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nuclear actin assembly in mammalian cells

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Für meine Eltern und Kuchen

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List of Abbreviations

3C	Chromosome conformation capture
3D	three-dimensional
aa	amino acid
ADP	Adenosine diphosphate
AFM	Atomic force microscopy
AJ	Adherens junction
APC	Adenomatous *polyposis coli*
APC/C	Anaphase-promoting complex/cyclosome
Arp	Actin-related protein
ATP	Adenosine triphosphate
ATPase	ATP hydrolase
BAF	Barrier-to-Autointegration Function
CaAR	Calcium-mediated actin reset
CaM	Calmodulin
CAP	Cyclase-associated proteins
Cc	critical concentration
CDK	Cyclin-dependent kinase
CRISPR	Clustered regularly interspaced short palindromic repeats
CSK buffer	Cytoskeleton buffer

Daam	Dishevelled-associated activator of morphogenesis
DAD	Diaphanous-autoregulatory domain
DID	Diaphanous-inhibitory domain
DMSO	Dimethyl sulfoxide
dn	dominant negative
DNA	Deoxyribonucleic acid
DRFs	Diaphanous-related formins
DSB	Double-strand breaks
ECM	Extra-cellular matrix
EM	Electron microscopy
EM-CCD	electron-multiplying charge-coupled device
ER	Endoplasmic reticulum
ERM	Ezrin, Radixin, Moesin
F-actin	Filamentous, fibrous actin
FAC	Focal adhesion complex
FH	Formin homology
Fhod	FH domain-containing protein
FLIM	Fluorescence lifetime imaging
Fmn	Formin
Fmnl	Formin-like
FN	Fibronectin
FRET	Förster Resonance Energy Transfer
G-actin	Globular actin
GaAsP	Gallium arsenide phosphide
GBD	GTPase-binding domain
GEF	Guanine nucleotide exchange factor
GPCR	G protein-coupled receptor
GTPase	GTP hydrolase
HAT	Histone acetyl transferase
HDAC	Histone deacetylase
hnRNP	heterogenous nuclear ribonucleoprotein
INF	Inverted formin
KASH	Klarsicht, ANC-1 and SYNE homology
LA	LifeAct
LINC	linker of nucleoskeleton and cytoskeleton
lncRNA	long non-coding RNA
LPA	Lysophosphatidic acid
mDia	mammalian Diaphanous
MNase	Micrococcal nuclease

mRNA	messenger RNA
MRTF	Myocardin-related transcription factor
myosin S1	myosin sub-fragment 1
n.d.	not detected
NES	Nuclear export sequence
NLS	Nuclear localization signal
NPC	Nuclear pore complex
NPF	Nucleation-promoting factor
PALM	Photoactivated localization microscopy
PDB	Protein Data Base
PFA	para-Formaldehyde
Pi	Inorganic phosphate
PML	Promyelocytic leukemia protein
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	nuclear localization signal
SCAI	Suppressor of cancer cell invasion
sCMOS	Scientific complementary metal-oxide-semiconductor
SD	Standard deviation
SEM	Standard error of mean
SRF	Serum response factor
STED	Stimulated emission depletion
STORM	Stochastic optical reconstruction microscopy
SUMO	Small ubiquitin-like modifier
SUN	Sad1- and UNC-84-domain containing protein
TAD	Topologically associated domain
TAN lines	Transmembrane actin-associated nuclear lines
TSA	Trichostatin A
WH2	Wiskott-Aldrich syndrome homology region 2

1. Introduction

1.1 Discovery, genetics and isoforms of actin

Although actin was first experimentally observed in an effort of W.D. Halliburton to enrich muscle myosin in 1887 and in this instance described as a non-myosin precipitate or “myosin-ferment" (Halliburton, 1887), the actual characterization of this protein occurred later, when the Hungarian biochemists Brunó Ferenc Straub and Ilona Banga coincidently purified actin in 1942. They characterized together with their principal investigator Albert von Szent-Györgyi Nagyrápolt the function of actin as a microfilament, as an ATP hydrolase (ATPase) as well as its role in muscle contraction (Odo et al., 1957; Straub & Feuer, 1950). Etymologically, the name “actin” is derived from its activating effect on myosins, which was first observed upon an increase in viscosity after adding actin to a myosin solution (Odo et al., 1957; Varga 1948). Both proteins in combination are referred to as actomyosin (Palacio et al., 2015).

Comparative analyses shows that actin can be found in every eukaryotic cell except nematode sperm (Roberts & Stewart, 1997), is highly conserved with more than 90% amino acid sequence similarity between human and yeast (*Saccharomyces cerevisiae*) (Hanukogle et al., 1983; Ng & Abelson, 1980) and comparable to its prokaryotic (i.e. MreB, FtsA) (Doi et al., 1988) as well as archaeal (Ta0583) (Roeben et al., 2006; Hara et al., 2007; Ghoshdastider et al., 2015) analogues. Several genes as well as pseudogenes (Moos & Gallwitz, 1982) developed during eukaryotic evolution (Bajusz et al., 2018) and their conserved amino acid sequence argues for a refined gene product. Mammalian actin genes evolved as derivates of an ancestral one by processive gene duplication (Jacobs, 2008) resulting in six slightly different actin isoforms with tissue-specific expression patterns (Perrin & Ervasti, 2010; Khaitlina, 2001).

The actin gene family summarizes three alpha actins; a skeletal muscle, an aortic smooth muscle and a cardiac muscle isoform (ACTA1, ACTA2 and ACTC1), two gamma actins, a cytoplasmic and an enteric smooth muscle isoform (ACTG1 and ACTG2, respectively) as well as beta actin (ACTB1) (Ponte et al., 1983; Miwa et al., 1991). All three alpha actins and the enteric smooth muscle gamma actin isoform are found in contractile tissue composing next to myosins the second basic element for muscle contraction described in the swinging cross-bridge model

(Huxley, 1957; Huxley & Niedergerke, 1954; Huxley, 2004). Both beta and cytoplasmic gamma actin co-exist in most mammalian cells, in which their ability to dynamically form microfilaments and their range of protein interactions impacts cellular processes at every level (Gunning et al., 2015).

1.2 G-actin

The actin gene encodes for a 42 kDa globular protein of 375 amino acids (aa) with an averaged diameter of 5 nm (Kabsch et al., 1990; Holmes et al., 1990) (Figure **1A**). Globular actin (G-actin) represents the monomeric subunit of actin filaments (microfilaments, filamentous or fibrous actin, F-actin) (Oda et al., 2009) and is part of the cytoskeleton next to intermediate filaments, microtubules, spectrins and septins. As one of the most abundant cellular proteins, the concentration of actin varies between 10 and 100 μM (Wu & Pollard, 2005), which corresponds to an amount of 12-120 million monomers in a mammalian cell. G-actin forms two asymmetrical lobes (classified as subdomains 1, 2 and 3, 4), which are separated by a cleft (Elzinga et al., 1973) (Figure **1A**). The cleft resembles a functionally active ATPase fold and binds ATP as well as bivalent cations to catalyze the hydrolyzation of ATP to ADP and P_i (Graceffa & Dominguez, 2003). Therefore, actin is an ATPase and the cycle of ATP via ADP + P_i to ADP is aligned with conformational changes in individual monomers and the structural integrity of a filament (Kirschner, 1980).

1.3 Actin filaments

Each actin subunit within a filament is adjacent to four others with a relative rotation of 166° on the helical axis appearing as a double-stranded right-handed helix with a diameter of 7 nm, which repeats its helical structure every 37 nm (Holmes et al., 1990) (Figure **1B**). Although the biochemical conditions to crystallize actin favor spontaneous actin polymerization, F-actin's symmetry is incompatible with topological requirements for crystal space groups (Hiller, 1986), which prevents the formation of clean F-actin crystals. The current three-dimensional (3D) F-actin model is therefore based on integrative efforts (Holmes et al., 2003; Reisler & Egelman, 2007; Holmes et al., 1990; Oda et al., 2009; Scoville et al., 2009) and describes two minor conformational changes between monomeric and incorporated actin subunits: in the

ATPase fold (explaining the increased ATP hydrolysis rate during the transition from G- to F-actin (Blanchoin & Pollard, 2002)) and in hydrophobic regions of the four subdomains (Scoville et al., 2009).

Within a cellular environment, F-actin occurs likely in distinct structural states with variable subunit rotation influenced by interacting proteins, such as Cofilin or Tropomyosin (Reisler & Egelman, 2007; Von Der Ecken et al., 2015). Early F-actin staining for electron microscopy was achieved with myosin molecules giving myosin-decorated F-actin a feather-like appearance, while the unidirectional arrangement of myosin sub-fragment 1 (S1) implies that actin filaments are polar structures (Begg et al., 1978) (Figure **1B**). The structural basis for actin treadmilling is provided by the exposed ATPase fold of actin subunits towards the pointed end (according to the feather-like appearance, also minus end), which is consequently masked at the opposite barbed end (plus end) (Holmes et al., 1990).

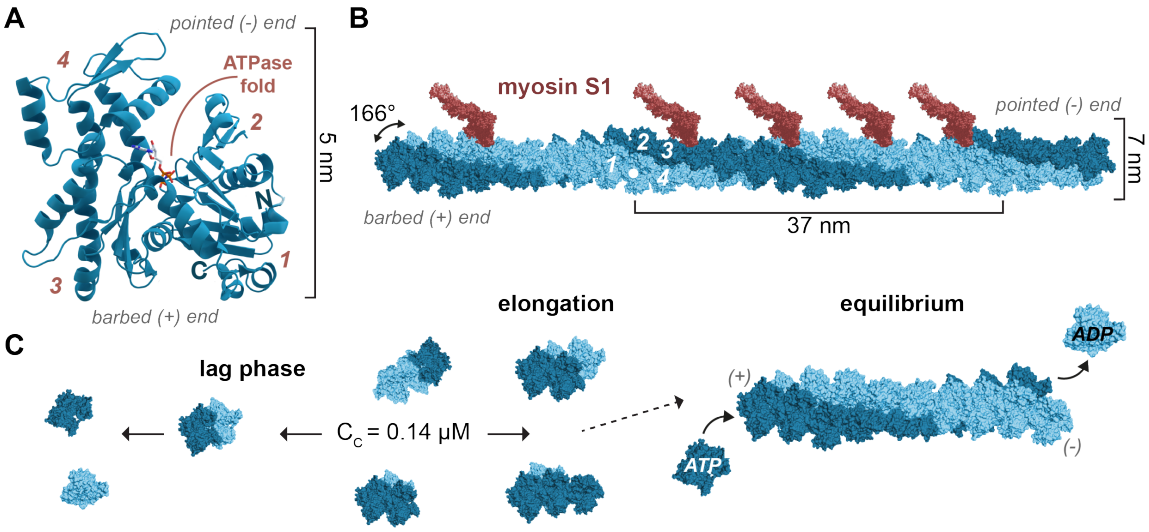

Figure 1: Features of G-actin, actin filaments and actin assembly.
(**A**) Protein structure of G-actin bound to ATP (PDB 1J6Z). Subdomains 1-4 and the ATPase fold are indicated. (**B**) 3D space-filling model of actin monomers (PDB 1J6Z) aligned to a filament with schematically bound myosin S1 (PDB 6C1H) to illustrate the feather-like appearance and polarity. Neighboring subunits are numbered for the indicated (•) monomer. (**C**) Representative schematic of individual actin polymerization steps in solution. C_C, critical concentration.

1.4 Treadmilling and assembly kinetics of actin filaments

Actin treadmilling and the nucleotide-bound state of G-actin influence endwise assembly kinetics of actin filaments. Beginning at the barbed end, actin is bound to ATP, but subunits along the actin filament hydrolyze ATP to ADP + P_i. P_i eventually dissociates and readily disassembling ADP-bound G-actin remains at the pointed end (Blanchoin & Pollard, 2002), whereas G-actin^{ATP} monomers are preferentially incorporated at the barbed end (Kirschner, 1980) (Figure **1C**). Nucleotide exchange from ADP to ATP in G-actin is catalyzed by CAPs (cyclase-associated proteins), Profilin or Thymosin, thereby regenerating the pool of polymerization-competent monomers (Goldschmidt-Clermont et al., 1992; Kotila et al., 2018).

The rate-limiting step of actin assembly (lag phase) is the formation of a trimeric actin complex (actin trimer or nucleus) due to its preference for additional binding of subunits over disassembly of present ones given a sufficient actin monomer concentration (critical concentration for actin polymerization (C_C)). Actin nuclei will begin to polymerize (elongation phase) leading to an equilibrium state characterized by balanced assembly and disassembly rates of actin monomers (Vavylonis et al., 2005). Experimental evidence of actin in solution shows barbed end assembly rates of 280 subunits s^{-1} under optimized conditions and a C_C of 0.14 μM in bulk actin assembly (Pollard, 1986) (Figure **1C**).

