[Frontiers in Bioscience 11, 1137-1146, January 1, 2006]

Role of NuMA in vertebrate cells: review of an intriguing multifunctional protein

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1. ABSTRACT

The 236 kDa large coiled-coil protein NuMA plays diverse important roles in vertebrate cells. It is an important component of the nuclear matrix in interphase cells, and is possibly involved in nuclear re-assembly after mitosis. In dividing cells, upon phosphorylation, NuMA disperses into the cytoplasm, associates with cytoplasmic dynein/dynactin to form a complex, and translocates along microtubules to the spindle poles where it organizes and tethers microtubules to spindle poles. It is thought that the stable complex of NuMA/dynein/dynactin is needed to focus microtubule minus ends to the spindle poles. But, it has also been reported that NuMA can organize microtubules in the absence of centrosomes and dynein. Another hypothesis suggests that once localized to the spindle poles, spindle-associated NuMA's exchange with cytoplasmic soluble pools and its stable crosslinking with the microtubule fibers are independent of dynein/dyactin. NuMA's function in spindle microtubule organization is regulated by RanGTP and Pins-related protein LGN. NuMA becomes dephosphorylated, loses its association with dynein/dynactin, and releases from spindle poles after anaphase onset to allow spindle disassembly and reformation of interphase daughter nuclei. The cell-cycle-dependent phosphorylation of NuMA is regulated by the balanced activities of protein kinases and phosphatases. It has been shown that phosphorylation of NuMA by cyclin B/cdc2 kinase allows NuMA to release from the nucleus and to associate with centrosomes and/or microtubules at the spindle poles, while NuMA's dephosphorylation due to the cyclin B degradation allows NuMA to dissociate from the spindle poles after anaphase onset. Overexpression of NuMA interferes with spindle-associated dynein localization and promotes multipolar spindle formation and cancer. On the other hand, NuMA is absent in many kinds of non-proliferating cells and highly differentiated cells. NuMA also functions during meiotic spindle organization in male and female germ cells. Degradation of NuMA results in the breakdown of normal nuclear structure, and has been used as a marker of cell apoptosis. The implications of NuMA protein in somatic cell animal cloning by nuclear transfer are discussed.

2. INTRODUCTION

The nuclear mitotic apparatus (NuMA) protein was first described by Lydersen and Pettijiohn (1), and it is named for its localization in the nucleus during interphase and at spindle poles during mitosis. NuMA is an abundant 236 kDa protein consisting of a globular head and tail domains separated by a discontinuous 1500 amino acid coiled coil spacer. Bands around 220 and 240 kDa most likely represent the hypophosphorylated interphase form and the hyperphosphorylated mitotic form of NuMA, respectively (2-4). A proteolytic fragment of 180 kDa NuMA peptide has also been detected at low levels during mitosis, as well as at the beginning of apoptosis (4,5). Over the past decade, a burst of interest in this intriguing protein has led to much documentation of its cell cycle redistribution, cell cycle-dependent targeting domains, phosphorylation/dephosphorylation and transport regulation, spindle pole tethering and maintenance, spindle assembly/disassembly, and nuclear reformation. In addition to its role in spindle pole formation and nuclear reformation, NuMA has also been implicated as a first target in programmed cell death. In the following chapters, we will summarize our recent understanding on the important roles of NuMA in various aspects of cell activity.

3. POSSIBLE INVOLVEMENT OF NuMA IN INTERPHASE NUCLEUS ASSEMBLY AND FUNCTION

NuMA is a highly insoluble nuclear matrix component binding to chromatin during interphase, and it disperses in the cytoplasm at the onset of mitosis, and finally redistributes to spindle poles in mitosis. NuMA is distributed in the interphase nucleus (2×10^5 copies/nucleus) (2). Immunofluorescence microscopy reveals that NuMA is resistant to DNA digestion and salt extraction, suggesting that it is an independent protein of the nuclear matrix (6). NuMA's distribution pattern in the interphase nucleus is different in different cell types. A number of investigators reported diffuse staining of NuMA in the nucleus excluding the nucleolus in various cell types, while punctuate and dotted patterns have also been shown (7). Our immunoelectron microscopy study reveals that NuMA is present in speckled and punctuate form, disperses in the cytoplasm at prophase, binds to spindle fibers at metaphase and anaphase, relocates to electron-dense areas around chromatin at telophase, and finally distributes in the reconstituted nucleus (8). Numerous studies have suggested that NuMA is involved in nuclear re-assembly after mitosis and it functions as a nuclear structural component in interphase, but this conclusion is still being debated. The cDNA sequence of NuMA shows its homology to some structural filament-forming proteins such as cytokeratins, nuclear lamins and myosin heavy chain (2,9). Transient overexpression of NuMA leads to three dimension lattice formation with a quasi-hexagonal organization, forming a scaffold in the nucleus (10), while overexpression of NuMA lacking the nuclear localization signals (NLS) results in cytoplasmic aggregates composed of 5 nm NuMA filaments (10,11). In addition, like other caspase targeted proteins, e.g., lamins, fodrin and actin, NuMA is cleaved when nuclear structure is disassembled during apoptosis (for detail, see later chapter) All these results suggest that NuMA functions in the establishment and/or maintenance of nuclear structure.

