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Minireview

Nuclear pore complex assembly through the cell cycle: Regulation and membrane organization

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Abstract In eukaryotes, all macromolecules traffic between the nucleus and the cytoplasm through nuclear pore complexes (NPCs), which are among the largest supramolecular assemblies in cells. Although their composition in yeast and metazoa is well characterized, understanding how NPCs are assembled and form the pore through the double membrane of the nuclear envelope and how both processes are controlled still remains a challenge. Here, we summarize what is known about the biogenesis of NPCs throughout the cell cycle with special focus on the membrane reorganization and the regulation that go along with NPC assembly.

Keywords: Nuclear pore complex; Nuclear assembly; Cell cycle; Nucleoporin

1. Introduction to the nuclear pore complex

The nuclear envelope in eukaryotic cells provides a physical barrier between the nucleoplasm, the site of DNA replication and transcription, and the cytoplasm, the site of protein translation. This separation allows and requires regulated transport of macromolecules such as proteins, RNA and ribonucleoprotein particles across the nuclear envelope. The sole sites of this transport are the nuclear pore complexes (NPCs). The traffic through the NPC is highly specific and regulated, nevertheless incredibly efficient with several hundred translocation events per second and NPC. How NPCs mechanistically mediate this enormous flux of macromolecules is still controversially discussed [1].

Although being indispensable to understand the mechanism of transport, a clear picture of the molecular structure of the NPC is still lacking and many questions about how it is assembled and maintained remain open. The assembly of the NPC must be accompanied with changes in membrane structure to generate the channel through the double membrane of the nuclear envelope in which the NPC is embedded and we will discuss here how this could be achieved. NPC assembly is regulated in space and time and some of the principles of this regulation have become evident in the last years. However, in addition to being a target of cell cycle regulation, it has emerged that the NPC itself controls several aspects of cell cycle progression and we will therefore review what is known about the crosstalk between the NPC and cell cycle regulation.

1.1. Overall structure

NPCs are very large macromolecular assemblies of an estimated mass of 66 Mega Dalton (MDa) in yeast and 125 MDa in vertebrates. Despite this mass difference, the fundamental architecture of NPCs is conserved between yeast and metazoa as shown by three-dimensional models of the NPC derived from electron microscopy.

Recently, state of the art cryo-electron tomography of NPCs in *Dictyostelium discoideum* has achieved a resolution of below 6 nm [2,3]. These studies confirm and extend previous structural observations (Fig. 1A): the complex has an eight-fold rotational symmetry and can be divided in three main parts: a central core in the plane of the nuclear envelope, a nuclear basket, and cytoplasmic filaments. The central core is formed by three distinct rings structures all of which contact the membranes of the nuclear envelope: Centrally, the spoke ring is composed of eight clamp-shaped structures that are attached to the membrane at two sites. This spoke ring is sandwiched between a cytoplasmic and a nucleoplasmic ring. The central core is decorated by eight extended filaments on the cytoplasmic side and by a basket-like structure connected with a distal ring on the nuclear side. The improved resolution of Beck et al. allowed the visualization of a luminal connector element that spans the space between outer and inner nuclear membrane and is attached to opposite sides of the membrane, where the cytoplasmic and nuclear rings make contact.

As informative as this cryo-electron tomography studies are, they do not provide sufficient resolution to study molecular details of the individual proteins of the NPC, the nucleoporins. Obtaining atomic resolution data of entire NPCs by X-ray crystallography seems an impossible undertaking for the near future. However, significant progress has been made in understanding the structures of single nucleoporins. Several high resolution structures of nucleoporins or fragments of nucleoporins are available [4–7, and references therein]. With more and more high resolution structures of individual

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Abbreviations: NPC, nuclear pore complex; ER, endoplasmic reticulum; MDa, Mega Dalton; ALPS, ArfGAP1 lipid packing sensor

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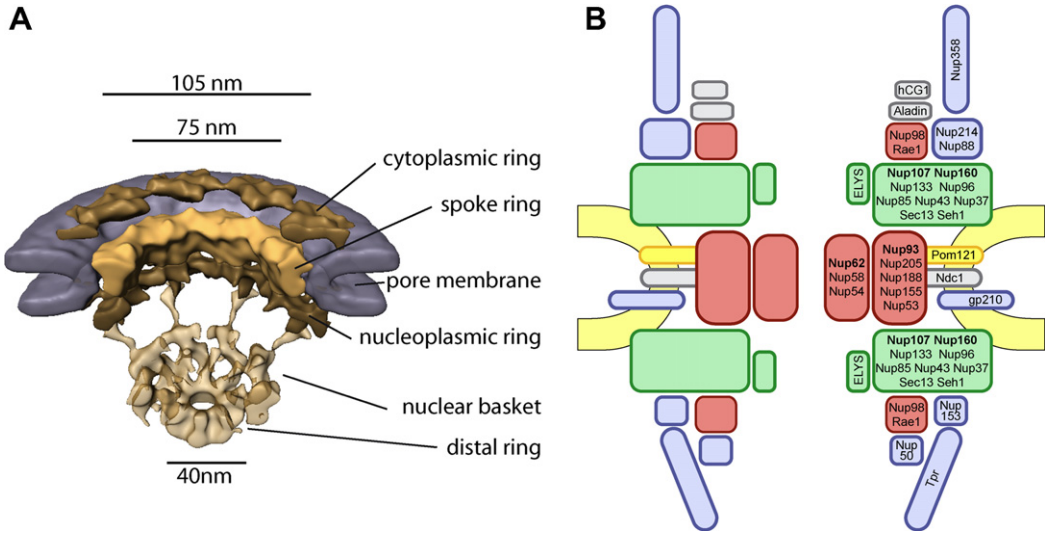

