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THE COMPLEX ORGANIZATION OF EUKARYOTIC CELL NUCLEUS (IV): THE NUCLEAR ENVELOPE

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Abstract: The nuclear envelope (NE), a double membrane structure, separates the nucleoplasm from cytosol. Each of the two membranes of the NE (the inner nuclear membrane, INM and the outer nuclear membrane, ONM) contain a particular protein complement, with specific domains, which accomplish various and critical functions: the lamin and chromatin anchoring at NE, the localization and movement of nucleus within cells, the control of transciption, etc. The nuclear pores complexes (NPCs) of the NE are large and complicated proteic structures, essentially involved in bidirectional transport of molecules between nucleus and cytoplasm. Some nuclear envelope molecular components are subjected to various genetic disorders known as envelopathies, which result in general syndroms, more or less severe.

INTRODUCTION

In the previous parts of this mini-review we tried to summarize the present knowledges regarding the structure and functions of some nuclear components: the nuclear bodies, the chromosome territories, the interchromatin domains, the nuclear matrix and the nuclear lamina. Although these components, defined as nucleoplasm (nuclear content), are essential, they could not properly function in eukaryotic nuclei without the presence of the nuclear envelope, which, primary, represent a physical barrier which separates the nucleoplasm from cytosol, but also interconnect them, allowing complex molecular exchanges. The advent of nuclear envelope in eukaryotic cells was a crucial landmark in cellular evolution and had enormous consequences in general systematics of all living beens.

THE NUCLEAR ENVELOPE

As it was shown in the first part of the minirewiew (*Overwiew on general organisation of the cell nucleus*) the nuclear envelope (also known as nucleolemma or karyotheca), is a complex structure consisting of two lipoproteic membranes – *the inner nuclear membrane* (INM) and the *outer nuclear membrane* (ONM).

These membranes are fused together at the site of *nuclear pores complexes* (NPCs), which create a discontinous surface of nuclear surface. The INM and the INM are parallel one to another and are separated by a 10-50 nm wide space (*perinuclear space*), which expands inside the endoplasmic reticulum (the lumen of ER).

The outer membrane, facing the cytosol, is continous with the membrane of *rough endoplasmic reticulum* (RER) and, similar to this, carries tightly bounded ribosomes; the INM indirectly contacts the nuclear chromatin by means of *nuclear lamina*.

As specified in the previous part of this minirewiew (*The Nuclear Matrix and the Nuclear Lamina*) the inner nuclear membrane contains a large variety of peripheral and integral membrane proteins (about 60 proteins), most of which are poorly characterized. Yet, few of these proteins are better known, such as: *emerin*, *lamina-associated polypeptides 1 and 2* (LAP1 and LAP2), the *lamin B receptor* (LBR), the *LEM domain-containing protein 3* (MAN1) and *nurim*. Many of the INM proteins are *lamin-binding proteins,* interacting with lamins; some of them are related with chromatin. Some data suggest that these proteins could also be involved in gene regulation and, possibly, in sterol metabolism (Holmer and Worman, 2001).

All the lamina-associated protein 2 (LAP2) isoforms (β, γ, δ, ε), emerin and MAN1 have a homologous N-terminal domain called the *LEM domain,* which is a 45 AA residue motif that

folds as two α-helices (Laguri et al., 2001). The LEM domains bind to BAF (the **B**arrier to **A**utointegration **F**actor), a chromatin-associated protein, linked to nuclear lamina and to nuclear envelope proteins mentioned above, as well.

The *Lamina-associated polypeptide 1* (LAP1) is a single-spanning integral membrane protein (isoforms A,B,C) expressed in most cells and tissues. LAP1 physically interacts with lamins, torsin A and emerin in nuclear envelope, suggesting that it may act as a crucial node in signal transduction across the inner nuclear membrane (Ji-Yeon-Shin et al., 2014).

