Nesprin-3 as a LINC between the nucleus and intermediate filaments
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Preface
Background

Eukaryotic cells can be roughly divided into two compartments: the nucleus and the cytoplasm. Whereas the nucleus contains the DNA and therefore harbors the blueprint for tissue development, cellular integrity and a stable organ structure are not possible without one of the main components of the cytoplasm, i.e. the cytoskeleton. The cytoskeleton consists of actin filaments, intermediate filaments and microtubules. These three types of filaments cooperate to provide shape and structure to cells. In addition, actin filaments and microtubules have important functions in cell movement and intracellular transport, respectively.

The nucleus is surrounded by the nuclear envelope (NE). This double-layered membrane consists of an outer nuclear membrane (ONM) facing the cytoplasm and an inner nuclear membrane (INM) facing the nucleoplasm. Situated between these membranes is the perinuclear space, a lumen that is continuous with the cisternal space of the rough endoplasmic reticulum. Like the cytoplasm, also the nucleus is strengthened by a nucleoskeleton. One of the main determinants of the shape and structure of the nucleus is the nuclear lamina. This meshwork of lamin intermediate filaments underlies the INM and functions as a scaffold for transmembrane and chromatin binding proteins (Gruenbaum et al., 2005).

Although the nucleus and the cytoplasm are separate entities within the cell, they are actually the subjects of extensive cross-talk. Communication between these two compartments primarily takes place through nuclear pore complexes (NPC). These macromolecular protein assemblies form aqueous channels that span the NE at sites where the ONM and INM are fused (D’Angelo and Hetzer, 2008; Vasu and Forbes, 2001). Whereas ions and small metabolites can passively diffuse through the NPCs, larger molecules can only pass the NE via active transport mechanisms (Cook et al., 2007; Görlich and Mattaj, 1996). As a consequence, the NE is an important regulator for the export of RNA from the nucleus and the import of proteins into the nucleus.

Besides the above-described chemical method of communication, the nucleus can also interact with the cytoplasm by more structural means. In fact, it has already been demonstrated in the early 1980s that intermediate filaments can associate with the nucleus (Capco et al., 1982; Lehto et al., 1978; Staufenbiel and Deppert, 1982; Woodcock, 1980). This nuclear-cytoskeletal connection forms the basis of a filamentous protein scaffold that extends from the nuclear interior to cell-cell and cell-matrix junctions at the plasma membrane (Fey et al., 1984). Moreover, the functional significance of this protein scaffold was demonstrated several years later, when Maniotis and coworkers showed that forces applied to the cell surface could induce nuclear deformation (Maniotis et al., 1997).

Integrins and dystroglycan were well known at that time for their ability to connect the extracellular matrix to the cytoskeleton (Geiger et al., 2001; Michele and Campbell, 2003). In contrast, the proteins responsible for the association of the cytoskeleton with the nuclear interior remained to be identified. As vertebrate NPCs can bind to B-type lamins and are anchored in the NE by their interaction with the nuclear lamina (Hutchison, 2002), they have previously been suggested to contribute to the connection between the nucleus and the cytoskeleton (Ingber, 2006). However, extensive research in the past decade has identified a novel protein complex, the LINC complex, that establishes the LInk between the Nucleoskeleton and the Cytoskeleton (Crisp et al., 2006).
LINC complexes consist of two main components: SUN proteins present in the INM and Klarsicht/ANC-1/Syne homology (KASH) domain-containing proteins located in the ONM. By interacting with each other in the perinuclear space, the proteins of these two families can bridge the NE (Razafsky and Hodzic, 2009; Starr and Fridolfsson, 2010). SUN proteins extend their N-termini into the nucleoplasm, where they can interact with both nuclear lamins and chromosomes (Crisp et al., 2006; Ding et al., 2007; Haque et al., 2006; Schmitt et al., 2007). KASH domain-containing proteins, in turn, have their N-termini in the cytoplasm and can bind either directly or indirectly to different components of the cytoskeleton (Padmakumar et al., 2004; Roux et al., 2009; Wilhelmsen et al., 2005; Zhen et al., 2002). Thus, based on their localization and binding partners, SUN and KASH domain-containing proteins form the major complex that connects the nuclear interior to the cytoskeleton.

