Microtubule-associated nuclear envelope proteins in interphase and mitosis

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Abstract
The LINC (linker of nucleoskeleton and cytoskeleton) complex forms a transcisternal bridge across the NE (nuclear envelope) that connects the cytoskeleton with the nuclear interior. This enables some proteins of the NE to communicate with the centrosome and the microtubule cytoskeleton. The position of the centrosome relative to the NE is of vital importance for many cell functions, such as cell migration and division, and centrosomal dislocation is a frequent phenotype in laminopathic disorders. Also in mitosis, a small group of transmembrane NE proteins associate with microtubules when they concentrate in a specific membrane domain associated with the mitotic spindle. The present review discusses structural and functional aspects of microtubule association with NE proteins and how this association may be maintained over the cell cycle.

Introduction
The nucleus is enclosed by the NE (nuclear envelope), which is an extension of the endomembrane system (for a review, see [1,2]). The NE consists of two concentric membranes separated by the PNS (perinuclear space). The membranes are perforated by nuclear pores joining the two membranes. The pores in turn are occupied by the NPCs (nuclear pore complexes), enabling selective nucleocytoplasmic transport of macromolecules. The NPC also allows transport of integral membrane proteins between the ONM (outer nuclear membrane) and the INM (inner nuclear membrane) [3–5]. The ONM is continuous with the rough ER (endoplasmic reticulum) and the PNS in turn forms a continuum with the lumen of the ER. In higher eukaryotes, the proteins of the INM connect to the nuclear lamina, an intermediate filament structure that gives the nucleus physical rigidity and protects against mechanical DNA damage.

The endomembrane system is well known to interact with the cytoskeleton, and it has long been known that the ER closely follows the microtubule network (for a recent review, see [6]). In addition, the Golgi apparatus is organized by the microtubules [7] and often localizes in close proximity to the centrosome. This interaction is strikingly apparent during mitosis, when the genetic material has to be properly segregated. At the same time, the endomembrane system is also segregated. In fact, the Golgi apparatus is a licensing factor for mitotic progression [8].

In mitosis of higher eukaryotes, the defining step separating prophase from metaphase is NEBD (nuclear envelope breakdown). During NEBD, the NE ruptures due to the minus-end-directed pulling of centrosomes combined with loss of interactions between the NE and structural components of the nucleus [1,9]. According to the traditional view, this rupture is suggested to allow the NE to be efficiently resorbed into the ER, leaving the mitotic machinery devoid of membranes and accessible for the required mitotic components. Lower eukaryotes, mitosis is a closed process, and NEBD does not occur, although partial rearrangement of the NPC are known to occur [10,11].

Given the multitude of interactions between the endomembrane system and the cytoskeleton, it is perhaps not surprising that nucleoplasmically localized NE proteins also interact with the cytoskeleton during interphase. These interactions are mediated by the recently discovered family of LINC (linker of nucleoskeleton and cytoskeleton) complexes [12–14]. The LINC complex consists of SUN domain-containing integral membrane proteins situated in the INM and KASH (Klarsicht/ANC-1/SYNE homology) domain containing transmembrane proteins (in human termed nesprins) in the ONM. A physical link forms by the interaction of the SUN domain within the KASH domain with the nuclear lamina [12–14]. The LINC complex has been shown to be involved in vital processes, such as cell migration and division.

It has been shown that centrosome attachment to nuclei is mediated by adaptor-dependent interactions between microtubules and nesprins and is affected by the presence of other NE proteins. Emerin [15], lamin A/C [16], nesprin 1 and 2 [12–14] and Samp1 [17] (Figure 1) have been shown to be essential for the proximal localization of the
centrosome to the NE, suggesting that these proteins are part of a common functional network. Centrosome dislocation is observed in Emery–Dreifuss muscular dystrophy, suggesting a disease mechanism involving aspects of microtubule–NE interaction [15]. In addition, the cytoskeleton is suggested to be of importance for chromosome organization [18] and INM proteins have been shown to affect radial positioning of chromosomes [19]. Furthermore, mechanosensation and the tensegral structure of the cytoskeleton are suggested to depend on the interactions between the NE and the cytoskeleton [20–22].

**Protein networks in the NE in interphase**

The relatively small number of studies of NE protein distribution that have been performed using high-resolution fluorescence microscopy show that INM proteins are not uniformly distributed, but rather form distinct patterns with variable degrees of overlap [23–26]. A- and B-type lamins form separate, but interconnected, networks [24]. SUN1 and SUN2 are partially co-localized and partially form separate networks [25,26], where a significant population of SUN1 co-localized with nuclear pores. In HeLa cells, Samp1 displayed a low degree of overlap with a number of NE markers, but a partial co-localization with SUN1 [23]. Because relative nuclear pore positions as well as bleach marks in the nuclear lamina were maintained despite elastic deformations of the NE observed in live cells [27], the NE protein distribution pattern is likely to be relatively stable and may serve as anchoring sites for the cytoskeleton. Support for this idea was recently provided by Luxton et al. [28], using live-cell imaging to show concerted retrograde movements of TAN (transmembrane actin-associated nuclear) lines, linear arrays of SUN2 and nesprin2 assembled on actin cables, during nuclear migration in polarized fibroblasts.

The LINC complex can vary in its composition, allowing for a multitude of interactions with cytoskeletal and nucleoskeletal components. In humans, to date, four nesprins with several isoforms have been characterized, allowing for interactions with microtubules, actin and intermediate filaments [12–14]. Nucleoskeletal interactions of the LINC complex, in turn, depend on the identity of the different SUN proteins, of which five have been characterized in humans to date. Furthermore, NE proteins (e.g. lamin proteins, emerin or Samp1) and proteins of the PNS (e.g. torsin A) may also influence LINC complex composition. Full understanding of NE protein patterning and its functional significance may have to include studies of all physiologically relevant permutations of the LINC complex at nanoscopic resolution.

