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THE COMPLEX ORGANIZATION OF EUKARYOTIC CELL NUCLEUS (III): THE NUCLEAR MATRIX AND THE NUCLEAR LAMINA

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Abstract

A large variety of nuclear fibrous proteins (such as actin, myosin, lamin B, transcription factors, topoisomerases, etc) represent constitutive elements of complex structures present in the eukaryotic nuclei: the nuclear matrix and the nuclear lamina, repectively. These nuclear compartments, with fibrous network-like structure, play crucial roles in structural organization of nuclei, chromatin remodeling, DNA transcription, signals transduction, cell cycle regulation, embryonic development and other nuclear basic processes.

Introduction

In the first two parts of this minireview we synthesized the present knowledge regarding the structure and functions of some essential nuclear compartments: the nuclear bodies, the chromosome territories and the interchromatin domains. Yet, these nucleoplasmatic compartments could not properly function without the participation of other nuclear components. Among these components, the *nuclear matrix* and *the nuclear lamina* act, primary, as a nuclear framework for chromatin, nucleols, different proteinaceus bodies and the inner nuclear membrane; their structural and functional characterization will be discuss further in the part III of this minireview.

THE NUCLEAR MATRIX

The nuclear matrix appears as a concept capable to explain, at least in a theoretical manner, the existence of a nuclear compartment with fibrous network-like structure, which can organize other nuclear compartments (such as chromatin and nucleoli) and can also serve as attachment frame for some of the nuclear bodies.

Although a cell fraction equivalent to what we designate today as the **nuclear matrix** was first obtained and described long time ago (1948) by Russian researchers (Pederson, 2000), it’s just in 1974 this fraction was rediscovered, isolated and characterized as a nuclear framework (Berezney and Coffey, 1974).

The nuclear matrix represents the major residual part of isolated nuclei (by centrifugation); after combined treatment of these nuclei with DNase and RNase and extraction of 90% of the nuclear proteins and phospholipids results an internal protein predominant structure, termed the *nuclear protein matrix.*

The electron microscopy images of this final nuclear matrix reveals that it forms an internal framework composed of remaining nucleolar structures, connected to a granular and fibrous internal matrix structure, which appear to be associated with the inner layer of the surrounding nuclear envelope containing residual nuclear pore complexes; biochemically, the nuclear matrix consists of large amounts of proteins and smaller amounts of DNA, RNA, phospholipids and carbohydrates (Berezney and Coffey, 1977).

Later, other structures, more or less similar with the nuclear matrix have been isolated by different research laboratories, using various experimental methods. These structures were called: *scaffold*, *nucleoskeleton*, *karyoskeleton* and *nuclear endoskeleton* by different authors (Tsutsui et al. 2005).

Whether its particular structure and cell related aspects, the nuclear matrix appears as a complex proteinaceous structure, similar with the fibrous architecture of the cytoskeleton, when observed in electron microscopy, after its isolation from other nuclear components.

Various researchers have been shown that a large variety of proteins could be associated with the nuclear matricial network (e.g. in the mature spermatic cells): actin, myosin, lamin B and also transcription factors or

topoisomerases (Moss et al. 1993, Carrey et al. 2002, Ocampo et al. 2005, Har-Vardi et al. 2007, cited by Johnson et al., 2011).

The existence of *nuclear actin* was contested at first, its presence in the nuclei being considerated as a contamination with cytosolic actin. Nowadays, the supramolecular organization, intranuclear locations and functions of actin constitutes major issues (and no longer its nuclear component status, which is a fact).

Beyond its resemblances with cytoplasmatic actin forms (monomeric, globular - G-actin and polymeric, filamentous - F-actin), the nuclear actin presents a larger range of conformations and oligomeric and polymeric forms (Pederson and Aebi, 2002).

The actin and the *actin - related proteins* (Arps) found in nuclei are members of the actin superfamily and are highly conserved throughout evolution, similar to their cytoplasmatic counterparts (Chen and Shen, 2007).

Nuclear actin plays various and crucial roles in nuclear structure organization, chromatin remodeling, DNA transcription and signal transduction.

As a part of *chromatin - remodeling complexes*, nuclear actin participates in gene expression.

In a large variety of organisms β-actin and different Arps have been identified as components of various chromatin remodeling and histone acetyltransferase (HAT) complexes, although the mechanisms by which such molecules carry out these functions are not fully understood. On the other hand, not all chromatin - modifying complexes contain actin or ARPs (Visa and Percipalle, 2010).

One of the most important functions of nuclear actin consists in its participation both in *DNA transcription* and *regulation of transcription*. Actin is associated with all three RNA polymerases (Pol I, II, and III) and togheter with *nuclear myosin 1* (NM1) acts in driving the transcription (Grummt, 2006).

