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LINC complex independent perinuclear actin organization and cell migration

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Abstract: The link of the metazoan nucleus to the actin cytoskeleton is highly important for actin polymerization and migration of multiple cell types as well as for mechanotransduction and even affects the cellular transcriptome. Several mechanisms of organization of actin filaments next to the nuclear envelope have been identified. Among these mechanisms the most studied one is the Linker of nucleoskeleton and cytoskeleton (LINC) complex-dependent perinuclear actin organization. However, recently additional mechanisms have been identified: an Actin-related protein-2/3 (Arp2/3)-dependent perinuclear actin polymerization during migration of dendritic cells and a perinuclear actin rim that is formed in response to external force application or migration cues. In parallel, there are also reports on cancer cells that migrate in a LINC complex independent manner and on cancers with reduced expression of the LINC complex components. Thus, suggesting that LINC complex independent migration may be associated with tumour formation.

Abbreviations

2/3D:	Two/three-dimensional
MRTF-A:	Myocardin related
ARP2/3:	Actin-related protein-2/3 transcription factor-A
CaAR:	Calcium-mediated actin reset
NPC:	Nuclear pore complex
INF2:	Inverted formin-2
ONM:	Outer nuclear membrane
INM:	Inner nuclear membrane
PNS:	Perinuclear space
KASH:	Klarsicht, ANC-1, Syne Homology domain
SUN:	Sad1p, UNC-84
LINC:	Linker of nucleoskeleton and cytoskeleton
TAN:	Transmembrane actin-associated nuclear

Introduction

The nuclear envelope separates the eukaryotic nucleus from the rest of the cell. Apart from generating a physical barrier, the nuclear envelope is actively involved in several processes including organization of nuclear architecture, transcriptional control, and migration of both the nucleus and the cell. The nuclear envelope is composed of the Nuclear Pore Complexes (NPCs) and the nuclear membrane, encompassing an inner nuclear membrane (INM, facing the inside of the nucleus) and an outer nuclear membrane (ONM, facing the cytoplasm) (Hetzer, 2010; Wilson and Berk, 2010). The ONM can interact with various cytoskeletal elements, including actin filaments, while in metazoans the INM is connected to a filamentous network termed the nuclear lamina (de Leeuw *et al.*, 2018; Gruenbaum and Medalia, 2015). The nuclear lamina is composed of lamins, which are type V intermediate filament proteins classified into A-type and B-type lamins. They are key components for determining the structure and the mechanostability of the nucleus, but they have many other roles, for example in chromatin organization and DNA damage repair (Donnaloja *et al.*, 2020; Gruenbaum and Foisner, 2015; Ho and Lammerding, 2012; Patil and Sengupta, 2021).

The nuclear envelope is connected to the various cytoskeletal networks in the cytoplasm including actin filaments. Actin association with the nuclear envelope is important for correct positioning of the nucleus in polarized cells, cell migration, mechanotransduction and transcriptional control (Davidson and Cadot, 2021).

The LINC Complex

The major known nuclear envelope component that facilitates interaction between the nucleus and the cytoskeleton is the

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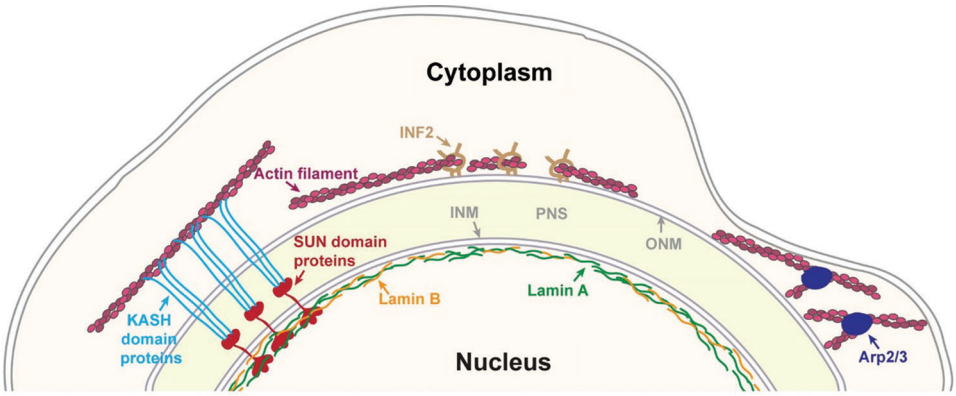

