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COMMENTARY

The LINC and NPC relationship – it's complicated!

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ABSTRACT

The genetic information of eukaryotic cells is enclosed within a double-layered nuclear envelope, which comprises an inner and outer nuclear membrane. Several transmembrane proteins locate to the nuclear envelope; however, only two integral protein complexes span the nuclear envelope and connect the inside of the nucleus to the cytoplasm. The nuclear pore complex (NPC) acts as a gateway for molecular exchange between the interior of the nucleus and the cytoplasm, whereas so-called LINC complexes physically link the nucleoskeleton and the cytoskeleton. In this Commentary, we will discuss recent studies that have established direct functional associations between these two complexes. The assembly of NPCs and their even distribution throughout the nuclear envelope is dependent on components of the LINC complex. Additionally, LINC complex formation is dependent on the successful localization of inner nuclear membrane components of LINC complexes and their transport through the NPC. Furthermore, the architecture of the nuclear envelope depends on both protein complexes. Finally, we will present recent evidence showing that LINC complexes can affect nucleo-cytoplasmic transport through the NPC, further highlighting the importance of understanding the associations of these essential complexes at the nuclear envelope.

KEY WORDS: LINC complex, Nuclear pore complex, Nups, SUN–KASH complex, Lamin, Nuclear envelope

Introduction

The genomic information in eukaryotic cells is protected by a double-layered nuclear membrane, which compartmentalizes the cell into two regions, the nucleus and the cytoplasm. Despite the spatial separation of the cytoplasm and the nucleus, their functions are tightly coupled and regulate various cellular functions. This coupling is achieved through two different protein complexes: (i) nuclear pore complexes (NPCs), as the biochemical gateway for nucleocytoplasmic traffic, and (ii) the linkers of the nucleoskeleton and cytoskeleton (LINC) complexes, which act as a physical linkage (Fig. 1A). Although NPCs and LINC complexes are often considered as separate entities that link the nucleus and the cytoplasm, various studies have shown that some functions of NPCs and LINC complexes are intimately associated.

NPCs are the unique gateways for the bidirectional transport of cargo between the nucleus and the cytoplasm. As the largest macromolecular complexes in the nuclear envelope, NPCs are responsible for translocation of vital cargos, ranging from different types of RNAs to various proteins (Raices and D'Angelo, 2012). NPCs are made up of almost 30 different types of proteins called nucleoporins (Nups) (Fig. 1B), which can be generally categorized into two major groups, FG Nups (Nups rich in phenylalanine–glycine repeats) and non-FG Nups (Nups that have no or a few phenylalanine–glycine repeats) (Jamali et al., 2011). FG Nups possess disordered regions (lacking secondary structure), which are responsible for the transport of cargos through the NPC. In addition to the overall structure of the NPC that has been conserved through evolution, recent bioinformatics studies have identified specific evolutionarily conserved features in the disordered regions of the sequences of Nups, which are believed to regulate cargo transportation (Ando et al., 2013; Peyro et al., 2015a). Other Nups primarily constitute the building blocks of the NPC scaffold, which is tightly connected to transmembrane proteins and the nuclear envelope. Beside controlling the nucleocytoplasmic traffic processes, NPCs are involved in many other vital processes in the cell, such as DNA repair, gene expression, aging, apoptosis, determination of cell differentiation (Nagai et al., 2008), and nucleo-cytoskeletal coupling and mechanobiology (Soheilypour et al., 2016). However, the underlying mechanism of transport is still under much debate, despite the fact that a multitude of computational and experimental studies have been performed with the aim of shedding light on how exactly different cargos are transported through the NPC (Yamada et al., 2010; Moussavi-Baygi et al., 2011; Labokha et al., 2012; Azimi et al., 2013; Ghavami et al., 2014; Zhao et al., 2014; Peyro et al., 2015b; Hough et al., 2015; Milles et al., 2015; Ma et al., 2016; Moussavi-Baygi et al., 2016).

Aside from the NPC, another type of protein complex provides a physical linkage between cytoskeletal and nucleoskeletal elements and so allows a direct transmission of mechanical signals through the nuclear envelope. In 2006, two independent studies presented components of these complexes, which are now known as LINC complexes (Crisp et al., 2006; Padmakumar, 2005; McGee, 2006). In metazoans and fission yeast, the main components of LINC complexes include proteins of the Klarsicht, ANC1 and Syne homology (KASH), and the Sad1p/UNC (uncoordinated)-84 (SUN) families, which interact in the perinuclear space (PNS) (Fig. 1C). SUN proteins contain a SUN domain that resides in the PNS and is preceded by a coiled-coil region that spans the remaining space towards the inner nuclear membrane. Finally, the transmembrane and nucleoplasmic domains of SUN proteins link the interior of the nucleus to the PNS (Sosa et al., 2013). SUN1 and SUN2 interact with elements of the nucleoskeleton and their nucleoplasmic domains bind to lamin A (Liu et al., 2007), as shown in Fig. 1. In *Caenorhabditis elegans*, the SUN protein UNC-84 is known to bind to the lamin LMN-1 (Bone et al., 2014). The nuclear anchors for SUN proteins are not limited to lamins. In yeast, which lack lamins, LINC complexes are anchored to chromosomes and promote chromosome movements (Morimoto et al., 2012). Furthermore, the nucleoplasmic domain of SUN proteins directly binds to chromatin in mammalian cells (Xiong et al., 2008).

KASH-domain-containing proteins comprise a short luminal peptide that resides in the PNS where it binds to the SUN domain of

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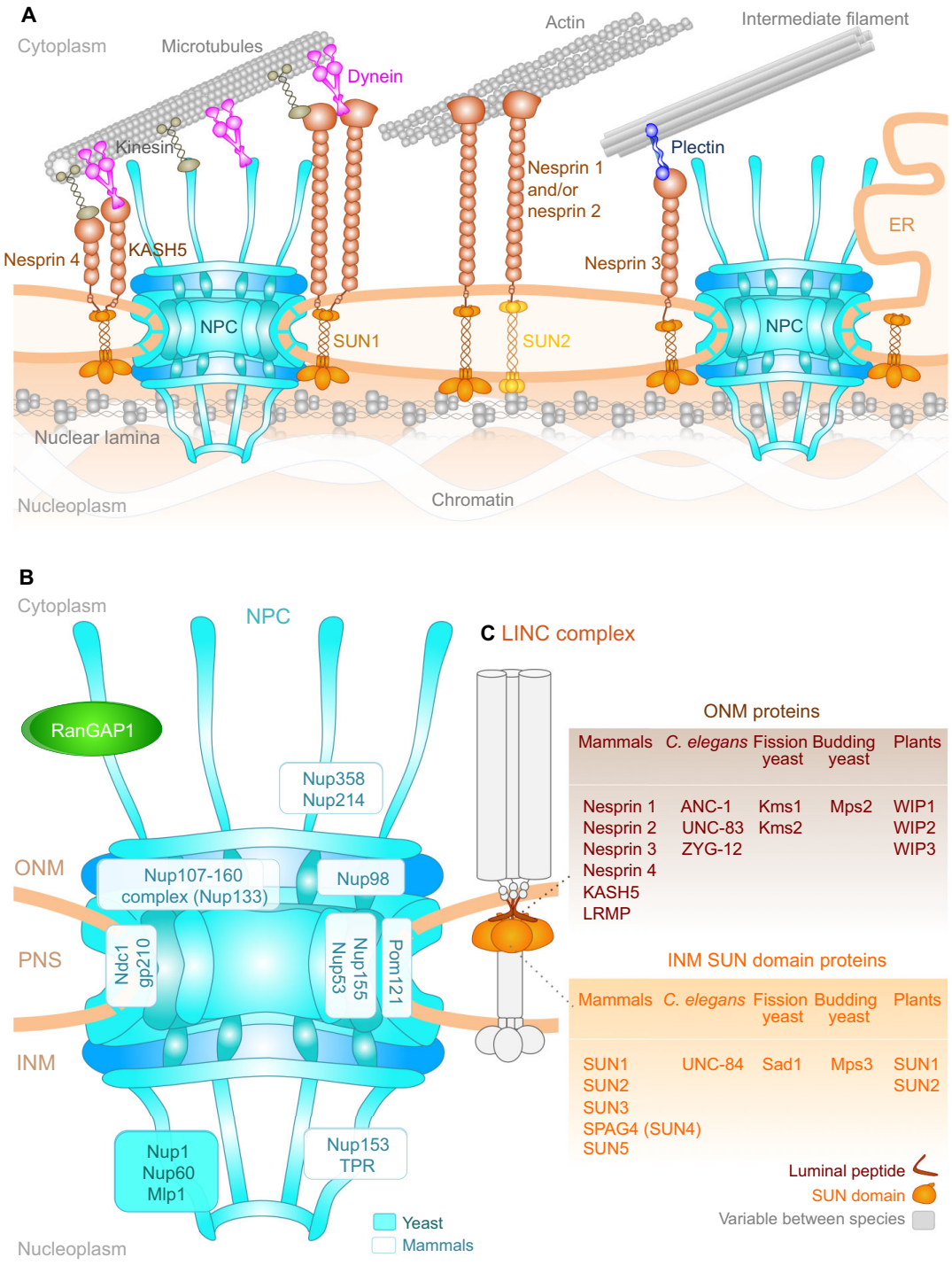

