



The Nuclear Mitotic Apparatus (NuMA) Protein: A Key Player for Nuclear Formation, Spindle Assembly, and Spindle Positioning

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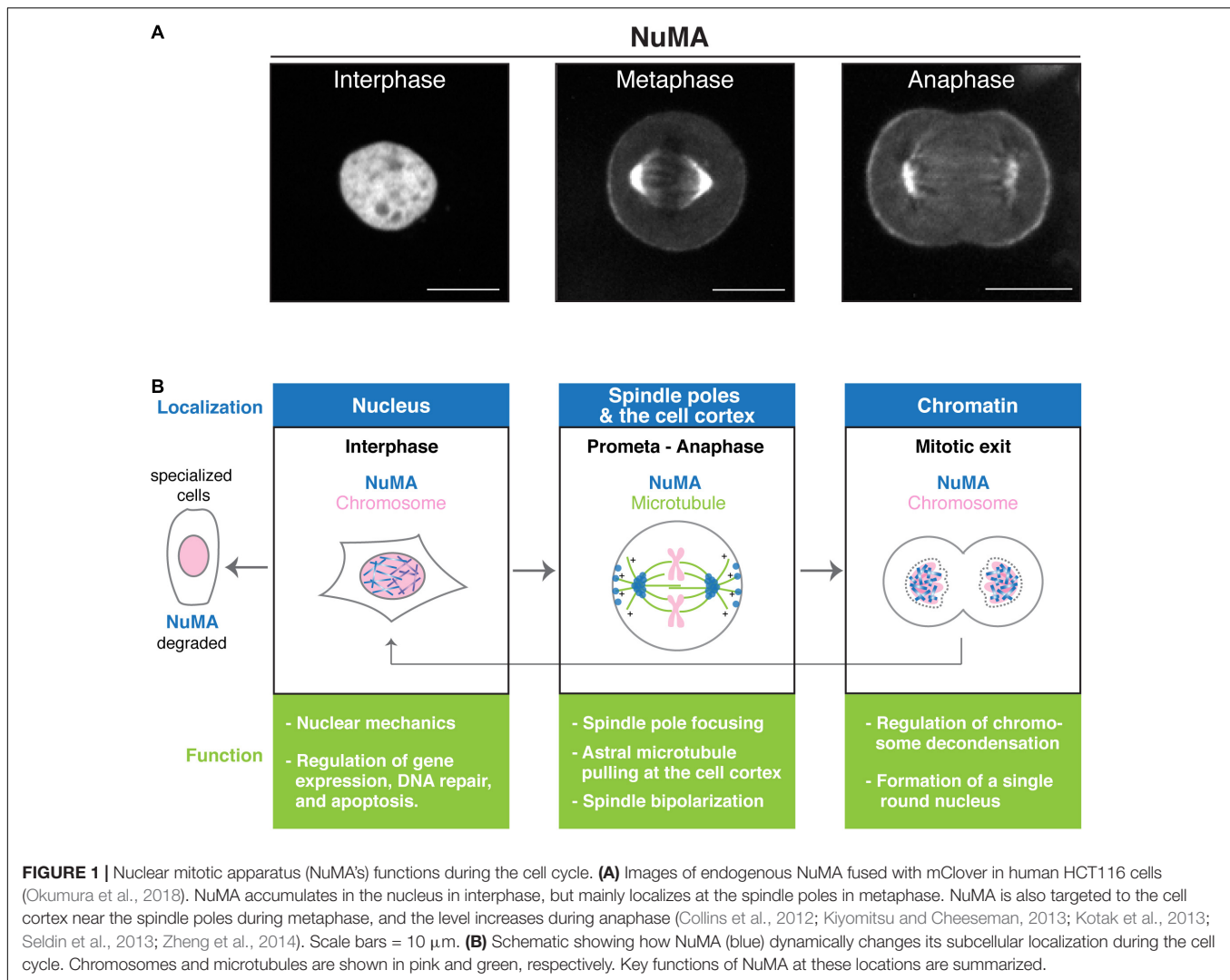
The nuclear mitotic apparatus (NuMA) protein is well conserved in vertebrates, and dynamically changes its subcellular localization from the interphase nucleus to the mitotic/meiotic spindle poles and the mitotic cell cortex. At these locations, NuMA acts as a key structural hub in nuclear formation, spindle assembly, and mitotic spindle positioning, respectively. To achieve its variable functions, NuMA interacts with multiple factors, including DNA, microtubules, the plasma membrane, importins, and cytoplasmic dynein. The binding of NuMA to dynein via its N-terminal domain drives spindle pole focusing and spindle positioning, while multiple interactions through its C-terminal region define its subcellular localizations and functions. In addition, NuMA can self-assemble into high-ordered structures which likely contribute to spindle positioning and nuclear formation. In this review, we summarize recent advances in NuMA's domains, functions and regulations, with a focus on human NuMA, to understand how and why vertebrate NuMA participates in these functions in comparison with invertebrate NuMA-related proteins.

Keywords: NuMA, spindle, nuclear formation, dynein, Ran-GTP

INTRODUCTION

The nuclear mitotic apparatus (NuMA) protein was initially identified as a non-histone chromatin protein in human cell lines (Lydersen et al., 1980) and named after its localization pattern to both the interphase nucleus and mitotic spindle poles (Lydersen and Pettijohn, 1980; **Figure 1A**). Since NuMA's dynamic translocation from the nucleus to the spindle poles was different from previously characterized nuclear components, NuMA was regarded as a novel class of nuclear protein, involved in both mitosis and nuclear reformation (Compton and Cleveland, 1994). Over the last 40 years, NuMA has been extensively studied in mammalian cultured cells, *Xenopus* egg extracts, and other vertebrate models (Cleveland, 1995; Sun and Schatten, 2006; Radulescu and Cleveland, 2010). One of the key findings early on was that NuMA interacts with cytoplasmic dynein to tether microtubules to spindle poles (Merdes et al., 1996). Later studies confirmed and expanded upon this result, positioning NuMA as a mitotic dynein adaptor, as described below.

