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The multi-faceted role of the actin cap in cellular mechanosensation and mechanotransduction

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Abstract

The perinuclear actin cap (or actin cap) is a recently characterized cytoskeletal organelle composed of thick, parallel, and highly contractile acto-myosin filaments that are specifically anchored to the apical surface of the interphase nucleus. The actin cap is present in a wide range of adherent eukaryotic cells, but is disrupted in several human diseases, including laminopathies and cancer. Through its large terminating focal adhesions and anchorage to the nuclear lamina and nuclear envelope through LINC complexes, the perinuclear actin cap plays a critical role both in mechanosensation and mechanotransduction, the ability of cells to sense changes in matrix compliance and to respond to mechanical forces, respectively.

What is the perinuclear actin cap?

Three distinct filamentous proteins - actin filaments, intermediate filaments, and microtubules - constitute the cytoskeleton, an expansive array of polymers that span the cytoplasm of all eukaryotic cells and mediate a multitude of important functions, including cell division, morphology, migration, and differentiation¹⁻³. Globular actin (G-actin) polymerizes into semi-flexible polymers, actin filaments (F-actin)⁴⁻⁶ that bind myosin II to form contractile actomyosin stress fibers⁷⁻¹⁰. Bundles of cross-linked, rope-like intermediate filaments (keratins, vimentin, neurofilaments)^{11, 12} provide structural integrity to the cytoplasm and the nuclear lamina¹³⁻¹⁵, while thick, hollow, cylindrical-shaped rigid microtubules provide the guiding tracks for the intracellular transport of organelles, such as mitochondria and microvesicles^{16, 17}, and set the polarization axis of migrating cells^{18, 19}.

The perinuclear actin cap (or actin cap) is a recently characterized organelle composed of thick, parallel, and highly contractile acto-myosin filament bundles that are anchored to the apical surface of the interphase nucleus through highly conserved linker proteins in a wide range of adherent cells²⁰ (Fig. 1). Actin-cap fibers are functionally, molecularly, and topologically distinct from conventional actin stress fibers, which include basal stress fibers, transverse arcs, and dorsal fibers^{21, 22}. While conventional stress fibers are confined to the basal layer or cortex of the cell and are arranged in diverse directions, actin-cap fibers are typically aligned with the long axis of the cell and terminate at the leading and trailing edges of migratory cells. Critical to the distinct functions of the perinuclear actin cap is its specific

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anchorage to the nuclear envelope. In contrast, conventional stress fibers are directly or indirectly connected to the plasma membrane.

The actin cap has been confirmed in both fixed and live cells and has been so far identified and characterized in mouse embryonic fibroblasts, human lung and foreskin fibroblasts, mouse myoblasts, human umbilical vein endothelial cells, and human ovarian epithelial cells^{20, 23, 24}. The actin cap is completely absent from undifferentiated embryonic stem cells and induced pluripotent stem cells, but starts to form and organize progressively during the differentiation²³. The actin cap is also absent from epithelial sheets, but appears rapidly upon epithelial-to-mesenchymal (EMT) transition²⁵.

Strikingly, the actin cap is disrupted or totally absent in cells from laminopathic mice and patients^{26, 27}, a group of human diseases that stem from mutations in the *LMNA* gene that encodes the nuclear lamina protein lamin A/C (Fig. 1), an intermediate filament. The actin cap is also disrupted in human cancer cells such as the HeLa cervical cancer line²⁸, U2OS osteosarcoma, MDA-MB-231 breast carcinoma, and MCF-7 breast adenocarcinoma²⁴ (Fig. 2). While a defective nuclear shape is a common trait of cancer cells, the biological significance of the lack of organized actin cap in cancer cells remains to be elucidated.

The actin cap is highly dynamic. Live-cell microscopy of cells transfected with GFP-lifeact readily reveals high contractility and dynamics, as the actin-cap fibers continuously undergo cycles of extension and retraction²⁴, precisely positioning and actively deforming the nucleus²⁰. Fluorescence recovery after photobleaching (FRAP) analysis also indicates that actin-cap fibers undergo much faster turnover than basal stress fibers²⁴. In somatic cells, the actin cap does not appear for several hours following cell division²⁰, indicating that the actin cap is not a permanent fixture of cells.