The *Lamina-associated polypeptide 2* (LAP2) *isoforms*, via their LEM domains, which binds to BAF, interact both with B-type lamins and chromatin inside nucleoplasm. LAP2 is a single-spanning integral membrane protein, too, and possesses a large nucleoplasmic domain with multiple distinct regions; because the association of the AA situated at the second half of its N terminus (nucleoplasmic) with B-type lamins, LAP 2 may modulate the assembly of nuclear lamins (Furukawa and Kondo, 1998).

Similar to LAP 1 and LAP 2, *emerin* is a single-spanning integral membrane protein, rich in serine, composed of 254 AA. It is highly expreessed in skeletal and cardiac muscle. Together with several other proteins of INM, emerin may be involved in some important processes, such as: the regulation of certain genes activity, the control of cell division cycle, the reassembly of nucleus in telophase and the conservation of nuclear structure and stability. In humans, emerin is coded by the EMD (STA) gene; mutations in EMD gene produce the Emery-Dreifuss muscular dystrophy and heart disorders (dilated cardiomyopathy and cardiac conduction abnormalities).

The *lamin B receptor* (LBR) is a multi-spanning membrane protein of INM. It has eight segments of hydrophobic amino acids (transmembrane domains), weights around 73,375 Da (Worman et al., 1990) and is coded by the LBR gene in humans. Its N-terminal end is located in nucleoplasm and binds to lamin and heterochromatin, while the C-terminal end is situated within the inner nuclear membrane and still attached at ER membranes after NE breakdown durig mitosis (Olins et al., 2010).

Functionally, the LBR could mediate the interactions between lamin B and chromatin; mutations in LBR gene are associated with autosomal recesive Greenberg skeletal dysplasia (an abnormal cholesterol biosynthesis) and with Pelger–Huët anomaly (blood laminopathy).

LEM domain-containing protein 3 (LEMD3 or MAN1) is a 82,3 KDa integral protein with two transmembrane segments, a nucleoplasmic N-terminus which contain a LEM domain and a C-terminal domain also facing the nucleoplasm (Lin et al., 2005). Through its LEM domain, MAN1 attach to BAF and indirectly interact with the chromatin, whereas through the RNA recognition motif (RRM), present at the C-end, MAN1 interracts whith Smad protein family members (Smad2 and Smad3), thereby mediating the cellular response at several cytokines, such as the transforming growth factor beta (TGF-β). Thus, it regulates the expression of several fundamental downstream genes. Mutations in MAN1 gene (LEM3) are involved in several genetic diseases - ostopoikilosis, melorheostosis and Buschke-Ollendorff syndrome.

Nurim is a six transmembrane-spanning protein, with the N- and the C- termini residing on the same side of the membrane, containing 262 amino acids residues; it lacks an N-terminal domain, characteristic to other INM proteins (Hofemeister and O'Hare, 2005). Some experimental evidences (the expression of nurim in a broad range of cancers, corelated with tumour severity, the emergence of NE abnormal shapes and increases of apoptosis in HeLa cells, caused by its knock-down,) suggest that nurim has an important role in the suppression of apoptosis (Chen et al., 2012).

After their synthesis on RER, the integral proteins reach the INM membrane by lateral diffusion (because of the continuity of ER with nuclear membranes) and are retained here by means of the assocation with nuclear ligands (Holmer and Worman, 2001).

Although the majority of the NE proteins resides in the INM, the *outer nuclear membrane* (ONM) includes several specific proteins,.

Some of these proteins share different common features, such as: the presence of a single transmembrane domain followed by a short luminal sequence, the interaction with cytoskeletal elements and the existence of a conserved C-terminal KASH domain.

Among these proteins are: members of the mammalian *nesprin* family, *Klarsicht* and *Msp-300* in *Drosophila melanogaster*, *Anc-1*, *Zyg-12* and *Unc-83* in *Caenorhabditis elegans* and *Kms2* in the fission yeast *Schizosaccharomyces pombe* (Rouxet al., 2009).