Another important finding was that NuMA interacts with leucine/glycine/asparagine-repeat-containing protein (LGN) to form the evolutionarily conserved NuMA/LGN/Gai complex at the mitotic cell cortex (Du and Macara, 2004). This study led to the discovery that NuMA plays a conserved role at the mitotic cell cortex for spindle positioning like *C. elegans* LIN-5



(Lorson et al., 2000; Srinivasan et al., 2003) and *Drosophila* Mud [Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006; The first publication is Lorson et al. (2000)]. Building on this, and other pioneering work on asymmetric cell division in *C. elegans* and *Drosophila* (Galli and van den Heuvel, 2008; Gonczy, 2008; Siller and Doe, 2009), further studies have established the conceptual framework that cortical cues converge on a conserved ternary complex, NuMA/LGN/G α in vertebrates, Lin-5/GPR-1/2/G α in *C. elegans*, and Mud/Pins/G α in *Drosophila*, that recruits and activates dynein to position the spindle in asymmetric division (Lechler and Fuchs, 2005; Poulson and Lechler, 2010; Morin and Bellaiche, 2011; Williams et al., 2011). In symmetrically-dividing vertebrate cells, the NuMA/LGN/G α complex is also involved in recruiting dynein and controlling spindle position and orientation (Figure 1A; Woodard et al., 2010; Peyre et al., 2011; Collins et al., 2012; Kiyomitsu and Cheeseman, 2012; Kotak et al., 2012; Kiyomitsu, 2019).

Interestingly, the cortical function seems to be most conserved in NuMA-related proteins. For example, LIN-5 and Mud localize

at both spindle poles and the cell cortex, but only serve an essential function at the cell cortex for spindle positioning and are non-essential for bipolar spindle assembly (Lorson et al., 2000; Bowman et al., 2006; Izumi et al., 2006; Siller et al., 2006). In addition, yeast Num1 was recently proposed as a functional homolog of NuMA based on its functional similarities at the cell cortex (Greenberg et al., 2018). Intriguingly, plants lack a homolog of NuMA, as well as cytoplasmic dynein (Yamada and Goshima, 2017), suggesting that plants have developed alternative mechanisms to control nuclear formation, spindle assembly and positioning.

In the last 10 years, many functional domains of human NuMA were identified, providing useful information to understand NuMA's functions and regulations at the molecular level. In this review, we focus on vertebrate NuMA because several features, such as nuclear localization, appear to be specific to this group. We begin with an overview of the localization and the structural domains of the human NuMA protein, and then discuss how vertebrate NuMA participates in spindle assembly, spindle positioning and nuclear formation.

LOCALIZATION AND AN OVERALL STRUCTURE OF NuMA

In cultured human cells, NuMA accumulates in the nucleus in interphase and at the spindle poles and cell cortex during mitosis (**Figure 1A**). NuMA's spindle-pole localization is most likely conserved in all cell types, including meiotic cells (Taimen et al., 2004; Alvarez Sedo et al., 2011; Kolano et al., 2012), but its nuclear and cortical localization may vary in different developmental contexts: nuclear NuMA is degraded in some specialized cells, such as smooth and skeletal muscle fibers (Merdes and Cleveland, 1998; **Figure 1B** left). Furthermore, cortical NuMA targeting is differentially regulated between symmetric and asymmetric division in mouse epidermal cells (Poulson and Lechler, 2010).

Human NuMA is a large (~238 kDa) protein that consists of N-terminal and C-terminal globular domains and a central long coiled-coil domain (Compton et al., 1992; Yang et al., 1992; **Figure 2A**). Full length NuMA expressed in *E. coli* forms a homo-dimer using the coiled-coil region (Harborth et al., 1995; Forth et al., 2014). Purified NuMA shows a long rod-shaped structure that has globular ends and a central long (~210 nm) α -helical domain that appears more or less flexible (Harborth et al., 1995, 1999; **Figure 2A**). The globular domains interact with many factors, whereas the central region has structural and likely intramolecular regulatory roles as described below.

DYNEIN-BINDING DOMAINS IN THE N-TERMINAL REGION OF NuMA

The N-terminal region is required to interact with cytoplasmic dynein and dynactin complexes during mitosis (Kotak et al., 2012). A recent structural study revealed that NuMA^{1–153} contains a Hook domain that directly interacts with dynein light intermediate chain (LIC) 1 and 2 (Renna et al., 2020; **Figure 2B**; all domains are listed in **Table 1**). The authors also identified a CC1-box like motif, NuMA^{360–385}, adjacent to the Hook domain which forms part of the binding interface between NuMA and LIC (Renna et al., 2020; **Figure 2B**). In addition, NuMA^{417–422} contains a Spindly-like motif which is well conserved in vertebrates (Okumura et al., 2018; Tsuchiya et al., 2021), and may interact with the dynactin point-end complex (Gama et al., 2017; Lee et al., 2020). As NuMA^{1–505}, but not NuMA^{1–413} and NuMA^{214–705}, is sufficient for dynein recruitment to the cell cortex (Okumura et al., 2018), multiple interaction sites in the N-terminal region appear to be important to stably interact with the dynein-dynactin complex during mitosis. These studies indicate that NuMA acts as a dynein activating adaptor (Lee et al., 2020), but in contrast to other established dynein adaptors such as BICD2 (McKenney et al., 2014; Schlager et al., 2014), its ability to form a ternary complex with purified dynein and dynactin and activate dynein motility *in vitro* has not been shown; post translational modifications may be required to form dynein-dynactin-NuMA complexes during mitosis.