The role of LINC complexes is further exemplified by a number of in vitro studies in which a general disruption of the LINC complex appeared to be associated with a decrease in cytoskeletal stiffness and a reduction in the transfer of forces from the cytoskeleton to the nucleus (Lombardi et al., 2011; Stewart-Hutchinson et al., 2008). Furthermore, loss-of-function studies in animal models have illustrated the importance of LINC complexes in a wide range of cellular activities, such as anchorage of muscle nuclei underneath the neuromuscular junction (Puckelwartz et al., 2009; Zhang et al., 2010; Zhang et al., 2007) and migration of nuclei during development of the brain and retina (Yu et al., 2011; Zhang et al., 2009). The specific KASH domain-containing proteins involved in these latter studies are well described. However, as the function of other family members remains to be determined, future research is required to extend our knowledge of the LINC complex. To make a contribution to this work, we initiated the studies described in this thesis.

Outline of the thesis

In chapter 1, a broad overview is provided on the initial identification of KASH domain-containing proteins. Members of this protein family were first described in Caenorhabditis elegans and Drosophila melanogaster, and are required for both nuclear migration and anchorage. The vertebrate counterparts of the KASH domain-containing proteins are the nesprins (nuclear envelope spectrin repeat). To date, four nesprin family members have been described in the literature; three of which are discussed as part of this chapter.

One of the nesprins, nesprin-3, forms the main focus of this thesis. Nesprin-3α was initially identified as a binding partner of the cytoskeletal cross-linker protein plectin. Moreover, these two proteins are suggested to link the NE to the intermediate filament cytoskeleton (Wilhelmsen et al., 2005). The aim of our current studies was to gain insight into the function of nesprin-3 and to investigate its interactions with newly identified and established binding partners.

As described in chapter 2, we started our studies by determining how nesprin-3 is localized at the ONM. By utilizing siRNA-mediated knockdown and a KASH domain mutant of nesprin-3, we demonstrate that nesprin-3 is retained at the NE by interactions with both SUN1 and SUN2. Additionally, we provide evidence for a self-association of nesprin-3 and propose a model in which plectin dimers are recruited to the NE by dimers of nesprin-3α, thereby establishing the link between the nucleus and the intermediate filament system.
We continued our studies in chapter 3 by investigating the function of nesprin-3 in zebrafish. Nesprin-3-deficient zebrafish were isolated from an ENU-mutagenized zebrafish library. In line with our model, we observed a dissociation of intermediate filaments from the NE in these zebrafish. However, loss of nesprin-3 did not result in gross abnormalities, which suggests that nesprin-3 is dispensable for zebrafish development. Notably, the identification of zebrafish nesprin-3β allowed us to detect two residues in nesprin-3α that are crucial for plectin binding. Similar mutagenesis studies for plectin indicate that the binding sites for nesprin-3α and the integrin β4 subunit partially overlap.

In chapter 4, an extensive overview is given of the expression pattern and binding partners of nesprin-3. More importantly, we speculate about the function of nesprin-3 and point out the deficits in our current knowledge.

One of these deficits was further investigated in chapter 5. We analyzed the distribution pattern of nesprin-3 in mouse tissues and describe the generation of nesprin-3 knockout mice. Furthermore, we investigated the function of nesprin-3 at the cellular level. These studies demonstrate that nesprin-3 is strongly expressed in Sertoli cell of the testis and that nesprin-3 is required for the recruitment of both plectin and vimentin to the NE. However, as testicular morphology and tissue integrity in general were not affected in the nesprin-3 knockout mice, we conclude that nesprin-3 is dispensable under normal conditions in vivo.

The research described in this thesis is then summarized in chapter 6. We discuss the results and place them in a broader perspective by relating them to recent findings described in the literature.
References


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