Nesprins (Nuclear envelope spectrin-repeat proteins) represent a family of proteins originally described as components of mammalian cell ONM, which connect the nucleoplasmatic and cytosolic cytoskeleton elements, but encompassing a large diversity of tissue-specific isoforms localised in various cellular compartments (Rajgor and Shanahan, 2013).

More specific, different types of nesprins, through their C-terminal CASH domain connected with the SUN domain of some INM proteins (forming LINC complexes), associate the nuclear lamin network with various cytoskeletals fibrils: the *nesprin1* (synaptic nuclear envelope protein1, syne-1, enaptin, encoded by the SYNE1 gene) and the *nesprin2* (synaptic nuclear envelope protein2, syne-2, encoded by the SYNE2 gene) bind to actin filaments; the *nesprin3* associates with the intermediate filaments (IF) linker plectin; the *nesprin4* (nesp4) interacts with kinesin1 and can induce kinesin-mediated cell polarization. Due to their structure, cellular localisation and connections, the nesprins play important roles as intracellular scaffolds and linkers, interfering in nuclear localisation and nuclear movements within cells and in the maintenance of cellular spatial organization.

The SUN domains, which characterize the *SUN - domain* protein family, are a few hundred amino acids long regions, located at the C-terminus and conserved in several proteins thought to localize in the INM. Usually, the SUN regions follow a transmembrane domain and a less conserved AA region. A large variety of SUN proteins, from very different organisms, is now known: *SUN-1/matefin* and *UNC-84* (*Caenorhabditis elegans*), *Klaroid* and *Spag4* (*Drosophila melanogaster*), *SUN1*, *2* and *3* and *SPAG4* (mammals), *Sad1p* (*Schizosaccharomyces pombe*) and others.

The KASH domains (**K**larsicht **A**NC-1 **S**yne **H**omology) of *KASH- domain* protein family, similar to SUN domains, are C-terminal protein regions which follow a transmembrane domain and contain ~ 30 AA. The large majority of KASH proteins are situated in ONM, although some of them are reported to be components of the INM. Within perinuclear space, the proteins with KASH domains interact with SUN domain proteins, giving rise to a *LINC complex* (Linker of Nucleoskeleton and Cytoskeleton). Associating the nucleoplasmic and cytosolic cytoskeleton elements, via the nuclear envelope, the LINK complexes participate in many cell activities: nuclear relocations and movements, the attachment of centrosomes to the ONM, the response to mechanic extern stimuli, etc.

Whereas the ONM protein quality control is carried out by endoplasmic-reticulum-associated protein degradation pathways (ERAD),with the help of E3 ubiquitin ligases Hrd1 and Doa10 and E2 ubiquitin-conjugating enzymes Ubc6 and Ubc7 (in yeasts), the same processes are less known for INM proteins. However, it was demonstrated that in yeast (*Saccharomyces cerevisiae*) the degradation of INM soluble and integral membrane proteins is mediated by the Asi

complex (the RING domain proteins Asi1 and Asi3) which functions in conjunction with ubiquitin-conjugating enzymes Ubc6 and Ubc7; this pathway is distinct from ERAD pathway, but comlementary to it (Khmelinskii et al., 2014).

The Nuclear Complexes (NPCs) and the Transport Through NPCs

The nuclear pores are not simple apertures or discontinuities of nuclear surface at the fussion sites of INM and ONM; in fact, the nuclear pores are large protein complexes (*nuclear pore complexes*, *NPC*s), that cross the nuclear envelope and are of crucial importance in the bidirectional transport of molecules between nucleoplasm and cytosol.

Observed in electron microscopy, the NPC appears as a complex structure with cylindric shape and octogonal symmetry; it measures between 100-150 nm in diameter and 50-70 nm in thickness, depending of organism (Wente and Rout, 2010). The number of NPC per nucleus is submitted to large variations (from a few hundred in glial cell to almost 20,000 in some neurons, with an average of 2,000 NPCs in the NE of the vertebrates cells), depending mainly on the cell type and the stage of the cell cycle. The total mass of NPCs ranges, for instance, between 66 MDa in yeast (*Saccharomyces cerevisiae*) to about 124 MDa in mammals.