Others argue that NuMA may act as a non-essential nucleoskeletal element at least in some types of cells. There is evidence that a morphologically normal looking nucleus can form in the absence of NuMA when human sperm DNA is subjected to egg extract (12). Further, it was recently suggested that nuclear localization of NuMA is just a simple mechanism to store NuMA until the next cell division to avoid NuMA-microtubule interaction during interphase, since certain types of cells lacking NuMA such as early primary spermatocytes, neurons, granulocytes etc. still maintain a normal looking nucleus (13,14).

While much of the recent research has been focused on our understanding of NuMA's role in re-assembly of the nucleus after mitosis and its role in apoptosis (as discussed in a later chapter), nuclear functions have been explored to a lesser extent. NuMA has been shown to be associated with small nuclear ribonucleoproteins and with splicing factors that are involved in recycling and phosphorylation of RNA processing factors. NuMA has been implicated in the regulation of DNA replication and transcription (3, 8, 15).

4. DYNEIN/DYNACTIN-DEPENDENT TRANSPORT OF NuMA TO SPINDLE POLES

After nuclear membrane breakdown, NuMA is aggregated within the interchromatin space while chromosome condensation takes place. It is enriched in a crescent area at the spindle poles to focus microtubules and to control the size and shape of the mitotic spindle. Several lines of evidence have shown that NuMA transport to spindle poles is a dynein/dynactin-dependent process (16, 17). In prometaphase HeLa cells, NuMA aggregates are associated with the actin-related protein Arp1, a subunit of the motor-cargo-mediating complex dynactin, using the minus end-directed motor activity to accumulate at spindle poles. Time-lapse tagging of GFP-NuMA revealed that NuMA frequently stretched along spindle fibers, and moved poleward along spindle fibers. At 37°C, the average transport velocity is 2.6 μm/min, in agreement with the dynein-dependent movement of microtubule seeds measured in spindles. Disruption of microtubules by nocodazole caused solubilization of pole-accumulated NuMA (16). Immunoprecipitation of NuMA from metaphase-arrested frog egg extracts further revealed an association of NuMA with cytoplasmic dynein and its activator dynactin. (17). Furthermore, disruption of dynactin complex by dynamitin subunit or dynactin action by antibody caused the failure of NuMA's accumulation at the minus ends of the spindle microtubules, providing direct evidence for dynein/dynactin mediated transport of NuMA to spindle poles (16).

5. INVOLVEMENT OF NuMA IN SPINDLE POLE FORMATION AND MAINTENANCE: TWO HYPOTHESES

In vertebrate somatic cells, centrosomes are located at spindle poles and function as microtubule-organizing centers, but they are not absolutely necessary for spindle pole formation and minus end microtubule focusing, since there is evidence showing that laser removal of centrosomes does not affect bipolar spindle assembly (18). Instead, the non-centrosomal protein dynein is essential for spindle pole organization (7). It has been shown that the formation of spindle poles is inhibited by an antibody against the dynein intermediate chain (19, 20). Dynein's function to tether spindle pole microtubules into bundles requires other proteins with microtubule binding sites. Dynactin and NuMA are two such molecules. NuMA is necessary for pole formation whether in the presence or absence of centrosomes. NuMA has microtubule-binding domains and can directly bind to microtubules to form filamentous structures, and it is the most likely candidate to serve as structural component in the motor complex. Electron microscopy reveals that, once NuMA localizes to spindle poles, it becomes part of insoluble matrix closely associated with microtubule minus ends (21). Immunodepletion of NuMA or disruption of dynein and dynactin results in failure of spindle pole formation or disruption of spindle poles (16). The evidence is strong that NuMA associates with cytoplasmic dynein/dynactin and these proteins form a stable complex to focus microtubule minus ends to the spindle poles using the minus-end-directed motor activity of dynein.