Fig. 1. See next page for legend.

SUN proteins (Fig. 1C). *In vitro* structural and biochemical characterization of the SUN–KASH complexes shows that three KASH proteins can simultaneously bind to a SUN trimer (Zhou et al., 2012a,b; Sosa et al., 2012; Nie et al., 2016) (Fig. 1C). To date, six mammalian KASH-domain proteins have been identified, including a family of four nuclear envelope spectrin-repeat proteins (nesprins), KASH5 and lymphocyte-restricted membrane protein (LRMP) (Lindeman and Pelegri, 2012; Morimoto et al., 2012; Horn et al., 2013; Rajgor and Shanahan, 2013; Luxton and Starr, 2014; Padmakumar, 2005) (Fig. 1C). Nesprin 1 and nesprin 2 are the largest isoforms of the nesprin family and are the only two that are known to directly interact with actin through their calponin homology actin-binding domain (Crisp et al., 2006; Sosa et al., 2013; Lombardi et al., 2011) (Fig. 1A). LINC complexes are also attached to the microtubule network; this is indirect and mediated through motor proteins. Nesprin 1 and nesprin 2, as well as nesprin 4 and KASH5 interact with microtubules through kinesins and dynein (Roux et al., 2009; Horn et al., 2013; Fan and Beck, 2004;

Fig. 1. Schematic representation of LINC complexes and NPCs, and their associations at the nuclear envelope. (A) Illustrated here are interactions of LINC complexes and NPCs with the cytoskeleton, nuclear membranes, the nucleoskeleton, as well as each other, at the nuclear envelope. In the cytoplasm, both NPCs and LINC complexes associate with microtubules through kinesin and dynein motor proteins. LINC complexes also interact directly with the actin cytoskeleton and with intermediate filaments through plectins. In the nucleoplasm, both NPCs and LINC complexes interact with nuclear lamina and chromatin. LINC complexes and NPCs also associate directly or indirectly with each other through interactions between Nup153 and the nucleoplasmic domain of SUN proteins. SUN1, but not SUN2, is directly associated with NPCs. (B) Schematic representation of the NPC architecture based on recent X-ray crystallographic analyses of the NPC structure (Lin et al., 2016). The location of nucleoporins (Nups) that are involved in interactions of the NPC with the cytoskeleton, the nucleoskeleton, the nuclear envelope or LINC complexes are indicated. Nup358 (also known as RANBP2) and Nup214 are localized to the cytoplasmic filaments of NPCs of vertebrates. Ndc1, gp210 (also known as Nup210) and Pom121 are transmembrane proteins that are mainly involved in integration of the NPC into the nuclear envelope. Nup153 (Nup1 and Nup60 are yeast homologs) and TPR (Mlp1 is the yeast homolog) are nuclear basket Nups. The Nup107-160 complex is one of the building blocks of the NPC scaffold. (Please note that only the Nups discussed in this review are labeled. Moreover, some nucleoporins such as Nup98, appear both at the cytoplasmic and the nucleoplasmic sides, and are symmetrically distributed in the NPC structure; however, only one occurrence of each nucleoporin is depicted in the figure.) (C) LINC complexes are formed through the interaction of SUN-domain proteins located at the INM and a short luminal peptide of a number of ONM proteins in the PNS. The inset provides a list of LINC components in various species. In metazoans and fission yeast, the ONM component of LINC complexes comprises KASH-domain proteins that reside mostly in the cytoplasm; these have a short KASH domain that extends into the PNS where it interacts with SUN-domain proteins. In budding yeast and plants, ONM-residing LINC constituents do not contain the conserved KASH domain but perform a similar biological role. SUN proteins are shown as trimers based on structural studies of human SUN2 in complex with the KASH domain of nesprin 2 (Protein Data Bank IDs 4FI9 and 4DXS; Zhou et al., 2012; Sosa et al., 2012). However, the oligomer state of other SUN proteins has not yet been determined. It should also be noted that it is not yet known whether two different KASH proteins can bind to the same SUN trimer, or whether KASH proteins bound to a SUN protein can associate with different cytoskeletal elements.

Morimoto et al., 2012) (Fig. 1A), whereas nesprin 3 associates with intermediate filaments through plectin (Wilhelmsen, 2005). LINC complexes are also present in budding yeast and plants; however, the luminal peptide of the outer nuclear membrane (ONM) component of these complexes lacks the conserved KASH domain found in metazoans (Fig. 1C). For a full list of LINC interacting partners please refer to Rothballer and Kutay (2013b).

The composition of the nuclear envelope is highly tissue specific, and more than 215 nuclear envelope transmembrane proteins have been identified to date (for a complete review see Wilson and Berk, 2010). In this Commentary, we focus specifically on the roles and functions of the NPC and LINC complexes that are associated with the nuclear envelope, as the structural integrity of the nuclear envelope depends on both complexes. Because LINC complexes directly interact with various cytoskeletal elements, we argue that they play an important role in the association of NPCs with the cytoskeleton. Furthermore, the association of both these complexes with the nucleoskeleton is likely to be co-dependent. Although early studies have shown no significant role of LINC complexes in the nuclear transport of proteins through NPCs (Liu et al., 2007), more-recent studies discussed here have drawn connections between nucleocytoplasmic transport through NPCs and LINC complexes, further highlighting the importance of better understanding the functional and structural links between these two essential components of the nuclear envelope.