However, in regard to actually determined actin concentrations in cells and the requirement for spatiotemporal regulation, monomeric actin needs to be bound to other factors to prevent spontaneous polymerization and actin nucleation has to be controlled for regulated filament assembly. Accordingly, different actin-binding proteins and nucleation factors were identified steering spatiotemporal actin dynamics.

1.5 Mammalian actin nucleators and actin-binding proteins

The actin-related protein (Arp) 2/3 complex, formins, and tandem-monomer-binding nucleators, such as JMY or Spire (Campellone & Welch, 2010) (Figure **2A, B**) represent three distinct families of actin nucleators in vertebrates. More recently, actin nucleation factors with different mechanisms were identified, i.e. Adenomatous *polyposis coli* (APC) (Juanes et al., 2017; Okada et al., 2010) or Leiomodins in muscle cells (Chereau et al., 2008).

The Arp2/3 complex first discovered in *Acanthamoeba* (Machesky et al., 1994) consists of seven subunits and has the unique ability to nucleate actin filaments on the surface of pre-existing ones. Daughter filaments are nucleated at a 70° angle on Arp2/3 complexes bound to already present actin filaments (Mullins et al., 1998). The pointed end of daughter filaments is capped by the Arp2/3 complex, which leaves the barbed end free for elongation or binding of capping proteins (Figure **2A**), creating Y-branched networks of actin filaments found in membrane ruffles or lamellipodia (Goley & Welch, 2006) (Figure **3**). By itself, the Arp2/3 complex nucleates actin poorly, but filament binding, different nucleation-promoting factors (NPFs) containing actin monomer-binding WH2 (Wiskott-Aldrich syndrome homology region 2) domains (i.e. WASP or Scar/WAVE) or phosphorylation of the Arp2 subunit can enhance this quality (Monfregola et al., 2010; Padrick et al., 2008; Padrick et al., 2011; LeClaire et al., 2008).

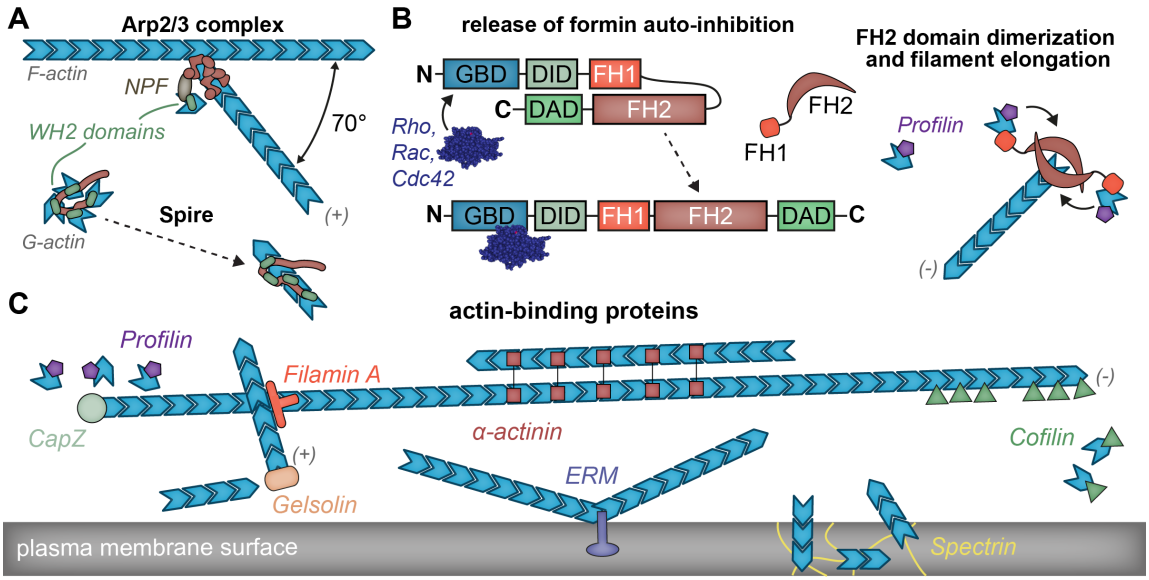

Figure 2: Functions of exemplary actin-binding proteins and nucleation factors. **(A)** Actin nucleation by NPF-activated Arp2/3 complex or Spire with indicated WH2 domains. NPF, nucleation promoting factor; WH2, Wiskott-Aldrich syndrome homology region 2. **(B)** Prototypical formin domain structure and release of auto-inhibition by Rho, Rac or Cdc42 binding (PDB 1FTN), FH2 domain dimerization, Profilin-G-actin^{ATP} binding by FH1 domains and filament elongation. GBD, GTPase-binding domain; DID, Diaphanous-inhibitory domain; FH1/2, formin homology domain 1/2, DAD, Diaphanous-autoregulatory domain. **(C)** Exemplary actin-binding proteins interact schematically with actin to perform illustrated functions. ERM, Ezrin, Radixin, Moesin.

In contrast to the Arp2/3 complex, formins elongate linear filaments and can further act as capping or bundling proteins (Baarlink et al., 2010). Mammalian cells express about 15 different formins, characterized by two defining domains, termed formin homology (FH) 1 and 2. The current model based on crystal structures of yeast formins indicates that two crescent FH2 domains form a donut-shaped head-to-tail dimer with intertwined connections, which adds actin subunits in a stair-stepping fashion while processively capping the barbed end (Xu et al., 2004). FH1 domains can bind Profilin and G-actin^{ATP}, thereby recruiting polymerization-competent actin monomers to the FH2 domains (Paul & Pollard, 2008) (Figure **2B**). One feature defining Diaphanous-related formins (DRFs, mDia1-3), inverted formin 2 (INF2), formin-related proteins (Fmnl1-3), Dishevelled-associated activator of morphogenesis (Daam1 and 2) as well as FH1/2-domain-containing proteins (Fhod1 and 3) is an intrinsic protein interaction between the N- and C-terminus mediated by the Diaphanous-inhibitory and the Diaphanous-autoregulatory domain (DID-DAD interaction, DID is sometimes referred to as FH3) (Breitsprecher & Goode, 2013), which sterically blocks FH2 domain dimerization and thereby its activity (Schönichen & Geyer, 2010). Rho GTPases (i.e. RhoA, Rac1 or Cdc42) (Rao et al., 2013) can bind to GTPase-binding domains (GBD, a region in front of the N-terminal DID) to prevent DID-DAD interactions and release the self-mediated inhibition allowing for actin filament elongation (Li & Higgs, 2003; Grikscheit et al., 2015) (Figure **2B**). Apart from formin autoinhibition, the DAD likely exerts additional functions in actin nucleation extending the current stair-stepping model (Gould et al., 2011) and intrinsic, biophysical properties of actin filaments such as force or torque further influence formin-mediated actin polymerization (Yu et al., 2017). Other formins lacking the DID-DAD interaction are Delphilin, inverted formin 1 (INF1) as well as Formin (Fmn) 1 and 2 (Faix & Grosse, 2006). FMN1 and 2 contain unique formin-spire interaction motifs, allowing for a direct cooperation between these two factors (Vizcarra et al., 2011). Some formins such as mDia2 and 3 or INFs are shown to interact with other cytoskeletal components, such as microtubules during kinetochore attachment or cell migration (Cheng et al., 2011; Daou et al., 2014; Bartolini & Gundersen, 2010). Various events such as phosphorylation (Wang et al., 2015) or lipid modifications (Grikscheit & Grosse, 2016) also regulate formin activity and their subcellular localization, allowing formins to fulfill a function in various actin structures, i.e. stress fibers (Gasteier et al., 2003;

Baarlink et al., 2010) or Fhod1-dependent TAN (transmembrane actin-associated nuclear) lines (Kutscheidt et al., 2014).

Spire, COBL or JMY (Zuchero et al., 2009) belong to a third class of tandem-monomer-binding actin nucleators and nucleate linear filaments. JMY is regulated in a p53-dependent manner during DNA damage and either acts as an Arp2/3 complex activator or nucleates actin by itself (Zuchero et al., 2009). Specific arrangements of WH2 or other G-actin-binding domains within these proteins nucleate actin by directly forming stable actin multimers as indicated by X-ray scattering studies, thus omitting the lag phase of actin assembly (Rebowski et al., 2008) (Figure **2A**). Spire can interact with Fmn to polymerize actin synergistically (Vizcarra et al., 2011); although the precise mechanism is not fully understood (Montaville et al., 2014; Baum & Kunda, 2005), its functional relevance has been demonstrated *in vivo* during asymmetric cell division of oocytes (Pfender et al., 2011) or in multiciliated cells (Yasunaga et al., 2015).

Other actin-binding proteins interact with the monomeric form of actin (i.e. Profilin with G-actin^{ATP} and Cofilin with G-actin^{ADP} (Kotila et al., 2018)), which is involved in actin treadmilling, or with actin filaments performing different functions such as cross-linking, bundling, capping or severing (i.e. Filamin A, α-actinin, CapZ, or Gelsolin) (Figure **2C**). As such, Cofilin interacts with actin filaments and generates a rotational twist resulting in a higher disassembly rate towards the pointed end (Bamburg 1999), but also displays filament severing and other activities dependent on the subcellular signaling context (Bravo-Cordero et al., 2013). The motor protein Myosin binds to actin filaments (Figure **1B**) and is crucial for mediating intracellular transport (Titus, 2018) and the generation of contractile force (Cramer, 2008). Other important interacting proteins are Tropomodulin, Tropomyosin or Thymosin, which were originally found in muscle or thymic tissue (Von Der Ecken et al., 2015; Bonello et al., 2016; Goins & Mullins, 2015; Pollard et al., 2000; Goldschmidt-Clermont et al., 1992). Spectrins (Machnicka et al., 2014; Young & Kothary, 2005; Simon & Wilson, 2011; Weber et al., 1994) are abundant in erythrocytes as well as neuronal cells (He et al., 2016) and mediate alongside of ERM proteins (Ezrin, Radixin, Moesin) (Hinojosa et al., 2017; Vilmos et al., 2016; Kristó et al., 2017; Fehon et al., 2010) direct membrane interactions of actin filaments (Figure **2C**).

Taken together, the dynamic assembly and disassembly of actin (actin dynamics) underlies a complex regulation in cells, but functionally distinct actin structures could be characterized biochemically and by imaging efforts.

1.6 Actin structures in mammalian cells

Actin structures influence cell motility, cell division, vesicle and organelle movement (i.e. clathrin-mediated endocytosis and mitochondrial fission) (Schook et al., 1979; Boldogh et al., 2001; Rehklau et al., 2017; Chakrabarti et al., 2018) signaling (Grosse et al., 2003), cell junctions and shape (Grobe et al., 2018; Grikscheit & Grosse, 2016). Distinct structures are vitally important for embryogenesis or wound healing (Colin et al., 2016; Martin & Lewis, 1992), but also involved in pathophysiological processes such as cancer cell invasion (Nürnberg et al., 2011), specific muscular, cardiac and auricular defects (Clarkson et al., 2004; Tang et al., 2009) or associated with intracellular pathogens, i.e. *Listeria monocytogenes*, which utilizes the Arp2/3 complex to polymerize comet tails for intracellular motility (Welch et al., 1997).

In general, actin filaments are in close contact to membranes and enable versatile processes with rapid dynamics (Doherty & McMahon, 2008; Hinojosa et al., 2017). Changes in cell shape due to interactions with the plasma membrane represent a basic function of F-actin (Bezanilla et al., 2015), i.e. at the leading edge of migrating cells (lamellipodia) described in an elastic Brownian ratchet model (Weed et al., 2000; Mogilner & Oster, 1996) or in the formation of the cytokinetic ring for abscission (Watanabe et al., 2008) (Figure **3A, B**). Other actin-dependent structures can be present in microvilli, adherens junctions (AJs), the cell cortex (cortical actin rim), filopodia and stress fibers (Figure **3A, B**); all of which disassemble during cell division for the formation of the cytokinetic ring and consequently re-assemble during interphase (Figure **3C**). Additional functions of actin filaments include scaffolding as well as generating polymerization-dependent force and tension (Feric & Brangwynne, 2013). Aberrant filamentous structures found in actin rods and patches are pathophysiological (Figure **3B**) and a byproduct of cellular stress, i.e. in neurodegenerative diseases (Minamide et al., 2000). These and other cell cycle- or signal-regulated actin structures are also formed in the nuclear compartment.

