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Review

Nuclear pore complex tethers to the cytoskeleton

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ABSTRACT

The nuclear envelope is tethered to the cytoskeleton. The best known attachments of all elements of the cytoskeleton are via the so-called LINC complex. However, the nuclear pore complexes, which mediate the transport of soluble and membrane bound molecules, are also linked to the microtubule network, primarily via motor proteins (dynein and kinesins) which are linked, most importantly, to the cytoplasmic filament protein of the nuclear pore complex, Nup358, by the adaptor BicD2. The evidence for such linkages and possible roles in nuclear migration, cell cycle control, nuclear transport and cell architecture are discussed.

Contents

1. Introduction

There was a time when the nucleus was considered a straightforward protective container for the cell's genes. Later it became evident that the chromosomes had to be organized specifically within the nucleus [1] and that this organization is dynamic on a long (epigenetic) or short (gene regulation) timescale. An important structure in these processes is the nuclear envelope, a double membrane system, continuous with the endoplasmic reticulum, that encloses the genome and partially separates it from the cytoplasm. The nuclear lamina and other components of the inner nuclear membrane, are primarily involved in epigenetic regulation, whereas the nuclear pore complex (NPC), or proteins of the NPC (nucleoporins) that are not necessarily associated with the NPCs, have roles in short timescale regulation (see [2] for review).

Recently it has become evident that the role of the nucleus can also extend out into the cytoplasm. To a varying degree, the nucleus is the predominant, and usually by far the most massive, organelle in the cell. It is therefore not surprising that it should have a dominant influence, as well as be used as a platform, for determining cell architecture and function. The LINC complex (LInker of Nucleoskeleton and Cytoskeleton) is the best known anchor of cell architecture to the nucleus [3]. It contains SUN domain pro-

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teins which are integral membrane proteins, primarily of the inner nuclear membrane, which interact with the nuclear lamina, chromatin and other "nucleoskeletal" structures. The SUN domain of these proteins is in the intramembrane lumen of the nuclear envelope (NE) and binds to KASH domains of the so-called nesprins. Within the LINC complex, nesprins are primarily outer nuclear membrane proteins. There are five human nesprins and multiple isoforms of these [4], which differ in their ability to bind to microtubules, F-actin and intermediate filaments. Therefore the primary function of the LINC complex appears to be to link the structural organization of the nuclear interior to the cytoskeleton. Due to the diversity of interactions, as well as the diverse roles of each of the elements of the cytoskeleton (determined by a plethora of binding proteins), the functions of the LINC complex are complex, diverse and poorly understood, but they appear to include mechnotransduction [5,6], polarity [7], and involvement in a variety of diseases [8].

The other link between the nuclear interior and the cytoplasm is the NPC, the subject of this review. The nuclear membranes present a more-or-less chemically impermeable barrier. Ion channels are present in both inner and outer membranes providing communication between the NE lumen and nucleus or cytoplasm, respectively [9]. NPCs provide a size dependent selective permeability barrier between the nucleus and cytoplasm [10]. NPCs allow diffusion [11] of small molecules (solutes to small proteins) but are impermeable to larger macromolecules, unless they bare signals that allow them to bind nuclear transport factors. Transport factors then facilitate movement of primarily RNAs and proteins through the selective barrier by interacting with the FG domains and affecting the functional organization of the barrier. Directionality in protein, and some RNA, transport is determined by the asymmetrically located Ran system that disassembles import complexes in the nucleus and export complexes in the cytoplasm [12]. mRNA export is more complex because it is coupled to RNA processing and translation, but similarly involves displacement of transport factors on the cytoplasmic side of the NPC [13].

The NPC is arguably the largest protein complex in eukaryotic cells, with an estimated molecular mass of about 100 MDaltons, and 500–1000 individual proteins. It consists of a scaffold, whose structure has been extensively investigated at close to atomic detail [14]. The scaffold consists of a series of stacked rings [15] which stabilize the membrane annulus and provide a platform for organizing the selective barrier. The barrier consists of fully or partially disordered "FG-domains", in the central channel [16]. Peripheral structures such as the nucleoplasmic basket and cytoplasmic filaments [17,18,19,86] are also anchored to the nucleoplasmic and cytoplasmic rings, respectively. In lower eukaryotes, such as yeasts, NPCs are thought to be mobile and move in the plane of the double membrane. There movement may be directed by microtubules, at least during cell division [20]. In more complex metazoa, however, interphase NPCs appear to be anchored to the nuclear lamina [21] Fig. 1), a complex intermediate filament network that lines the inner nuclear membrane. How, and whether, NPCs are anchored in plants [22] and other organisms is not so clear. The nuclear lamina, amongst other roles, is a major component of a mechanically robust inner shell for the NE [23]. Together with other inner nuclear membrane proteins, the lamina also has crucial roles in organizing the interphase chromosomes, such as tethering telomeres, peripheral localization of inactive heterochromatin, determining chromosome and gene positioning and gene activation [2].