On the other hand, the interphase role of the N-terminal region of NuMA is not clear. Although both N-terminal and

C-terminal globular domains contain several S/TPXX motifs which are supposed to contribute to DNA binding (Suzuki, 1989), the N-terminal NuMA fragments do not show clear DNA or chromatin binding compared to NuMA's C-terminal (Serra-Marques et al., 2020). However, the N-terminal domain may contribute to the lattice formation of NuMA oligomers in the nucleus (Gueth-Hallonet et al., 1998; Harborth et al., 1999), as described below.

THE CENTRAL LONG COILED-COIL DOMAIN OF NuMA

Electron micrographs of recombinant human NuMA indicate that the central region forms a long flexible rod-shaped structure (Harborth et al., 1995; **Figure 2A**). One of most important functions of the central region is homo-dimerization. *In vitro* studies suggest that either the N-terminal (199–432 or 1–400 a.a) or C-terminal part (670–1700 a.a) of the central coiled-coil is sufficient to form a homo-dimer (Harborth et al., 1995; Forth et al., 2014). Dimerization is most likely critical for most NuMA functions, including dynein binding via its N-terminal Hook domain (Renna et al., 2020) and microtubule cross-linking activities via the C-terminal domain (Forth et al., 2014).

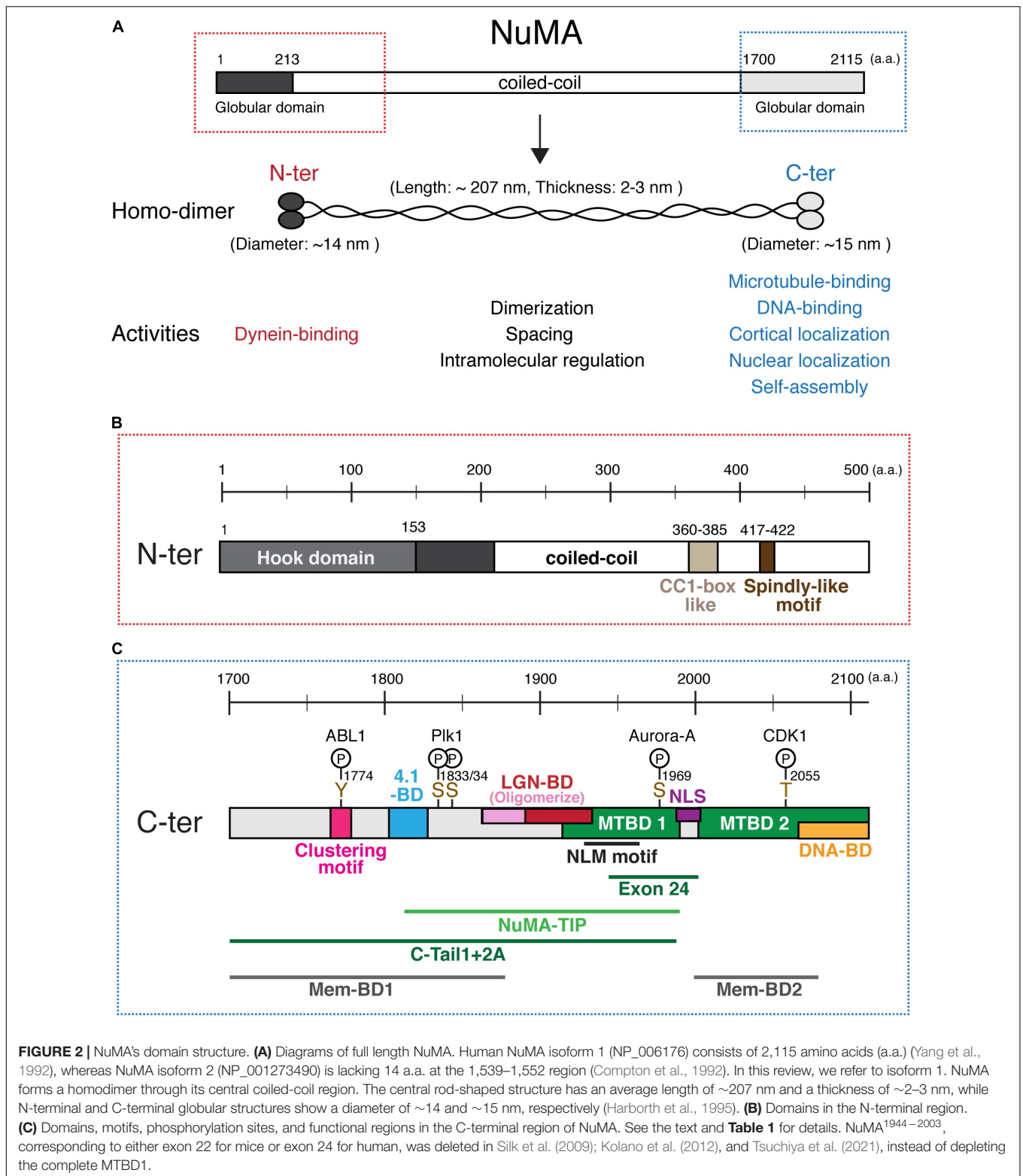
The predicted long coiled-coil region of vertebrate NuMA proteins, which exceeds 1,000 a.a. in length, is likely to perform other important functions. Transient overexpression of wild type NuMA in HeLa cells induced a regular nuclear lattice structure which has quasi-hexagonal organization (Gueth-Hallonet et al., 1998). Interestingly, an addition or deletion in the coiled-coil domain changed the spacing of the hexagons, suggesting that the central coiled-coil defines the length of the nuclear lattice structure (Gueth-Hallonet et al., 1998; Harborth et al., 1999).

