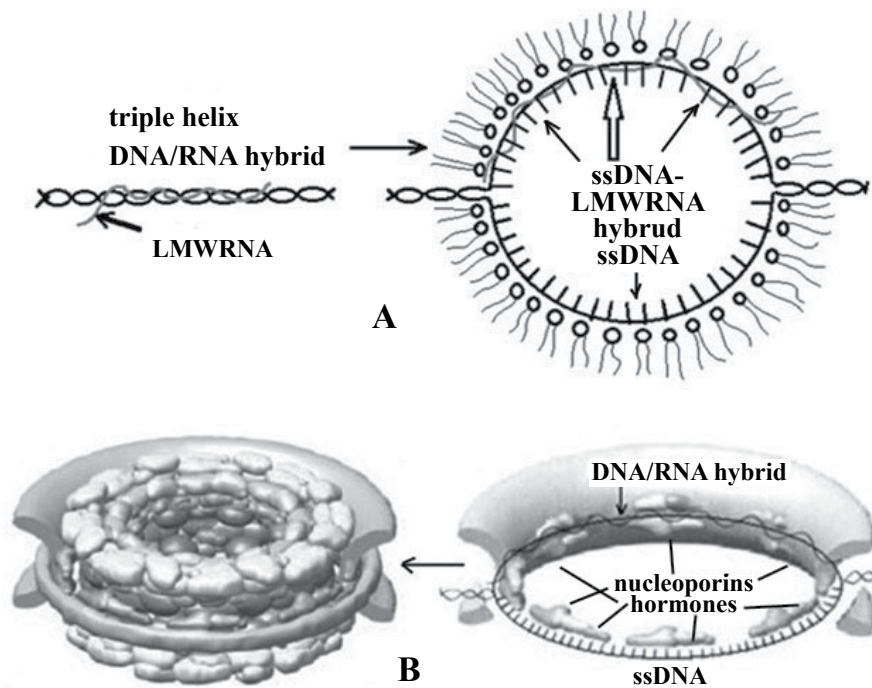


Figure 2: Model of DMC a) Triple helix DNA/RNA hybrids located on chromatin interact with membrane vesicles, which after DNA induced fusion interact with nuclear envelope to form nuclear pores. In annules of pore this hybrid unwinds forming ssDNA and double-stranded DNA/l.m.w.RNA hybrid; b) Possible ssDNA and DNA/RNA hybrid location in nucleoporins based models of nuclear pore complex.

due to DNA-lipid interactions, which provides most strong binding of chromatin and the nuclear membrane, and nucleoporins.

As follows from the above the issue on the existence of DMC is rather complicated. This issue can be referred to biophysics' one. The existence of DNA-lipid interactions *in vitro* is a proof of it [1-9]. We proposed several models of the formation of nuclear pores taking into consideration only DNA-lipid interactions [10,13,15,17,18]. The latest model of the formation of nuclear pores [41] even without the involvement of nucleoporins is rather complex and has a number of stages (Figure 3).

If these stages are excluded from consideration, then it is the model the majority of scientists study trying to build a pore complex using only nucleoporins. Probably it will be easier to obtain the desirable model using also all stages nuclear pore assembly shown on Figure 3. There are rather many facts which could not be ignored to support our model.

Conclusion

In conclusion, the problem of nuclear envelope formation requires not merely development of the existing approaches and inclusion of additional factors but complete change of the paradigm in this question. DNA-lipid interactions are currently the most obvious and deeply investigated area of biophysics [17] being in good agree-

ment with the "nucleoporins approach" [41] to solution of the problem of nuclear pore formation.

The problem of nuclear pore formation involves the factors that cannot be overcome within the limits of the existing paradigm, e.g., the fusion of two very distant membranes with formation of a hole between them. There are numerous electron-microscopic observations of nuclear pore stages formation [42], that confirm our model and, as a consequence, the role of nuclear pores in transcription [43], which researchers try to solve in the framework of the nucleoporin model of the pores, whereas it can be solved only with our nuclear pore model.