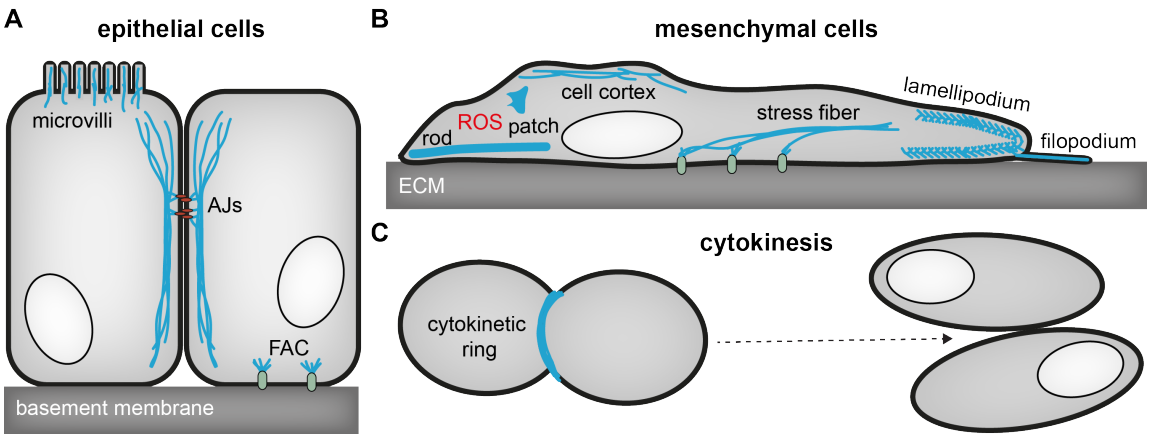

Figure 3: Actin structures in mammalian cells.
Depiction of actin structures (blue) in (**A**) epithelial, (**B**) mesenchymal or (**C**) cytokinetic cells. AJs, adherens junctions; ECM, extracellular matrix; FACs, focal adhesion complexes; ROS, reactive oxygen species.

1.7 Actin inside cell nuclei

While the general presence of actin in mammalian cell nuclei has been established in 1963 after discovering ATP- and $MgCl_2$-dependent actomyosin-like behavior in nuclear extracts (Ohnishi & Kawamura, 1963), the discussion about its present polymerization states was inconclusive and only resolved later. Initially, only monomeric forms of actin (Gonsior et al., 1999) and stress-induced nuclear actin rods (Iida et al., 1986) were readily detectable (Figure **4A-C**) inspiring the premise that dynamic actin filaments do not form within this compartment (Kapoor & Shen, 2014). The dot-like staining pattern of the 2G2 actin antibody (Gonsior et al., 1999) and the lack of a phalloidin-positive signal (conventional F-actin marker (Lengsfeld et al., 1974; Vandekerckhove et al., 1985)) (Belin et al., 2013) lead to the conclusion that nuclear actin filaments do not exist or adopt novel, unconventional conformations (Munsie et al., 2009; Gonsior et al., 1999). Only recent technical and methodological advances enabled confident visualization of intranuclear actin filaments (Baarlink & Grosse, 2014), which appear to form as short-lived event-mediated responses regulated by signaling cascades (Plessner & Grosse, 2015).

Indirect kinetical analyses refer to differently behaving pools of nuclear actin, i.e. a nucleocytoplasmic shuttling fraction of polymerization-competent G-actin, stably incorporated actin monomers as well as subunits in potentially filamentous structures (about 20% of total nuclear actin) (Belin et al., 2013; McDonald et al., 2006). In

somatic cell nuclei, the presence of a polymerization-competent pool of actin underlies nucleocytoplasmic shuttling dynamics (Grosse & Vartiainen, 2013). The import and export of G-actin is mediated in a Ran-dependent manner by Importin 9 as well as Exportin 6 and respectively coupled to Cofilin or Profilin (Stüven et al., 2003; Dopie et al., 2012) (Figure **4A**).

1.8 Diverse functions of monomeric nuclear actin

Next to polymerization-competent nuclear actin monomers, another fraction is stably incorporated in different chromatin-remodeling complexes such as SWI/SNF (Schubert et al., 2013; Rando et al. 2002), NuA4 (Wang et al., 2018) or INO80 (Kapoor et al., 2013; Ayala et al., 2018) (Figure **4B**). In the INO80 complex, actin is constrained by Arp4, Arp5 and Arp8, sterically blocking barbed end elongation and ensuring its monomeric state (Brahma et al., 2018; Kapoor et al., 2013). Herein, the ATPase activity of G-actin is thought to act as a molecular switch, inducing conformational changes within the complex (Kapoor & Shen, 2014; Bajusz et al., 2018; Kadoch & Crabtree, 2015).

Nuclear G-actin is also important for the transcriptional activity and localization of all three RNA polymerases (Serebryannyy et al., 2016; Miyamoto & Gurdon, 2011; Miyamoto et al., 2011; Grosse & Vartiainen, 2013) and plays a role in recruitment of the transcriptional elongation factor P-TefB (Qi et al., 2011). Pre-mRNA processing and gene splicing is affected by the interaction of actin with hnRNPs (heterogenous nuclear ribonucleoproteins) (Sjölinder et al., 2005; Percipalle, 2002; Percipalle, 2013), which can lead to a recruitment of histone acetyl transferases (HATs) (Obrdlik et al., 2008) and other histone-modifying complexes (i.e. Nu4A) to affect the epigenetic landscape around actively transcribed genes (Zheng et al., 2009) (Figure **4B**). Another interaction is found between DNase I and G-actin (Kabsch et al., 1990) rendering the enzyme inactive and regulating DNase I function during apoptosis (Eulitz & Mannherz, 2007; Sinxadi et al., 2016) (Figure **4C**); the picomolar affinity of this interaction was also effective for early crystallization efforts of G-actin (Kabsch et al., 1990). Of note, a fraction of the nuclear G-actin pool appears to be post-translationally modified by SUMOylation (Figure **4**), a covalent linkage with a small ubiquitin-like modifier (SUMO) (Hofmann et al., 2009). While further functional implications of this post-translational modification are still unclear (Alonso

et al., 2015), SUMOylations on Lys-68 and -284 appear to influence nucleocytoplasmic shuttling of actin by favoring its retention in the nuclear compartment (Hofmann et al., 2009).

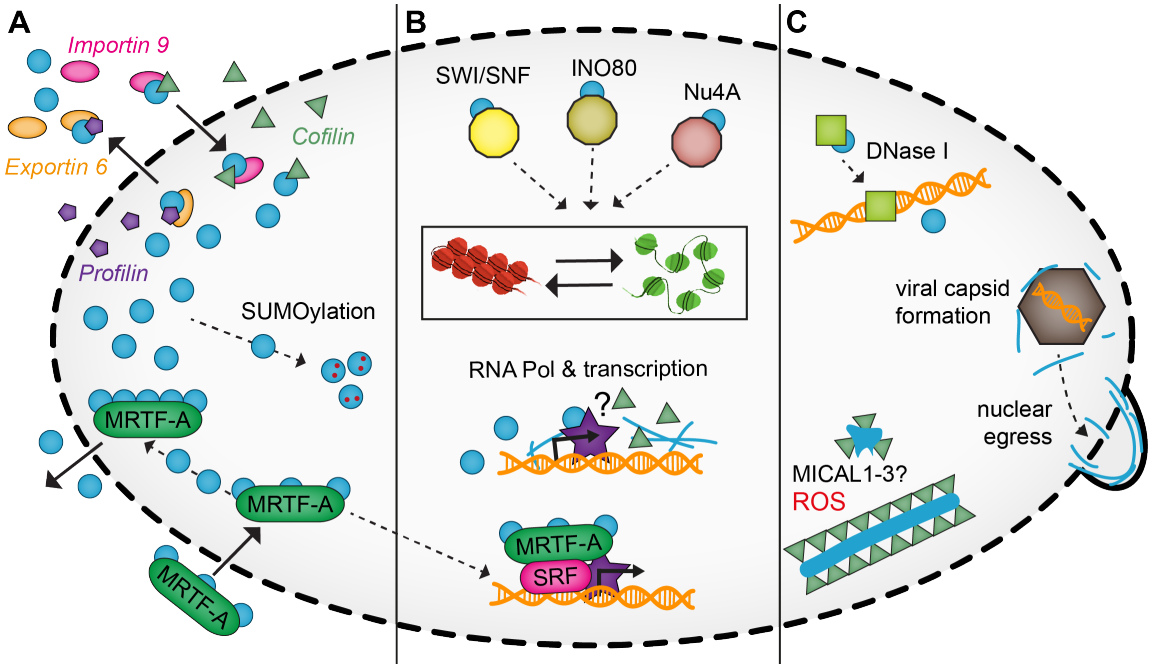

Figure 4: Monomeric actin and pathophysiological actin filaments in somatic cell nuclei.
(**A**) Nucleocytoplasmic shuttling of polymerization-competent monomers, actin-dependent translocation of MRTF-A and SUMOylation of actin. (**B**) Stably incorporated monomers in chromatin remodeling complexes as well as monomers and filaments associated with transcription. (**C**) Pathophysiological actin patch and rod formation, DNase I and viral processes. ROS, reactive oxygen species.

1.9 Evidence for nuclear actin filaments

Although the requirement of G-actin for numerous fundamental nuclear functions has been established, its polymeric form is still insufficiently investigated, but recently receiving more attention. Indications for nuclear F-actin functions in somatic nuclei could be derived from studies in germline cells of non-mammalian model organisms, in which nuclear F-actin has been experimentally confirmed since 1973 (Clark & Rosenbaum, 1979; Parfenov & Galaktionov, 1987). Frog, starfish and other oocytes lack the nuclear actin export factor Exportin 6 (Schuh & Ellenberg, 2006; Stüven et al., 2003; Bohnsack et al., 2006; Mogessie & Schuh, 2017) (Figure **4A**) allowing nuclear actin concentrations of above 100 μM and increasing the possibility for nuclear

actin assembly (Samwer et al., 2013; Bohnsack et al., 2006; Stüven et al., 2003). Observed actin filaments participate in different contexts, i.e. at the nuclear envelope to facilitate chromatin binding (Oda et al., 2017), at nuclear pore complexes (NPCs) (Kiseleva, 2004) or in a perinuclear rim and contractile network to promote nuclear envelope breakdown and chromosome congression in the early stages of meiosis (Lénárt et al., 2005; Mori et al., 2014; Bun et al., 2018; Mori et al., 2011; Burdyniuk et al., 2018; Mogessie & Schuh, 2014). Xenotransplantation of muscle cell nuclei into frog oocytes revealed that actin polymerization by nucleation factors Arp2/3 and Wave1 (Miyamoto et al., 2013) are required to induce pluripotency genes such as *Oct4*, giving nuclear F-actin a role in transcriptional reprogramming (Miyamoto et al., 2011; Scheer et al., 1984).

Further indirect evidence could be derived from application of drugs interfering with actin filaments (such as Cytochalasins (Casella et al., 1981; Spudich, 1973) or Latrunculins (Spector et al., 1983)) and observing intranuclear movement of i.e. chromosomal loci (Dundr et al., 2007; Spichal et al., 2016) or PML bodies (Muratani et al., 2002; Majewski et al., 2018). Interestingly, the majority of actin-binding proteins underlie nucleocytoplasmic shuttling dynamics (Kristó et al., 2016), partially by unconventional, NPC-independent mechanisms such as lateral diffusion through the nuclear envelope as shown for Myo1C (Nevzorov et al., 2018). Although visualization of actin filaments cannot always be presented, functional readouts in relation to actin-binding proteins should include rescue experiments performed with deficient mutants after knockdown of the endogenous proteins to specify the requirement for functional actin nucleation. Following this procedure, the FH2 domain of nuclear mDia2 was shown to be critical for CENP-A loading onto centromeres in G1 (Liu & Mao, 2016). Live cell imaging data of centromere-associated actin filaments recently confirmed this model (Liu et al., 2018). Of further interest, nuclear Cofilin interacts with actin and RNA polymerase II to allow transcription by hypothetically regulating local actin dynamics (Obrdlik & Percipalle, 2011; Xie & Percipalle, 2017), although such filaments remain to be observed (Figure **4B**).