Role of the LINC complex in the assembly of NPCs and in their distribution on the nuclear envelope

The first observation of a functional association between LINC and NPCs came from a study in 2007 in Hela cells (Liu et al., 2007); there, the authors showed that both the luminal and nucleoplasmic domains of SUN1 are involved in and required for its association with NPCs. Interestingly, many studies have shown partially redundant roles for SUN1 and SUN2 in several cellular functions, including anchoring of nuclei (Lei et al., 2009), DNA damage response (Lei et al., 2012) and centrosome–nucleus coupling (Zhang et al., 2009), as well as in the development of the retina outer nuclear layer (Yu et al., 2011). However, SUN1 and SUN2 have independent roles at NPCs. Liu et al. have shown that only SUN1, and not SUN2, colocalizes with Nup153. Furthermore, the distribution of NPCs across the nuclear envelope is altered only upon disruption of SUN1 (Liu et al., 2007). The authors also show that SUN1 proteins affect the distribution of NPCs independent of KASH-domain nesprin proteins, which couple SUNs to the cytoskeleton (Liu et al., 2007). A later study has also shown that NPCs colocalize with SUN1, but not with SUN2 (Lu et al., 2008), suggesting that not all SUN proteins colocalize with NPCs. This has since been confirmed when it was reported that SUN1 only associates with NPCs that are formed during interphase, and not with postmitotic NPCs (Talamas and Hetzer, 2011).

Interphase NPCs assemble through different mechanisms to those of postmitotic NPCs. In the initial postmitotic phase, some NPCs reassemble before the nuclear envelope is completely formed; however, later on during interphase, NPCs assemble *de novo* into a highly organized nuclear envelope and thus are limited by the spacing between the inner nuclear membrane (INM) and ONM (D'Angelo et al., 2006). Thus, an early step in NPC assembly during interphase is the local fusion of the INM and ONM at sites of NPC assembly, a step that SUN1 is likely to be involved in (shown in Fig. 2). Through such *de novo* NPC assembly at the site of membrane expansion when the nucleus grows after mitosis, the density of NPCs on the nuclear envelope remains constant (Fig. 2). SUN1 associates with the transmembrane Nup Pom121 to reduce membrane spacing, which might promote membrane fusion (Talamas and Hetzer, 2011; Jaspersen and Ghosh, 2012) (Fig. 2). In agreement with previous work (Liu et al., 2007), these studies have also shown that both the nucleoplasmic and transmembrane domains of SUN1 are required for the localization and association of SUN1 with the NPC. This suggests that the interaction between the nucleoplasmic domain of SUN1 and the NPC is indirect or transient, as any biochemical interaction between SUN1 and NPCs has not been formally proven yet. The effect of SUN1 on the distribution of NPCs is likely to be due to an inhibition of *de novo* NPC assembly, which reduces the total number of NPCs on the nuclear envelope, thus explaining the apparent clustering of NPCs (Fig. 2). Furthermore, although SUN1 is essential, its interaction with KASH proteins does not have a substantial role in NPC assembly (Talamas and Hetzer, 2011).

Lamins also have a vital role in maintaining the mechanical integrity of the nuclear envelope and in positioning the NPCs by acting as their anchoring points; indeed, both Nup53 and Nup153 associate with A- and B-type lamins, and Nup153 has multiple binding sites for A- and B-type lamins (for a full review see Fiserova and Goldberg, 2010). Interestingly, both the N- and C-terminal domains of Nup153 directly interact with lamins; however, some studies suggest that this interaction is partially dependent on SUN1 (Liu et al., 2007; Fiserova and Goldberg, 2010). Furthermore, owing to the strong association between Nup153 and lamins,

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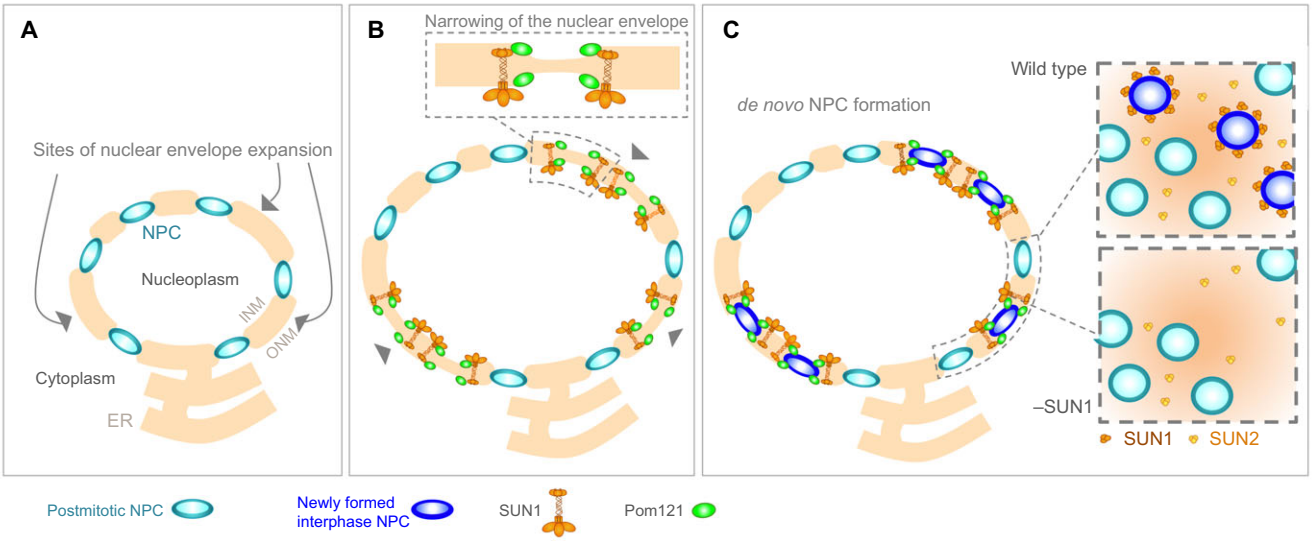

Fig. 2. SUN1-mediated *de novo* NPC assembly. (A) During interphase, the nucleus grows and the nuclear envelope expands at specific locations (gray arrows); however, the density of NPCs remains constant. (B) The nuclear envelope narrows at sites of nuclear envelope expansion; here, the interaction of SUN1 with nucleoporin Pom121 helps to narrow the nuclear envelope and so promotes *de novo* NPC assembly. (C) Assembly of NPCs at sites where the nuclear envelope has narrowed; these newly formed NPCs remain associated with SUN proteins. In the absence of SUN1, there is no *de novo* NPC assembly, resulting in an overall reduction in the total number of nuclear pores; therefore, NPCs appear clustered in SUN1-depleted cells (lower inset). Note that SUN1 and SUN2 are both shown as trimers; however, SUN1 and SUN2 oligomeric states during various cellular processes have yet to be validated *in vivo*.

Nup153 might have a role in laminopathies (Al-Haboubi et al., 2011), a set of diseases that have been highly correlated with disruptions of the nucleo-cytoskeletal coupling that is mediated through LINC complexes (Hale et al., 2008; Yang et al., 2013; Méjat and Misteli, 2010). Furthermore, lamins are required for the even distribution of NPCs; in their absence, cytoskeletal forces exerted by dynein can pull NPCs toward the centrosome, resulting in their clustering (Guo and Zheng, 2015).