The second model suggests that once NuMA has used the motor activity of dynein to become localized at spindle poles, it forms a matrix to hold microtubule minus ends together following dissociation from dynein (22). NuMA can organize the spindle poles by stable crosslinking of the microtubule fibers independent of dynein/dynactin (23). Depletion of NuMA rather than dynein causes the failure of microtubule focusing into poles (16). Both *in vivo* and *in vitro* methods show that there are three distinct pools of NuMA in mitotic cells: one is associated with spindle poles and does not undergo exchange with the other two pools. Analysis of GFP-NuMA in living cells indicates that this pool of NuMA constitutes less than 20% of total NuMA and a portion of this pool is associated with centrosomes. The second pool of NuMA is also associated with spindle poles, but this pool readily exchanges with the third pool of freely soluble NuMA. In living cells, continuous exchange exists between soluble and spindle-associated pools of NuMA, and the half time of dynamic exchange is about 3 min. These dynamics require cellular energy, but not NuMA's binding to dynein (22).

6. NuMA'S INVOLVEMENT IN SPINDLE MICROTUBULE ASSEMBLY

During cell division, the spindle apparatus is formed to move and separate sister chromatids into two daughter cells. Spindles consist of two half asters composed of microtubules, anchored with their minus ends at the poles and their plus ends at kinetochores. Two different mechanisms are employed by various organisms to assemble spindle microtubules. One is nucleation and anchorage of microtubules at preexisting centers such as centrosomes or spindle pole bodies, and the other is microtubule growth off the surface of chromosomes, followed by sorting and focusing into spindle poles (24). After release from the nucleus, NuMA associates with centrosomes at the spindle poles where it also appears to have an intimate association with pericentriolar material (PCM). It has been well documented that NuMA is a mitotic MTOC component, and plays an essential role in the early stages of microtubule assembly or microtubule nucleation (7). In cell-free systems, assembled mitotic asters are composed of microtubules organized in a radial array that contain NuMA aggregations at the central core (18, 25). When one or two centrosomes are removed from living cells, bipolar spindles are formed. The acentrosomal focused spindle pole contains NuMA, but lacks centrioles, gamma-tubulin and pericentrin (18).

The GTPase Ran has also been shown to stimulate spindle microtubule polymerization in mitotic extracts. RanGTP discharges NuMA from importin β (an inhibitor of microtubule aster assembly) and thus induces the formation of spindle-like structures in the absence of typical centrosomes (26,27). A human Pins-related protein called LGN is a key regulator of spindle organization. It regulates spindle organization through inhibition of NuMA function (28). The LGN-binding domain of NuMA triggers microtubule aster formation, while NuMA-binding domain of LGN blocks aster assembly in *Xenopus* egg extracts treated with taxol (29). All these results suggest that NuMA may play a functional role in the organization of the microtubules of the mitotic spindle. A recent computer simulation proposes that microtubule organization in spindles involves both motile forces from motors and static forces from non-motor cross-linking proteins including NuMA (30).

7. NuMA IS RELEASED FROM THE SPINDLE POLES AFTER ANAPHASE ONSET AND IS PRESENT IN THE FORMING DAUGHTER NUCLEI IN TELOPHASE

NuMA loses its association with dynein/dynactin and releases from spindle poles after anaphase onset to allow spindle disassembly and remodeling of the microtubule network. In spindles assembled in *Xenopus* egg extract, NuMA disappears from the poles after anaphase chromosome separation. In HeLa cells and other cell types, NuMA remains at the spindle poles in anaphase, and re-localizes to the surface of reforming daughter nuclei in telophase. NuMA was also shown to release from the anaphase spindle poles after microtubule depolymerization, and re-localized to the chromosome surface in all cells observed (31). Our immunoelectron microscopy studies showed that in MCF-7 and LNCaP cells NuMA labeling was associated with spindle fibers at anaphase and relocated to electron-dense areas around chromatin at telophase, and finally was present in the reconstituted nuclei (8).