The involvement of actin molecules in RNA transcripts synthesis manifests in several ways: they can bind transcription factors, determining their localization in cells, they can associate directly with the RNA polymerases and they couple with with nascent mRNPs, in order to select the histone modifiers to transcribed genes (Miralles and Visa, 2006).

In Pol I transcription, actin-NM1 interactions act as molecular motors; the same interactions play also a role in the transition of the initiation complex into the elongation complex (Grummt, 2006).

During the process of transcription controlled by Pol II, β-actin participates at the formation of the preinitiation complex; in Pol III transcription, β-actin functions as a subunit of this enzyme.

Two possible models which explain the involvement of actin in transcription elongation stage were conceived.

One model states that the actin-NM1 complex allows the transcription machinery to slide along the DNA molecule, the elongation molecular motor being in permanent contact with both the polymerases and the DNA (chromatin). The second model assumes that actin concurs at the recruitment of histone modifiers to protein-coding genes; the actin binds to hnRNP proteins and finally becomes a component of the nascent pre-mRNPs (Visa and Percipalle, 2010).

Some studies demonstrates the involvement of nuclear actin in the *nuclear export* of some molecules (e.g. retroviral RNAs and proteins such as protein kinase inhibitor (PKI) because of its association with the nucleoplasmic filaments of nuclear pore complexes (Hofmann et al., 2001).

THE LAMINS AND THE NUCLEAR LAMINA

The *lamins* are fibrous proteins and represent elements of cytoskeleton – Class V intermediate filaments, present in most animal cell nuclei.

Inside nucleus the lamins are distributed and concentrated in two main regions: in close contact with the inner nuclear membrane (INM) of the nuclear envelope, where they form the **nuclear lamina** – a layer with proteinaceous structure, and throughout the nucleoplasm where, together with other proteins (such as actin , myosin, etc.) compose the nucleoplasmic "veil" of the nuclear matrix.

The nuclear lamina is a dense structure, with a fibrillar network oganization and ~30 to 100 nm thickess.

In the early seventies, the nuclear lamina was a matter of uncertainties concerning its architecture, functions as well its presence in different cells; these uncertainties were also reflected in the names gave by different cell biologists to the concept of nuclear lamina: “dense lamella”, “fibrous lamina” , “zona nucleum limitans” or just plain “lamina” (Leslie, 2005).

Furthermore, the studies carried out in the same period (immunofluorescent microscopy and electron microscope immunoperoxidase staining of antibodies to each of the three predominant pore complex-lamina bands, applied on a centrifugal fraction from rat liver nuclei consisting of nuclear pore complexes associated with the proteinaceous lamina which underlies the INM) showed that the major polypeptides of these structures are localized at the periphery of the interphase nucleus and have little or no cytoplasmic presence (Gerace et al. 1978).

Besides lamins, the nuclear lamina comprises other important components - *the lamin-associated membrane proteins.*

According some criteria (such as the sequence homology, biochemical properties and cellular localization during the cell cycle) the lamins can be classified in two major types: the *A-type* (lamin A, C), which are splicing variants of the *LMNA* gene, present at 1q21 and the *B-type* (lamin B_1, B_2), coded by *LMNB1* and *LMNB2* genes, situated on 5q23 and 19q13, respectively.

The lamin intermediate filaments weight from 60 to 80 kDa and display an almost complete α-helical conformation, with numerous α-helical domains which are separated by non-α-helical linkers; both the C-terminus (with a domain containing the nuclear localization signal (NLS) and the N- terminus have not an α-helical conformation.

The *lamin-binding proteins* are among the most important lamin-associated membrane proteins. They are either integral or peripheral membrane proteins of INM, bound to the fibrilar elements of the lamina and many of them are well known and characterized nowadays: the *nesprin*, the *emerin*, *lamina-associated proteins 1 and 2* (LAP1 and LAP2), the *lamin B receptor* (LBR) and the *LEM domain-containing protein 3* (MAN1). These proteins mediate the attachment of the nuclear lamina to the nuclear envelope.

Other lamin-binding proteins are transcription factors: the *retinoblastoma transcriptional regulator* (RB), *germ cell-less* (GCL), *sterol response element binding protein* (SREBP1), FOS and MOK2. The *barrier to autointegration factor* (BAF) is a chromatin-associated protein linked to the nuclear lamina and to several of the nuclear envelope proteins mentioned before. *Heterochromatin protein* 1 (HP1) binds both chromatin and the LBR (Coutinho et al., 2009).

Due to its position inside nucleus, its structure, spatial organization and the complex relations with other molecular components (lamin-binding proteins and chromatin), the nuclear lamina plays multiple and essential roles in the accurate run of nuclear functions, such as: chromatin organization, cell cycle regulation, DNA replication, cell differentiation and apoptosis.