Trafficking of SUN proteins across the NPC for INM localization

The first step in LINC complex formation is the transport of SUN-domain proteins from their site of synthesis in the endoplasmic reticulum (ER) to their final destination in the INM. This process is NPC dependent and involves three major steps (Fig. 3). First, SUN-domain proteins, which are inserted into the ER membrane must be translocated from the ER to the ONM where NPCs reside (Fig. 3A, step 1). Next, these proteins must move across the NPC to reach the INM (Fig. 3A, step 2) and finally be retained in the INM where they can bind to KASH-domain proteins (Fig. 3A, step 3). The mechanisms of the nuclear import of soluble proteins are well known. Small molecules and ions (<∼9 nm or 40 kDa) can freely diffuse through NPCs, however larger molecules (up to ∼40 nm or 25 MDa) contain a nuclear localization signal or sequence (NLS), which is recognized by transporter proteins called importins (Pante and Kann, 2002; Jamali et al., 2011). Importins then mediate the transport of cargo through the central pore of the NPC.

SUN-domain proteins traverse the NPC through mechanisms that are similar to those of large soluble molecules. With the exception of UNC-84, the nucleoplasmic domains of most SUN proteins are within the 40-kDa free diffusion range (see Fig. 3B); however, these proteins are unlikely to freely diffuse through the NPC owing to their anchorage to the membrane and their large luminal domains. Indeed, several SUN proteins have experimentally been proven to contain nuclear-targeting sequences that play roles in their INM localization (Fig. 3B) (Daryabeigi et al., 2016; Tapley et al., 2011; Hasan et al., 2006; Graumann et al., 2010; Chen et al., 2014). Human SUN2, UNC-84 and plant SUN1 and SUN2 all contain an NLS (Turgay et al., 2010; Graumann et al., 2010; Tapley et al., 2011). The NLS of human SUN2 has been shown to directly bind to the importin-α–importin-β heterodimer, which might mediate its transport through the NPC (Fig. 3B,C) (Turgay et al., 2010). SUN1 and UNC-84 contain a SUN-specific nuclear envelope localization sequence named SUN-NELS; however, the importin which recognizes this signal has yet to be determined (Hasan et al., 2006). Another nuclear-targeting sequence found in several INM proteins, including the SUN-domain protein UNC-84, is a short positively charged sequence adjacent to the transmembrane domain called the INM-sorting motif (INM-SM). As INM proteins are inserted into the ER, their INM-SM is recognized by a small membrane-associated isoform of importin α, Impα-16 (encoded by *KPNA4* in mammals), which remains associated with these proteins and potentially mediates their transport across the NPC (Braunagel et al., 2007; Tapley et al., 2011). Finally, the budding yeast SUN protein Mps3 contains a nuclear-targeting domain (Mps3-NTD) that directly binds to yeast histone variant Htz1 (H2A.Z), which contains an NLS-like sequence recognized by several yeast importins including Kap123 (Gardner et al., 2011) (Fig. 3C). We have not found any information on the nuclear-targeting mechanisms of the fission yeast SUN protein Ndc1, or human SUN3, SUN4 or SUN5 proteins.

Although the role of nuclear-targeting sequences of SUN proteins in INM localization is evident, the mechanisms of SUN protein trafficking across the NPC are poorly understood (Fig. 3A, step 2). Recent structural models of the NPC reveal that a considerable disruption of the NPC structure is required for these relatively large proteins of the INM to diffuse across the NPC (Lin et al., 2016). Specifically, the luminal domains of SUN proteins must overcome the POM layer within NPCs (see Box 1). One hypothesis is the existence of ∼10-nm peripheral channels within each spoke of the NPC through which INM proteins could be transported (Maimon et al., 2012; Katta et al., 2014). Conversely, a more recent structural

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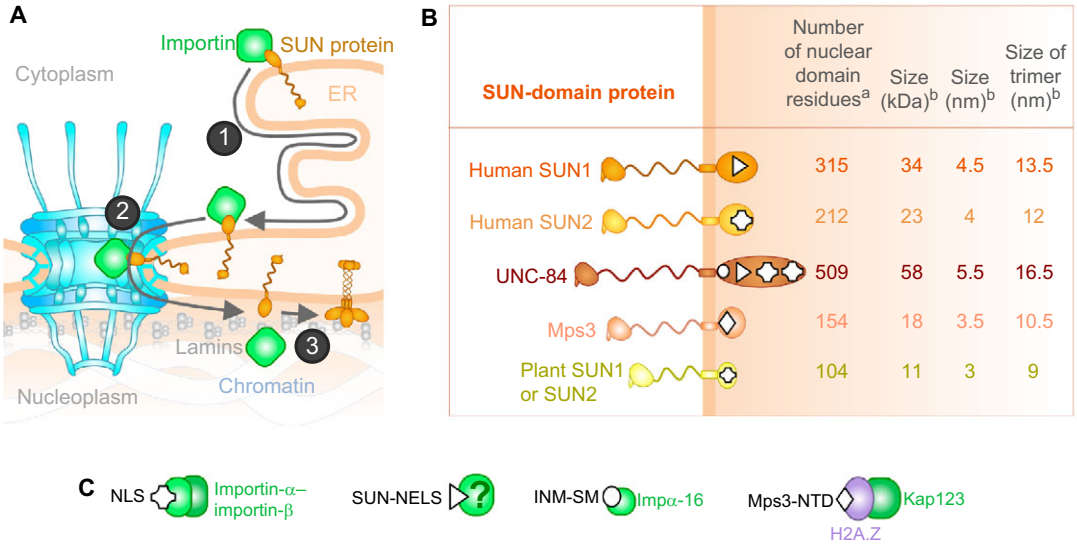

Fig. 3. Transport mechanisms of SUN-domain proteins across the NPC for INM localization. (A) SUN-domain proteins are translocated from their site of synthesis in the ER to the ONM (1) and across the NPC to the INM (2), where they are retained and perform most of their known functions (3). SUN-domain proteins are likely to be transported across the NPC by transporters (importins) that recognize nuclear-targeting sequences on the nucleoplasmic domains of SUN proteins. (B) The size of the nucleoplasmic domains of SUN-domain proteins, including the number of residues, molecular mass (size, kDa) and diameter (size, nm). Sizes of the nucleoplasmic domains of SUN trimers have also been estimated. Each SUN protein contains a nuclear-targeting sequence on its nucleoplasmic domain, as shown. [a]The number of nucleoplasmic residues for each SUN-domain protein was extracted from uniprot.org. [b]All molecular weights were estimated using the ExPASy Compute pI/Mw tool (Gasteiger E et al., 2005). Sizes in nm were calculated using the 'Protein size from MWt' tool (http://www.calctool.org/). Note that we found no information on the nuclear-targeting mechanisms of fission yeast SUN-domain protein Sad1 and hence did not include it. (C) Importin proteins recognize the nuclear targeting sequences on SUN proteins and mediate their transport across the nuclear pore. NLSs can bind to the importin-α–importin-β heterodimer (Turgay et al., 2010). The transporter protein that recognizes SUN-NELS is unknown (Tapley et al., 2011; Hasan et al., 2006). INM-SM is recognized by a small isoform of importin α, Impα-16 (encoded by *KPNA4* in mammals). Mps3-NTD binds directly to yeast histone variant Htz1 (H2A.Z); H2A.Z contains an NLS recognized by the yeast importin Kap123 (Gardner et al., 2011). NLS, nuclear localization signal (cross) (Turgay et al., 2010; Tapley et al., 2011; Graumann et al., 2010); SUN-NELS, SUN-specific nuclear envelope localization signal (triangle) (Tapley et al., 2011; Hasan et al., 2006); INM-SM, inner nuclear membrane sorting motif (circle) (Tapley et al., 2011); Mps3-NTD, budding yeast SUN-domain protein Mps3 nuclear targeting domain (diamond) (Gardner et al., 2011).

analysis of the NPC showed that Nups are tightly packed within each spoke of the NPC, and no such channels were observable (Lin et al., 2016). However, the authors did observe small gaps between the spokes in the central channel, which they suggest could be a potential uninterrupted path for transport across the INM. Future studies are needed to investigate the Nups involved in the trafficking of SUN-domain proteins through the NPC.