Earliest reports of polymeric actin in mammalian cells include stress-induced pathophysiological nuclear actin rods or patches (Figure **4C**). These are partially associated with reactive oxygen species (ROS) potentially implicating redox modification of actin by MICAL1-3 (Lundquist et al., 2014; Grintsevich et al., 2016; Hung et

al., 2011; Aberle, 2013; Hung et al., 2010) (Figure **4C**) and can be observed during stress responses such as heat (Iida et al., 1986), neurodegeneration (Minamide et al., 2000), distinct chemical compounds i.e. DMSO (Fukui & Katsumaru, 1980) and myopathies (Stenzel et al., 2015; Serebryannyy et al., 2016). Pathophysiological nuclear F-actin can also be induced by viral infections (Cibulka et al., 2012), performing different functions such as viral capsid formation (Feierbach et al., 2006) or nuclear egress by rupturing the nuclear envelope (Hepp et al., 2018; Ohkawa & Welch, 2018) (Figure **4C**).

Increased nuclear envelope permeability, alteration of nucleocytoplasmic actin distribution (Belin et al., 2013) or deregulated actin dynamics (i.e. by overexpressed actin-binding proteins (Kelpsch et al., 2016; Du et al., 2015; Dopie et al., 2015)) aid in the formation of Cofilin-enriched nuclear actin rods (Nishida et al., 1987; Munsie et al., 2012) (Figure **4C**). Specific mutations stabilizing actin (Kokai et al., 2014; Stern et al., 2009) can disturb chromatin organization via alteration of histone modifications, i.e. by deregulation of HDACs and other histone-modifying enzymes (Serebryannyy et al., 2016). Altering nuclear actin levels by ectopic expression of actin variants results in defects of basic cellular functions such as transcription (Dopie et al., 2012) and mitosis (Kalendová et al., 2014) or induces cellular quiescence (Fiore et al., 2017); either due to a lack of monomeric actin in the nucleus or because of spatially diminished chromatin accessibility implicating a possible regulation of various processes by nuclear actin filaments as well as the requirement for tightly controlled nuclear actin levels.

1.10 Physiological nuclear actin assembly in somatic cells

In steady-state interphase conditions, nuclear G-actin levels are above the critical concentration, but filamentous structures cannot be readily observed (Belin et al., 2013; Bajusz et al., 2018). Advancements in nuclear actin probing concepts have led to the discovery of transient nuclear actin assembly upon serum stimulation (Melak et al., 2017). Apart from prior technical limitations, the apparent event-mediated nature of nuclear actin polymerization makes their experimental detection more difficult; signal-regulated nuclear actin assembly induced by stimulation with serum or one of its components LPA (Baarlink & Grosse, 2014) requires either rapid fixation or high frame rates for visualization (Baarlink et al., 2013). This GPCR-associated pathway

converges on nuclear formin activity (Figure **5**), reduces nuclear G-actin levels and thereby retains MRTF-A (myocardin-related transcription factor A; also, MAL or MKL1), the transcriptional co-activator of SRF (serum response factor) in the nuclear compartment for MRTF-SRF-dependent gene expression (Figure **4A, B**). The ability of formins (namely mDia1 and 2) to polymerize nuclear actin filaments is also observed when optogenetically releasing their auto-inhibited state, which leads to immediate and reversible nuclear actin assembly (Baarlink & Grosse, 2014; Baarlink et al., 2013).

Several studies indicate a function for polymeric nuclear actin during DNA damage responses, such as a critical involvement of actin-binding proteins (i.e. JMY (Zuchero et al., 2009) or SCAI (Hansen et al., 2016; Kreßner et al., 2013; Brandt et al., 2009)) or the impact of actin-depolymerizing drugs (Seeber & Gasser, 2017). Although nuclear actin filaments could be directly visualized upon DNA damage induced by multiple agents, the precise molecular mechanisms need to be further elucidated and expanded by cell type- and signaling context-dependent analyses, especially in relation to the distinctive actin nucleation factors, Fmn2 and Spire1/2 (Belin et al., 2015; Aymard et al., 2017) or Arp2/3 (Caridi et al., 2017; Caridi et al., 2018; Schrank et al., 2018) (Figure **7**). Interestingly, myosins are readily found in the nuclear compartment with different, functional implications (Onganía & Pomar, 2018; Xie & Percipalle, 2017) and involved in double-strand break (DSB) relocalization in *Drosophila* (Caridi et al., 2018), but this mechanism still needs to be addressed in mammalian cells (Figure **7**).

So far, physiological nuclear actin filaments in somatic cell nuclei could be visualized and characterized during GPCR or integrin signaling, DNA damage or reassembly of daughter nuclei after mitosis (Figure **7**) and are discussed in more detail as part of the *Results* and *Discussion* sections.

1.11 Cell-matrix adhesion and LINC complex

The extracellular matrix (ECM) in connective tissues consists of proteoglycans, hyaluronic acid, collagens, elastins, laminin as well as fibronectin in varying ratios (Iozzo, 1998) and provides the platform for cell-matrix adhesion. Individual cells connect to this substrate via the formation of focal adhesion complexes (FACs) (Horzum et al., 2014). FACs are a multi-protein complex, consisting of different receptors directly

binding to ECM components (i.e. integrins to RGD motifs found in fibronectin (Campbell & Humphries, 2011)) and adapter molecules linking these receptors to the cytoskeleton (Paxillin, Talin, Vinculin, Zyxin and α-actinin) (Bertocchi et al., 2017). *De novo* FAC formation is initiated upon contact of a detached cell to a substrate, which in turn activates specific signaling events (initially via activation of focal adhesion kinase (FAK) (Humphries et al., 2019)) for assembly of actin stress fibers (classified as dorsal or ventral stress fibers and transverse arcs (Hotulainen & Lappalainen, 2006; Young & Higgs, 2018)) as well as actin-based protrusions (Humphries et al., 2019). FACs are organized as units of fixed dimensions and undergo longitudinal splitting together with interacting stress fibers (Young & Higgs, 2018) (Figure **3B**).

Overall alterations in cell morphology are accompanied by nuclear shape changes resulting in specific gene expression profiles dependent on surface pattern-based force distribution (Jain et al., 2013). In particular, the transcription factors MRTF and YAP/TAZ are influenced by various parameters during cell adhesion, such as substrate stiffness, ECM composition (Meng et al., 2018) or actin dynamics (Grosse et al., 2003), regulate each other (Foster et al., 2017) and drive the expression of cytoskeletal target genes, which resembles a feed-forward loop (Olson & Nordheim, 2010).

Eukaryotic cells developed a connective module between the nuclear and cytoplasmic compartment, termed LINC (linker of nucleoskeleton and cytoskeleton) complex with specific proteins residing in the outer as well as inner nuclear membrane. The core interaction is composed of three Nesprin proteins in the outer membrane, which bind SUN1/2 trimers (Sad1- and UNC-84-domain containing proteins) via KASH (Klarsicht, ANC-1 and SYNE homology) domains in the transmembrane space (Sosa et al. 2012). In addition, Nesprins are connected to cytoskeletal filaments, which enable mechanotransduction by directly transmitting physical force to the nuclear compartment (Guilluy et al., 2014); SUN proteins are able to receive these signals by interacting with different nuclear proteins, i.e. Emerin, A- and B-type lamins, Myo1C and actin (Simon & Wilson, 2011; Holaska et al., 2004; de Leeuw et al., 2018; Dechat et al., 2010; Nevzorov et al., 2018; Dzijak et al., 2012). Disease-relevant implications of the LINC complex are laminopathies (Hatch & Hetzer, 2016; Starr, 2012) and specific myopathies, such as Emery-Dreifuss muscular dystrophy

(Puckelwartz et al., 2009; Morris, 2001; Holaska et al., 2004). Recently, the LINC complex has been implicated in chromatin organization and DNA repair (Aymard et al., 2017). Nesprins further interact with TAN lines (Kutscheidt et al., 2014) facilitating nuclear re-positioning, an active process required for efficient cell migration or differentiation (Saunders et al., 2017). Direct force engagement on the LINC complex was shown to activate nuclear Src kinases for a wide range of nuclear phosphorylation events as well as nuclear RhoA (Guilluy et al., 2014); however, activation of formins by nuclear small GTPases is an open question (Dubash et al., 2011; Baarlink & Grosse, 2014; Staus et al., 2014).

Complementing each other, cell adhesion, signaling via the LINC complex and the transcriptional output have to be considered in more complex settings, such as mesenchymal or amoeboid migration during cancer cell invasion (Labernadie et al., 2017; Chambliss et al., 2013; Ballestrem et al., 2000) or confined migration, which occurs during metastasis and diapedesis (Olson & Sahai, 2009) and readily causes nuclear envelope ruptures associated with micronuclei formation and DNA damage (Hatch & Hetzer, 2016; Denais et al., 2016; Ungricht & Kutay, 2017), culminating in genomic instability.

1.12 Mitotic exit in mammalian cells; nuclear re-assembly and volume

Mitotic exit in mammalian cells is defined as the time frame from nuclear re-assembly during telophase and cytokinesis to the presence of a functional interphase nucleus. The characteristic feature of this cell cycle phase completing mitosis (Schooley et al., 2012) is the re-assembly of the nuclear envelope on BAF (barrier-to-autointegration factor)-coated chromatin surfaces (Samwer et al., 2017) by the formation of tubular membrane structures from the endoplasmic reticulum (ER) (Lu et al., 2011; Anderson & Hetzer, 2007; Anderson & Hetzer, 2008; Lu et al., 2009), which further leads to the reformation of the nuclear lamina (Moir et al., 2000) and is accompanied by decondensation as well as spatial organization of mitotic chromosomes (Schooley et al., 2012). Precursors of nuclear pore complexes (NPCs) are inserted into the fenestrated nuclear envelope (Otsuka et al., 2018) and assembled in a temporally defined manner (Iino et al., 2010; Dultz & Ellenberg, 2010; Hampoelz et al., 2016) rapidly reinstating nucleocytoplasmic shuttling (Ungricht & Kutay, 2017). Spastin and

ESCRT-III interact with membranes and mediate sealing of the reformed nuclear envelope and disassembly of the mitotic spindle (Vietri et al., 2015). Signaling events governing these complex series of events primarily include dephosphorylation of CDK1 (cyclin-dependent kinase 1) substrates (Wu et al., 2010; Petrone et al., 2016; Hein et al., 2017) mediated by protein phosphatase PP2A-B55α (Schmitz et al., 2010) or PP1γ with Repo-Man (Vagnarelli et al., 2011), while other CDK1 substrates are subjected to proteasomal degradation during anaphase by APC/C (anaphase-promoting complex/cyclosome) (Chang et al., 2014).

During mitosis, chromatin takes on a compact conformation due to an ATP-dependent process facilitated by Condensins I and II, which resemble mechano-chemical motors to drive DNA loop extrusions and thereby condense chromatin to its characteristic chiasmatic shape during mitosis. Super-resolution microscopy (Cremer et al., 2018; Cremer & Cremer, 2001) and the genome-wide extension of chromosome conformation capture (3C) termed Hi-C (Cullen et al., 1993; Hakim & Misteli, 2012) revealed a complex chromatin architecture in interphase nuclei consisting of different topologically associated domains (TADs) and the A/B compartments, which summarize actively transcribed and silenced genes comparable to accessible eu- as well as condensed heterochromatin. In addition, chromosome territories initially defined by fluorescence *in situ* hybridization (FISH) as established, relative positions for decondensed chromosomes in interphase nuclei are propagated to daughter cells after mitosis (Manders et al., 2003). These arrangements are facilitated in the first quarter of the G1 phase (Nagano et al., 2017), cannot be obtained by mere removal of Condensin-mediated DNA loop extrusion and therefore require an active process. So far, RuvB-like ATPases associated with the INO80 chromatin remodeling complex (Ayala et al., 2018) are implicated in chromatin decondensation as assessed *in vitro* by inducing decondensation of mitotic chromosomes with *Xenopus* egg extracts (Magalska et al., 2014; Strzelecka & Heald, 2014). Other than the activity of chromatin remodelers and general dephosphorylation events (Landsverk et al., 2005; Manders et al., 2003), no processes are linked to chromatin decondensation leaving the reformation of complex nuclear architecture unexplained.

An inherently linked property to chromatin decondensation and nuclear reassembly is nuclear volume expansion, which is achieved due to the loss of Condensin-mediated compaction (Walther et al., 2018), formation of sub-nuclear structures (i.e. nucleoli or PML bodies) (Orlova et al., 2012), establishment of general nuclear architecture (TADs and A/B compartments) (Nagano et al., 2017) and nuclear import. Nuclear volume is coupled to cell size in yeast (Kume et al., 2017) and generally correlates with genome size among different organisms (Webster et al., 2009). In *Xenopus laevis*, nuclear volume is largely controlled by nuclear import via NPCs (D'Angelo et al., 2006), which could also be partially observed in mammalian interphase cells, whereas the expansion of daughter nuclei at mitotic exit is less well defined (Kume et al., 2017; Khalo et al., 2018; Jevtić et al., 2014).

