The role of nesprins as multifunctional organizers in the nucleus and the cytoskeleton

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Abstract

Nesprins (nuclear envelope spectrin repeat proteins), also known as SYNE (synaptic nuclear envelope protein), MYNE (myocyte nuclear envelope protein), ENAPTIN and NUANCE, are proteins that are primarily components of the nuclear envelope. The nuclear envelope is a continuous membrane system composed of two lipid bilayers: an inner and an outer nuclear membrane. Nesprins are components of both nuclear membranes and reach into the nucleoplasm and the cytoplasm, where they undergo different interactions and have the potential to influence transcriptional processes and cytoskeletal activities.

Introduction

The nature of the NE (nuclear envelope) is of fundamental interest in cell biology as it forms an interface between the nuclear interior and the cytoplasm. The NE is a highly specialized and complex entity formed by two juxtaposed membranes, the ONM (outer nuclear membrane) and the INM (inner nuclear membrane). The two membranes are separated by the PNS (perinuclear space), connected by the NPCs (nuclear pore complexes) and bridged by ONM–INM protein interactions which are primarily formed by the NE components nesprins (nuclear envelope spectrin repeat proteins) and SUN proteins, also known as SYNE (synaptic nuclear envelope protein), MYNE (myocyte nuclear envelope protein), ENAPTIN and NUANCE. Nesprins comprise a large family of SR (spectrin repeat)-containing type II transmembrane proteins with evolutionarily conserved orthologues in lower eukaryotes including Saccharomyces cerevisiae (Kms1) and Dicyostelium discoideum (interaptin) [1–3].

To date, four different nesprins have been described in mammals (nesprin-1, -2, -3 and -4). Each nesprin is encoded by a single gene that, owing to differential splicing and initiation of transcription, gives rise to a multitude of isoforms differing in size and domain composition. The largest, so-called giant, isoforms of nesprin-1 and -2 have molecular masses of 1014 and 796 kDa respectively. They resemble each other with respect to their domain composition, containing an N-terminal actin-binding domain formed by paired calponin homology domains that is followed by a rod domain and a C-terminal transmembrane domain. The most obvious structural differences between the different nesprins, besides differences in their molecular masses, are in their N-termini. Nesprin-3 harbours at the N-terminus, instead of an actin-binding domain, a plectin-binding domain that mediates the binding to intermediate filaments [4]. The nesprin-4 N-terminus interacts via motor protein complexes with the microtubule network [5]. The rod domain is composed of a large number of SRs. SRs are present in proteins that play a role in cytoskeletal organization such as spectrin, α-actinin and dystrophin. They are three-helix bundles and known sites for protein–protein interactions [6]. Work by Simpson and Roberts [7] revealed that almost the entire rod domain of nesprins is formed by SR-like structures, with 74 in nesprin-1 and 56 in nesprin-2. The C-terminal transmembrane domain is called the KASH (Klarsicht/SYNE homology) domain on the basis of its presence in Caenorhabditis elegans (ANC-1), Drosophila (Klarsicht) and mammalian (SYNE) NE proteins. The KASH domain is sufficient for targeting the nesprins to the NE and mediates their interactions with the INM SUN proteins [8].

In the present article, we focus primarily on the interactions of nesprin-2 and on its in vivo role by analysing a mouse model.

Interaction partners of nesprin

The role of NE proteins as organizers of various cellular processes has been underestimated for a long time, in which the NE has been depicted as a simple barrier allowing the genetic material to be kept separate from the cytoplasm. Today, an increasing number of proteins are known that specifically localize to one or both nuclear membranes. Intriguingly, some of these proteins form high-molecular-mass complexes known as the LINC (linker of nucleoskeleton and cytoskeleton) complex. In this complex, nesprins, together with further NE proteins, form an assembly traversing the NE to connect the nuclear interior with different cytoskeletal aspects. The core of the LINC complex consists of nesprins and SUN domain proteins that interact in the PNS [8,9]. Like nesprins, SUN domain proteins

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Abbreviations used: INM, inner nuclear membrane; KASH, Klarsicht/ANC-1/SYNE homology; LINC, linker of nucleoskeleton and cytoskeleton; NE, nuclear envelope; ONM, outer nuclear membrane; PNS, perinuclear space; SR, spectrin repeat; SYNE, synaptic nuclear envelope protein.