Because NPCs appear immobile during interphase [21], and because NPC proteins have been shown to interact with lamins [24], it is assumed that NPCs are fixed in space by stable tethering to the lamina [25]. Indeed depletion of lamins *in vitro* [26] and in cells [27] leads to NPC clustering and redistribution. However, Nup153, a lamin interacting nucleoporin, is dynamic [21] and is therefore not a good candidate for a protein that maintains a stable interaction. There is also growing evidence for attachments of the NPCs to other cytoskeletal components of the cell, which may be either stable or dynamic. This includes indirect connections via the LINC complex, or direct association of microtubule motor complexes, which mediate the association of NPCs with microtubules. It is these NPC-cytoskeleton linkages that will be the focus of this review.

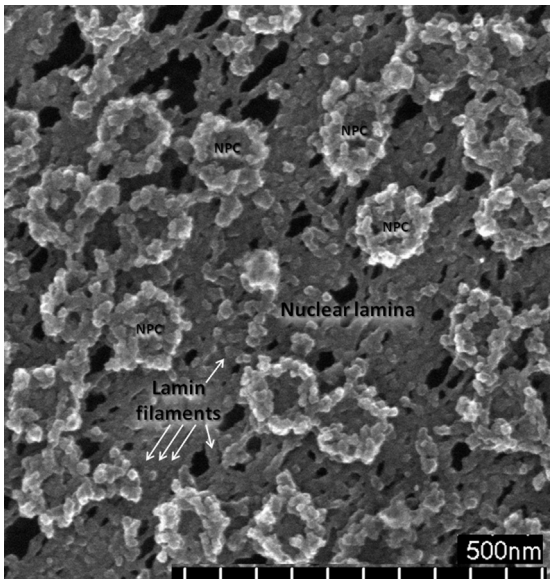

Fig. 1. NPCs are anchored to the nuclear lamina. Isolated *Xenopus laevis* nuclear envelope was extracted with non ionic detergent, Triton X-100 to remove the membranes and imaged by high resolution scanning electron microscopy, showing NPCs embedded in and linked to the filamentous lamin network (white arrows).

2. NPCs and the LINC complex

The relationship between the NPC and the LINC complex has been recently reviewed [28] and therefore will not be the focus of this review which will instead concentrate on the direct linkages between the NPC and cytoskeleton. However if the LINC Complex does provide an indirect NPC-cytoskeleton association, this must be considered from a functional and experimental point of view. The only published association of LINC complex components with NPC components, is that SUN1 (not SUN2) localizes to the NPCs [29] and that SUN1, tagged with GFP at the C-terminus, co-localizes with immunofluorescence of Nup153 [30]. Nup153 is a nucleoplasmic nucleoporin [31,32], that may be part of the basket [33] or nucleoplasmic ring [34]. Nup153 interacts with, and is recruited to the NPC *in vitro* by lamins [24]. Anti-GFP immuno-electron microscopy showed that SUN1-GFP localized very close to the outer edge of the NPC [30], consistent with the location of Nup153 [34]. SUN1 depletion results in clustering of NPCs [30], similar to Nup153 depletion [34], suggesting that SUN1 is required either for tethering NPCs or to correctly organize NPCs within the NE.

As SUN1 is a component of the LINC complex, and SUN1 binds strongly to KASH domain proteins [35], it is possible that NPC-associated SUN1 forms a LINC complex that locates one or more elements of the cytoskeleton to the NPC via one or more nesprin isoforms. Although it is reasonable to suppose that at least one nesprin isoform would therefore be located as part of a LINC complex to the NPC, there is no published data to support this and it is possible that SUN1 acts alone, or with different partners, at the NPC.

Surprisingly, Nup153 was also shown to interact directly with adenomatous polyposis coli (APC), promoting the association with