2. Results

2.1 Premise and Outline

The objective of this dissertation was the characterization, manipulation and functional analysis of nuclear actin structures in mammalian cells, which included the observation of nuclear actin filaments during cell spreading, fibronectin (FN) stimulation and mitotic exit by live cell imaging. The consecutive application of different methods to interfere with identified nuclear actin structures in order to analyze their functional impact revealed further mechanistic insights into these processes.

In the following part of this dissertation, published results are summarized by explaining concepts used for visualization of nuclear actin followed by the description of studies on nuclear actin structures during cell adhesion and mitotic exit. References to published figures or data are indicated with a normal font, whereas summary figures are referred to in **bold**. Permission for reprints of published figures was obtained and is available upon reasonable request. Individual author contributions in regard to experimental data presented in the dissertation-relevant publications are noted in a separate paragraph for each publication using an *italic* font. The following lists show publications relevant to this dissertation in chronological order as well as other peer-reviewed publications. Publications (1-5) and a separate declaration are attached to this version of the dissertation.

2.1.2 Peer-reviewed publications relevant to this dissertation

1. ***M. Plessner***, M. Melak, P. Chinchilla, C. Baarlink, and R. Grosse, Nuclear F-actin formation and reorganization upon cell spreading. J. Biol. Chem. 290, 11209–11216 (2015).

2. ***M. Plessner*** and R. Grosse, Extracellular signaling cues for nuclear actin polymerization. Eur. J. Cell Biol. 94, 359–362 (2015). (Plessner & Grosse 2015).

3. *M. Melak*, **M. Plessner** and R. Grosse, Actin visualization at a glance. J. Cell Sci. 130, 1688–1688 (2017).

4. *C. Baarlink*,* ***M. Plessner****, A. Sherrard**, K. Morita, D. Virant, S. Misu, E.-M. Kleinschnitz, R. Harniman, D. Alibhai, S. Baumeister, K. Miyamoto, U. Endesfelder, A. Kaidi and R. Grosse, A transient pool of nuclear F-actin at mitotic exit controls chromatin organization. Nat. Cell Biol. 19, 1389–1399 (2017). *, shared contribution.

5. *M. Plessner* and R. Grosse, Dynamizing nuclear actin filaments. Curr. Opin. Cell Biol. 56, 1–6 (2018).

2.1.3 Other peer-reviewed publications

1. *A. Jelinek*, L. Heyder, M. Daude, **M. Plessner**, S. Krippner, R. Grosse, W. E. Diederich and C. Culmsee, Mitochondrial rescue prevents glutathione peroxidase-dependent ferroptosis. Free Radic. Biol. Med. 117, 45–57 (2018).
2. **M. Plessner**, J. Knerr and R. Grosse, Centrosomal actin assembly is required for proper mitotic spindle formation and chromosome congression. iScience, editorial assessment of revision.
3. *M. Abdellatif*, L. Hipp, **M. Plessner**, P. Walther and B. Knöll, Visualization of endogenous nuclear actin by immunoelectron microscopy. HACB, submission.

2.2 Actin Visualization at a Glance (3)

The summary of literature and research on visualization of F-actin in living or fixed cells revealed preferential choices for individual model organisms, a uniform design principle of most actin probes and the necessity for carefully maintaining expression levels to avoid changes in actin dynamics (Melak et al., 2017). In regard to visualization of actin structures in fixed mammalian cells, the fungal toxin and bicyclic heptapeptide phalloidin is defined as a gold standard and binds to junctions between individual subunits within an actin filament (Lengsfeld et al., 1974; Coluccio & Tilney, 1984).

Ideal phalloidin staining requires the preservation of native actin structures without fixative-induced alterations. Pre-extraction and fixation with glutaraldehyde or para-formaldehyde (PFA) (Leyton-Puig et al., 2016; Baarlink et al., 2013) in CSK buffer (Wilson et al., 2016) results in optimal fixation of actin filaments largely avoiding artifacts. Glutaraldehyde fixation requires $NaBH_4$ post-treatment to reduce free aldehyde groups (Leyton-Puig et al., 2016), thus avoiding autofluorescence in the 500-530 nm range upon blue light irradiation. The strong protein cross-linking effect of glutaraldehyde impairs epitope binding of most immunofluorescence antibodies, but can be alleviated by treatment with ethanolamine or lysine (McClung & Wood, 1982). In contrast to fixation with formaldehyde and glyoxal (pH = 6) (Richter et al.,

2017), which readily preserve actin filaments as well as antibody epitopes, methanol fixation is not appropriate due to its actin-depolymerizing effect (Prast et al., 2006). Nevertheless, glutaraldehyde still provides the most accurate appearance compared to high-pressure freezing of cryo-substituted cells (Leyton-Puig et al., 2016).

Novel detection methods or super-resolution approaches combined with careful sample preparation (Virant et al., 2018; Traenkle & Rothbauer, 2017; Baarlink et al., 2017; Plessner & Grosse, 2015) can facilitate the detection of phalloidin-sensitive nuclear actin filaments in native cells (Baarlink et al., 2013). Nuclear F-actin visualization requires confocal imaging coupled to sensitive detection (enabling low excitation) with a high dynamic range to detect faint nuclear actin fluorescence while avoiding interference due to the overexposed signal obtained from cytoplasmic actin, which is generally achieved by current applications such as GaAsP detectors (Michalet et al., 2007) or sCMOS and EM-CCD cameras (Broughton, 1993; Huang et al., 2013).

Considering live cell imaging, conventional probes for endogenous actin (Belin et al., 2014; Melak et al., 2017) are largely restricted to the cytoplasm due to a comparably low abundance of actin in nuclei. The common design of these probes relies on the presence of an actin-binding domain to locally enrich the fluorophore-conjugated or differently tagged probe at actin filaments while creating a uniform cytoplasmic background signal due to free or actin monomer-bound configurations. Fusion of cytoplasmic probes with nuclear localization sequences (NLS) (Melak et al., 2017; Baarlink et al., 2013) enables faithful visualization of polymeric nuclear actin upon stimulation, whereas additional fusion with an NES (nuclear export sequence) allows simultaneous visualization of the cytoplasmic actin pool. These concepts have been applied in the discovery of nuclear actin assembly during serum or fibronectin stimulation, mitotic exit and DNA damage (Caridi et al., 2018; Schrank et al., 2018; Belin et al., 2015; Plessner et al., 2015; Baarlink et al., 2017; Baarlink et al., 2013).

The 3D space-filling models of the actin filament and actin-binding probes were created by M. Melak. Live cell imaging data showing different actin probes was generated by M. Plessner.

2.3 Nuclear F-actin Formation and Reorganization upon Cell Spreading (1)

In continuity to my bachelor thesis “Nuclear Actin Polymerization in Cell Motility” (Plessner, 2014), which summarized initial findings on nuclear F-actin formation after cell spreading (Figure 2D; **5A**), the nuclear Actin-Chromobody (nAC, a single chain nanobody directed against actin conjugated with a fluorescent protein (Actin-Chromobody) fused to an NLS (Fig. 1A)) was introduced as a novel actin probe to visualize nuclear actin dynamics in living cells (Figure 1B, C), allowing for faithful visualization of spreading-induced nuclear F-actin formation (Figure 2B, C; 3A; **5B**).

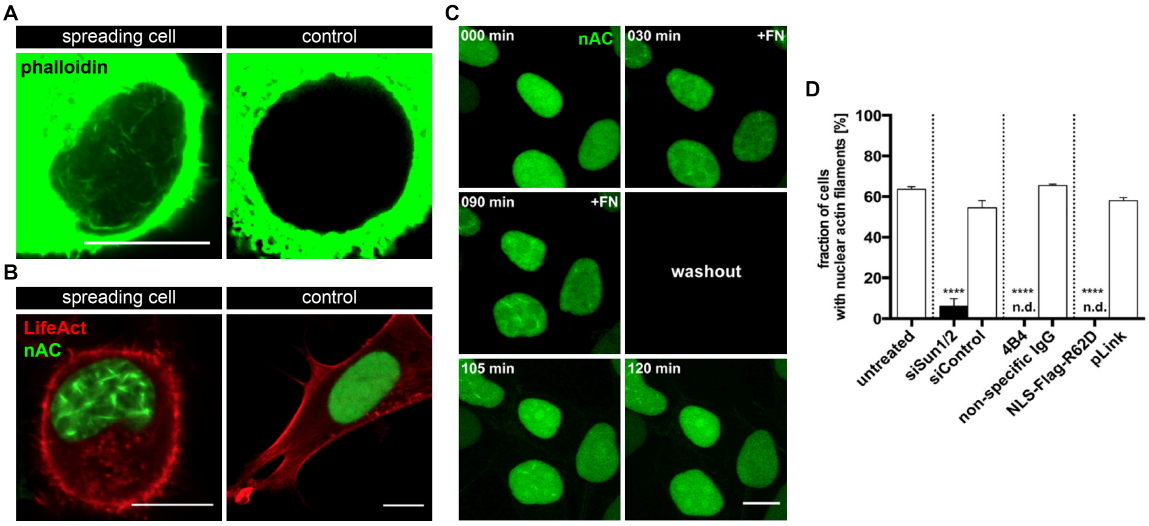

Figure 5: Summarized data adapted from “Nuclear F-actin Formation and Reorganization upon Cell Spreading” (1).

(**A**) Phalloidin staining of NIH3T3 cells, fixed with glutaraldehyde at 2 (spreading cell) or 12 (control) hours after spreading on an uncoated glass surface. Scale bar, 10 μm. (**B**) According to (**A**), NIH3T3 cells transfected with LifeAct-mCherry and nAC-GFP were imaged during and after cell spreading. Scale bar, 10 μm. (**C**) NIH3T3-nAC cells were stimulated with soluble FN (000 min) and imaged over time. Washout of FN-containing medium was performed after 90 min and shows reversibility of nuclear F-actin formation. Maximum intensity projections of acquired z-stacks are shown. Scale bar, 10 μm. (**D**) Quantification of FN-induced nuclear F-actin in NIH3T3-nAC cells under indicated conditions after 90 min. Data were collected from 3 independent experiments and shown as mean ± SEM. Related conditions were analyzed by a two-sided, unpaired Students t-test; ****, $P < 0.001$. n.d., not detected; 4B4, integrin β1-blocking antibody.

In contrast to the rapidly induced nuclear actin network after serum stimulation, cell spreading resulted in bundled filaments, which were detectable in about 75% of analyzed nuclei within 60-90 min (Fig. 3A, B). As the applied cell spreading assay requires *de novo* FAC formation, we performed further mechanistic investigations by stimulation with soluble FN to directly engage integrin β1, revealing reversible nuclear F-actin formation (Figure 4A; **5C**). Pre-treatment with the integrin β1-blocking antibody (4B4, Beckham-Coulter) abolished formation of nuclear actin filaments, identifying integrin-based signaling as the initial trigger mechanism. This experimental design allowed further the assessment of mechanotransduction by manipulating LINC complex components (i.e. SUN1/2 RNAi (RNA interference) experiments), which was critically required to induce FN-mediated nuclear F-actin (Figure 4B, E; **5D**). Ectopic expression of the actin mutant Arg-62-Asp (actin^{R62D}) acts presumably dominant-negative on actin assembly by prohibiting further barbed end incorporation of actin monomers (Posern, 2002; Kokai et al., 2014) and consequently inhibits as a nuclear-targeted version the FN-induced nuclear actin response (Figure 4D, E; **5D**). In addition, nuclear mDia formins are required as shown by RNAi against mDia1/2 as well as expression of a nuclear-targeted dominant-negative mDia (dnDia.NLS) (Figure 5A-D) (Baarlink et al., 2013). Knockdown of nuclear envelope proteins (A-type lamins and Emerin) (Fig. 5C) resulted in decreased nuclear F-actin formation without affecting cell spreading *per se* (Fig. 5D). Cell adhesion or FN stimulation lead to an increase in MRTF-A-dependent gene expression as shown by endogenous MRTF-A immunostaining as well as SRF-dependent reporter gene assays, which depend on nuclear formin activity (Figure 5E-G). In summary, this study uncovered a signaling pathway connecting integrin signaling through the LINC complex to nuclear F-actin formation and MRTF-A-dependent transcriptional activity (Figure 6).