Molecular architecture of the actin cap and accessory proteins

The perinuclear actin cap is physically, yet dynamically, connected to the nuclear envelope through linkers of nucleoskeleton and cytoskeleton (LINC) complexes²⁹ and is terminated by actin-cap associated focal adhesions (ACAFAs) at the basal surface of the adherent cell²⁴ (Fig. 1). LINC-complex proteins nesprins bridge fibers of the actin cap to the nuclear envelope directly through the actin-binding domain of nesprin2giant^{30, 31} and indirectly through the multi-functional actin-binding protein plectin, which itself binds nesprin3^{32, 33} (Fig. 1). Nesprins bind SUN (for Sad1p, UNC-84) proteins via KASH (for Klarsicht, ANC-1, Syne Homology) domains in the periplasmic space between the inner and outer membranes of the nuclear envelope^{34, 35}, and SUN proteins connect to major nuclear lamina protein lamin A/C³⁵, which interacts directly and indirectly with chromosomal DNA³⁶ (Fig. 1). Fibers of the actin cap contain more phosphorylated myosin II, the active form of the motor and F-actin-bundling protein myosin II, and the F-actin crosslinking/bundling protein α -actinin than basal stress fibers²⁴. Fibers of the actin cap are terminated by particularly large focal adhesions, which are located at the edges of the cell and contain high density of phospho-FAK, the active form of focal adhesion kinase (FAK)²⁴.

Nuclear shaping by the perinuclear actin cap in health and disease

The first established function of the perinuclear actin cap was nuclear shaping²⁰. When the actin cap of adherent cells is disrupted with a low-dose treatment of the F-actin depolymerizing drug latrunculin B or by disruption of the LINC complexes, nuclear lobulation ensues and the nucleus can bulge to almost twice its original height²⁰ (Fig. 2). The actin cap serves to maintain the thin, disk-like morphology as well as the translocation and rotation of the interphase nucleus during the migration and shear-response of adherent cells³⁷.

Defects in nuclear shape are a salient, commonly shared feature of nuclei in laminopathic cells^{26, 27}. Embryonic fibroblasts collected from a mouse lacking *LMNA* (*Lmna*^{-/-}), which displays an Emery-Dreyfus muscular dystrophy-like phenotype, do not show a significant difference in the organization of basal actin fibers from that of *Lmna*^{+/+} cells, but lamin A/C deficiency severely decreases the fraction of cells that contain an organized, intact actin cap (see the status of actin cap in healthy vs. diseased cells in Fig. 2). Additionally, cells with the homozygous mutation *Lmna*^{1530p/1530p}, which causes an accelerated aging (progeria) phenotype in mice³⁸, show even fewer actin caps than *Lmna*^{-/-} cells²⁰. Both *Lmna*^{-/-} and *Lmna*^{1530p/1530p} cells display disrupted LINC complexes at the nuclear envelope²⁸, which could explain their lack of organized actin caps since specific disruption of LINC complexes causes specific disruption of the actin cap (Fig. 2) and abrogated nuclear-shaping ability²⁰.

Cellular mechanosensation vs. cellular mechanotransduction

In addition to nuclear shaping, the perinuclear actin plays a central role in the ability of cells to sense changes in matrix compliance and to respond to mechanical forces. Here, we distinguish cellular mechanosensation from cellular mechanotransduction. The ability of cells to recognize, analyze, and respond to consistently changing extracellular substrate rigidity, termed mechanosensation, plays a crucial role in various physiological and pathological processes *in vivo*. For instance, the mechanical rigidity of the extracellular matrix seems to play an important regulatory factor during embryogenesis: mesenchymal stem cells grown on matrices of controlled stiffness mimicking brain tissue, striated muscle, and bone are differently differentiated towards neurogenic, myogenic, and osteogenic lineages, reflectively^{39, 40}. Enhanced tissue stiffness by increased collagen cross-linking density also correlates with progression of malignant breast cancer⁴¹⁻⁴³. Mechanosensation of extracellular physical cues indeed determines function and ultimate fate of cells⁴⁴, while abnormality in mechanosensation is considered a distinct feature of diseased cells.

