Cyclin B degradation leads to NuMA release from dynein/dynactin and from spindle poles

Katja Gehmlich[†], Laurence Haren[‡] & Andreas Merdes⁺

Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, UK

The protein NuMA localizes to mitotic spindle poles where it contributes to the organization of microtubules. In this study, we demonstrate that NuMA loses its stable association with the spindle poles after anaphase onset. Using extracts from *Xenopus laevis* eggs, we show that NuMA is dephosphorylated in anaphase and released from dynein and dynactin. In the presence of a nondegradable form of cyclin B ($\Delta 90$), NuMA remains phosphorylated and associated with dynein and dynactin, and remains localized to stable spindle poles that fail to disassemble at the end of mitosis. Inhibition of NuMA or dynein allows completion of mitosis, despite inducing spindle pole abnormalities. We propose that NuMA functions early in mitosis during the formation of spindle poles, but is released from the spindle after anaphase, to allow spindle disassembly and remodelling of the microtubule network.

EMBO reports (2004) 5, 97–103. doi:10.1038/sj.embor.7400046

INTRODUCTION

Cell division requires faithful DNA replication and symmetric distribution of the genetic material. To achieve this, a spindle apparatus moves chromosome pairs towards the equator of the cell in the first stage of mitosis, and subsequently separates them into sister chromatids at opposite spindle poles. Chromosome separation takes place in anaphase and is initiated by cleavage of the cohesin SCC1 by the protease separase. Separase itself is regulated by an inhibitor, securin, and by phosphorylation dependent on cdc2/cyclin B (Stemmann *et al*, 2001). Both securin levels and cdc2/cyclin B kinase activity drop at anaphase onset due to ubiquitin-dependent proteolytic degradation, mediated by the anaphase-promoting complex. Work by several groups has shown that a nondegradable form of cyclin B ($\Delta 90$), missing the amino-terminal destruction box, is able to maintain cdc2 kinase activity while anaphase chromosome separation takes place

(Holloway *et al*, 1993; Murray *et al*, 1996; Wheatley *et al*, 1997). However, although cyclin $\Delta 90$ permits chromosome separation, it prevents proper mitotic exit, inhibiting chromosome decondensation, nuclear re-formation and spindle disassembly. In the presence of cyclin $\Delta 90$, separated chromosomes are grouped in rosettes around stable microtubule asters at the former poles, while the rest of the spindle disassembles (Holloway *et al*, 1993). In this report, we investigate the dynamics of spindle poles late in mitosis, following inactivation of cdc2/cyclin B. The spindle protein NuMA has been previously characterized as an important factor for pole formation early in mitosis (Compton, 1998). Multiple groups have shown that NuMA is phosphorylated in mitosis (Saredi *et al*, 1997, and references therein). The present study investigates the molecular mechanisms that lead to NuMA assembly at the poles, and how its dephosphorylation affects spindle disassembly and mitotic exit.

RESULTS

To study the mechanisms of spindle pole assembly and disassembly, we investigated NuMA localization in anaphase. In spindles assembled in CSF-arrested frog egg extracts (Fig 1A), we noticed that NuMA disappeared from the poles after anaphase chromosome separation was triggered by the addition of CaCl_2 (Fig 1B). In contrast, NuMA remained at the spindle poles in anaphases of cultured HeLa cells and only re-localized to the surface of the re-forming daughter nuclei in telophase (Fig 1C–E). Other cell types from a variety of vertebrate species displayed the same characteristics as HeLa (supplementary information online, Fig S2A–F). However, NuMA was irreversibly released from HeLa anaphase spindle poles when microtubules were depolymerized by cold treatment. During this treatment, NuMA re-localized to the chromosome surface, even though cytokinesis or re-formation of nuclei had not taken place (Fig 1H). NuMA was distributed along the chromosome surface in all cells that had entered anaphase, and could not be transported back to the spindle poles after microtubules recovered in re-warmed cells (Fig 1I, arrows). In contrast, NuMA was able to re-localize to re-forming spindle poles in prometaphase and metaphase cells in the same cultures (Fig 1F,G). Two other spindle pole proteins, TPX2 and pericentrin, were investigated in this assay (supplementary information online, Fig S1): TPX2 was found to diffuse into the cytoplasm upon cold treatment but re-localized to re-forming spindle poles, whereas

Wellcome Trust Centre for Cell Biology, ICMB, University of Edinburgh, Edinburgh EH9 3JR, UK