Recently, Serra-Marques et al. (2020) found that the long coiled-coil is also required for the formation of a single, round nucleus, and that this role is independent from NuMA's role in spindle formation. In addition, the authors revealed that a small portion of the coiled-coil^{213–705} is sufficient to prevent NuMA's C-terminal region from binding chromosomes during late metaphase and anaphase (Serra-Marques et al., 2020).

At the mitotic cell cortex, the coiled-coil region of NuMA^{706–1699} is required to generate proper spindle pulling forces when NuMA constructs are targeted to the membrane during mitosis (Okumura et al., 2018). However, the coiled-coil region is dispensable for bipolar spindle formation (Hueschen et al., 2017). At the cell cortex, the long coiled-coil may be used to separate dynein from the actin-rich cell cortex and/or to increase the efficiency of astral microtubule capture by cortical NuMA-dynein complexes during mitosis (Kiyomitsu, 2019).

MICROTUBULE-BINDING DOMAINS IN NuMA'S C-TERMINAL REGION

The C-terminal globular region of NuMA^{1700–2115} contains several important domains that determine its localization and function (**Figure 2C**). First, this region contains two



microtubule binding domains (MTBDs); here we refer to them as MTBD1^{1914–1985} (Du et al., 2002) and MTBD2^{2002–2115} (Gallini et al., 2016; Chang et al., 2017; **Figure 2C**). Although MTBD1 has weaker microtubule-binding affinity than MTBD2

in vitro (Chang et al., 2017), MTBD1 contains the conserved NuMA-LIN-5-Mud (NLM) motif^{1922–1957} (Siller et al., 2006) and acts to establish and maintain spindle-pole focusing (**Figure 3A**) in mouse fibroblasts (Silk et al., 2009), mouse

TABLE 1 | A summary of NuMA's domain and modifications.

Region (a.a.)	Domain and modification	References
1–153	Hook domain that interacts with LIC 1 and 2	Renna et al., 2020
1–213	Globular domain	Radulescu and Cleveland, 2010
1–505	Sufficient for cortical dynein recruitment	Okumura et al., 2018
360–385	CC1-box like motif	Renna et al., 2020
417–422	Spindly-like motif	Okumura et al., 2018; Tsuchiya et al., 2021
199–432	Dimerization	Harborth et al., 1995
670–1700	Dimerization	Harborth et al., 1995
1–400	Dimerization	Forth et al., 2014
Coiled-coil region (706–1699)	Required for spindle pulling force generation. Inhibits chromatin binding during anaphase and promotes the formation of a single round nucleus	Okumura et al., 2018; Serra-Marques et al., 2020
1699–1876	Membrane binding region (Mem-BD) 1	Kotak et al., 2014
1701–1725	Cleavage site during apoptosis	Gueth-Hallonet et al., 1997
1701–1981	C-tail1 + 2A: sufficient for minus-end targeting	Hueschen et al., 2017
1768–1777	Clustering domain	Okumura et al., 2018
1788–1925	Sufficient for metaphase cortical localization	Seldin et al., 2013
1802–1824	4.1 protein binding region	Mattagajasingh et al., 2009
1811–1985	NuMA-TIP	Seldin et al., 2016
1861–1928	Longer binding region of LGN ^{7–367}	Pirovano et al., 2019
1900–1926	Minimal binding region of LGN	Zhu et al., 2011b
1914–1985	Microtubule binding domain (MTBD) 1	Du et al., 2002
1922–1957	NLM motif	Siller et al., 2006
1944–2003	Human exon 24 (=mouse exon 22)	Silk et al., 2009; Gallini et al., 2016
1996–2074	Membrane binding region (Mem-BD) 2	Zheng et al., 2014
1988–2005	NLS sequence	Tang et al., 1994; Chang et al., 2017
2002–2115	Microtubule binding domain (MTBD) 2	Gallini et al., 2016; Chang et al., 2017
2058–2115	DNA binding domain	Rajeevan et al., 2020
Y1774	Phosphorylation residue by ABL1	Matsumura et al., 2012
SS1833/34	Phosphorylation residues by PIK1	Kettenbach et al., 2011
S1969	Phosphorylation residue by Aurora-A kinase (at spindle pole)	Gallini et al., 2016; Kotak et al., 2016
T2055	Phosphorylation residue by CDK (during metaphase)	Compton and Luo, 1995; Kotak et al., 2013
Full length NuMA	MARs (DNA sequence) binding	Luderus et al., 1994