In contrast to mechanosensation, mechanotransduction is the mechanism by which cells respond to and convert externally applied mechanical forces into biochemical signals⁴⁵. Cells *in vivo* are subjected to a variety of external forces, such as compressive and tensile forces caused by fluid shear stresses. These forces come into play in a multitude of physiologic and diseased conditions, such as blood flow through the vasculature⁴⁶ and interstitial flow in connective tissues and tumor cells¹. Shear stress applied to endothelial progenitor cells that freely circulate in blood or tissue and contribute to vascular regeneration, termed angiogenesis, promotes the formation of capillary-like tubule structures⁴⁷. The absence of mechanical stimulation can be a major cause of bone loss in osteoporosis, another debilitating and progressive disease of aging⁴⁸, and microtubule-based primary cilia regulate bone density by responding to fluid flow^{49, 50}.

Two main cellular pathways are activated following mechanical stimulation. Ion channels open and close in response to changes in force and, in turn, alter ion flux across the cell membrane, activating signaling pathways and ultimately affecting gene transcription⁵¹. Additionally, focal adhesions are involved in transducing stress signals to the genome^{52, 53}. Focal adhesions are large and dynamic protein clusters organized at the basal surface of the cell, through which cells are mechanically anchored to the extracellular substratum and biochemical signals are transmitted^{24, 54, 55}. The dynamic assembly and disassembly of focal adhesion complexes regulate binding between transmembrane adhesion molecules (integrins) and the extracellular matrix, modulating cell migration and mechanosensation⁵⁶⁻⁵⁹. Both ion channel and focal adhesion-mediated signaling implicate the actin cytoskeleton. Cytoskeletal tension may regulate ion channel conductance in response to force^{51, 60}, while actin stress fibers are anchored by focal adhesions, and actin-focal adhesion dynamics rely on transmission of force⁶¹.

Below we highlight recent experimental evidence showing how the perinuclear actin cap and the focal adhesions that terminate actin-cap fibers play a central role in both mechanosensation and mechanotransduction.

Cellular mechanosensation through actin-cap associated focal adhesions (ACAFAFs)

Since actin-cap fibers are organized along the side and apical surfaces of the interphase nucleus and terminated by focal adhesions organized at the basal surface of adherent cells, the focal plane of a light microscope can be progressively lowered from the apical surface of the cell, identifying the actin cap, to the basal surface of the cell revealing ACAFAFs, by following individual actin-cap fibers (Fig. 3A).

Quantitative microscopy analysis in human and mouse fibroblasts and endothelial cells reveals that around ~30% of all focal adhesions per cell are ACAFAFs (labeled in red in Fig. 3A), which are significantly larger and more elongated than conventional focal adhesions (CFAs, labeled in yellow in Fig. 3A), which terminate basal stress fibers. Because actin-cap fibers are typically aligned with the long axis of the cell²⁰, ACAFAFs are typically located at the leading edge and trailing edges of motile cells, far from the nucleus²⁴. ACAFAFs are also more dynamic than CFAs, as measured by a significantly faster recovery of photobleached GFP-tagged focal-adhesion proteins (shorter halftime recovery, ~20s vs. ~30s) and much higher translocation speed than other focal adhesions²⁴.

The morphological and dynamical differences between ACAFAFs and CFAs are caused in part by the specific LINC-mediated connections of the actin-cap fibers to the nucleus. Due to the underlying nucleus and its internal pressure, actin-cap fibers and corresponding ACAFAFs are under significantly higher tension than conventional actin structures and corresponding CFAs of the cell²⁴. This increased tension may promote the higher turnover dynamics of ACAFAFs^{58, 62}. Binding of actin-cap fibers to their corresponding focal adhesions not only distinguishes ACAFAFs from CFAs, but also mediates their enhanced mechanosensing response to changes in mechanical properties of the microenvironment. For instance, variation of matrix compliance mimicking different stiffnesses of the microenvironment *in vivo* (i.e., bone, muscle, adipose tissue, and brain tissue) reveals a significantly more sensitive response of the actin cap and associated ACAFAFs than conventional actin structures and CFAs (Fig. 3B). All actin structures and focal adhesions are disorganized on extremely compliant substrates (<1 kPa, close to the elasticity of brain tissue⁶³), which confirms that CFAs also participate in mechanosensation⁶⁴⁻⁷¹, but a larger amplitude of physical stimulation is required to match the level of ACAFA-mediated mechanosensation (Fig. 3C). Consequently, ACAFAFs dominate mechanosensing response to changes in matrix compliance.