The upper limit for the size of the nucleoplasmic domains of INM proteins to ensure their successful transport through the NPC has been suggested to be 10 nm (Lin et al., 2016; Turgay et al., 2010). We estimate the size of the nucleoplasmic domains of SUN proteins (Fig. 3B) to be less than 10 nm. We have also estimated the size of SUN trimers to see whether transport of the trimer would be feasible, as it is not yet known whether SUN proteins form trimers in the ER or ONM, or only after they are targeted to the INM. Based on these estimates, SUN proteins are more likely to be transported as monomers and form higher-order oligomers later on inside the INM, as shown in Fig. 3A.

Roles of NPCs and LINC complexes in nuclear envelope architecture

Cells maintain an even spacing between the INM and ONM, which typically ranges from 30 to 50 nm, through mechanisms that are not fully understood. The only known structures through which the INM and ONM are connected are NPCs and the LINC complexes, and it is thus not surprising that both are involved in the architecture of the nuclear envelope (see Box 1). The first structural insights into SUN–KASH complexes were used to predict that the luminal coiled-coil regions of SUN proteins, which span the nuclear envelope, could act as rulers that adapt to, or determine, the thickness of the PNS (Sosa et al., 2013). The predicted length of SUN1 and SUN2 proteins in the PNS matches the typically observed PNS thickness of 30 to 50 nm (Sosa et al., 2013), and, according to this hypothesis, the thickness of the PNS might be reduced in cells that express SUN proteins with shorter luminal domains, such as testis-specific SUN3, SUN4 and SUN5 (Sosa et al., 2013) (see Fig. 4A). However, there is currently no evidence that sperm cells or other cell types with shorter SUN proteins have reduced PNS spacing. Furthermore, shortening of the SUN protein UNC-84 in *C. elegans* does not result in any observable narrowing of the PNS and, interestingly, also did not disrupt nucleo-cytoskeletal coupling (Cain et al., 2014; Cain and Starr, 2015). The mechanisms by which shorter SUN proteins overcome the PNS gap to bind to KASH-domain proteins and mediate nucleo-cytoskeletal coupling remains unclear. Currently, two potential mechanisms have been proposed: (i) LINC complexes composed of shorter SUN proteins either locally pinch the nuclear envelope (Fig. 4A) or (ii) SUN proteins adapt to the existing PNS thickness by extending their luminal domains (Cain and Starr, 2015) (Fig. 4B). In agreement with the second proposed mechanism, our recent molecular dynamics simulations suggest that tensile cytoskeletal forces acting on KASH proteins can be directly transmitted to the luminal domain of SUN proteins without disrupting the SUN–KASH domain interactions, and thus might represent a possible mechanism underlying this extension (Jahed et al., 2015). However, cytoskeletal forces can only be transmitted

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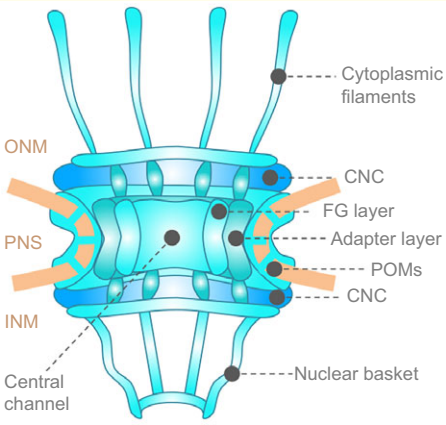
Box 1. Structure of NPCs and LINC complexes, and their anchorage to the nuclear membranes

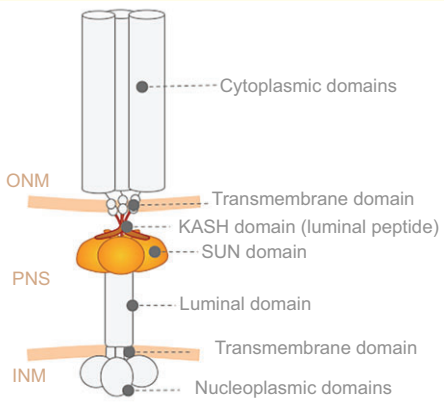
NPCs



The NPC features an eightfold symmetric structure where each repeating unit is called a spoke (Alber et al., 2007). This eightfold symmetry is conserved across different species and is suggested to maximize the bending stiffness (i.e. resistance against bending deformation) of the NPC (Wolf and Mofrad, 2008). Although the detailed molecular architecture of the NPC is yet to be understood, recent studies have provided us with a more comprehensive architectural model of the NPC (von Appen et al., 2015; Lin et al., 2016). The eight spokes form three distinct parts of the NPC structure, namely the central channel, the cytoplasmic filaments, which emanate into the cytoplasm, and the nuclear basket (see figure). A specific complex of Nups, referred to as the coat Nup complex (CNC), forms an outer ring that is symmetrically located on the cytoplasmic and nuclear faces (Lin et al., 2016). Two distinct layers of Nups, namely central channel Nups (also referred to as the FG layer) and adapter proteins (forming the adapter layer, also referred to as the scaffold layer) form concentric cylinders in the central channel (Jamali et al., 2011; Lin et al., 2016). A third layer of Nups, i.e. POMs (also referred to as the membrane layer), comprises transmembrane proteins and plays an important role in anchorage of the NPC scaffold into the nuclear envelope.

The NPC is fused to the nuclear envelope through different types of nucleoporins (Jamali et al., 2011). Various proteins interact with each other and with the nuclear envelope at different stages of NPC formation and assembly. For example, the transmembrane NPC proteins gp210 (also known as Nup210) and Pom121 have a role in anchoring the NPC to the membrane in vertebrates (Rothballer and Kutay, 2013a). Other nucleoporins, such as Nup53 and Nup155 are also involved in NPC assembly into the nuclear envelope. Nup53, for instance, directly interacts with the nuclear envelope both in yeast and *Xenopus* (Marelli et al., 2001; Vollmer et al., 2012). In addition, several studies have identified interactions between Nup53 and transmembrane protein Ndc1 (Mansfeld et al., 2006; Onischenko et al., 2009; Eisenhardt et al., 2014; Hawryluk-Gara et al., 2008). Furthermore, recent experiments have revealed that the nuclear basket Nups Nup1 and Nup60 (as well as the mammalian homolog Nup153) might also be involved in integrating NPCs into the nuclear envelope (Mészáros et al., 2015; Vollmer et al., 2015). However, these are only examples of the interactions with the nuclear envelope that are required for NPC assembly; for more comprehensive papers on NPC structure, see Jamali et al. (2011); Alber et al. (2007); Kabachinski and Schwartz (2015).