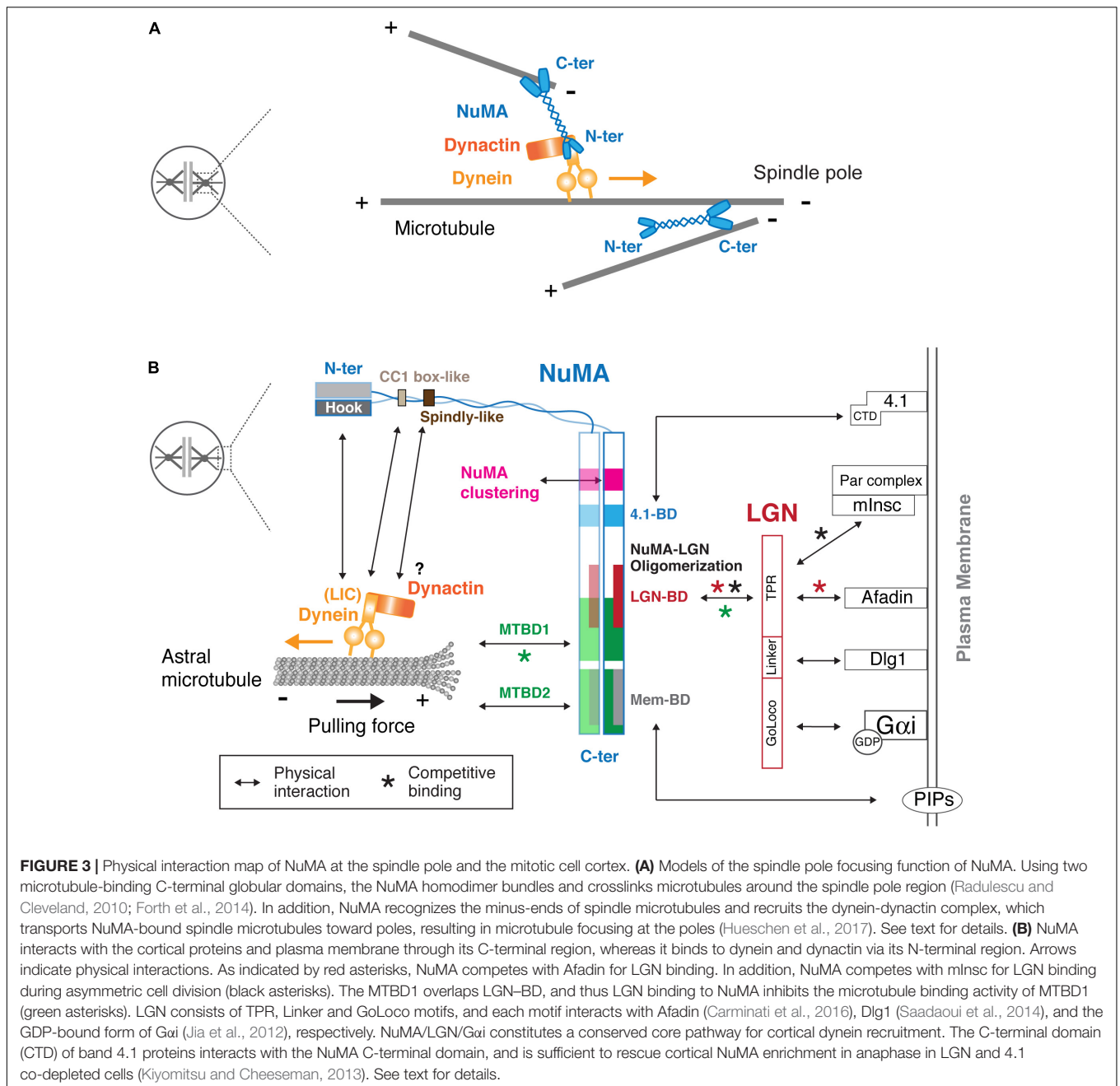
meiosis I spindle (Kolano et al., 2012), and human HCT116 cells (Tsuchiya et al., 2021).

In some cells, such as mouse keratinocytes, MTBD1 is not required for spindle pole focusing, but is required for spindle orientation (Seldin et al., 2016). Full length NuMA accumulates at the minus-end of microtubules, and the NuMA^{1701–1981} fragment, called C-Tail1 + 2A, is sufficient for minus-end recognition (Hueschen et al., 2017). In addition, NuMA's dimerized C-terminal fragment that crosslinks two parallel microtubules tends to move in the minus-end direction under forces (Forth et al., 2014). However, intriguingly, the NuMA^{1811–1985} fragment, called NuMA-TIP, appears to preferentially accumulate at the curling microtubule ends, and can remain attached to the depolymerizing microtubule plus-end (Seldin et al., 2016). The authors propose that this unique property of MTBD1 may be important to facilitate the interaction of astral microtubule plus-ends with NuMA at the cell cortex during spindle orientation (Seldin et al., 2016). However, in human cells, MTBD2, but not MTBD1, is required for spindle pulling activity when NuMA constructs are targeted to the cell cortex (Okumura et al., 2018). Interestingly, MTBD2 can bind both the microtubule lattice and tubulin dimers (Pirovano et al., 2019), indicating that MTBD2 may act not only for astral microtubule binding, but also for regulating plus-end dynamics of astral microtubules during the cortical pulling-force generation. How and when the MTBDs come into play during spindle pole focusing and orientation likely depends on the cellular context (Borgal and Wakefield, 2018).

CORTICAL TARGETING DOMAINS IN NuMA'S C-TERMINAL REGION

NuMA's C-terminal domain also defines its cortical localization during mitosis by binding to LGN, band 4.1 proteins and the plasma membrane. Whereas LGN is targeted to the cell cortex by binding to GDP-bound Gai through its C-terminal GoLoco motif, LGN binds and links NuMA to the cell cortex using its N-terminal TPR motif (Figure 3B). LGN and Gai are indispensable for cortical localization of NuMA in metaphase (Du and Macara, 2004; Woodard et al., 2010; Kiyomitsu and Cheeseman, 2012; Kotak et al., 2012). Zhu et al. (2011b) identified the NuMA^{1900–1926} peptide as the minimal region required to bind to the inner groove of LGN^{TPR}. This LGN-binding domain partially overlaps with MTBD1 (Figure 2C), and LGN binding thus inhibits the microtubule binding activity of NuMA *in vitro* (Du et al., 2002; Figure 3, green asterisk). Recently, Pirovano et al. (2019) revealed that a longer NuMA^{1861–1928} region forms a hetero-hexamers with LGN^{7–367}, in which an extended NuMA^{1861–1880} region hooks onto an adjacent LGN to form oligomers. Consistently, expression of a longer NuMA^{1788–1925} fragment is targeted to the LGN-localizing metaphase cell cortex, whereas a shorter one, NuMA^{1892–1925}, is not (Seldin et al., 2013).

However, given the complicated cortical protein network, additional mechanisms might contribute to cortical NuMA-LGN targeting and stability. In fact, it has been shown that



cortical NuMA-LGN is affected by disrupting cortical actin networks (Kaji et al., 2008; Carminati et al., 2016) or their regulators (Machicoane et al., 2014; Kschonsak and Hoffmann, 2018). Recently, Carminati et al. (2016) demonstrated that F-actin binding protein Afadin, which also binds LGN competitively with NuMA, is required to facilitate NuMA-LGN complex formation at the metaphase cell cortex (Figure 3B). On the other hand, Dlg1, that directly interacts with the phosphorylated LGN linker region (Zhu et al., 2011a) is also required for cortical LGN and NuMA localization (Saadaoui et al., 2014).