[†]Present address: Department of Cell Biology, University of Potsdam, Lennéstr. 7a, 14471 Potsdam, Germany

[‡]Present address: CNRS-Pierre Fabre, 205 route de Narbonne, 31077 Toulouse, France

⁺Corresponding author. Tel: +44 131 650 7075; Fax: +44 131 650 7360; E-mail: a.merdes@ed.ac.uk

Received 8 May 2003; revised 8 September 2003; accepted 10 October 2003; published online 12 December 2003

Fig 1 | NuMA is released from anaphase spindles. (A,B) Spindles assembled in *Xenopus* egg extract, in metaphase and anaphase, respectively. (C–E) HeLa cells in metaphase, anaphase and telophase. Microtubules in red, DNA in blue and NuMA in green. (F) HeLa cell in prometaphase, after 1 h incubation on ice, and (G) after cold treatment and subsequent re-warming to 37 °C for 10 min. Immunofluorescence of tubulin reveals that the spindle is re-forming, although a high background of soluble tubulin is still visible. (H) HeLa cell in anaphase, after 1 h cold treatment, and (I) after cold treatment and re-warming for 10 min. Few spindle microtubules at the poles are visible (arrows). Scale bar, 10 μm.

most of the pericentrin remained bound to the centrosome throughout microtubule disassembly and reassembly.

To investigate molecular changes of NuMA at the metaphase/anaphase transition, we isolated NuMA by immunoprecipitation from frog egg extracts at different stages: extracts that were cycled from S-phase into metaphase, and extracts at 10 min after stimulation of anaphase onset by Ca^{2+} . In addition, we immunoprecipitated NuMA from extracts supplemented with a nondegradable form of cyclin B (cyclin $\Delta 90$) and subsequently stimulated to undergo anaphase chromosome separation (Holloway *et al*, 1993). As expected, histone kinase activities dropped in anaphase extracts, but remained high in anaphase extracts that contained cyclin $\Delta 90$ (Fig 2A). Although equal amounts of NuMA were isolated from all three extracts, phosphorylated NuMA could only be found in metaphase and in anaphase extracts with nondegradable cyclin $\Delta 90$, whereas in regular anaphase extracts NuMA was almost completely dephosphorylated (Fig 2C). We then tested whether dynein and dynactin, known to form a complex with NuMA in frog egg cytoplasm (Merdes *et al*, 1996, 2000), were present in our immunoprecipitated material. As shown in Fig 2D,E, the amount of NuMA-associated dynein and dynactin dropped significantly after induction of anaphase, but remained high when nondegradable cyclin $\Delta 90$ was present. The reciprocal experiment showed that NuMA co-immunoprecipitated with dynein in metaphase and cyclin $\Delta 90$ extracts, but not in anaphase (Fig 2F,G). We then investigated whether spindle-pole-associated dynein was seen in metaphase or anaphase cells (Fig 2H,I). Consistent with our biochemical data, HeLa cells in metaphase showed a significant amount of dynein at the poles, colocalizing with NuMA (Fig 2H). Additional dynein was seen at the kinetochores. In anaphase, the dynein signal at the poles diminished, and most dynein was found diffuse in the cytoplasm

or associated with the central spindle (Fig 2I). Comparable data were seen for dynactin p150 (not shown).

As cyclin $\Delta 90$ prevented dephosphorylation of NuMA and dissociation of dynein and dynactin, we wanted to test whether NuMA was kept at anaphase spindle poles with high cyclinB/cdc2 activity. We investigated samples of frog egg extracts at various time points after anaphase onset. Mitotic spindles in both extracts with or without added cyclin $\Delta 90$ were able to undergo anaphase chromosome separation (Fig 3), as reported previously (Holloway *et al*, 1993). However, in control extracts, NuMA disappeared from most spindle poles at approximately 30 min after Ca^{2+} addition, and spindles became fragile (Fig 3E). In contrast, spindles in cyclin $\Delta 90$ -treated extracts remained stable and retained pole-associated NuMA (Fig 3F). After 50 min, spindle pole microtubules in controls disappeared nearly completely and NuMA was re-imported into nuclei (Fig 3G), in contrast to cyclin $\Delta 90$ -treated samples that showed either intact spindles or focused microtubule asters, with NuMA at their centre, resembling remnants of spindle poles (Fig 3H). When we quantified the numbers of NuMA-positive spindles at 30 min in anaphase, we counted 14% ($N=189$) in controls and 95% ($N=37$) in cyclin $\Delta 90$ -treated extracts. At 50 min, differences were even more evident, with mostly re-formed nuclei in controls (2% NuMA-positive spindles/337 chromatin arrays) and 95% NuMA-positive spindles ($N=61$) in cyclin $\Delta 90$ -treated extracts. The concentrated NuMA staining at the spindle poles was also lost when metaphase spindles were treated with the CdK inhibitor roscovitine: NuMA was found diffuse along spindle microtubules only 5 min after the addition of the drug to egg extracts, and spindle pole microtubules lost their focus (Fig 3J,K). This phenotype was reminiscent of spindles treated with antibody against dynein (Fig 3L), suggesting that continuous NuMA transport to the poles is required to maintain pole integrity,