FIGURE 1. Major mechanisms of perinuclear actin organization. The nucleus is separated from the cytoplasm by the nuclear envelope, which is composed by an outer nuclear membrane (ONM) and an inner nuclear membrane (INM) that are separated by the perinuclear space (PNS). The INM is connected to the nuclear lamina that is generated by A and B-type lamins. One mechanism to link actin filaments to the nucleus is by SUN domain and KASH domain proteins that interact inside the PNS to form the Linker of nucleoskeleton and cytoskeleton (LINC) complex. Perinuclear actin filaments can be generated by Inverted formin 2 (INF-2) to form a perinuclear actin rim. Perinuclear actin rim was also found to be sensitive to increased lamin B1 levels. In dendritic cells that pass through small constraints Actin-related protein-2/3 (Arp2/3) promotes the formation of perinuclear actin filaments.

Linker of nucleoskeleton and cytoskeleton (LINC) complex. This complex is formed by Sad1p/UNC-84 (SUN) domain proteins and Klarsicht, ANC-1, Syne Homology (KASH) domain proteins, which cross the INM and the ONM, respectively. SUN domain proteins were shown to form trimers while their C-termini interact with the C-termini of three KASH domain proteins to form stable hexamers. These hexamers may further interact with each other to generate higher order complexes (Hao and Starr, 2019; Jahed *et al.*, 2021). The N-terminus of the SUN domain proteins extends into the nucleus to interact with nuclear factors such as the nuclear lamina, nuclear membrane proteins and chromosomes. The N-terminus of the KASH domain proteins extends into the cytoplasm to interact with various cytoskeletal elements including actin filaments and microtubule motor proteins (Fig. 1) (Jahed and Mofrad, 2018; Rothballer and Kutay, 2013; Stewart-Hutchinson *et al.*, 2008).

The LINC complex was shown to be crucial for linking several cytoplasmic actin structures to the nucleus including the actin cap and the transmembrane actin-associated nuclear (TAN) lines (Davidson and Cadot, 2021). The actin cap is a structure composed of thick and highly contractile bundles of actomyosin filaments loaded on top of the nucleus in polarized primary cells that functions in maintaining nuclear shape and in pulling the nucleus towards the front of the cell during migration (Khatau *et al.*, 2009; Kim *et al.*, 2014; Maninova *et al.*, 2017). TAN lines are linear arrays of nuclear envelope membrane proteins composed of the LINC proteins Nesprin-2G and SUN2, which are formed on the dorsal surface of the nuclear envelope. TAN lines are formed along actin cables that move towards the rear end of the cell. The link of the actin cables to the nucleus by the LINC complex and lamin A results in rearward nuclear movement during early stages of cell migration to polarize the cell (Kutscheidt *et al.*, 2014; Luxton *et al.*, 2010).

As the major linker of the cytoskeleton to the nucleus, the LINC complex was found to have a key role in multiple processes including mechanotransduction, chromosome organization, DNA repair, cell migration and cytoskeleton organization (Bone and Starr, 2016; Hao and Starr, 2019; Hieda, 2019; Jahed and Mofrad, 2018; Khilan *et al.*, 2021; Osorio and Gomes, 2014; Wong *et al.*, 2021). However, in recent years it appears that in some cases actin filaments may accumulate next to the nucleus by LINC complex independent mechanisms.

Perinuclear Actin Independent of the LINC Complex

One type of a LINC complex independent perinuclear actin structure is a transient actin polymerization around the nucleus by the Arp2/3 complex. This phenomenon was found in mouse dendritic cells migrating through small constraints in both *in vitro* and *in vivo* systems (Fig. 1). Knockdown of SUN1 in SUN2 knockout cells did not interfere with this perinuclear actin accumulation, whereas knockdown of lamin A did lead to reduced accumulation of actin around the nucleus (Thiam *et al.*, 2016). Notably, lamin A is known to associate with increased nuclear rigidity (Goldberg *et al.*, 2008; Harada *et al.*, 2014; Lammerding *et al.*, 2006; Stephens *et al.*, 2017; Swift *et al.*, 2013; Zhang *et al.*, 2019). In addition, the observation that internalized large beads accumulated similar actin filaments around them during confined cell migration (Thiam *et al.*, 2016) suggests that the trigger for actin polymerization is just the relative high stiffness of the nucleus. It was shown that these perinuclear actin filaments deform the nucleus to enable its passage through small constraints, though it is still not clear how Arp2/3 is activated (Thiam *et al.*, 2016).