In asymmetrically dividing epithelial or mammary stem cells, the situation is more complex. Par3-binding mInsc (mammalian

homolog of Inscuteable) and NuMA compete for binding to LGN^{TPR}, with mInsc showing a more than fivefold higher affinity (Culurgioni et al., 2011; Yuzawa et al., 2011; Zhu et al., 2011b). Previously, mInsc-bound LGN-Gai was supposed to be transferred to NuMA, but Culurgioni et al. recently reported that the Inscuteable-LGN tetramer is so stable that LGN cannot be dissociated from Inscuteable by NuMA (Culurgioni et al., 2018). The authors proposed that the Inscuteable-LGN tetramer generates a localized pool of Gai-GTP molecules, which upon GTP-hydrolysis recruits a distinct population of LGN that subsequently recruits NuMA and dynein to orient the spindle (Culurgioni et al., 2018).

Although the LGN pathways are critical for cortical NuMA localization during metaphase in both symmetric and asymmetric divisions (Morin and Bellaiche, 2011), recent studies revealed that NuMA can be targeted to the anaphase cell cortex independently of LGN in symmetrically dividing mammalian cells (Collins et al., 2012; Kiyomitsu and Cheeseman, 2013; Kotak et al., 2013; Seldin et al., 2013; Zheng et al., 2014). Band 4.1 proteins link the plasma membrane to the actin cytoskeleton (Baines et al., 2014), and interact with NuMA via their C-terminal domain (CTD) and NuMA's 4.1-binding domain (1802–1824) (Mattagajasingh et al., 2009; **Figure 3B**). This NuMA-4.1 interaction appears to be important for the cortical stability of NuMA in metaphase keratinocytes (Seldin et al., 2013), but also has an important role for anaphase NuMA localization: double depletion of LGN and 4.1 proteins eliminates cortical NuMA in anaphase human cells (Kiyomitsu and Cheeseman, 2013). Importantly, this phenotype was rescued by the expression of the membrane-targeted C-terminal domain (CTD) of band 4.1 (Kiyomitsu and Cheeseman, 2013), which does not bind to actin, suggesting that 4.1 proteins contribute to anaphase NuMA localization independently of LGN and cortical actin structures. How 4.1 proteins regulate NuMA remains unclear; one possibility is that they increase cortical NuMA retention by linking it with the cell cortex and/or transferring NuMA to the plasma membrane (as described below). In addition to these cortical proteins, other proteins may be involved in cortical NuMA recruitment in developmental contexts. For example, NuMA's C-terminal region interacts with Disheveled, which controls spindle orientation during Zebrafish gastrulation (Segalen et al., 2010).

Importantly, NuMA can directly interact with the plasma membrane. The C-terminal region contains two membrane-binding domains, NuMA^{1699–1876} (Kotak et al., 2014) and NuMA^{1996–2074} (Zheng et al., 2014), which overlap with 4.1-BD and MTBD2, respectively, (**Figure 2C**) and are referred to as Mem-BD1 and 2 in this review (**Figure 2C**). Both membrane-binding domains preferentially bind to phosphorylated forms of phosphatidylinositol (PIPs) and are required for efficient cortical accumulation of NuMA (Kotak et al., 2014; Zheng et al., 2014). Mem-BD2, which partially overlaps with the DNA-binding domain (**Figure 2C**), is required for proper chromosome separation during anaphase (Zheng et al., 2014).

DNA-BINDING DOMAIN IN NuMA'S C-TERMINAL REGION

In contrast to invertebrate NuMA-related proteins, vertebrate NuMA proteins analyzed so far localize in the nucleus (Lydersen and Pettijohn, 1980; Compton et al., 1992; Yang et al., 1992; Merdes et al., 1996). Previously, human NuMA was reported to interact with defined DNA sequences called matrix attached regions (MARs) *in vitro* (Luderus et al., 1994). Recently, two studies demonstrated that the C-terminal region of human NuMA interacts with DNA *in vitro* and chromatin in cells (Rajeevan et al., 2020; Serra-Marques et al., 2020). Rajeevan et al. (2020) showed that the C-terminus NuMA^{2058–2115} fragment

is sufficient to bind DNA *in vitro*, and the basic amino acids within the region are critical for its interaction with chromatin in cells (**Figure 2C**). When endogenous NuMA was replaced with mutated versions lacking its DNA-binding ability, cells showed improper chromosome decondensation during mitotic exit and an abnormal nuclear shape (Rajeevan et al., 2020), suggesting that NuMA–DNA interactions are critical for proper regulation of chromosome decondensation during nuclear reformation.

NUCLEAR LOCALIZATION SEQUENCE/SIGNAL IN NuMA'S C-TERMINAL REGION

NuMA has a nuclear localization signal/sequence (NLS) between the MTBDs in its C-terminal region (Tang et al., 1994; **Figure 2C**). When Chang et al. (2017) solved the crystal structure of the importin- α -NuMA-C-terminus complex, they found that NuMA–NLS exhibits a novel, non-classic interaction mode with importin- α , and that importin- β sterically inhibits NuMA's MTBD2 *in vitro*. The NLS sequence is well conserved from *H. sapiens* to *X. laevis* (Chang et al., 2017), but not in *C. elegans* LIN-5, or *drosophila* Mud (Lorson et al., 2000; Siller et al., 2006). In fish, the highly conserved KR, H and KK residues of the NLS, which interact with the minor-, linker and the major-NLS-binding site on importin- α , respectively (Chang et al., 2017), are not identical and several amino acids are inserted between the H and KK residues (Tsuchiya et al., 2021); yet the Zebrafish NuMA C-terminal region can be targeted to the nucleus (Segalen et al., 2010). It would be interesting to understand whether NuMA localizes to the nucleus in other fish species, and why the NLS was acquired in vertebrates.