LINC complexes



The crystal structure of the SUN domains of SUN2 in complex with the luminal KASH peptide of nesprin 2 has recently been identified in two independent studies (Protein Data Bank IDs 4FI9 and 4DXS, respectively) (Zhou et al., 2012; Sosa et al., 2012). Both structures reveal a trimeric SUN2 complex that simultaneously forms strong interactions with three KASH peptides in the PNS. The second, more complete structure (4DXS), also reveals a covalent disulfide interaction between two highly conserved cysteine residues of SUN2 and KASH2; this strongly stabilizes the interaction and is potentially crucial for the transmission of cytoskeletal forces to the nucleus (Jahed et al., 2015). LINC complexes interact directly with the nuclear envelope. First, KASH-domain-containing proteins are anchored to the ONM through their short 21-amino-acid transmembrane domain. Second, trimers formed by SUN proteins contain hydrophobic transmembrane domains that span the INM. This transmembrane domain is variable between various SUN isoforms and can contain one to three loops. Both SUN1 and SUN2 contain a single 21-amino-acid transmembrane domain that interacts directly with the INM. However, some studies predict that three additional hydrophobic sequences within SUN1 could also interact with the INM or ONM.

after the LINC complex is formed, so even if these complexes can extend in response to force, it remains unclear how shorter INM-anchored SUN proteins are able to interact with the KASH domain at the ONM to initiate complex formation.

As discussed above, both the LINC complexes and NPCs are anchored to and span the nuclear envelope, and are therefore likely to be involved in maintaining an even distance between the INM and ONM, even if they do not determine the exact distance. In fact, electron microscopy images of the nuclear envelopes of Hela cells that have been depleted of SUN1 and SUN2 revealed frequent and discrete expansions of the PNS (Crisp et al., 2006) (Fig. 4C). In regions where these expansions were not observed, the integrity of the nuclear envelope may have been maintained by NPCs (see Fig. 4C). In *C. elegans*, a similar increase in nuclear envelope thickness has been observed in UNC-84 mutant cells; this, however, was limited to muscle cells from the body wall, suggesting that SUN proteins only determine nuclear envelope thickness in force-bearing cells (Cain et al., 2014). The increases in nuclear envelope spacing due to LINC disruptions observed in these two studies are, however, distinct. In Hela cells lacking SUN1 and SUN2, frequent increases in the PNS spacing of up to ~100 nm are seen, whereas the membrane around the NPCs remains intact (Fig. 4B). In contrast, in the *unc-84*-mutant *C. elegans* muscle wall cells, large local distortions of up to ~520 nm were observed, but only in regions that are assumed to be under high mechanical stress – i.e. the two ends of each nuclei. This difference is possibly due to the different magnitudes of force that are exerted on the ONM of Hela cells compared to the forces that act on the end of each muscle wall nucleus in *C. elegans*. In either case, the nature of the forces that act on the nuclear envelope remain unclear, because the disrupted LINC complexes should no longer be able to transmit forces from the cytoskeleton to the PNS. Forces could be exerted onto the ONM through the dynamic rough ER, which expands into the cytoplasm, given that the ONM is continuous with and

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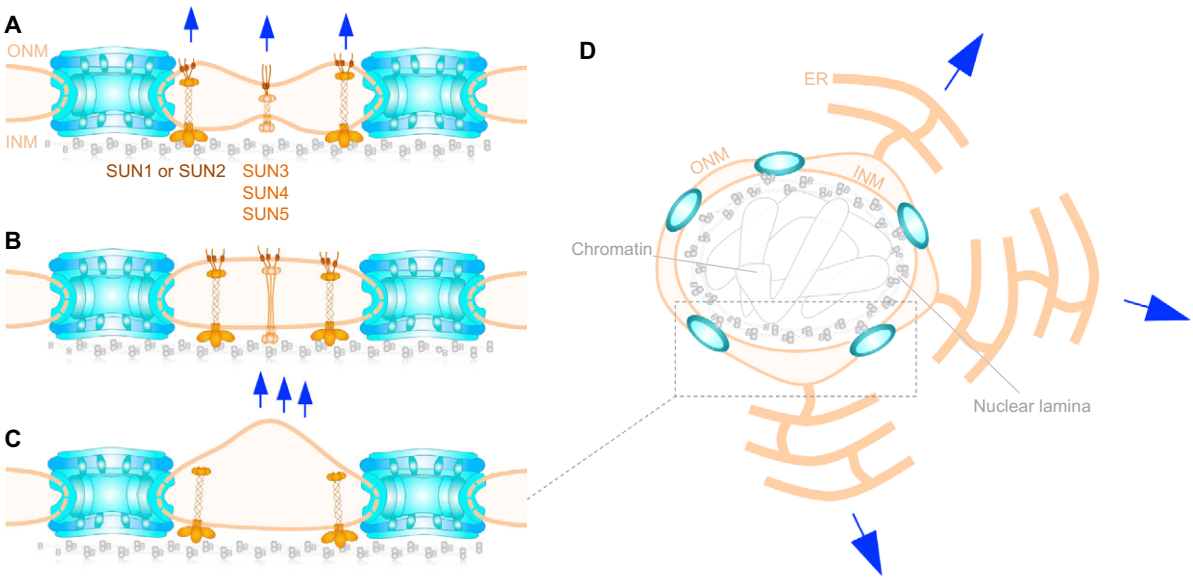

Fig. 4. Possible models for the maintenance of the nuclear envelope thickness by LINC complexes. (A) First model. LINC complexes entirely determine the thickness of the nuclear envelope. Therefore, a locally reduced nuclear envelope thickness should be observed with shorter SUN proteins, such as testis-specific SUN3, SUN4 or SUN5 compared with that containing SUN1 and SUN2, although it might not be possible to resolve this using current microscopy techniques (Sosa et al., 2013; Cain and Starr, 2015; Cain et al., 2014). The integrity of the nuclear envelope is maintained despite the cytoskeletal forces (blue arrows) that act on LINC complexes. (B) Second model. LINC complexes adapt to the pre-determined thickness of the nuclear envelope by extending their luminal regions so they span the entire nuclear envelope thickness (Cain and Starr, 2015; Cain et al., 2014). (C) Increased nuclear envelope spacing is observed upon disruption of LINC complexes, while the nuclear envelope remains intact around NPCs (Crisp et al., 2006; Cain et al., 2014). Mechanical forces (blue arrows) might separate the ONM from the INM in cells where LINC complexes are not able to form and, therefore, are also unable to maintain the connection between the ONM and INM. These forces might be exerted onto the ONM through the dynamic rough reticulum (ER), as shown in D. (D) A potential mechanism of nuclear envelope separation upon SUN depletion is depicted. Here, the ONM, which is continuous with the rough ER is pulled away from the INM, which remains associated with the nuclear lamina and chromatin in LINC-disrupted cells.

connected to the ER in several areas (English and Voeltz, 2013). Furthermore, in the absence of LINC complexes, the INM remains intact and connected to the nuclear lamina and chromatin through NPCs and other INM proteins (see Fig. 4D). This could result in a separation and thus increased distance between the INM and ONM in the absence of LINC complexes (Fig. 4C,D).

NPCs are also likely to be involved in determining and maintaining nuclear envelope architecture because the INM and ONM are fused at the periphery of each NPC. Indeed, Nup153 depletion leads to a plasticity of the nuclear envelope and structural defects, such as membrane invaginations that span throughout the nucleus (Zhou and Panté, 2010). Furthermore, some Nups that form the nuclear basket in yeast (see Box 1), such as Nup1 and Nup60, have been found to interact with the INM and induce curvature (Mészáros et al., 2015). Here, bipartite motifs comprising an amphipathic helix (residues 16–27 in Nup1 and residues 31–40 in Nup60) accompanied by an α-helical region (residues 85–123 in Nup1 and residues 91–162 in Nup60) have been suggested to facilitate induction of curvature through a combination of both hydrophobic insertion and scaffolding (Mészáros et al., 2015). Such a mechanism is supported by various observations, including the *in vitro* bending of membranes by Nup1 and Nup60, and the *in vivo* reshaping of the nuclear membrane when Nup1 is overexpressed (Mészáros et al., 2015). In addition, it has been shown previously that Nup60 has some affinity to phospholipid bilayers, providing additional support for a role of Nup60 in inducing membrane curvature (Patel and Rexach, 2008). Similarly, the mammalian homologue Nup153 directly binds to the INM through an N-terminal amphipathic helix (Vollmer et al., 2015).