8. CELL-CYCLE DEPENDENT DISTRIBUTION AND FUNCTION OF NuMA IS REGULATED BY PHOSPHORYLATION/DEPHOSPHORYLATION MEDIATED BY CDC2/CYCLIN B KINASE

It has been shown that NuMA undergoes cell-cycle dependent phosphorylation/dephosphorylation, which controls its distribution and function. NuMA is a phosphoprotein in interphase and undergoes additional phosphorylation at the onset of mitosis (7). In dividing cells, upon phosphorylation, NuMA disperses into the cytoplasm during nuclear envelope breakdown (3) and forms a complex with dynein/dynactin as mentioned above. This allows NuMA's association with microtubules and translocation along microtubules to spindle poles where it tethers spindle microtubules (8). Phosphorylation of NuMA is regulated by the balanced activities of protein kinases and phosphatases. The identities of the kinases and phosphatases that act on NuMA are currently not well defined. NuMA's carboxyl-terminal globular tail has four predicted cdc2 phosphorylation sites and NuMA is a downstream target (substrate) of cyclin B/cdc2 kinase. Cyclin B/cdc2 kinase is associated with NuMA in mitotic extracts but not in the dephosphorylated interphase state (32). P34cdc2 domain on NuMA determines its action with microtubules. Point mutations in NuMA that prevent phosphorylation in mitosis abolish its spindle pole localization (33). Therefore, hyperphosphorylation of NuMA by cyclin B/cdc2 kinase appears to allow NuMA to associate with centrosomes and/or microtubules at the spindle poles.

Spindle poles are dynamic structures that are under the control of cyclin B/cdc2 kinase, NuMA and a variety of other centrosomal and non-centrosomal proteins. It has been reported that, after anaphase onset, NuMA is dephosphorylated and released from the spindle poles due to the cyclin B degradation. In *Xenopus* egg extracts, NuMA is dephosphorylated in anaphase and released from the dynein/dynactin complex. In the presence of non-degradable cyclin B (Δ90), NuMA remained phosphorylated, associated with dynein/dynactin, and remained localized to spindle poles (31). Another publication also indicates that NuMA is dephosphorylated during interphase nucleus reassembly induction in mitotic extracts, and this process is controlled by protein kinases and protein phosphatases (32).

9. OVEREXPRESSION OF NuMA PROMOTES SPINDLE MULTIPOLARITY ASSOCIATED WITH CANCER

Supernumerary centrosome formation can lead to an increase in spindle poles and is linked to aneuploidy and cancer. As mentioned above, NuMA is located in spindle poles and functions as a centrosome protein that is critical for spindle assembly. NuMA1 gene maps to one of the most frequently amplified chromosomal segments in cancer cells. It is a conceivable candidate for predisposition to a variety of forms of cancer. NuMA-retinoic acid receptor alpha (PARalpha) fusion proteins exist in acute promyelocytic leukemia (34), and disruption of NuMA function plays a role in the development of myeloid leukemia (35). Multiple spindles were linked to various degrees in the oral squamous cell carcinoma lines (36). An untimely and abnormal division of NuMA has been shown to be an early step in splitting of the spindle poles. Recently, NuMA gene was localized to chromosome 11q13 and variations in the NuMA gene are likely responsible for increased breast cancer risk (37). The oral cancer cell lines UPCI:SCC103 and UPCI:SCC078 exhibit relatively high amounts of NuMA expression and display about 20% multipolar spindles. Transfection with NuMA siRNA reduced the NuMA amounts and nearly eliminated multipolar spindles (38). The same group also observed similar results for the SK-HEP-1 liver adenocarcinoma cell line. When NuMA level was allowed to recover, the frequency of multipolarity returned to that of untreated cells (38). It is suggested that overexpression of NuMA perturbs the ability of these cells to coalesce supernumerary centrosomes into a single pole. In various cancer cells, dynein is lost in spindle poles. Overexpression of NuMA interferes with spindle-associated dynein localization, promoting multipolarity; while when NuMA amount is reduced, dynein is detectable in nearly all the spindles in some (not all) kinds of cancer cells (38).

Hyperphosphorylation of centrosome proteins has been reported in cancer cells and tissue and may be associated with progressive stages of breast cancer (39). Phosphorylation plays a significant role in NuMA's mitotic functions and increased phosphorylation contributes to the formation of multipolar centrosomes (40). Further studies are required to test whether abnormal phosphorylation of NuMA may play a role in the formation of multipolar mitosis.