Fig. 1. Structure of the NPC. (A) Reconstruction of the *Dictiostylium* NPC from cryo-electron tomography (adapted by permission from Macmillan Publishers Ltd.: Nature, Beck et al., copyright 2007). Cytoplasmic filaments are not visible and a mass density in the centre of the NPC which is at least partially ascribed to transported substrate has been omitted. Membranes are shown in grey. (B) Diagram of the modular structure of the metazoan NPC composed of different biochemically characterized subcomplexes. The exact position of many nucleoporins in the NPC is unknown and therefore drawn schematically. Nucleoporins and complexes recruited during the first, second, third and forth step of NPC assembly as described in the text are colored in green, yellow, red and blue, respectively. For nucleoporins colored in grey, the assembly time points have not been analyzed so far.

nucleoporins and eventually NPC subcomplexes of the NPC being solved and further improvements in electron microscopy techniques it will hopefully become feasible to fit atomic resolution structures into the overall low resolution structure of the NPC in the not too distant future.

Recently, Alber et al. took an alternative approach to bridge the gap between the overall structural and biochemical information [8,9]: integrating data about the stoichiometry, localization, shape of and protein–protein interactions between each of the nucleoporins they modeled the best fit to place each nucleoporin in the frame of the known size and symmetry of the yeast NPC. This model predicts that the central core is composed of two inner and two outer rings. The outer rings could relate to the nucleoplasmic and cytoplasmic rings described previously by electron microscopy. It remains to be seen to which extent the inner rings would correspond to the one spoke ring seen before. The authors also propose that each of the eight symmetrical spokes is split into two very similar columns formed by duplicated or homologous nucleoporins thus suggesting that the entire complex may have a 16-fold rotational symmetry. However, this interesting predicted feature awaits confirmation by direct structural investigation.

1.2. Composition

Given their enormous size, NPCs are built by a surprisingly small number of approximately 30 nucleoporins. Nucleoporins can be quite large with up to 360 kDa and due to the eight-fold symmetry of the NPC are thought to come in multiples of eight. Nucleoporins can be roughly categorized into "unstructured" and "structured" based on the presence or absence of hydrophobic phenylalanine–glycine (FG) repeats. FG repeat containing stretches of nucleoporins presumably line the channel with unstructured filaments that are assumed to constitute the permeability barrier of the NPC. In contrast, nucleoporins or stretches of them devoid of FG repeats are thought to be structured and form the backbone of the overall architecture of the NPC. Bioinformatic analysis predicts that the structured parts of nucleoporins consists of only two different fold types, repeating alpha helixes (in an alpha-solenoid fold) and zigzagging beta sheets (in a beta-propeller fold) [10]. Many of the solved structures of nucleoporins support this prediction, but recent data shows that the structural repertoire of the NPC is more complex. Although yeast Nic96p and Nup145 do contain the predicted alpha-helices, those are not arranged in a typical alpha solenoid fold [5,6,11].

The NPC is firmly anchored in and has extensive contact with the nuclear membrane, however, only three nucleoporins, the pore membrane proteins (Poms) contain transmembrane domains and only one of them, Ndc1, is conserved [12]. Pom152p in yeast is speculated to have its equivalent in gp210 in metazoa as both contain a single transmembrane region and expose the largest part of the protein to the intermembrane space of the nuclear envelope. The third, Pom34p is only found in yeast and has no structural similarity to the vertebrate Pom121.

The modular organization of the NPC is not only evident in its overall eight-fold symmetry with central spokes, nucleoplasmic basket and cytoplasmic filaments. The NPC can also be dissected biochemically into distinct subcomplexes both in yeast and higher eukaryotes ([8], see [7] for review), which are thought to act as building blocks of the NPC (Fig. 1B). Three major complexes make up the major mass of the NPC, in vertebrates these are the Nup107–160, Nup93 and Nup62 complexes. These are stably bound within the NPC, whereas several more peripheral nucleoporins exchange rapidly with a free pool [13]. Although the individual nucleoporins often show only limited sequence conservation between yeast and vertebrates, the overall structure and composition of these subcomplexes appear to be conserved during evolution.