Fig 2 | NuMA is dephosphorylated and released from dynein and dynactin in anaphase. (A) Autoradiograph of a histone H1 kinase assay, (B) immunoblot of immunoprecipitated NuMA from *Xenopus* egg extracts, (C) autoradiograph of ^{32}P -phosphorylated NuMA after immunoprecipitation, and immunoblots of (D) co-immunoprecipitated dynactin p150 and (E) dynein intermediate chain. (F,G) Reciprocal experiment using antibody 70.1 for dynein immunoprecipitation, showing immunoblots of (F) dynein intermediate chain and (G) NuMA. Experiments were performed using extracts cycled from S-phase into metaphase (meta), extracts stimulated to enter anaphase for 10 min (ana), and equivalent extracts in anaphase in the presence of cyclin B $\Delta 90$ (ana c $\Delta 90$). (H,I) Dynein staining of the spindle poles is reduced in anaphase. HeLa cells in (H) prometaphase and (I) anaphase, stained for DNA, NuMA and dynein intermediate chain. Scale bar, 10 μm .

and that this is mediated by an interaction between the phosphorylated protein and the dynein/dynactin complex.

As our data indicate that anaphase is executed while NuMA disassembles from the poles, we tested directly whether inhibition of NuMA or dynein with specific antibodies interferes with chromosome separation and completion of mitosis. First, we added antibodies to *Xenopus* egg extracts cycling from S-phase into mitosis. As seen previously for NuMA or dynein inhibition, microtubules formed spindle-like structures around chromosomes aligned at the equatorial plane, but these structures lacked focused poles (Fig 4C,E; Heald *et al*, 1996; Merdes *et al*, 2000). Extracts were subsequently treated with CaCl_2 to induce anaphase. As shown in Fig 4B,D,F, chromosome separation took place in control spindles as well as in structures without intact poles (controls 96% ($N=92$), anti-NuMA 94% ($N=106$), anti-dynein 67% ($N=43$)). Although chromosome separation in extracts appeared less symmetric than in anaphases of cultured cells (see also Murray *et al*, 1996), we think that the majority of these separation events were anaphase-specific and not due to incomplete prometaphase chromosome congression, as the

starting material prior to anaphase onset showed equatorial alignment (controls 71% ($N=246$), anti-NuMA 76% ($N=46$), anti-dynein 91% ($N=46$)). Further, separation of the kinetochores was seen in spindles from anaphase extracts, following staining of the centromere-binding protein CENP-E (Fig 4G–J). Our data suggest that chromosome separation can occur *in vitro* despite inhibition of NuMA and loss of intact spindle poles. To investigate this problem *in vivo*, we tested whether mitosis could be completed normally in cultured cells microinjected with antibodies against NuMA. We injected *Xenopus* A6 cells late in prometaphase, when we could see condensed chromosomes that were aligning at the metaphase plate. Whereas injection of control antibodies had no visible effect on spindle morphology (Fig 5A–C), those cells that were injected with anti-NuMA showed dramatic abnormalities, with unfocused spindle poles and centrosomal microtubule asters often disconnected from the body of the spindle (Fig 5D–F, arrowheads). Such defects were seen in 87% of the cells in prometaphase or metaphase 30 min after injection ($N=62$). The overall spindle shape was more barrel-like, with an increased width-to-length ratio. These metaphase spindles

Fig 3 | Stable spindle poles, containing NuMA, in anaphase in the presence of nondegradable cyclin B $\Delta 90$. (A,C,E,G) Spindles were formed in *Xenopus* egg extracts and stimulated to enter anaphase. (B,D,F,H) Spindles in extracts in the presence of cyclin B $\Delta 90$. (A,B) Metaphase spindles in extracts prior to CaCl_2 addition (0 min). (C–H) Progression through anaphase at various times after CaCl_2 addition (15, 30 and 50 min). (H) Microtubule asters persist in extracts containing cyclin B $\Delta 90$ and contain NuMA at their organizing centre (arrows). (I–K) Metaphase spindles in (I) control extract and in (J,K) extracts treated for 5 min with $13 \mu\text{M}$ roscovitine. (L) Metaphase spindle in extract subsequently treated with anti-dynein antibody. Microtubules in red, DNA in blue and NuMA in green. Scale bar, $10 \mu\text{m}$.