10. NuMA IS ABSENT IN SOME QUIESCENT CELLS AND HIGHLY DIFFERENTIATED CELLS

NuMA is not expressed in all cell types. It is expressed in actively proliferating cells, but not in some non-proliferating or end-differentiated cells. Merdes and Cleveland (12) reported absence of NuMA in a number of cell types, including spermatozoa, blood granulocytes, and differentiated smooth and skeletal muscle fibers. During culture of myoblasts, NuMA is degraded during muscle cell differentiation. Taimen *et al* (41) also reported other NuMA-negative cell types, including superficial keratinocytes, neutrophil granulocytes, syncytiotrophoblasts, and some neurons. In addition, the disappearance of nuclear NuMA was observed in ROS 17/2.8 rat osteosarcoma cell and in quiescent human breast cancer cells after extended culture. The percentage of NuMA-positive human breast cancer cells diminished from an initial approximately 100 to 60% during 6 days of culture. We recently found that the proportion of NuMA-positive pig cumulus cells decreased after serum starvation and contact inhibition when cultured *in vitro* (our unpublished data). As mentioned in the next chapter, NuMA is absent in transiently cell cycle-arrested leptotene, zygotene and pachytene primary spermatocytes as well as non-proliferative elongating spermatid and mature sperm in rats (14). It was also reported that the

presence of NuMA correlates positively with the presence of proliferation marker Ki-67 antigen and negatively with increased culture time, confluence, and size of the cell islets (41).

11. NuMA CLEAVAGE IS AN EARLY STEP IN PROGRAMMED CELL DEATH

Programmed cell death or apoptosis is morphologically characterized by shrinking of the cell, chromatin cleavage and condensation, blebbing of cell membrane, and finally formation of apoptotic bodies. Biochemical changes include the activation of caspases, followed by cleavage or degradation of various target proteins. One of these proteins is NuMA. NuMA acts as a nuclear structural target for a death protease during apoptosis (4). Immunofluorescence analysis has shown that the normal diffuse distribution of NuMA is changed and NuMA is excluded from the condensed chromatin during apoptosis (5). NuMA first condenses, then concentrates in the center of the nucleus, and finally enriches in the nuclear fragment within the apoptotic bodies (13). In heat-treated mouse splenic T lymphocytes, many bright spots of NuMA were observed in the diffusely labeling nucleoplasm, and this pattern was retained throughout subsequent stages of apoptotic nuclear collapse (42). The cells with distinctively large NuMA spots are targeted for, or have initiated the death program (43).

Several studies have shown that NuMA is an early target protein of caspases and it is specifically degraded in early apoptosis (44,45). It is redistributed and/or specifically degraded prior to, or coincident with the onset of DNA degradation. NuMA is cleaved coincidently with poly (ADP-ribose) polymerase-1 (PARP-1) and nuclear lamin B in Jurkat T and Hela cells (13). A ~180 kDa form of NuMA was described in HeLa cells treated with 5,6-dichloro-1-ß-D-ribofuranosyl-benzimidasole and in HL60 cells treated with camptothecin, staurosporine, cycloheximide and A23187 (4). The incubation with Fas receptor antibody resulted in the cleavage of NuMA into ~180 and ~190 kDa fragments in both Jurkat T and HeLa cells. The 190 kDa fragment was further proteolyzed into a 180 kDa fragment. In addition, the ~160 kDa fragment was detected thereafter in Jurkat T cells. In HeLa cells the 160 kDa fragment was not detected, which suggests that different proteolytic enzymes are activated in HeLa and Jurkat T cell lines (13). In the presence of caspase inhibitors and in apoptotic caspase-3-deficient MCF-7 cells NuMA was not cleaved, suggesting that NuMA is cleaved by caspase-3 and/or another protease downstream caspase-3 in vivo (13). Although recombinant caspase-3, -4, -6 and -7 all cleave recombinant NuMA or NuMA in HeLa cells, only recombinant caspase-6 results in the production of a 160 kDa NuMA fragment (13, 46). Therefore, it seems probable that caspase-3 produces the 180/190 kDa fragments and caspase-6 the 160 kDa fragment (13). In hydroxyurea- and staurosporine-treated BHK cells, NuMA is cleaved between residues 1701 and 1725, releasing the C-terminal tail domain, which contains a functionally important nuclear localization signal (5). In addition to caspases, granzyme B has been shown to cleave NuMA (47). The degradation of NuMA resulted in breakdown of the normal nuclear structure, and has been used as a marker of cell apoptosis.

12. NuMA PLAYS IMPORTANT ROLES IN MEIOTIC CELL DIVISION

The study of NuMA in germ cells is still relatively new and largely unexplored.