The involvement of lamina in *chromatin organization* is strongly supported by the evidence of the non-random organization of the nuclear genome. The lamins and the lamin-binding proteins - lamina-associated polypeptide (LAP2 beta) and the lamin B receptor bind to DNA or interact with chromatin via histones, BAF-1 and HP1 chromodomain proteins, respectively; thus, they may provide anchorage sites for chromatin fibers at the nuclear envelope (Gotzmann and Foisner, 1999).

The lamin polypeptides have an affinity for binding chromatin at specific DNA sequences called *matrix attachment regions* (MAR), through their α-helical domains.

The crucial role of nuclear lamina in results in its participation at the onset of mitosis (prophase, prometaphase), when disassembly of various cellular structures (such as nuclear envelope, nuclear lamina and nuclear pore complexes) occurs.

These events are triggered by lamin phosphorylation by the cyclin B/Cdk1 protein kinase complex (Mitosis-Promoting Factor, MPF), which drives the disorganization of the lamina and the nuclear envelope. At the end of mitosis, (anaphase, telophase), after chromosome segregation, dephosphorylation of nuclear lamins induces the reassembly of the nuclear envelope and thus, the reformation of nucleus.

The role of nuclear lamina in *embryonic development* and cell differentiation was studied on various animal models (*Xenopus laevis*, the chicken and mammals).

These studies demonstrate that the presence and proportion of B-type and A-type lamins in higher vertebrates (or their counterparts in *Xenopus*) vary during development, according to a similar pattern: in the early embryonic stages, the B-type lamins are the only (or prevailing) lamins present; in further stages, as different tissues differentiate, the expression of lamin B decreases (or is relatively constant), whereas an increase in the levels of lamin A and lamin C is observed.

A possible inference of these findings is that a functional nuclear lamina, in its most basic form, could contain only B-type lamins.

The nuclear lamina also participates in the elongation phase of *DNA replication.*

This function is accomplished by lamins because they act as a structural scaffold which support the correct assembly of the elongation complexes in nucleus; another hypothesis postulates that the lamina could be the initiation point for the assembly of this scaffold.

The disorganization of nuclear lamina is one of central events which occurs in early stages of *apoptosis.* Unlike the disassembly of lamina during mitosis, which is a phosphorylation-induced process, the degradation of lamina (both of lamins and nuclear lamin-associated membrane proteins) at the beginning of apoptosis is carried out by proteolytic cleavage. This proteolytic activity is performed by enzymes who belongs to caspase-protein family.

The participation in *anchoring the nuclear pore complexes* (NPC) in the nuclear envelope is another important function of nuclear lamina.

The interference of nuclear lamina in *cell movements* is now an evidence: the lamina provides a supporting structure for the 3D migrations of single animal cells, via linker complexes (LINC). These linkers connect the

nucleoskeleton (the nuclear lamins A/C) to the cytoskeleton (the actin bundles which form a perinuclear cap) allowing the formation of lamellipodia and determining the successive protrusion and pulling of cells; the disruption of LINC complexes disorganizes the actin cap and affect the 3D cell migration.

The structural and functional defects of lamins (or lamin-associates membrane proteins) which lead to emergence of abnormal nuclear lamina behaviour are designated as **laminopathies**; they belong to a more generic nuclear pathology class termed *envelopathies* and represent a group of rare genetic disorders produced by multiple causes: mutations (nonsense and missense mutations or point mutations) in genes encoding proteins of the lamina, splicing defects of LMNA mRNA, processing defects, gene dosage defects (e.g. the duplication of lamin B gene LMNB1) and autoimune antibodies.

Because the normal lamina form a nuclear scaffold which confer mechanical strenght to nuclear structures it appears to be an adaptation to motility of animal cells; thereby, the animals manifest muscle defects diseases (myophaties) as symptoms of different envelophaties, whereas other organisms (such as plants or fungi), which are sessile, do not move and lack the nuclear lamina.

At present, a large number of different types of laminophaties is known: muscular distrophies, cardiomyopathies, dysplasies, progeria, etc.

For instance, the Emery-Dreifuss muscular dystrophy is a muscular wasting disease, progeria causes premature aging, the restrictive dermopathy is a disease associated with extremely tight skin and other severe neonatal abnormalities.

Conclusion

Various studies carried on during the last decades have revealed the biochemical, structural and functional complexity of the nuclear matrix and the nuclear lamina; their features explain the roles of these components in supporting and connecting the other nuclear compartments. The specific disorders of nuclear cytoskeleton (especially the envelopathies) which manifest not only at micoscopic level, but also at whole body level, emphasize the importance of the highly organized proteinaceus nuclear network in the economy of eukaryotic cells.

In the last part (IV) of this minireview a presentation of boundary nuclear structures (the nuclear envelope whith nuclear pores) will be made, in order to complete the informations about the complex organization of eukaryotic cell nucleus.

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