The effect of LINC complexes on NPC-mediated nucleocytoplasmic transport

A few recent studies have proposed an interesting and direct connection between LINC complexes and transport through the NPC. Transport of biomolecules through the NPC is dependent on the gradient of the small GTPase Ran between the nucleus and the cytoplasm. Two proteins, RanGTPase-activating protein 1 (RanGAP1) in the cytoplasm and RCC1 in the nucleus, maintain this gradient in mammals (Grossman et al., 2012; Schmidt and Görlich, 2016). In mammals, NPCs are responsible for anchorage of RanGAP1 at the nuclear envelope. Specifically, RanGAP1 is modified by SUMO, which activates it for binding to cytoplasmic Nup538 (Matunis et al., 1998; Kalab and Heald, 2008) (Fig. 5A). In plants, however, RanGAP1 has a WPP domain that interacts with the WPP-interacting tail-anchored proteins WIT1 (also known as WT1-AS) and WIT2 at the ONM (Zhao et al., 2008). WPP-domain-interacting proteins (WIPs), in turn, interact with WITs and anchor RanGAP1 to the nuclear envelope. Recently, WIPs have been suggested to be plant-specific KASH-like proteins that interact with SUN proteins (Zhou et al., 2012a,b). Therefore, in plants, the LINC complex is responsible for anchoring RanGAP1 to the ONM, which facilitates an efficient regulation of Ran-dependent transport through the NPC. Therefore, plant LINC complexes are likely to be responsible for positioning RanGAP1 at the periphery of the NPC as they are able to rapidly capture shuttling Ran-bound GTP and induce hydrolysis to Ran-bound GDP (Fig. 5B). No such roles have yet been identified for mammalian LINC complexes.

However, a more recent study has explored a direct role for SUN1 in messenger (m)RNA export through the NPC in mammalian cells (Li and Noegel, 2015). Before export, mRNA transcripts bind to a

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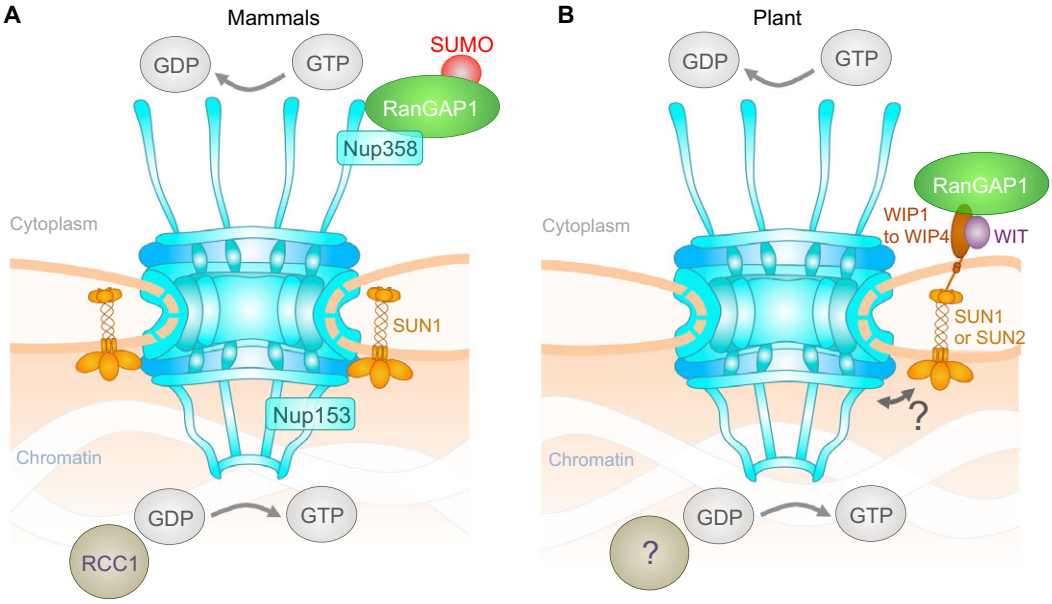

Fig. 5. Role of mammalian and plant LINC complexes in the transport of biomolecules through the NPC. (A) In mammals, the concentration gradient of Ran between the cytoplasm and the nucleus is maintained by the localization of RanGAP1 and RCC1 at the NPC periphery in the cytoplasm and the nucleus, respectively. Mammalian RanGAP1 is modified by SUMO, which activates it for binding to Nup538 (also known as RANBP2) (Matunis et al., 1998). No roles for mammalian SUN1 have been identified in this process thus far, although SUN1 is known to associate with the NPC in mammals. (B) In plants, LINC complexes recruit RanGAP1 to the ONM to maintain the gradient of the small GTPase Ran between the nucleus and the cytoplasm. The plant RanGAP1 protein lacks a SUMO-binding domain and are instead anchored to the ONM through WIPs (WIP1 to WIP4) and WIT proteins. Although WIP proteins lack a KASH domain, they are suggested to perform a similar biological function as KASH proteins and bind to plant SUN1 and SUN2 to link the nucleoskeleton and cytoskeleton (Zhou et al., 2012). Plant RanGAP1 has been shown to localize to the NPC, which might be mediated through an interaction between plant SUN proteins and Nups in the nuclear basket (see Box 1); similar to the association of Nup153 and SUN1 in mammals. No Homolog of RCC1 in plants has yet been identified.

multitude of factors and cofactors, and form a messenger ribonucleoprotein particle (mRNP) that is then exported. By using co-immunoprecipitation and pulldown experiments, Li and Noegel have recently shown that both SUN1 and SUN2 directly interact with some mRNP components *in vitro* (Li and Noegel, 2015). Moreover, the N-terminus of SUN1 (residues 1–239) directly interacts *in vitro* with nuclear export factor 1 (NXF1), the main transporter for the export of the bulk of mRNA, and accordingly, knockdown of SUN1 results in an accumulation of mRNAs in the nucleus. As SUN1 also interacts with Nup153, the authors propose a regulatory mechanism for mRNP export, whereby mRNPs associate with SUN1 and then are handed over to Nup153 for export through the NPC (Li and Noegel, 2015). Taken together, these observations not only point to an intriguing connection between LINC complexes and nucleocytoplasmic transport through the NPC but also lend further support for the existence of physical and functional links between the mechanical and biochemical pathways connecting the nucleus and the cytoplasm.

Cooperation between NPCs and LINC complexes during mitosis

During open mitosis, the nuclear envelope breaks down to allow spindle microtubules to access mitotic chromosomes (for a full review on nuclear envelope breakdown see Güttinger et al., 2009). This process includes the dissociation of the nuclear envelope and NPCs from lamins and chromatin (Güttinger et al., 2009). NPCs have a primary role in the mitotic process. Through selective transport, nuclear pores mediate the proper localization of the different kinases that participate in mitosis (Alvarez-Fernández and Malumbres, 2014). For instance, cyclin-dependent kinase 1 (Cdk1), which interacts with cyclin B, is involved in disassembly of the nuclear envelope through phosphorylation of Nup98 and lamins (Laurell et al., 2011). Cdk1 is transported bidirectionally through the NPC during interphase; however, from the beginning of prophase, Cdk1 is only transported from the cytoplasm into the nucleus (Gavet and Pines, 2010).