The NPCs are composed of about 30 different species of proteins called *nucleoporins* (Nups), each of them present in multiple copies (numerous nucleoporins have 8, 16 or 32 copies); in total, a mature NPC could contain between 500 and1000 protein molecules. In fact, the NPCs proteins contain just a few different types of repetitive domains, generated through extensive gene duplication: *solenoid domains* (alpha solenoid or beta- propeller fold) – about a half of them and *intrinsically disordered domains* – the other half (highly flexible proteins devoided of ordered secondary structure).

Beside its composition and molecular weight, the complexity of NPC also express by its double symmetry: an *eightfold rotational symmetry*, visible on both sides of the NE (nucleoplasmic and cytosolic) and a *twofold transverse symmetry*, as a result of the simmetric orientation of the central portion of NPC proteins, which leads to the identity of nuclear and cytosolic NPC parts.

According their position toward the two nuclear membranes and the relative localization from outside to inside of the NPCs, the nucleoporins could be classified in three distinct types (Alberts et al., 2015):

- *Transmembrane ring proteins*, that anchor the NPC to the nuclear envelope;
- *Scaffold nucleoporins,* with a multilayered transversal architecture and ring morphology; they possess solenoid domains and some of them are membrane-bending proteins, involved in the stabilisation of NE membrane curvature at the site of nuclear pores; from each of the eight subunits of the outermost and innermost scaffold nucleoporin layers emerge microfibrils: since the fibrils facing the cytosol are free-ended, those who face the nucleoplasm converge at their distant end and form a structure similar to a basket;
- *Channel nucleoporins,* which occupy the innermost position and line the central pore of the NPC. These proteins have folded anchoring domains; in addition, some of them present intrinsically disordered domains, with unstructured polypeptide chains; the central NPC channel is filled with a mesh of these unstructured chains, which behave like a filter against the passage of large macromolecules (passive diffusion). The central region of the NPC is in fact an aqueous

channel between the nucleoplasm and the cytoplasm, with a diameter ranging from 5.2 nm in humans to 10.7 nm in *Xenopus laevis.*

The primal function of the NPCs is the control of *NE permeability*: they must to allow the free difussion of small molecules (water, ions, sugars), the passage of macromolecules (proteins, RNAs, ribosomal subunits) and, at the same time, to prevent the passage of nonspecific molecules. All these exchanges are multi-level and highly regulated.

The measured particles diffusion rates at NPCs varies mainly according the size of particles: for instance, if the NE is freely permeable for small molecules (metal ions, small metabolites, etc., weighting 4,000 Da or less), the diffusion rate decreases for larger proteins and, for proteins larger than 60,000 Da, the passive diffusion stops.Yet, for more complex particles (including DNA polymerases and RNA polymerases, ribosomal subunits, etc.), with molecular masses up to 200,000Da, the transport through nuclear pores relies on binding to specific protein receptors that actively pass the molecules through NPCs (Alberts et al., 2015). Overall, the NPCs are capable of transporting particles up to 39 nm in diameter (Wente and Rout, 2010).

Similar to all permeable biologic membranes, the transport across the NE consists of two opposite bidirectional and continous processes: the *import* of molecules (mainly proteins, such as histones, DNA and RNA polymerases, tanscriptional regulators, carbohydrates, lipids, signaling molecules, etc.) and the *export* (including different types of RNA and ribosomal subunits). The import of molecules from cytoplasm, as well as the export of molecules synthesized into nucleus in cytoplasm are highly selective processes.

The *import of proteins*, even the very large ones, requests the fullfilment of several essential conditions: the presence of a particular sorting sequence in transpoted proteins (cargo), called *nuclear localization signal (NLS),* involved in the selection of molecules which reach the nucleoplasm and the existence of *nuclear import receptors* (called *karyopherins* in general and, more secific, *importins,* in vertebrates), which recognize and bind to both NLS and NPC proteins.