C. Baarlink generated the nuclear Actin-Chromobody (nAC) as a derivative of the Actin-Chromobody-TagGFP2 (ChromoTek, Planegg-Martinsried). Other actin probes depicted in Figure 2A-C were provided by M. Melak. All experimental data in this manuscript were generated by M. Plessner with the exception of mDia1/2 data shown in Figure 5C, which was provided by P. Chinchilla.

2.4 A transient pool of nuclear F-actin at mitotic exit controls chromatin organization (4)

Analysis of fixed nAC-expressing NIH3T3 cells on glass coverslips showed an overrepresentation of nuclear actin filaments in neighboring cells, which were identified as daughter cells at mitotic exit and led to the hypothesis of cell cycle-dependent nuclear F-actin formation after cell division. Initial validation of this hypothesis was performed by long-term live cell imaging of NIH3T3-nAC cells, which showed nuclear actin assembly as a transient, self-mediated process during re-assembly of daughter nuclei at the end of mitosis.

Nuclear actin assembly at mitotic exit was studied with different actin probes and in relation to other cellular structures by confocal microscopy of reforming daughter cell nuclei (Figure 1a-e; **6A, B**). This process appears to be conserved among different mammalian cell lines with slight variations in appearance as well as duration (Figure S1c-e) and forms independent of A-type lamins, Emerin or the LINC complex (Figure S1f-i). Initial efforts of interfering with nuclear actin polymerization included the application of actin-depolymerizing drugs (Latrunculin, Cytochalasin or Swinholide A (Bubb et al., 1995)), which showed distinctively smaller daughter nuclei compared to DMSO-treated control cells implying failures in chromatin decondensation and nuclear organization (Figure **6C**). Live 3D imaging data (z-stacks) of the chromatin marker histone H2B allowed nuclear surface reconstructions using the image processing software IMARIS (Bitplane, Andor Technology, Belfast), from which the respective nuclear volume was measured as a surrogate to quantify a defective nuclear architecture (Mora-Bermúdez & Ellenberg, 2007). In accordance with visual impressions (Figure 4a; **6B, C**), nuclear volume measurements show an overall decrease upon inhibition of global actin polymerization during mitotic exit (Figure S3a, b; **6D**).

As RNAi-based searches for a specific nucleation factor (including most formins and the Arp2/3 complex) were inconclusive (Table S1) and to avoid effects on cytoplasmic actin dynamics by actin-depolymerizing drugs, reliable inhibition of nuclear actin assembly was obtained by overexpression of the nuclear actin export factor Exportin 6, which substantially lowers nuclear actin levels ultimately prohibiting polymerization (Figure 3b; S3a, b), or by nuclear non-polymerizable actin^{R62D} (Figure 3e; S3c-f). In addition to fluorophore-conjugated actin^{R62D}, self-cleaving Flag-actin-

T2A-SNAP constructs were generated to omit potential disturbances in formin-mediated actin assembly (Chen et al., 2012). Establishing the necessity of nuclear and not cytoplasmic actin polymerization, manipulations with nuclear actin^{R62D} or Exportin 6 resulted in defective volume expansion, which emerged only after mitosis (Figure 3c, d, f, g), but not in arrested interphase cells (Figure S3g). This was complemented by assessing chromatin densities of daughter cell nuclei, which is defined as the integrative sum of H2B fluorescence intensities divided by nuclear volume (Figure 4b-e; **6E, F**).

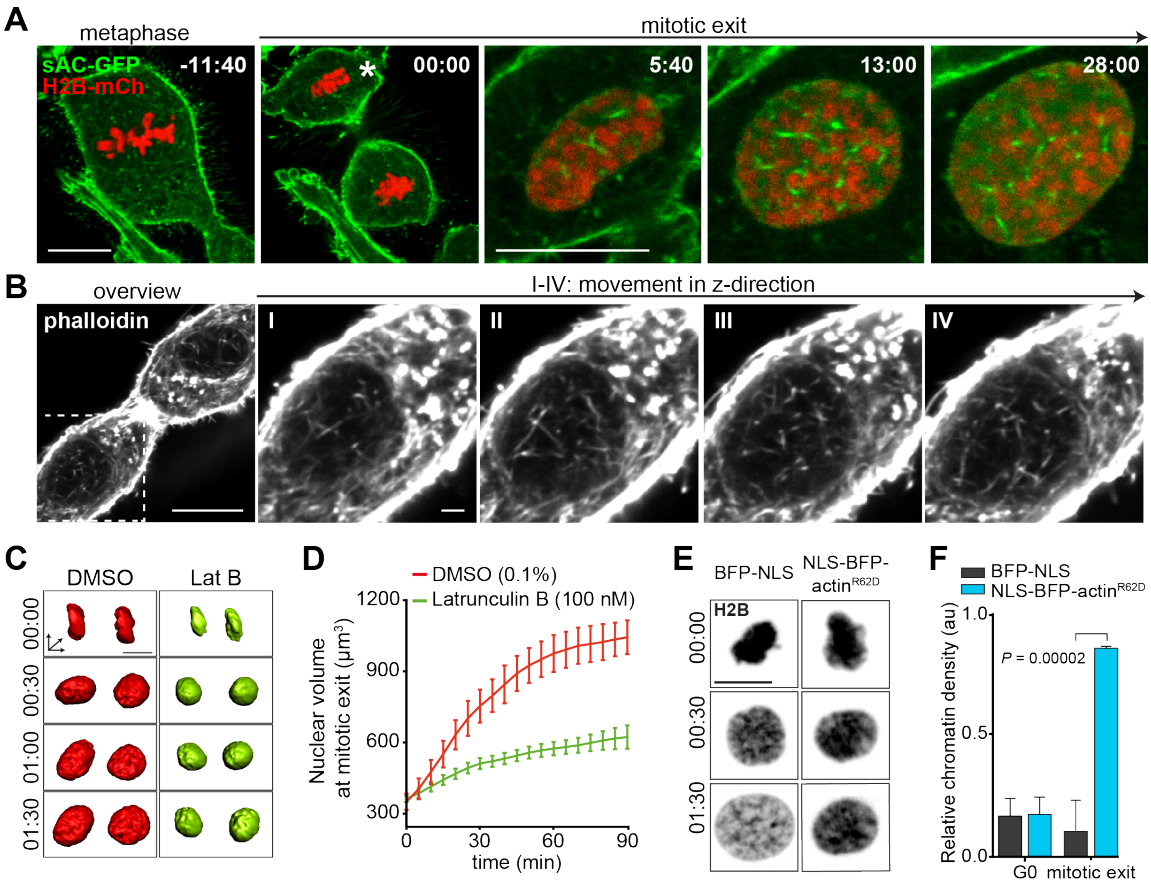

Figure 6: Summarized data adapted from "A transient pool of nuclear F-actin at mitotic exit controls chromatin organization" (4).

(**A**) NIH3T3 cells stably expressing sAC-GFP and H2B-mCherry were imaged during mitosis. The nucleus indicated by an asterisk is shown magnified for additional time points to emphasize nuclear volume expansion. Scale bar, 10 μm; time stamp, min:s. (**B**) Glutaraldehyde-fixed NIH3T3 cells at mitotic exit were stained with phalloidin. The area indicated by a dashed rectangle is shown magnified for individual confocal slices with a step size of 0.37 μm. Scale bars, 10 and 1 μm. (**C**) 3D surface reconstructions of NIH3T3-H2B-mCherry cells treated with DMSO (0.1%, control) or Latrunculin B (Lat B, 100 nM) at mitotic exit. Scale bar, 10 μm; time stamp, h:min. (**D**) Quantification of nuclear

volume corresponding to (**C**). n = 50 nuclei, data are shown as mean ± SD and pooled from at least 3 independent experiments. (**E**) NIH3T3-H2B-mCherry cells were induced to express either BFP-NLS or NLS-BFP-actin^{R62D} and imaged at mitotic exit. Inverted maximum intensity projections are shown to illustrate chromatin densities. Scale bar, 10 μm. (**F**) Quantification of relative chromatin density as the integrative sum of H2B fluorescence intensities divided by nuclear volume in interphase (G0, serum starvation) or 90 min after anaphase (mitotic exit). n = 60 nuclei, data are presented as mean ± SEM pooled from at least three independent experiments and analyzed by two-way ANOVA.

Nuclear F-actin at mitotic exit forms independent of transcriptional activity (Figure S3h, i), as filament formation was unperturbed by application of Flavopiridol (Bensaude, 2011), and is composed of single and bundled actin filaments as assessed by super-resolution microscopy of phalloidin staining (Figure 2; S2). Of note, single actin filaments are not discernable by PALM of nAC-Dendra2 due to the size of the photoconvertible protein (Figure 2b, d). Investigations into the functional relevance of this nuclear F-actin pool at mitotic exit were performed by assessing RNA Pol II-dependent transcription (Figure 5a; S5a) and general proliferation (Figure 5b), showing a significant decrease upon inhibition of nuclear actin assembly. Furthermore, nuclear F-actin was observed in fertilized mouse oocytes during pronuclei formation as well as after the first mitotic division and regulates nuclear volume expansion (Figure 5c-f), which resembles a requirement for early embryonic development, as expression of Exportin 6 leads to developmental defects (Figure 5g; S5b).

While the impact of inhibited nuclear actin assembly on nuclear volume expansion was striking, indirect mechanisms apart from chromatin decondensation are able to influence this feature (Webster et al., 2009). Thus, more precise investigations of chromatin decondensation were conducted by different assays on histone modifications (H3S10ph defines mitotic chromatin, H4K16ac is related to transcriptionally active chromatin in interphase (Johansen & Johansen, 2006; Wang & Higgins, 2013)) (Figure S4c-e) and by MNase digestion of chromatin from post-mitotic nuclei (Figure S4f), providing further evidence of defective chromatin decondensation upon nuclear F-actin inhibition. Other sophisticated analyses included electron microscopy of cryo-substituted cells, which depicts the electron density of chromatin and allows for classification into eu- and heterochromatin by trainable image segmentation (Figure 4j-l; S4g, h), as well as fluorescence lifetime (FLIM) measurements of fluorescence resonance energy transfer (FRET) between GFP- and mCherry-

tagged histone H2B (Llères et al., 2009; Lou & Hinde, 2018; Sherrard et al., 2018) (Figure 4f-i) to indicate nucleosome spacing. Control experiments with the HDAC inhibitor TSA (Trichostatin A) confirmed the validity of this approach, since increased histone acetylation leads to more accessible chromatin, which is reflected in a higher GFP-H2B fluorescence lifetime (Fig. S4a, b).

In search of mechanistic regulation, a phalloidin-based pulldown of F-actin (Samwer et al. 2013) in nuclear extracts from RO-3306-synchronized cell populations (Petrone et al., 2016; Vassilev et al., 2006) showed different actin-binding proteins (Figure 6a-d; Table S2). Among others, this proteomic search revealed the actin-depolymerizing factor Cofilin, which is inactivated by phosphorylation on Ser-3 (Moriyama et al., 1996). Phosphorylation kinetics show increasing Ser-3 phosphorylation levels at 70 min after mitosis, arguing for the presence of active, actin-depolymerizing Cofilin during the time frame of nuclear actin assembly at mitotic exit (Figure 6e, S6a-d). Accordingly, knockdown of endogenous Cofilin resulted in stabilized nuclear actin filaments (Figure 6f, g) and is not rescued by ectopic expression of an NES-tagged Cofilin variant (Figure 6h-j; S6e), demonstrating a function specific to the nucleus. Equivalent to inhibition of nuclear actin filaments, filament stabilization impairs chromatin decondensation as well as nuclear volume (Figure 6k, l) and overexpression of nuclear-targeted Cofilin conversely inhibits actin polymerization in this compartment during mitotic exit (Fig. 7a, b; S6f). Fusion of Cofilin to the optogenetic LOV2-based LEXY module (Niopek et al., 2016) enabled light-activated nuclear export of Cofilin (Figure 7c, d; S6g). While actively maintaining export of Cofilin, nuclear actin filaments are stabilized, but a dynamic rearrangement is induced by cessation of illumination allowing nuclear re-import of Cofilin, which immediately translates into an increase in nuclear size (Figure 7e, f) and implicates actin dynamics rather than filament formation for volume expansion and chromatin decondensation. Compatible with a requirement for nuclear actin dynamics, transient and F-actin-associated nuclear envelope protrusions are discernable during mitotic exit (Figure 1b, 3a) and AFM-based imaging reveals nuclear actin-dependent changes of nuclear surface topology (Figure 3h, i). Of note, such changes in nuclear surface topology were not detected upon TSA-mediated HDAC inhibition and thereby induction of chromatin decondensation in interphase (Fig 3h, j), arguing for an active process specifically during the early G1 phase.