Our model assumes the loop's organization of interphase chromatin and nucleoid. Such interphase chromatin organization gradually turn into the loop organization of mitotic chromosomes, and all this follows from acknowledgement of the real existence of DMC, which were thrown out of the arsenal of biology 30 years ago. Many problems of the nuclear matrix study can be explained on the basis of our DMC model and detailed analysis of the procedure of nuclear matrix isolation. The fact that nuclear pore formation involves hydrodynamic forces that appear during the DNA-induced fusion of cell membrane vesicles cannot be explained in principle within the limits of the nucleoporin's-based pore model. The time has come to combine our model with the dom-

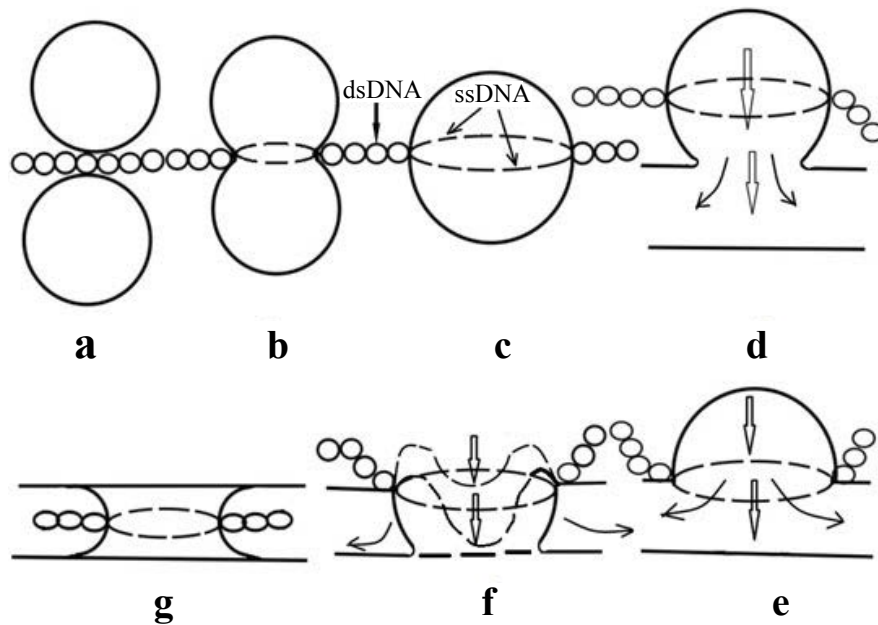


Figure 3: The model of nuclear pore complex assembly: the first stage a,b) Fusion of two membrane vesicles with active involvement of DNA chromatin with the formation of large liposome surrounded along their diameter by single-stranded DNA and DNA/RNA hybrid; c) These structures resemble “string of pearls” structures founded in chromatin. The second stage - interaction of a “string of pearls” structures and nuclear envelope fragments where the annulus between two nuclear membranes is formed. This stage includes fusion of one half of a membrane vesicle and the membrane, turning the second half of membrane vesicle inside out and its fusion with other nuclear membrane; d-g) The third stage - interaction of nucleoporins with single-stranded DNA and DNA/RNA hybrids in the pore annulus and the formation of native structure of the pore complex.

inant “nucleoporin model” of nuclear pores assembly for completion it.

Outlook

So, we got the latest idea about the structure of nuclear pores and should find a place for hormones in the pore structure. Since many of them are hydrophobic, it is logical to assume that hormones can interact with single-stranded DNA in a pore complex located in the lipid environment of the nuclear envelope. It can be assumed that the addition of certain hormones will positively affect on the transcription. It is logical to expect a similar mechanism of interaction of hormones with transcription factories; however, in this case the localization of the hormone will be intra-nuclear type [43]. There are hormones that inhibit transcription in nuclear pores, then they can be a good help in cancer treatment.

Accepting a new paradigm will result in increase of research activity in the field of TC, because the efforts of 4-5 laboratories engaged now in this problem worldwide are clearly insufficient. The author has succeeded, using an original technique, to isolate “membrane DNA”, and there is a necessity of its further analysis including sequencing [44]. It should be acknowledged that the isolation and analysis of DMC and “membrane DNA” is a difficult problem, and we understand the previous unsuccessful attempts of its isolation [45]. The analysis of

“membrane” ssDNA and LMW RNA sequence being a component of DMC will make it possible to analyze the most transcriptional active genes in the cell by analyzing the LMW RNA as part of pore complexes. The interaction between numerous nuclear receptors and DMC components will provide for a better understanding of their influence on genome expression.

Investigation of the role of TC in DMC and nuclear pore formation must also involve additional forces. The computer simulation of TC and first stages of nuclear pore assembly is the task of the nearest future. We have already spoken about the potential role of DMC in the processes of replication and transcription, hormone action, cancer progression and ageing of cells, as well as evolution of eukaryotes. The existing concept of the structure and functions of cell nucleus will be radically revised, what will result in the progress in treatment for many diseases that have been incurable up to now.

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