12.1. Female germ cells

In *Xenopus* oocytes, a transient microtubule array (TMA) is nucleated from a novel MTOC near the base of the germinal vesicle. The MTOC-TMA transports the meiotic chromosomes to the animal cortex, where it serves as the precursor to the first meiotic spindle. NuMA was localized to the base of the MTOC-TMA and the meiotic spindle, and microinjection of anti-NuMA antibody disrupted the organization of the MTOC-TMA and subsequent assembly of the meiotic spindles (48). During meiotic progression in pig oocytes, NuMA was localized in the nucleus at germinal vesicle stage. After germinal vesicle breakdown (GVBD), NuMA was aggregated in the vicinity of the chromosomes, and then translocated to both poles of a barrel-shaped metaphase I spindle. Further, nocodazole, an inhibitor of microtubule polymerization, induced disappearance of the pole staining of NuMA in pig metaphase II oocytes. All these observations suggest that pig meiotic spindle poles are formed by the bundling of microtubules at the minus ends by NuMA (49). In mouse oocytes, NuMA was also concentrated in the germinal vesicle at interphase and then concentrated at the poles of the meiotic spindle (50). But another report indicated that NuMA was localized along the meiotic spindle, but not the meiotic spindle poles (49). In contrast to the stable localization of NuMA at the spindle poles in mitosis, NuMA becomes re-localized to the spindle midzone during anaphase I and telophase I in pig oocytes. It is postulated that in the centrosome-free meiotic spindle, NuMA aggregates the spindle microtubules at the midzone during anaphase and telophase (49). After fertilization, NuMA was relocated into the reformed pronuclei in fertilized mouse and bovine eggs (50,51). Further studies are needed to clarify whether NuMA is involved in pronuclear formation.

12.2. Male germ cells

In rats, proliferating type A and B spermatozoa expressed abundant NuMA, but leptotene, zygotene, and early pachytene spermatocytes lost their nuclear NuMA at the beginning of a long-lasting prophase. NuMA concentration was again observed at the end of prophase, and a redistribution of NuMA into meiotic spindle poles was observed in first and second meiotic divisions. Early spermatids displayed NuMA staining, but late spermatids and mature spermatozoa lost NuMA. Thus, NuMA may play important roles in meiotic spindle pole formation during spermatogenesis (14). Because mature spermatozoa lack NuMA, it is maternal NuMA that contributes to the organization of the mitotic spindle after fertilization.

13. IMPLICATION OF NuMA IN ANIMAL CLONING BY NUCLEAR TRANSFER

Since the birth of “Dolly”, somatic nuclear transfer (SCNT) offspring have been cloned in many species. However, births of SCNT offspring, including

transgenic SCNT offspring are rare, with average efficiency rates just 1–3% of SCNT constructs (52). In mammals, mature spermatozoa do not contain NuMA; while in mature MII eggs, NuMA is an important component that participates in acentriolar meiotic spindle pole formation. In fertilized eggs, maternally inherited NuMA is involved in first mitotic spindle formation as mentioned in the previous chapter.

Reprogramming of somatic nuclear function by transplantation of nuclei into recipient oocytes is associated with morphological remodeling of the somatic nucleus. In non-human primates, NuMA concentrates at centrosomes in unfertilized meiotic oocytes and fertilized mitotic cells. During nuclear transfer, meiotic spindle removal depletes NuMA from the oocyte. After nuclear transfer, NuMA is not detected on the abnormal mitotic spindles. It is suggested that the failure of primate cloning is largely the result of depletion of microtubule motors and centrosome proteins (e.g., NuMA) during nuclear transfer (53). Recently, by using new approaches, non-human primate nuclear transfer embryo development was significantly improved, but embryo transfers of 135 nuclear transfer embryos into 25 staged surrogates still did not result in convincing evidence of pregnancies. NuMA protein is either not detected or at barely detectable concentrations in the NT mitotic spindle. The residual NuMA, retained within the oocyte cytoplasm following spindle extrusion and imported into the interphase nucleus following nuclear transfer, is not effectively targeted to the spindle poles. These results show that the mechanism recruiting NuMA to the microtubule minus-end is inefficient in non-human primate nuclear transfer eggs (52). However, this conclusion needs further clarification.

Another recent report indicated that, in mouse somatic cell nuclear transfer, the transplanted nuclei display enhanced concentration of NuMA as a result of translation from maternal mRNA and *de novo* transcription. The one-cell stage cloning embryos also exhibit differentiated-cell specific A-type lamins. The authors propose that somatic nuclear remodeling deficiencies by nuclear transfer might emanate, at least in part, failure to remodel the somatic nucleus morphologically into a functional embryonic nucleus (54). Our recent observations showed that NuMA staining was absent in all donor cell fibroblast nuclei (0 h) immediately after nuclear transfer but NuMA staining was detected by 6 h in most pronucleus-like structures of the reconstructed pig eggs (our unpublished data). We propose that NuMA may serve as molecular marker for remodeling of donor cell nuclei.