Taken together, transient nuclear actin assembly at mitotic exit is dependent on Cofilin activity and possibly drives nuclear envelope protrusions to facilitate chromatin decondensation as well as the establishment of nuclear architecture at mitotic exit. Interfering with nuclear actin dynamics leads to defects in these processes as well as basic nuclear functions during the subsequent interphase.

Data in Figure 1a, b; 2a; 6e-j; 7c-f; S1a, b, i; S3c-f and S6a, b, e-g were provided by C. Baarlink. M. Plessner generated experimental data shown in Figure 1c-e; 3b-g; 4a-e; 5a, b; 6k, l; S1c-h; S3a, b, g-l; S5a; S6c, d as well as Table S1 and further analyzed data from other co-authors in Figure 5e, f; 7f and S1a. A. Sherrard was involved in generating AFM data in Figure 2h-j and S3a, b with R. Harniman, FLIM-FRET data (Figure 4f-i and S4a, b) with D. Alibhai, electron microscopy data in Figure 4j-l; 7a, b; S3h, and data from other chromatin decondensation assays in Figure S4c-f with A. Kaidi. Experiments on biological relevance in mouse oocytes were performed by K. Morita, S. Misu and K. Miyamoto (Figure 5c-g and S5b). Generation of super-resolution microscopy data in Figure 2 and S2 was a collaborative effort of E.-M. Kleinschnitz, M. Plessner, D. Virant and U. Endesfelder. Proteomic data in Figure 6a-d and Table S2 were generated by M. Plessner (sample preparation) with S. Baumeister (mass spectrometry).

2.5 Extracellular signling cues for nuclear actin polymerization, Dynamizing nuclear actin filaments (2, 5)

Relevant topics of published reviews (2, 5) (Plessner & Grosse, 2015; Plessner & Grosse, 2018) will be part of the *Discussion* and not separately summarized.

STED microscopy of phalloidin-stained serum-induced nuclear F-actin in Figure 1 (2) was kindly provided by H. Ta. Experimental data showing optogenetically induced nuclear actin assembly with mCherry.nuc.LOV-DAD (196) in Figure 2 (2) was generated by M. Plessner. No original data is included in (5).

3. Discussion

Unravelling prior skepticism on the existence of nuclear actin filaments in somatic cell nuclei, current research faithfully established that filamentous actin structures polymerize as a result of different signaling events (Figure 7). Visualization of actin in living cells is now able due to more refined actin probes, NLS-mediated nuclear targeting and technological advances (Belin et al., 2013; Plessner et al., 2015; Baarlink et al., 2013). Several general mechanisms were developed to negatively interfere with nuclear actin polymerization (actin^{R62D}, Exportin 6 or nuclear Cofilin) (Shav-Tal & Lammerding, 2015; Plessner et al., 2015; Baarlink et al., 2017) enabling the study of functional implications upon nuclear F-actin inhibition. Currently, the precise composition of chromatin and membrane interactions of nuclear actin filaments need to be elucidated and integrated with data from other experimental approaches (such as Hi-C or super-resolution microscopy of nano-scale chromatin contacts (Nagano et al., 2017; Belton et al., 2012; Nozaki et al., 2017)) in order to phenomenologically and mechanistically model the actin-dependent behavior of the nuclear compartment.

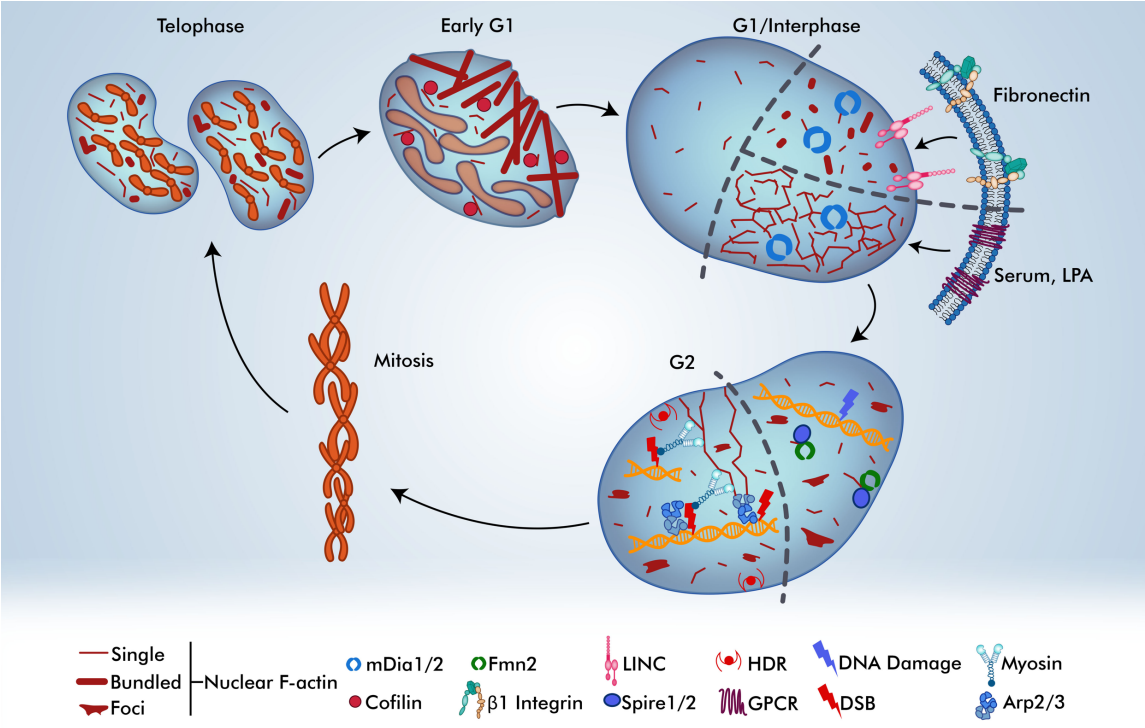

Figure 7: Cell cycle- and signal-regulated nuclear actin assembly in mammalian cells.
This artistic representation illustrates different forms of nuclear F-actin assembled during mitotic exit (cell cycle) or by stimulation with fibronectin, GPCR ligands and DNA-damaging agents (signal) (from (Plessner & Grosse 2019)).

3.1 Nuclear actin visualization and GPCR-mediated nuclear actin assembly (2, 3, 5)

The main concern of currently used actin probes is the artificial induction of nuclear actin structures (such as nuclear actin rods), which is readily observable upon high expression levels of LifeAct- or Utrophin-NLS, but not with the nAC (Melak et al., 2017). Nuclear actin rod formation can depend on probe-mediated filament stabilization or on alterations in nucleocytoplasmic shuttling dynamics of actin, which elevate nuclear actin levels and therefore induce unregulated filament formation. In line with this, nuclear actin levels appear unchanged upon stable expression of the nAC as determined by immunoblotting of cytoplasmic and nuclear fractions (Plessner et al., 2015) and by analysis of compartment-specific mCherry-actin fluorescence intensities (Baarlink et al., 2017). Although it is yet to be determined whether the nAC affects actin filament stability on a basic level, i.e. by altering the subunit rotation angle, this probe does not induce nuclear actin rods and has likely a lower binding affinity to actin than LifeAct. Theoretically, co-import of actin with NLS-tagged actin probes is conceivable, but does not result in increased nuclear actin levels for the nAC and therefore simply might not occur or is compensated by other cellular mechanisms. Additional fusion of an NES should enable co-export of actin in a similar manner and thereby directly antagonizes an increased import of actin.

In consideration of nuclear actin visualization in fixed cells, glutaraldehyde or PFA fixation with phalloidin staining are comprehensive techniques for the study of nuclear actin filaments. To exclude any exogenous influence of the applied fixatives or other reagents, native cells need to be fixed by cryo-substitution after high-pressure freezing and optimally analyzed by cryo-EM, which has recently been performed to provide atomic resolution of the nuclear lamina *in situ* (Turgay et al., 2017; Taimen et al., 2009). Disregarding the resolution of cryo-EM, extensive techniques and a Vimentin knockout are necessary to remove other cellular material in order to access the nuclear compartment, which potentially alters present actin structures. Thus,

phalloidin staining appears acceptable for characterization of novel nuclear actin structures, but should be performed without an actin probe present, otherwise artificial probe-induced filaments will be visualized with phalloidin leading to a false-positive assessment of nuclear F-actin (Belin et al., 2015; Du et al., 2015). In accordance, functional assays while interfering with nuclear actin polymerization should be conducted without an actin probe present to exclude any probe-derived perturbance of actin dynamics. Of interest, the missing visualization of transcription-associated nuclear actin filaments in light of the plethora of indirect evidence could be due to rapid dynamics within the milli-second range or due to complicated image acquisition in transcription-associated biochemical microenvironments, which potentially involve phase separation (Hnisz et al., 2017).

Serum and LPA-mediated signaling converge on nuclear formin activity and are regulated by GPCRs. Notably, the rapid activation of nuclear formins in the context of serum stimulation is reminiscent of calcium-dependent second messenger kinetics and further depends on calmodulin (CaM), CaM kinase and INF2 (*Y. Wang; personal communication*) to modulate chromatin dynamics, i.e. nucleosome spacing (Llères et al., 2009; Lou & Hinde, 2018). In line with these findings, mechanical or calcium-dependent stimulation induces changes in the actin cytoskeleton (calcium-mediated actin rest, CaAR) including the formation of a perinuclear actin rim (Wales et al., 2016; Shao et al., 2015), which likely influences general nucleocytoplasmic shuttling dynamics via NPCs and could be assessed by analyzing fluorescent Dextran distribution upon stimulation. T cell activation also initiates a striking cytoplasmic actin rearrangement comparable to CaAR (Wales et al., 2016) and involves Arp2/3-mediated nuclear actin filaments (Tsopoulidis et al., 2019), which are also implicated in DSB relocalization and might matter in more general forms of intranuclear transport (Caridi et al., 2018). Cofilin inactivation by phosphorylation at Ser-3 (Moriyama et al., 1996) or rapid nuclear export of Cofilin are potentially required to balance elevated nuclear Cofilin levels arising from the co-import of actin with Cofilin, otherwise the activated form of Cofilin would likely prevent efficient actin filament elongation. Nuclear Cofilin and p-Cofilin levels should be assessed after inducing store-mediated calcium release, i.e. by A23187, as CaM kinases were shown to influence Cofilin activity (Zhao et al., 2012). Since formin activation by nuclear small

GTPases is still a matter of debate, endogenous GTPases should be visualized without ectopic expression by CRISPR/Cas-mediated protein tagging at genomic loci and further experimentally pursued by immunostaining and immunoprecipitation in nuclear extracts.

3.2 Nuclear F-actin Formation and Reorganization upon Cell Spreading (1)

This study uncovered an integrin and LINC complex-dependent pathway for nuclear actin assembly, which subsequently influences gene expression by MRTF-A/SRF. It is tempting to speculate about other implications, such as additional downstream effects of nuclear actin filaments on chromatin organization, nuclear shape and positioning as well as on activating upstream events, such as force transduction during cell adhesion, which could be experimentally assessed by using a force biosensor during live cell imaging and FN stimulation (Grashoff et al., 2010). In turn, force transmission due to cytoplasmic shape changes and FAC formation should directly influence the nuclear envelope at LINC complex sites (Versaevel et al., 2014), which remains to be further investigated by AFM and live cell imaging of nuclear envelope dynamics.

Follow-up experiments show a potential link between transient nuclear F-actin formation upon ECM contact and MRTF-A translocation during directed cell migration in a 3D collagen-FN matrix (*M. Geißler, personal communication*). Specialized modes of cell migration involving spatial confinement stress the nuclear envelope and readily induce ruptures, which correlate with the induction of nuclear actin filaments (*M. Piel, personal communication*), possibly due to a transient unregulated exchange of cytoplasmic and nuclear content. However, it is still unclear whether this nuclear F-actin response is required to maintain genomic stability, although important functions for nuclear actin-mediated DSB clearance were identified (Schrank et al., 2018; Caridi et al., 2018).

3.3 A transient pool of nuclear F-actin at mitotic exit controls chromatin organization (4)

Comprehensive analyses already revealed the presence and function of nuclear actin filaments at mitotic exit in various cell lines, although the assembly mechanism is less well studied. A parallel study investigating the same phenomenon in U2OS and *Xenopus* cells describes a formin dependency of these nuclear actin filaments, as assessed by application of the pharmacological FH2 domain inhibitor SMIFH2 (Parisis et al., 2017). However, SMIFH2 does not influence nuclear volume expansion (*unpublished data*) and SMIFH2-treated nuclei still show residual, more bundled nuclear actin filaments (Parisis et al., 2017), although the DRF mDia2 is required for CENP-A loading in early G1 (Liu & Mao, 2016; Liu et al., 2018).