ACAFA-mediated cellular mechanosensation relies on the physical connections between the nucleus and the actin cap mediated by the LINC complexes (Fig. 1). When the LINC complex is disrupted by introducing KASH2 constructs³⁵ that displace nesprin2-giant and nesprin3 from the nuclear envelope, actin-cap fibers are disrupted (top-right panels of Fig. 2) such that either no actin-cap associated focal adhesions are present, or any remaining actin-cap fibers and associated focal adhesions are less responsive to changes in matrix compliance. α -actinin²⁴, which crosslinks and bundles actin filaments of the actin cap, synergistically with myosin II regulate cellular mechanosensation by the actin cap²⁴. A recent theoretical model explains why ACAFAFs, which are significantly larger than CFAs, are more mechanosensitive than CFAs, as it predicts that the level of mechanosensation scales with focal adhesion size⁵⁸.

The actin cap mediates fast mechanotransduction

The actin cap, which along basal actin fibers is disrupted by serum starvation, is reformed and organized in response to fluid shear stresses as low as 0.01 dyn/cm^2 within 5 min in mouse embryonic fibroblasts and myoblasts⁷² (left panel, Fig. 4). In contrast, basal actin fibers can only form past a threshold shear stress level 50-fold higher than required for the formation of the actin cap. Biochemical stimulation by addition of serum mediates actin-cap formation, but only at timescales orders-of-magnitude longer than required for fluid shear stresses. Similarly, the rate of actin cap disappearance following shear cessation is significantly faster than following the switch from serum-rich to serum-starved conditions⁷². Therefore, the response of the actin cap to mechanical forces is much faster and more dynamic than with biochemical stimulation, indicating that the actin cap could be part of an extremely fast and efficient, physical pathway of transmitting mechanical stimuli from the extracellular milieu all the way to the nucleus.

Our recent studies have revealed two main regulators on either end of the actin-cap fibers that are critically required for shear-induced actin cap formation: focal adhesion protein zyxin (but not FAK and vinculin) in ACAFAs, lamin A/C, the LINC complex protein nesprin3, and to a lesser extent the LINC complex protein nesprin2giant (right panel, Fig. 4). The important role of these specific proteins in providing a cellular response to external shear stresses is consistent with the requirement of zyxin for chemokines interleukin-8 and CXCL1 to be upregulated after mechanical stretching of human primary endothelial cells⁵³. Zyxin is mobilized from focal adhesions to actin filaments in response to cyclic stretching or shear stress, while FAK and other focal adhesion components remain at focal adhesion sites⁷³. Nesprin3 is required for proper flow-induced MTOC re-positioning and polarization in human aortic endothelial cells⁷⁴. Moreover, disruption of the LINC complex by way of nesprin-displacing dominant negative KASH2 or SUN1 constructs cause fibroblasts to lessen their displacement in response to microneedle manipulation of the cytoskeleton⁷⁵. Together, these results suggest that the actin cap provides a completely continuous linkage between the extracellular milieu and the nucleus for effective and fast mechanotransduction.

Studies of the actin cap in mechanotransduction have predominantly been performed in isolated cells. For cardiovascular function and diseases, in particular, it remains to be determined how the actin cap organizes and functions in confluent monolayers of endothelial cells exposed to shear flow forces. Moreover, where actin-cap fibers are nucleated remains unknown: Actin-cap fibers could be formed at the nuclear surface or at focal adhesions. How the formation of the actin cap affects chromosomal organization, epigenetic modifications, and gene expression is currently under study.