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proteins are type II integral transmembrane proteins with a C-terminus facing the PNS and an N-terminus reaching into the nucleoplasm. The nesprin N-terminus can reach into the nucleoplasm as well as the cytoplasm based on the location in the ONM or INM. Both nesprins and SUN proteins interact with lamins. Lamins are intermediate filament proteins residing along the nucleoplasmic surface of the INM and have been thought to be responsible for maintaining nuclear architecture. They represent the best analysed members of NE proteins and are now known to regulate the organization of chromosomes, transcription, cell-cycle progression and nuclear migration [10].

Laminopathies and nesprins
Mutations in NE proteins such as lamins, emerin or nesprins lead to a group of human diseases that are collectively known as laminopathies or nuclear envelopathies. The first disease described in this context was the X-linked recessive form of Emery–Dreifuss muscular dystrophy that results from mutations in the STX gene encoding emerin [11]. Diseases that are known to arise from mutations in NE proteins reach from progeria, neuropathy and lipodystrophy to cardiac and muscular dystrophies [12]. Most of these rare diseases are due to mutations in the LMNA gene, which encodes lamin A/C. Even though lamins are ubiquitously expressed, mutations in these proteins manifest themselves in elaborate phenotypes, differing with respect to the affected tissue, the age of onset and severity. At the cellular level, laminopathies are characterized by nuclear deformations and nuclear or even cellular fragility [13].

Nuclear deformations have also been observed in primary cells from mice lacking the giant isoform of nesprin-2. Nesprin-2 giant-deficient primary fibroblasts and keratinocytes display an increase in nuclear size and show shape alterations. Additionally, loss of nesprin-2 giant results in a redistribution of emerin along the NE [14]. This indicates that nesprin-2 plays crucial roles in maintaining nuclear integrity and organization.

The lamin A/C mutation S143F causes a phenotype resembling aspects of myopathy and progeria. At the cellular level, this mutation is characterized by a typically high prevalence of nuclear deformations. The nuclear deformations are accompanied by reduced nesprin-2 presence at the NE and altered emerin distribution, as well as a redistribution of nesprin-1 to the cytoplasm. Remarkably, the presence of nesprin-2 giant in these cells counteracts the nuclear shape deformation, a finding that supports the central role of these proteins as core components of nuclear architecture [15]. Not too surprisingly, laminopathies can also be caused by mutations in nesprin-1 or nesprin-2 [16].

Nesprin–nesprin interactions
How the different nesprins and their numerous isoforms assemble along the NE remains an open question. For SR-containing proteins, such as α-actinin or spectrin, it has been known that they can form homodimers through interactions of their SRs. Likewise, nesprin-1, for example, can oligomerize via C-terminal SRs [17,18]. Similar interactions of C-terminal nesprin-2 SRs have not been shown until now; however, the finding that a loss of nesprin-2 giant from the NE is associated with a down-regulation of smaller C-terminal isoforms, may be due to the interconnectivity and interdependency of different nesprin isoforms.

The complexity of NE protein assemblies continues at the level of the INM with the SUN proteins, which are able to form dimers, tetramers and even higher homo- or hetero-oligomeric structures [19]. Additionally the luminal domains of SUN1/2 promiscuously interact with the KASH domains of nesprin-1, -2 and -3, which offers further flexibility and complexity [19,20].

Starting from the NE, nesprin functions reach into the cytoplasm and the nucleoplasm. On the cytoplasmic aspect of the NE, nesprins connect the nucleus to different cytoskeletal constituents such as actin filaments, microtubules and the intermediate filament system. Loss of nesprins results in a disorganization of these cytoskeletal filamentous systems, as shown in case of actin and intermediate filaments, and microtubules [21,22]. An intact cytoskeletal organization is essential for cell polarization and cell migration. Additionally, cell polarization requires the reorientation of the centrosome (also MTOC [microtubule-organizing centre]) towards the direction of migration. Loss of nesprins results in a failure to reposition the centrosome and in an increased nucleocentrosomal distance in primary nesprin-2 giant-deficient fibroblasts and in cells in which nesprin-2 was depleted by RNAi (RNA interference) [14,22]. A possible role of such processes in the establishment of laminopathies remains to be determined.