A NLS, which designes a protein for import, is, typically, a single (or double) short sequenceof amino acids, rich in lysine and arginine, positively charged and possibly forming loops or patches on the molecule surface; it can be located anywhere in the polypeptidic chain, the precise location of the NLS not being important in its functions. Different nuclear proteins share identical NLSs.

The first NLS sequence discovered was PKKKRKV (of the SV40 Large T- antigen), which is considered a classical NLS. Now, many classical (monopartite or bipartite) and non-classical NLSs are known in various nuclear proteins.

The nuclear import receptors (generally called *Kap* in *S. cerevisiae* and *importins* in vertebrates) ensure the import of proteins by their double recognition and binding ability: to the NLSs of cargo proteins and to the phenylalanine-glycine repeats (FG) of the unstructured regions of channel nucleoporins. The importins are soluble cytosolic proteins.

The cargo proteins do not always directly interact with the appropriate importins: in some cases the interactions between the two types of molecules are mediated by an additional class of proteins – the *adaptor proteins*, which recognize and bind to the NLS of cargo, activates and expose their own NLSs, which, in turn, bind to the nuclear import receptors. The combinations of import receptors and adaptors provide the cell recognition capacity of a large number of NLSs and thus, the nuclear import of various proteins.

Two main types of importins are known: the *importin-β family,* which bind and can transport the cargo proteins alone or can react and form heterodimers with the other type, the *importin-α.* As part of a heterodimeric complex, the importin-β play the role of a typical importin,

while the importin-α functions as an adaptor protein; in the last case, a trimer NLS-importin-α-importin-β forms.

The translocations of NLS proteins through the NPC into the nucleoplasm (the protein import cycle) could be divided in three main steps:

- The cargo proteins *bind* to the appropriate importin, directly or via an adaptor protein;
- The complex cargo-importin-β (or the trimer cargo-importin-α-importin-β) is *tranlocated* into cytoplasm, with a rate of diffusion depending mainly of concentration and binding affinity of importin-α. In this stage, the presence of Ran-GTP, a Ras family GTP-ase is of particular importance: the gradient of the two coformational forms of Ran (Ran-GTP and Ran-GDP) in cytosol and nucleoplasm directs the protein transport through NE (import and export). The Ran-GTP is more concentrated inside the nucleus, while Ran-GTP concentrates outside nucleus, following the GTP hydrolisis by Ran in cytosol.

When the cargo-nuclear import receptors complexes reach the nuclear side of the NPC, after passing through the poral channel, the Ran-GTP binds to them;

- The attachment of Ran-GTP modifies the conformation of importin-β of transporting complexes, leading to dissociation of the complex and the releasing of the cargo inside the nucleus; the importin-β stills bounded to the Ran-GTP, which is ready to be recycled: the complex is tansported back in cytosol through the NPC and here, under Ran-GAP catalysis, the Ran-GTP is transformed in Ran-GDP, by GTP hydrolisis. The Ran-GDP dissociates from the carrier importin and the receptor became available for another import cycle.

The *export of proteins* from nucleus (e.g. those who compose the ribosomal subunits) is a process which works in a reverse order to protein import. Similar to proteins import into nucleus, the export of cargo proteins in cytsol relies on the existence of the *nuclear export signals (NESs)* on macromolecules to be exported, on *nuclear export receptors* (also called *exportins*), which are karyopherins, and on Ran-GTP enzymatic transport system. Generally, the nuclear export receptors are related to nuclear import receptors and are coded by the same gene family (with a large number of members in animal cells). The nuclear export receptors have double binding affinity: for nuclear proteins NESs and for NPC proteins, leading the passage of their cargo through the NPC to the cytoplasm.

It was demonstrated that, in some cases, the export of proteins containing leucine-rich NESs (for instance the human immunodeficiency virus type 1 (HIV-1) Rev-mediated nuclear export and Mason-Pfizer monkey virus (MPMV) constitutive transport element (CTE) - mediated nuclear export in *Xenopus laevis* oocytes), carried by the export receptor CRM1/exportin1, needs additional protein factors which interact with these specific NESs (Hofmann et al., 2001).