Another perinuclear actin structure that is LINC-independent is the perinuclear actin rim (Fracchia *et al.*, 2020; Shao *et al.*, 2015a; Shao *et al.*, 2015b) that was also termed Calcium-mediated actin reset (CaAR) (Wales *et al.*, 2016). A transient formation of perinuclear actin filaments around the cytosolic side of the nuclear envelope of cells in two-dimensional (2D) culture was found in several cell

types including mouse fibroblasts, breast cancer cells and kidney epithelial cells (Shao *et al.*, 2015a; Shao *et al.*, 2015b; Wales *et al.*, 2016). This actin structure was found to form for a period of 1-5 minutes in response to application of external force that led to calcium ions influx, which in turn activated the actin polymerization promoting factor Inverted formin 2 (INF-2) (Fig. 1) (Shao *et al.*, 2015b; Wales *et al.*, 2016). In mouse fibroblasts inhibition of the LINC complex by overexpression of a dominant negative KASH domain did not interfere with the perinuclear actin rim formation (Shao *et al.*, 2015b). Overexpression of the KASH domain of either Nesprin 1 or Nesprin 2 saturates the endogenous SUN domain proteins, thus it interferes with the formation of the endogenous LINC complex (Lombardi *et al.*, 2011; Stewart-Hutchinson *et al.*, 2008).

We were able to detect a similar perinuclear actin rim in mouse melanoma cells migrating in a 2D system or embedded in a 3D system of collagen fibers that was also LINC complex-independent but had a much longer lifetime, on a time scale of hours. This stable perinuclear actin rim was induced by migration cues and was sensitive to elevated levels of lamin B1, while it was not affected by either higher levels of lamin A or inhibition of the LINC complex by overexpression of a dominant negative KASH domain (Fig. 1) (Fracchia *et al.*, 2020). The ability of lamin B1 that is localized at the inner side of the nuclear envelope, to affect LINC complex independent actin filaments at the outer side of the nuclear envelope suggests an inside-out signal transduction mechanism. It may be based on force transmission due to altered nuclear envelope stiffness upon changes in lamin B1 levels. Alternatively, it may indicate the existence of a protein-based physical linkage between the nuclear lamina and perinuclear actin filaments that is not based on KASH domain and SUN domain proteins.

The role of the perinuclear actin rim is not clear. The transient perinuclear actin rim was suggested to promote cell migration by supporting changes in transcription that occurred due to translocation of the transcription coactivator Myocardin related transcription factor-A (MRTF-A) from the cytoplasm to the nucleus (Wales *et al.*, 2016). On the other hand, we found that the stable perinuclear actin rim in melanoma cells reduced the cellular migration rate (Fracchia *et al.*, 2020). Intensive nuclear envelope stretching during cell migration can disrupt its integrity and lead to DNA damage (Denais *et al.*, 2016; Raab *et al.*, 2016). Thus, we hypothesize that the stable perinuclear actin rim may be involved in protection of chromatin from a mechanical damage during the migration process by increasing the rigidity of the nuclear envelope and limiting nuclear envelope folding.

LINC Complex Independent Cell Migration

The findings of perinuclear actin structures that are LINC complex independent suggest that there should be cells that migrate without the support of the LINC complex. Indeed, migration of cells in a LINC complex independent manner or even in a faster rate upon inhibition of the LINC complex was reported for mouse melanoma cells (Fracchia *et al.*, 2020), human lung cancer cells (Lv *et al.*, 2015) and rat mammary adenocarcinoma cells (Sharma *et al.*, 2021). Still, it should be noted that there are other cancer cells that do require the LINC complex to support their migration (Colón-Bolea *et al.*, 2020; Imaizumi *et al.*, 2018; Infante *et al.*, 2018). Interestingly, in parallel there are accumulating reports on down-regulation of LINC complex components in various cancer types such as breast cancer, lung cancer, prostate cancer and liver cancer (Cartwright and Karakesisoglou, 2014; Lv *et al.*, 2015; Marmé *et al.*, 2008; Matsumoto *et al.*, 2015; Sharma *et al.*, 2021; Sur-Erdem *et al.*, 2020; Tessema *et al.*, 2008; Yajun *et al.*, 2017). Taken together, these recent data may suggest that some cancer cells acquire alternative mechanisms that are LINC complex independent to connect actin filaments to their nuclei or to disconnect their nucleus from the actin network to enhance their migration capabilities. These mechanisms are still to be discovered.

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