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Fig. 1. Subcellular organization of the perinuclear actin cap and LINC complexes

Perinuclear actin-cap fibers (green) are functionally and topologically distinct from conventional basal actin fibers (gray) due to tight coupling to the side and apical surfaces of the nuclear envelope through linkers of nucleoskeleton and cytoskeleton (LINC) complexes. LINC complexes consist of KASH-domain-containing nesprin isoforms (i.e., nesprin2giant and nesprin3) that are connected to the actin cap through actin binding domains (ABD) and plectin binding domains (PBD), respectively, localized in the outer nuclear membrane (ONM) and SUN proteins that interact with the nuclear lamina located underneath the inner nuclear membrane (INM). SUN proteins and Nesprins are connected through KASH-SUN interactions in the perinuclear space (PNS). (Reproduced from ref. ^{24, 28}).

Fig. 2. Organization of the perinuclear actin cap in disease

Actin cap (left panels) and basal actin filament organization (right panels) for healthy primary cells (MEFs: mouse embryonic fibroblasts, HFFs: human foreskin fibroblasts, HUVECs: human umbilical vein endothelial cells), compared to diseased cells (U2OS: osteosarcoma, MDA-MB-231: breast carcinoma, MCF-7: breast adenocarcinoma, and MEF *LMNA*^{-/-}: a model for laminopathic diseases). Transfection of EGFP-KASH2 in MEFs (top right panels) displaces outer nuclear membrane-embedded LINC complex proteins nesprin2giant and nesprin3 from the nuclear envelope and disorganizes the actin cap. This in turn disrupts cellular mechanosensation and mechanotransduction. Insets magnify the red-boxed regions for detail. Filled red arrowheads point to organized, intact actin-cap fibers; empty arrowheads point to disrupted or absent actin cap. (Images of MEF, HFF, HUVEC, and U2OS taken from ref.²⁴)

Fig. 3. Actin-cap associated focal adhesions mediate cellular mechanosensation

A. Actin-cap associated focal adhesions (ACAFAFs). Gradual lowering of the focal plane of the light microscope, from the apical surface of a cell, where actin-cap fibers are readily detected, to the basal surface, reveals that actin-cap fibers are draped over the nucleus (DAPI, blue) and terminated by a subset of focal adhesions termed actin-cap associated focal adhesions (ACAFAFs, red), while all other focal adhesions that are not linked to the actin cap are termed conventional focal adhesions (CFAs, yellow). Segments of actin-cap fibers in the plane of focus are traced in green. Six individual actin-cap fibers and corresponding twelve actin-cap associated focal adhesions (at both ends of the actin-cap fibers) are visualized in a vinculin-stained mouse embryonic fibroblast (MEF). **B.** Mechanosensing response of the actin cap and associated ACAFAFs to changes in substrate stiffness. On a stiff glass substrate, MEFs show highly ordered actin-cap fibers (top left panel) terminated by corresponding ACAFAFs (top right panel) and organized basal actin fibers (top middle panel) terminated by CFAs. When these cells are placed on a soft polyacrylamide gel, actin-cap fibers are partially or completely disrupted (bottom left panel), and ACAFAFs largely disappear (bottom right panel) without substantial changes in organization of basal actin fibers (bottom middle panel) and CFAs. Insets show a detail of the actin-cap fibers (red) and ACAFAFs (yellow). Filled arrowheads indicate the presence of organized actin cap or organized ACAFAFs; empty arrowheads indicate the absence of organized actin cap or CFAs. **C.** Schematic of cellular mechanosensation. Well-arranged actin-cap fibers (green) above the nucleus are terminated by large and elongated ACAFAFs (red) at the periphery of the adherent cell. While substrate compliance increases, actin-cap fibers and corresponding ACAFAFs disappear earlier than basal actin (gray) and CFAs

(yellow), but on the extremely soft matrix, both types of actin structures and focal adhesions are largely absent. (Reprinted from ref. ²⁴)

Fig. 4. LINC-mediated formation of the actin cap in cellular mechanotransduction

Schematic of an adherent cell exposed to fluid flow shear force. Left, micrograph shows a serum-starved control C2C12 mouse myoblast cell (which contains no organized actin structures before stimulation) that forms an organized actin cap (inset) in response to exposure of a shear stress of 5 dyn/cm² for 5 min. The right micrograph shows a serum-starved C2C12 cell that has been shRNA-depleted of LINC complex protein nesprin3. This cell is unable to form an organized actin cap after exposure to the same fluid shear stress.