In order to prove the hypothesis that NuMA from donor cells participates in the spindle assembly in nuclear transfer embryos, we observed the NuMA distribution in nuclear transfer reconstituted embryos. The NuMA antibody used in our experiment did not recognize NuMA protein of mouse oocytes but recognized NuMA protein of porcine granulosa cells at different stages, so this antibody was used for tracking the fate of NuMA protein that comes from the donor cell (porcine somatic cell), without interference from NuMA protein that comes from recipient mouse oocytes. In the pig-mouse interspecies reconstructed embryos, NuMA concentrated between the disarrayed chromosomes 15 min after activation and was translocated to transient spindle poles 1 h after activation. When pseudo-pronuclei formed, NuMA became translocated into pseudo-pronuclei. After pseudo-pronuclear envelope breakdown, NuMA was located between the chromosomes and then translocated to spindle poles at first mitotic metaphase. After NuMA antibody microinjection, all spindles observed were disorganized, showing blunt spindles and multipolar spindles. These observations suggest that NuMA from donor cells contributes to the mitotic spindle function in reconstituted embryos and pericentriolar material might not be species-specific in nucleating microtubules and assembling mitotic spindles (55).

14. PERSPECTIVE

NuMA's distribution, phosphorylation, translocation, and regulation during the mitotic cell cycle are summarized in Figure 1. Although NuMA is located in the nucleus and several lines of evidence have suggested that NuMA is a component of the nuclear matrix and that it participates in the re-formation of the nuclei after mitosis, NuMA's function in transcriptional activity remains largely unknown. In addition, NuMA is not present in some types of cells, requiring further studies on its roles in the interphase nucleus. After nuclear membrane breakdown, phosphorylated NuMA associates with dynein/dynactin and is transported along the microtubules to spindles where it tethers minus ends of microtubules. It is commonly accepted that NuMA organizes microtubule minus ends at spindle poles in a dynein/dynactin process, but a dynein-independent model is also proposed. After anaphase onset, NuMA is dephosphorylated and becomes dissociated from spindle poles. It has recently been shown that cdc2/cyclin kinase plays an important role in the cell cycle-specific NuMA phosphorylation/dephosphorylation, but NuMA's function may be regulated by a series of protein kinases and protein phosphatases. It is likely that more than one protein kinase is involved in these events. Further, there is still no direct evidence showing that cdc2/cyclin B acts on NuMA tail domain, and there is a possibility that cdc2/cyclin B may act on its downstream kinases to carry out its functions. Protein 4.1 has been shown to be a crucial factor for nuclear architecture and assembly/maintenance of mitotic spindle and spindle poles, as well as the formation of centrosome-nucleated and motor-dependent self-organized microtubule asters (56,57). Its interaction with NuMA needs further investigation. NuMA has been used as a marker of nuclear matrix breakdown in early apoptosis, but the significance of NuMA cleavage remains unclear and cleavage of NuMA is not essentially needed for nuclear structure breakdown at least in some types of cells. NuMA overexpression causes multipolar spindle formation and we are at the beginning of understanding NuMA's association with cancer, but the role of abnormal NuMA phosphorylation remains to be investigated. NuMA's significance during germ cell meiosis and animal cloning by nuclear transfer awaits further clarification.