In proteins nuclear export cycle, Ran-GTP associates with nuclear export receptors in nucleoplasm and stimulates the association of cargo carrying the appropriate NES to these receptors. The complex travels then via NPCs into the cytosol, encounters the Ran-GAP, whose GTP is hydrolyzed in GDP; following hydrolisis, the cargo and Ran-GDP are released from nuclear export receptors and the free receptors return into nucleus.

The *export of RNA* from nucleus have some general characteristics:

- The export pathways differs for each class of exported RNA,
- The extranuclear transport of cellular RNAs ((tRNA, rRNA, U snRNA, microRNA) and viral RNAs relies on Ran-GTP cycle;
- For some mRNAs transport, export factors are necessary; for example, in higher eukaryotes, the splicing of mRNA recruits a protein complex – TREX, which function as an adapter

for mRNA binding protein TAP; for other m RNA molecules, the export does non need splicing events.

The *assembly of NPCs* has a variable dynamics during the cell cycle stages and intesifies at the beginning of telophase.

There are several theories that try to explain the main events of NPCs formation.

- One theory affirms that a complex of nucleoporins (Nups), connected with chromatin, inserts in the double nuclear membrane and determines the fusion of the two membranes at the insertion site; gradually, other nucleoporins bind to the initial Nup complexes, until a full structured NPC is made;
- Other theory states an opposite order of NPC assembly events: first, a prepore formed by several Nup complexes attached at chromatin appears; later, the double nuclear envelope forms around the preporal complexes.

The *disassembly* of NPCs during mitosis is a multiple step process, initiated by the peripheral nucleoporins dissociation (such as Nup 153, Nup 98 and Nup 214), a step thought to be driven by the phosphorylation of Nups. The scaffold nucleoporins of NPCs, which constitute cylindrical ring complexes within NE, remain stable.

More specific, during the close mitosis of *Aspergillus nidulans*, the partial NPCs dissasembly consists in dispersion of at least five nucleoporins throughout cytoplasm, while at least three nucleoporins, with structural function, remains at the NPCs. These mitotic changes in NPCs architecture requires the activation of NIMA and Cdk1 kinases (De Souza et al., 2004).

Considering the lamina as a stuctural and functional part of the nuclear envelope, a series of human diseases are linked with NE protein defects (generally known as *envelopathies* and particullary *laminopathies*).

For instance, mutations in LMNA genes cause many clinical disorders which can be classified into several groups, according the predominantly affected tissues (Worman et al., 2010): the *striate muscle* (Emery-Dreifuss muscular dystrophy, cardiomyopathy dilated 1A, limb-girdle muscular dystrophy type 1B, etc.), the *adipose tissue* (Dunnigan-type familial partial lipodystrophy, lipoatrophy with diabetes and other features of insulin resistance, mandibuloacral dysplasia), the *peripheral nerve* (Charcot-Marie-tooth disease type 2B1) and *multiple tissues* (Hutchinson-Gilford progeria syndrome, atypical Werner Syndrome, variant progeroid disorders etc.).

Defects in other NE proteins could result in other laminopathies: for example, mutations in emerin (or Lamin A/C) may cause Emery-Dreifuss muscular dystrophy while mutations in lamin B receptor (LBR) cause the Pelger-Huët anomaly (PHA), an autosomal dominant disorder. Of all the laminopathies, the Hutchinson-Gilford progeria syndrome (HGPS) is considered one of the most severe syndromes, because the life expectancy of the affected individuals is very low, around 13 years (Chi et al., 2009).

CONCLUSIONS

Since its discovery, at the beggining of the ninetenth century, the nucleus appears as a major rank organelle in all cells life. Since then, countless microscopy, biochemical, functional and genetic studies of nuclei were made. All these researches conclude that nuclei are extremely complex structures, with a high ordered internal organisation and coordination of their subcomponents activities and which play a major role in all cellular activities control, by expressing the genetic information they encompass.

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