THE CLUSTERING DOMAIN IN NuMA'S C-TERMINAL REGION

The C-terminal globular domain has another key feature that facilitates NuMA's self-assembly into oligomers (Harborth et al., 1999). *In vitro*, 10–12 NuMA homo-dimers assemble through its C-terminal region to form multi-arm oligomers. Each oligomer has a central clustered core with projected arms, and may be connected to create 3D nuclear architecture during interphase (Harborth et al., 1999). NuMA's punctate signals at the cell cortex are most likely a result of its oligomerization/clustering. This clustering activity is attributed to a well-conserved 10 a.a. sequence of NuMA^{1768–1777} (Okumura et al., 2018). Mutant analyses indicated that NuMA's clustering is required for spindle pulling and spindle orientation at metaphase (Okumura et al., 2018), and for spindle bipolarization during prometaphase in acentrosomal human cells (Chinen et al., 2020), but is dispensable for spindle pole focusing (Okumura et al., 2018). It will be exciting to see what kind of structures are actually generated in cells by NuMA's clustering activity both in mitosis and interphase. Especially, it is important to understand how this clustering activity synergetically functions with NuMA–LGN oligomer formation to organize high-ordered functional structures that

capture and pull on astral microtubules at the mitotic cell cortex (Pirovano et al., 2019; **Figure 3**).

MITOTIC AND MEIOTIC REGULATION OF NuMA

Since NuMA's C-terminal domain binds to microtubules, the plasma membrane, and chromatin (**Figure 2C**), these interactions must be regulated throughout the cell cycle. In fact, the well conserved threonine at 2055 (T2055) is phosphorylated by CDK during metaphase (Compton and Luo, 1995; Kotak et al., 2013; Seldin et al., 2013), which inhibits NuMA's cortical association and thus promotes microtubule-binding. CDK-based phosphorylation of NuMA is also critical for releasing NuMA from the chromosomes at mitotic entry (Rajeevan et al., 2020). During anaphase, NuMA is dephosphorylated by the PPP2CA-B55gamma-PPP2R1B complex (Keshri et al., 2020), which dissociates NuMA from the spindle poles (Gehmlich et al., 2004) and promotes its cortical association.

The microtubule binding activity of NuMA is vital for its mitotic and meiotic functions (Silk et al., 2009; Kolano et al., 2012; Tsuchiya et al., 2021). Thus, importins that sterically inhibit C-terminal microtubule-binding (Chang et al., 2017) must be released during mitosis. Previously, it was demonstrated that the NuMA-importin interaction is disrupted by Ran-GTP binding to importin- β in *Xenopus* egg extracts (Nachury et al., 2001; Wiese et al., 2001), and that Ran-GTP is essential for acentrosomal spindle assembly in female meiosis (Dumont et al., 2007; Holubcova et al., 2015; Drutovic et al., 2020). However, recent evidence suggests that Ran-GTP is not essential for mitotic spindle assembly and mitotic progression in chicken DT40 (Furuta et al., 2016) and human HCT116 cells (Tsuchiya et al., 2021). In addition, Ran-GTP is not required to activate NuMA and TPX2 in HCT116 cells (Tsuchiya et al., 2021), suggesting that additional parallel pathways exist to activate these proteins at a distance from chromosomes (Wei et al., 2015; Eibes et al., 2018; Brownlee and Heald, 2019). As cellular concentrations of Ran-GTP are variable across cell types and organisms (Kalab et al., 2006; Hasegawa et al., 2013), and the size of spindles and cells change dramatically during early embryonic divisions (Courtois et al., 2012; Levy and Heald, 2012), it would be important to determine how NuMA is spatiotemporally activated by Ran and other factors during mitosis and meiosis.

MITOTIC REGULATION OF NuMA AT THE SPINDLE POLES AND THE CELL CORTEX

Once activated, the NuMA homo-dimer binds to microtubules independently of dynein (Heald et al., 1997; Hueschen et al., 2017) and cross-links two microtubules using its two C-terminal globular domains (**Figure 3A**; Forth et al., 2014). This microtubule crosslinking function may be facilitated at the spindle poles by binding to other microtubule associated proteins such as Rae1 (Wong et al., 2006), Eg5 (Iwakiri et al., 2013), and

dynein/dynactin complexes (Merdes et al., 1996, 2000). On the other hand, when new microtubules are created in the spindle, NuMA is targeted to their minus-ends and subsequently forms a complex with dynein and dynactin; when this complex binds adjacent microtubules and moves toward their minus end, it pulls the NuMA-bound microtubules along, resulting in a focused spindle pole (Hueschen et al., 2017; **Figure 3A**).

At the poles, NuMA is phosphorylated by Aurora-A kinase at S1969, which leads to its dynamic mobility from the spindle poles to the cell cortex during metaphase (Gallini et al., 2016; Kotak et al., 2016; **Figure 2C**). NuMA is also phosphorylated by Polo-like kinase 1 (Plk1) at S1833/34 (Kettenbach et al., 2011), which promotes NuMA's turnover rate at both spindle poles and the cell cortex, and when inhibited results in NuMA's accumulation at both locations (Sana et al., 2018). Plk1 localizes at the spindle poles, but also accumulates at kinetochores of misaligned chromosomes which locally diminish cortical LGN when they are located near the cell cortex (Tame et al., 2016). Since NuMA and LGN are inter-dependent (Du and Macara, 2004), the kinetochore-localized Plk1 may also target NuMA, which in turn reduces LGN. However, artificial membrane tethering of Plk1 dissociated dynein, but not LGN, from the cell cortex (Kiyomitsu and Cheeseman, 2012). In addition, when the immunoprecipitated GFP-LGN complexes were incubated with Plk1, Plk1 dissociated dynein and dynactin, but not NuMA, from LGN (Kiyomitsu and Cheeseman, 2012). Therefore, kinetochore- or centrosome-localized Plk1 may down-regulate the NuMA-LGN interaction synergetically in cooperation with other factors derived from chromosomes or centrosomes. Another kinase, ABL1, phosphorylates the well-conserved Y1774 residue of NuMA to control spindle orientation (Matsumura et al., 2012). Y1774 is located in the clustering motif (Okumura et al., 2018), but it remains unknown whether ABL1 regulates NuMA's clustering activity.