**Microtubule-associated NE proteins in mitosis**

Recently, transmembrane NE proteins have been shown to associate with microtubules of the mitotic spindle [17,29]. Using specific antibodies and expression of chimaeric fusion protein, we have demonstrated the existence of a novel membrane domain associated with the mitotic spindle containing the integral INM protein Samp1 [17] (Figure 2). We term this membrane domain the SE (spindle endomembrane). The existence of membranes along the spindle microtubules was totally unexpected in view of the traditional model where the metazoan mitosis is completely open and the spindle devoid of membranes. More recently, the existence of SEs attained further support when Lu et al. [30] were able to detect GFP (green fluorescent protein)-tagged integral ER proteins along spindle microtubules. These transmembrane ER proteins did not accumulate at the SE, but rather were present relative to their concentrations in the ER. Very recently, two novel integral INM proteins, WFS1 (Wolfram syndrome 1) and Tmem214 (transmembrane protein 214), were shown to be able to concentrate to the SE [29], like Samp1 [17].

The SE has to be accounted for when we view the evolution of the nuclear membrane and mitosis in metazoan cells and raises questions concerning the function of the SE and its relationship to other spindle components. The existence of an elusive structure known as the ‘spindle matrix’ in the mitotic spindle has been suggested by several different groups (reviewed in [31]). The spindle matrix is a non-microtubule matrix-like structure located in the central spindle region, but absent from the subcentrosomal region. The spindle matrix is envisioned to contribute in force generation and stability during chromosome segregation and/or recruitment of motor proteins and SAFs (spindle-assembly factors). The spindle matrix and the SE are two different structures that may have different functions in the mitotic process, and it is possible that the SE may serve as a scaffold for the spindle matrix. It is also well established that components of the nucleocyttoplasmic transport system have been found to

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**Figure 1** | Detachment of centrosome from the NE after post-transcriptional silencing of Samp1

Phase-contrast micrographs of HeLa cells treated with control siRNA (small interfering RNA) (a) and specific siRNA against Samp1 (b–d). Centrosomes are marked with white asterisks. Note the increased distance between the centrosome and the nuclear boundary after silencing of Samp1 expression. Scale bar, 10 μm.
localize to the mitotic spindle. For example, the nucleoporins Nup98/Rae1, RanBP2 (Ran-binding protein 2), the Nup107–160 complex and nucleocytoplasmic transport receptors have been shown to play important role in assembly and maturation of the mitotic spindle [32–34]. Many questions remain concerning the function and origin of the SE.

It is possible that the SE is formed from the collapsing NE during NEBD and that the SE is one of the first membranes to come in contact with chromosomes of the daughter nuclei during post-mitotic reformation of their NEs. The SE may thus be retained to ensure the proper partition of NE membranes and proteins to the daughter nuclei. Another possibility, as hinted above, would be conservation of some aspects of the functional protein networks of the interphase NE in order to transfer some structural information across the mitotic process. This may perhaps aid in the organization of chromatin and ensure the propagation of architectural epigenetic cues [35].

In order to seek an answer to the question of a function of the SE and how it is organized along the spindle microtubules, let us consider the evolution of the eukaryotic cell and the processes of mitosis and cytokinesis. Eukaryogenesis is still an unresolved mystery. For an extensive review of the origin of the nucleus and mitosis, see [36]. During the evolution of eukaryotes, novel methods to properly segregate a membrane-enclosed genome had to be formed, but also ways to ensure the proper segregation of various organelles and division of the endomembrane system. Most probably, a system for the segregation of the endomembrane system preceded the development of a nucleus. In early eukaryotes and in lower eukaryotes today (Figure 3A), the NE remains intact during mitosis and segregation depends on association of the genome to intranuclear spindles emanating from MNCs (microtubule-nucleating centres). The MNCs, in turn, are attached to the cytoplasmic microtubule network emanating from the centrosomes. There are several variations on the mitotic system present today [10], one being open mitosis that developed in metazoa.

With the discovery of the SE, some steps in the process of mitosis in higher eukaryotes might have to be revised. At the moment, it is not known how the SE is organized (Figure 3B). In the present review, we consider two major possible scenarios. First (Figure 3B, a), during mitosis after NEBD, tubular SE/ER membrane protrusions may either be caught or extended de novo along the spindle microtubule in much the same way that ER does along microtubules during interphase. Components of the SE, including a subset of INM proteins, might then be recruited by interactions with the mitotic spindles, thereby setting up the observed selective accumulation of spindle-associated membrane proteins. A second, and somewhat speculative, possibility is that, right after NEBD, the spindle microtubules projecting from the spindle pole start interacting with the residual NE, thus pushing and deforming the endomembrane now lacking the supportive nuclear lamina. This would lead to complete encasement of the spindle microtubules on the cytoplasmic side, thereby conserving the topology of closed mitosis in lower eukaryotes (Figure 3B, c). In this model, the points of microtubule interaction at the end of the SE cones might act as MNCs. This would be by analogy to the process of closed mitosis (Figure 3A), except that there has to be an MNC for each kinetochore microtubule. From the evolutionary perspective, this is an attractive model. Whichever of these two models that will come closest to reality has to await supportive evidence at the ultrastructural level or confirmed presence of specific markers at the MNCs. In either case, some NE protein may stay associated to the microtubule cytoskeleton throughout the cell cycle.
Funding

This work was supported by grants to E.H. from the Swedish Research Council [grant number 2010-4481] and the Swedish Cancer Foundation [grant number 10 0277].

References


Received 8 August 2011
doi:10.1042/BST20110680

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