SUN1 and SUN2 also have crucial but redundant roles in the dissociation of chromatin from the INM given that co-depletion of these two proteins delays membrane removal in Hela cells during nuclear envelope breakdown (Turgay et al., 2014). The phosphorylation of SUN1 at three residues within its nucleoplasmic domain during early mitosis weakens its interactions with lamins; this facilitates the dissociation of lamins from the INM to prepare for nuclear envelope breakdown (Patel et al., 2014). At the same time, the interactions between SUN proteins and dynein-linked KASH proteins are maintained, which allows for tearing of the nuclear envelope through forces that are exerted by molecular motors such as dynein (Patel et al., 2014). Nuclear envelope disassembly is dependent on the recruitment of dynein in order to mediate spindle assembly, and both LINC complexes and NPCs have been shown to be indispensable for this process. Two distinct pathways have been observed for dynein recruitment. Nucleoporins Nup133 (Bolhy et al., 2011) and Nup358 (also known as RANBP2) (Splinter et al., 2010) recruit dynein through direct interaction with BICD2, the mammalian homolog of *Drosophila* Bicaudal D, which acts as an adapter protein between dynein and its cargos in human cells at G2–prophase (Splinter et al., 2010). Although mammalian LINC complexes are important in recruiting dynein during cell migration (Zhang et al., 2009), NPC components appear to be responsible for G2-specific dynein interactions with the nuclear envelope (Splinter et al., 2010). However, recent studies show that dynein-mediated nuclear envelope breakdown continues even after NPCs have fully dissociated, suggesting that there is a second, nucleoporin-independent pathway in Hela cells that might involve LINC complexes (Turgay et al.,

2014). Indeed, components of LINC complexes interact with dynein during cell division in various species – the *C. elegans* KASH protein ZYG-12 in complex with SUN1 directly binds to dynein and recruits it to the membrane during cell division (Malone et al., 2003). Similarly, *Schizosaccharomyces pombe* KASH proteins Kms1 and Kms2, in complex with the SUN protein Sad1 associate with dynein (Anon, 1995; Miki et al., 2004). Finally, mammalian meiosis-specific KASH5 interacts with the microtubule-associated dynein–dynactin complex (Morimoto et al., 2012).

In a closed mitotic system such as in yeast, the factors responsible for chromosome segregation (i.e. the spindle pole body) must be inserted into the nuclear envelope in order to access the DNA. The yeast nucleoporin Ndc1 is required for the insertion of the spindle pole, and of NPCs, into the nuclear envelope (Winey, 1993; Chen et al., 2014). In addition, yeast SUN protein Mps3 forms a complex with nucleoporin Ndc1 in areas away from NPCs and the spindle pole and partitions it between the two (Chen et al., 2014). The duplication of the spindle pole, a process crucial for chromosome segregation, also depends on the interactions between Mps3 with nucleoporin Ndc1 (Chen et al., 2014).

As discussed above, the relationship between NPCs and LINC complexes at the nuclear envelope is highly complex, and only a limited number of studies have directly addressed any direct functional interactions between these two integral members of the nuclear envelope. However, as we have summarized in this Commentary, it is clear that the only two known linkages between the interior of the nucleus and the cytoplasm, namely NPCs and LINC complexes, cooperate in various cellular processes. Nevertheless, the molecular details underlying this association and the implications for their cellular functions remain largely unexplored.

Conclusions and open questions

Here, we have examined recent support for the physical and functional connections between the NPC and LINC complexes that contribute to the regulation of various cellular functions. However, despite the ample evidence for associations between these two molecular complexes, several important outstanding questions in the field remain, as outlined here.

Most importantly, the direct binding partners that link NPCs and LINC complexes have yet to be identified. Furthermore, it is unknown whether the SUN-domain proteins that are associated with NPCs are distinguishable from those associated with KASH-domain proteins of LINC complexes and whether SUN-domain proteins can simultaneously interact with NPCs, KASH proteins and the cytoskeleton. Is the subset of NPCs that is associated to LINC complexes through SUN domains functionally or structurally different from other NPCs? Further studies on the distribution of LINC complexes on the nuclear envelope might shed light onto some of these questions. The density and distribution of NPCs on the nuclear envelope during the cell cycle has been studied in several cell types; however, not much is known with regard to that of the LINC complexes. Furthermore, only a few studies thus far have directly addressed the functional and structural relationship between NPCs and LINC complexes. As highlighted in this Commentary, in order to fully understand the function of these two integral nuclear envelope assemblies, it will be important to examine their roles in greater detail and in the context of different cellular processes. Some unanswered questions to be addressed based on the functional relationship of LINC complexes with NPCs in the context of the cellular processes discussed in this review are as follows: what is the mechanistic role of SUN1 in the fusion of the INM and ONM during *de novo* NPC assembly? How are membrane-anchored SUN proteins transported across the NPC without large disruptions in the NPC structure? What are the mechanistic roles of LINC complexes and NPCs in maintaining the nuclear envelope spacing?

An association between LINC complexes and NPCs would provide an indirect coupling between the NPC and elements of the cytoskeleton, resulting in the exposure of NPCs to cytoskeletal forces. However, the implications of such coupling are only speculative at this point. The complex architecture of the NPC might allow the NPC to change its diameter in response to the transport of differently sized cargos (Solmaz et al., 2011, 2013; Koh and Blobel, 2015; Melcak et al., 2007). It remains to be determined whether any change in the diameter of the NPC is force-regulated and whether components of the LINC complex have a role in NPC dilation and constriction. It should be noted that the dilation and/or constriction of the NPC is still a matter of controversy and has been refuted by some experimental studies and observations (Stuwe et al., 2015; Chug et al., 2015). In addition, it is now well established that distinct structures (e.g. ion channels, NPCs and nucleoli) are mechanically linked throughout the cell such that mechanical stimuli acting on cell-surface receptors elicit the reorganization of molecular assemblies in the cytoplasm and the nucleus. This has led to the notion that regulatory information is transferred to different cellular structures, such as ion channels and the NPC (Maniotis et al., 1997). With regard to the NPC, this idea is further supported by the fact that NPCs expand and that transport rates through NPCs increase upon physical extension of cells (Feldherr, 1990). Therefore, one could speculate that the size of the NPC and transport through it could be regulated by cytoskeletal forces, which, in turn, depend on the external forces applied on the cell. These cytoskeletal forces are transmitted to NPCs through the interactions of their cytoplasmic constituents, such as Nup358, with microtubules. Forces from the actin cytoskeleton and intermediate filaments might also be transmitted to NPCs through their associations with LINC complexes. Because cytoskeletal forces also regulate LINC complex functions, we could hypothesize that some functionalities of NPCs and LINC complexes are coupled. Based on the recent evidence for a direct role of SUN1 in mRNA export through the NPC (Li and Noegel, 2015), this idea of force-regulated transport could be further expanded to include a direct role for cytoskeletal forces in nucleocytoplasmic transport, in addition to an indirect role through regulating the size of the NPC. However, further studies will be required to gain insights into the mechanisms of such a pathway.

Competing interests

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