In addition to these kinases, Ran-GTP gradients negatively regulate cortical NuMA-LGN localization near chromosomes in a distance dependent manner (Kiyomitsu and Cheeseman, 2012). Although molecular mechanisms by which Ran-GTP eliminates the NuMA-LGN complex from the cell cortex remain unclear, the cortical patterning created by chromosome-derived Ran-GTP is sufficient to explain why the mitotic spindle orients along its interphase cell axis (Dimitracopoulos et al., 2020). NuMA's continuous exclusion from the equatorial region of the cell cortex during anaphase is dependent on the signals downstream of the centralspindlin complexes (Kotak et al., 2014).

INTERPHASE FUNCTION OF NuMA

Several lines of evidence indicate that NuMA acts as a non-essential nucleoskeletal element in interphase (Zeng et al., 1994; Merdes and Cleveland, 1998; Harborth et al., 1999), which is nicely reviewed by Radulescu and Cleveland (2010). However, it is difficult to understand this role separately from the several roles it plays during mitosis since mitotic errors cause abnormal nuclei. Recently, Serra-Marques et al. (2020) nicely demonstrated that NuMA's contribution to building a single, round nucleus

is independent from its mitotic functions by inducing mitotic exit in NuMA KO Rpe1 cells without spindles (**Figure 1B**). Furthermore, they showed that NuMA keeps the decondensing chromosome mass compact during mitotic exit (Serra-Marques et al., 2020). Although the precise mechanisms are still unclear, evidence suggests that NuMA, like barrier-to-auto integration factor (BAF) at the chromosome ensemble surface (Samwer et al., 2017), may offer structural support throughout the nucleus by cross-linking chromosomes and preventing the nuclear envelop from penetrating into the chromosome mass, which results in a decrease of multinucleation and abnormally shaped nuclei during mitotic exit (Serra-Marques et al., 2020). Alternatively, NuMA may coordinate chromosome compaction and nuclear envelop assembly using its binding abilities to both chromosomes and membrane (Serra-Marques et al., 2020), as well as its binding to importins which can recruit membrane vesicles and nucleoporins (Lu et al., 2012).

In addition to its roles during mitotic exit, NuMA promotes the nucleus' mechanical robustness (Serra-Marques et al., 2020) and could contribute to several interphase events, including chromatin organization (Abad et al., 2007), gene expression (Harborth et al., 2000; Ohata et al., 2013), DNA repair (Vidi et al., 2014; Moreno et al., 2019), and apoptosis (Gueth-Hallonet et al., 1997; Kivinen et al., 2005; Lin et al., 2007). Since chromatin architecture is highly dynamic during different phases of the cell cycle (Shoab et al., 2020), acute protein depletion technologies, such as auxin-inducible degron (AID) (Natsume et al., 2016; Yesbolatova et al., 2020; Tsuchiya et al., 2021), would be useful to precisely understand the interphase functions of NuMA in future studies.

DISCUSSION

Vertebrate NuMA, and its related proteins in invertebrates, have been extensively studied using many techniques and model organisms. Over the past 10 years, much light has been shed on this multi-functional protein, revealing key domains, modifications and binding partners. However, we still do not know how NuMA contributes to spindle pole focusing, spindle positioning or nuclear formation at the molecular and structural level. In the next 10 years, it would be especially important to visualize the functional structures of NuMA and its complexes using biochemical reconstitution, high-resolution imaging and *in situ* structural analyses. In addition, most reported interactions and functions are not sufficiently validated in a physiological condition. Since many functions discussed in this review appear to be specific in vertebrates, vertebrate developmental models

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would be useful to obtain a comprehensive understanding of the diverse functions of NuMA. Furthermore, optogenetic manipulation of NuMA, or its related proteins, would serve as a powerful tool to control spindle position and orientation (Fielmich et al., 2018; Okumura et al., 2018), and could reveal physiological roles of division orientation and daughter cell size during development (Jankele et al., 2021).

Many key questions about NuMA remain to be answered. (1) How does NuMA recognize microtubule minus-ends and depolymerizing microtubule plus-ends? (2) How are dynein–dynactin–NuMA complexes formed during mitosis and meiosis and regulated at the spindle poles? (3) How are different cortical NuMA complexes assembled at the mitotic cell cortex and spatiotemporally regulated by intrinsic and extrinsic signals? (4) When does NuMA start to localize at the mitotic cell cortex to control spindle positioning during early vertebrate development? (5) What kinds of high-ordered structures are created by NuMA at the cell cortex and interphase nucleus to generate cortical spindle pulling forces and a mechanically robust nucleus, respectively? (6) How is NuMA dissociated from importins in a Ran-independent manner? (7) Why did NuMA acquire an NLS in vertebrates? (8) How has the NuMA gene evolved to achieve different functions in different organisms? Addressing these questions must provide new, exciting insights not only to advance our knowledge about nucleus formation, spindle assembly and spindle positioning, but also to understand how complex human cell architecture evolved.

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TK wrote the first draft, acquired the fund. TK and SB revised manuscript, figures, and Table. SB got the images of **Figure 1A**. Both authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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