Nesprin and signalling
Besides having roles in structural aspects, nesprins control signalling events in the nucleus. Two mechanisms have been suggested: proteins residing at the NE regulate signalling pathways by transferring mechanically induced signals from the cytoplasm into the nucleoplasm through the LINC complex bypassing the nuclear pore. Furthermore, components of the NE, such as the nesprins, might have a role in controlling the accessibility of transcription factors to the nucleus and in regulating the spatial organization of chromatin.

Signalling pathways are characterized by a sophisticated temporally and spatially controlled interplay of events such as protein modifications or changes in subcellular localization. On the way from the cytoplasm into the nucleus, transcription factors have to pass the NE. A prominent example is β-catenin, which has dual functions as a component of adherens junctions mediating the adhesion of neighbouring cells and as a component of the Wnt signalling pathway. If the Wnt pathway is inactive, cytoplasmic pools of β-catenin are
kept low by degradation. Upon activation of the signalling pathway, β-catenin is stabilized and enters the nucleus, where it acts as a transcription factor [23]. The current data predict different roles of NE proteins in this pathway. Emerin, which predominantly localizes along the INM, supports the export of β-catenin from the nucleus [24], whereas nesprin-2 is a positive regulator of Wnt signalling, since loss of nesprin-2 results in a decrease in nuclear β-catenin pools and a down-regulation of Wnt pathway activity [25].

In vivo role: mouse model
To elucidate nesprin-2 functions in vivo, we analysed wound healing in nesprin-2 giant-deficient mice [14]. Wound healing comprises several stages, such as the invasion of macrophages into the wound area at the early stages, the differentiation of fibroblasts into myofibroblasts and keratinocyte proliferation [26], and allowed us to address aspects of nesprin biology that, until now, have been studied in cell culture only, such as cytoskeletal organization, cell migration, reorientation of the centrosome, regulation of transcription factors and cell proliferation. When we followed the wound closure over a period of 10 days after wounding, we observed a significant delay in wound closure starting at day 7 that persisted until day 10, a time when the wild-type wound had nearly closed. These changes were accompanied by reduced cell proliferation, reduced formation of myofibroblasts and reduced expression of transcription factors. Furthermore, we noted alterations in the actin and microtubule cytoskeleton and altered focal adhesion sites. We conclude that the loss of nesprin-2 affects the cytoskeleton and associated cellular activities and also the transcriptional programme (Figure 1).

We have already shown that the Wnt pathway can be regulated by affecting β-catenin levels in the nucleus [25]. Another mechanism to regulate gene activity is by changing the spatial organization of chromosomes and the accessibility of genes for transcription factors. To explore the impact of nesprins on the organization of chromatin, we performed ChIP (chromatin immunoprecipitation) experiments using antibodies targeted against the N- and the C-terminus of nesprin-2 followed by the analysis of the precipitated DNA sequences. We found an association of nesprin-2 with DNA which, in the case of N-terminus-specific antibodies, was primarily of heterochromatic and centromeric origin. Support for such an interactions comes from analysis of heterochromatin staining in nesprin-2 giant-knockout or -knockdown cells where it is significantly altered. Changes in

Figure 1 | Scheme of the protein complexes formed at the NE and the interactions of nesprins
The LINC complex (nesprin-SUN) and associated proteins can transduce signals from the cytoplasm to the nucleus. Nesprins can transduce mechanical signals through the LINC complex to the nucleoplasm and are also involved in signal transduction processes through interaction with transcription factors. Furthermore, gene transcription may be affected through interactions of NE proteins with chromatin, thereby altering the status of chromatin from being inactive to being actively transcribed or vice versa. MTOC, microtubule-organizing centre.
heterochromatin staining were also observed in laminopathic cells, suggesting that this might be a general mechanism exerted by the NE [27].

In conclusion, we propose a model in which nesprins are an integral component of a protein complex which is located at the NE and reaches from the nucleoplasm to the cytoplasm. On the basis of their location and the many interactions that nesprins can undergo, they can affect a wide range of cellular activities (Figure 1).

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**References**


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