In line with missing evidence obtained by siRNA-mediated knockdown of individual actin nucleators, it is questionable whether *de novo* actin assembly occurs in the nuclear compartment or if already present actin filaments are engulfed during nuclear envelope re-assembly and reorganized during the early G1 phase. Of note, knockdown of actin nucleators likely influences cell division *per se* and therefore potential implications on nuclear actin assembly at mitotic exit can remain undetected. Thus, a more objective assessment is required, i.e. by a whole-genome siRNA-mediated screen coupled to automated microscopy and machine learning-based image classification.

The RanGEF RCC1 is stably associated with chromatin and leads to a local enrichment of GTP-bound Ran at mitotic chromosomes (Halpin et al., 2011), which facilitates cargo release from importin complexes, generating a distinct biochemical microenvironment and enabling i.e. chromatin-mediated microtubule nucleation (Roostalu & Surrey, 2017). This mechanism was described to induce actin polymerization (F-actin patches) around chromosomes in germline cells (Burdyniuk et al., 2018). Inhibiting importin cargo release with Importazole (Soderholm et al., 2011) during mitosis leads to severe defects and cell death (*unpublished data*). While this is feasible to explain nuclear actin assembly, further studies need to be performed addressing the effect of mitotic chromatin on actin assembly, i.e. in pyrene actin assembly assays, TIRF microscopy of actin filament formation and by proteomics of chromatin fractions upon expression of Ran mutants or treatment with Importazole.

Regarding nuclear volume, preliminary experiments show that chromatin decondensation and volume expansion are partially independent processes, occurring in the same time frame after mitosis (Nagano et al. ,2017). The application of Actinomycin D (a DNA-intercalating compound originally used to inhibit RNA polymerase activity) visibly blocks chromatin decondensation, as the shape of mitotic chromosomes is retained within a reformed nucleus after mitosis, but surprisingly does not influence nuclear volume expansion, further arguing for a chromatin-independent mechanism (*unpublished data*). The rate and extent of nuclear volume expansion differs between analyzed cell lines and correlates with the extent of nuclear actin assembly, which is overall decreased in various tumor cell lines (*unpublished data*). However, these initial findings need to be expanded to draw any conclusions and potentially open the possibilities for clinical applications in cancer therapy.

Our current model describes nuclear F-actin-dependent formation of protrusions in the nuclear envelope, which is compatible with the involvement of actin, Spectrin and protein 4.1R required for nuclear re-assembly in *Xenopus* egg extracts (Krauss et al., 2002; Meyer et al., 2011; Krauss et al., 2003). Similar processes occur during nuclear egress of replicating Baculoviruses (Hepp et al., 2018; Ohkawa & Welch, 2018). Further proteomic approaches need to be performed to assess binding partners of nuclear F-actin at mitotic exit, which will likely reveal adaptor molecules to chromatin or the nuclear envelope. It is tempting to speculate about potential interactions with long non-coding (lnc) or other small RNAs due to proteomic identification of several RNA binding proteins associated with actin (Iyer et al., 2015). This should then be accompanied by a more extensive study of nuclear envelope dynamics. Different actin-binding proteins such as bundling factors or myosins are likely involved in nuclear actin assembly and distinctively regulated during mitosis (Wu et al., 2010). Pharmacological inhibition of non-muscle myosin IIA with Blebbistatin (Képiró et al., 2014) prohibits nuclear volume expansion (*unpublished data*), potentially implicating actomyosin contractility as a nuclear force generator.

The analysis of inhibited nuclear actin assembly at mitotic exit could be expanded to better visualize nuclear organization, i.e. by CRISPR/Cas-mediated tagging of endogenous genomic loci. While general DNA replication defects upon nuclear F-actin inhibition were already observed (Parisis et al., 2017) and chromatin

decondensation eventually occurs upon further progression into interphase, preliminary data show a spatially disorganized assembly of pre-replication complexes in nuclear actin^{R62D}-expressing cells, arguing for defects in establishing A/B compartments despite eventual completion of chromatin decondensation (*A. Sherrard, personal communication*). Although the nuclear lamina is expendable for nuclear F-actin formation, it is still of interest whether nuclear F-actin influences the re-formation of the nuclear lamina or further insertion of NPCs into the nuclear envelope (shown for *Xenopus laevis* (Parisis et al., 2017)), which could potentially explain the observed defect in nuclear volume. For this, co-visualization of other cellular structures (ER, NPCs, nuclear lamina etc.) together with nuclear F-actin are required and should be assessed upon Exportin 6 or nuclear actin^{R62D} overexpression. Moreover, it should be established to what extent these manipulations affect chromatin remodeling complexes and how such complexes aid in establishing nuclear organization. Of interest, the chromatin-remodeling factor RUVBL1 was shown to interact with actin filaments (Taniuchi et al., 2014), but it is unclear whether this affects nuclear volume at mitotic exit. Notably, inducing chromatin condensation in interphase cells by pharmacological means (Tosuji et al., 1992; Miura & Blakely, 2011; Opsahl et al., 2013; Jossé et al., 2015) partially lead to nuclear actin filament formation (*unpublished data*), but induces cellular defects prohibiting further investigation. Since recent publications show actin-dependent intranuclear motility of DSBs, it is interesting to see whether actin filaments are involved in general nuclear transport mechanisms, which could be assessed upon CRISPR/Cas-induced changes in genome organization (Wang et al., 2018).

Overall, nuclear F-actin appears to have various effects on nuclear organization at mitotic exit, which sets the tone for subsequent interphase functions, primarily gene regulation and expression. This directly implicates post-mitotic nuclear F-actin in developmental contexts or in situations involving genomic instability. Hi-C experiments should be performed in cells at mitotic exit with or without nuclear actin polymerization, to precisely map the arising differences, which will lead to a better understanding of general nuclear architecture, while identification of adaptor molecules will lead to a better mechanistic understanding.

Summary

The filament-forming protein actin is abundant in eukaryotic cells and its rapid dynamics as well as versatile protein interactions result in a diverse array of functions to form important cytoskeletal structures. These influence among others shape, migration and organelle-associated processes, i.e. vesicle movement or mitochondrial fission. The study of such structures in the nuclear compartment was first successful in germline cells of non-mammalian model organisms with high nuclear actin concentrations. Somatic, mammalian cell nuclei show substantially lower actin levels and faithful visualization of nuclear actin assembly could only be achieved by actin-binding probes fused to nuclear localization sequences circumventing the otherwise saturated cytoplasmic signal. Although high expression levels can lead to artificially induced filaments, careful titrations allowed the discovery of two different types of nuclear actin assembly by live-cell imaging of mammalian cells, regulated either by extracellular signals or the cell cycle.

Extracellular signals for nuclear actin assembly can be induced by integrins and mechanotransduction, activation of other cell surface receptors or DNA damage and subsequent repair mechanisms. Mechanistic evaluation revealed that integrin-mediated nuclear actin filaments depend on the actin assembly factors mDia1 and 2 as well as the linker of nucleoskeleton and cytoskeleton complex positively influencing myocardin-related transcription factor A/serum response factor-dependent gene expression. Integrin-mediated nuclear actin assembly was also observed during cancer cell invasion trough collagen matrices.

In contrast, cell cycle-regulated nuclear actin assembly occurs together with the re-assembly of daughter nuclei after mitosis. Due to the breakdown of the nuclear envelope for open mitosis, daughter cells have to re-assemble this compartment at mitotic exit. However, the complex organization of interphase nuclei originating from mitotic chromosomes is not fully understood. Our data indicate an important role for nuclear actin dynamics in nuclear volume expansion and chromatin decondensation, which are necessary for a functional interphase nucleus and physiological cellular behavior as well as early embryonic development. We could visualize single and bundled actin filaments in inter-chromosomal spaces and at the nuclear envelope, which were negatively regulated by the actin-depolymerizing factor Cofilin.

However, multidisciplinary approaches are further required to study the precise influence of nuclear actin assembly on chromatin dynamics in more detail. Exploring this phenomenon by a combination of proteomics, Hi-C, super-resolution live-cell imaging and novel labeling methods for genomic loci and nucleosomes will aid our understanding of the complex and dynamic nuclear architecture. Further mechanistic studies into the upstream regulation and the influence of other actin-binding proteins are required to model nuclear actin assembly at mitotic exit.

Zusammenfassung

Die Abundanz von Aktin und der schnelle, regulierte Auf- und Abbau von Aktinfilamenten einhergehend mit einer Vielzahl von Proteininteraktionen resultiert in einer großen Bandbreite Aktin-abhängiger Funktionen, meist als wichtiger Bestandteil des Zytoskeletts. Dies beeinflusst unter anderem die Form und Migration von Zellen, aber auch intrazelluläre, Organell-assoziierte Prozesse, wie Vesikelbewegung oder das Verhalten von Mitochondrien. Die Untersuchung von Aktinfilamenten im Nukleus gelang zuerst in Oozyten von Wirbellosen, welche sehr hohe Aktinkonzentrationen in diesem Kompartiment aufweisen. Somatische, Säugertier-Zellkerne beinhalten substantiell geringere Mengen an Aktin, was die Visualisierung von Aktinfilamenten erschwerte. Die Fusion von Nukleus-Lokalisierungs-Signalen mit Aktin-bindenden Domänen und fluoreszierenden Proteinen ermöglichte die Beobachtung von dynamischen, nukleären Aktinfilament durch Vermeidung des ansonsten überexponierten, zytoplasmatischen Fluoreszenzsignals. Obwohl zu hohe Expressionslevel in einigen Fällen zur artifiziellen Induktion von Filamenten führen können, erlaubte die sorgfältige Titration dieser nukleären Aktinproben die Entdeckung von zwei unterschiedlichen Formen der Aktinassemblierung im Säugetierzellkern, welche zum einen über extrazelluläre Signale, zum anderen über den Zellzyklus reguliert werden.

Extrazelluläre Signale für nukleäre Aktinassemblierung sind Integrine sowie Mechanotransduktion, die Aktivierung weiterer Oberflächenrezeptoren oder DNA-Schäden und nachfolgende Reparaturmechanismen. Mechanistische Analysen zeigten, dass Integrin-vermittelte Aktinfilamente im Zellkern von den Aktinassemblierungsfaktoren mDia 1 und 2 sowie von dem *linker of nucleoskeleton and cytoskeleton*-Komplex abhängig sind. Dieser Prozess wirkt sich auch positiv auf die *myocardin-related transcription factor A/serum response factor*-abhängige Genexpression

aus. Weiterhin wurden ähnliche Formen von nukleärer Aktinassemblierung bei der Krebszellinvasion beobachtet.

Zellzyklus-abhängige nukleäre Aktinfilamente treten im Gegensatz dazu zeitgleich mit dem Wiederaufbau der Tochterzellkerne nach der Mitose auf. Aufgrund des Abbaus der Zellkernmembran für die offene Form der Mitose müssen Tochterzellen dieses Kompartiment am Ende des Teilungsprozesses wiederaufbauen. Wie sich die komplexe Organisation von Interphasezellkernen aus mitotischen Chromosomen entwickelt, ist bisher nicht ausreichend verstanden. Unsere Daten weisen auf eine wichtige Rolle für nukleäre Aktindynamik bei der Expansion des Tochterzellkernvolumens und der Chromatindekondensation hin, was nachfolgend die Funktionen eines Interphasezellkern und physiologisches Zellverhalten bestimmt. Wir konnten einzelne und gebündelte Aktinfilamente in Chromosomzwischenräumen sowie an der Zellkernmembran visualisieren und einen negativen Einfluss des Aktin-depolymerisierenden Faktors Cofilin herausstellen.

Nichtsdestotrotz sind multidisziplinäre Ansätze notwendig, um den Einfluss dieser nukleären Aktinfilamente auf die Chromatindynamik detaillierter festzustellen. Eine Untersuchung dieses Phänomens mithilfe von proteomischen Methoden, Hi-C, super-auflösender Lebendzellmikroskopie und neuartigen Verfahren zur Markierung genomischer Bereiche ist hierbei notwendig, um die komplexe, dynamische Architektur des Zellkerns aufzuschlüsseln. Weitere mechanistische Arbeiten in Hinblick auf vorgeschaltete Signalwegen sowie andere Aktin-bindenden Proteine sind für ein vollständiges Modell dieses Prozesses ebenfalls unabdingbar.

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