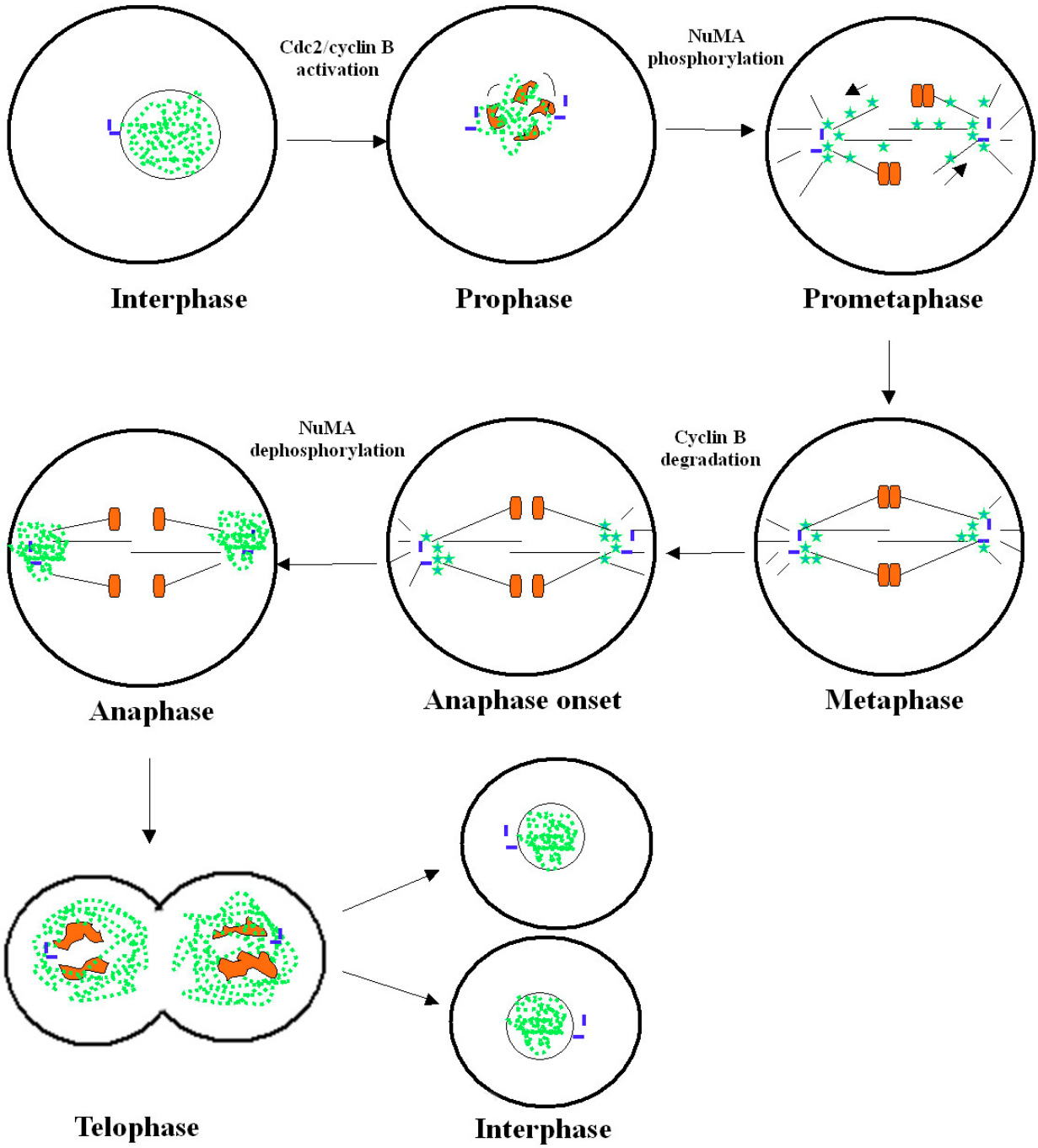

Figure 1. NuMA translocation, phosphorylation and regulation during mitotic cell cycle. At interphase, NuMA is distributed in the nucleus, and it is released into the cytoplasm during mitotic prophase after nuclear membrane breakdown stimulated by cdc2/cyclin kinase. NuMA is then phosphorylated by cdc2/cyclin kinase, associated with dynein/dynactin to form a stable complex, and transported to the spindle poles (centrosomes) where it organizes and tethers microtubules at the minus ends. Cyclin B degradation causes anaphase onset (chromatid separation), NuMA dephosphorylation, NuMA dissociation with spindle poles and spindle microtubule disassembly. The dephosphorylated NuMA is distributed to the forming nucleus area at telophase and finally becomes located in the nucleus of two daughter cells. Green granulated dots represent unphosphorylated NuMA; green stars represent phosphorylated NuMA associated with dynein/dynactin; and condensed chromatin/chromosomes and centrioles are indicated in red and blue, respectively.

15. ACKNOWLEDGMENT

Part of the authors' studies cited in this review were supported by NSFC (30225010, 30371037, 30430530**)** and 973 project (G1999055902) as well as NIH grant (R03-HD43829-02).

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Key words: NuMA, Mitosis, Spindle pole, Cyclin B, Meiosis, Nuclear transfer, Apoptosis, Cell cycle, Microtubule, Dynein, Programmed cell death, Apoptosis, Cloning, Review

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