($N=23$) were on average 1.3 times wider than in controls ($N=8$) or untreated cells ($N=34$). In all, 88% of the cells proceeding into anaphase A after microinjection showed abnormal spindle poles ($N=17$), but separated chromosome masses were visible (Fig 5G). Surprisingly, cells that progressed into anaphase B showed only 24% abnormal spindles ($N=25$), and these sometimes had twisted central spindle microtubules (Fig 5H–J). Cells in telophase ($N=37$) showed no obvious defects in microtubule organization (Fig 5K,L). Furthermore, nuclear re-formation and cytokinesis were completed normally. In another set of experiments, cells were injected while blocked in S-phase, and subsequently released into mitosis. Similar spindle phenotypes with abnormal poles were seen, but anaphase chromosome separation was achieved despite abnormal spindle morphology (supplementary information online, Fig S2G,H), indicating that the requirement of NuMA for mitosis is not absolute.

DISCUSSION

The finding by Holloway *et al* (1993) that anaphase chromosome separation can take place despite high levels of cyclinB/cdc2 kinase activity has raised the question of why cyclin B is degraded

at anaphase onset. Their initial work indicated that in the presence of nondegradable cyclin $\Delta 90$, chromosomes do not decondense at the end of mitosis and microtubules at the spindle poles persist, while controls show spindle disassembly and nuclear re-formation following cyclin B degradation. Furthermore, work by Wheatley *et al* (1997) demonstrated that cyclin $\Delta 90$ inhibits re-localization of the chromosomal passenger protein TD60 and of myosin II, and prevents contractile ring formation. In this work, we identify NuMA as a downstream target of cyclin B/cdc2. We show that the prolonged stability of spindle poles in the presence of nondegradable cyclin B correlates with NuMA binding to the microtubule organizing centre. It was reported earlier by Compton & Luo (1995) that NuMA is phosphorylated in mitosis, and that four predicted cdc2 phosphorylation sites exist in the NuMA tail region. However, direct proof for a dependence of NuMA on cdc2 kinase was still missing. Here we document that NuMA fails to become dephosphorylated in anaphase when cyclin B/cdc2 kinase activity is artificially kept high by nondegradable cyclin $\Delta 90$, suggesting that NuMA is either phosphorylated directly by cdc2 kinase, or by other kinases whose activity is dependent on cdc2. The present work further shows that NuMA is released from

Fig 4 | Anaphase chromosome separation in extracts takes place in the absence of intact spindle poles. (A) Control spindle in metaphase egg extract. DNA staining shows aligned chromosomes. (B) Spindle in extract stimulated to enter anaphase. Chromosomes are separated to opposite poles. (C) Spindle pole formation is inhibited in metaphase with anti-NuMA antibody. (D) Anaphase chromosome separation in a spindle inhibited with anti-NuMA. (E) Spindle pole formation is inhibited in metaphase with anti-dynein. (F) Anaphase chromosome separation in a spindle inhibited with anti-dynein. (G,H) Control spindles in (G) metaphase and (H) anaphase, stained for CENP-E (green). (I,J) Spindles assembled in the presence of anti-NuMA antibody, in (I) metaphase and (J) anaphase, stained for CENP-E (green). Paired arrowheads in (G,I) indicate sister kinetochores. (H,J) Mostly single kinetochores (arrowheads) were seen in anaphase. Scale bar, 10 μ m.

dynein and dynactin in anaphase, raising the question of whether poleward NuMA transport stops at this stage. Close inspection of early anaphase reveals that NuMA is still bound to the poles despite the absence of dynein/dynactin, both in extract spindles (Fig 3C) and in cultured cells (Fig 1D). This can be explained by our previous finding that NuMA binds constitutively to tubulin (Haren & Merdes, 2002). Further, NuMA has been shown to form a fibrous matrix at the poles (Dionne *et al*, 1999), which might take several minutes to disassemble while anaphase chromosome separation is already underway. Whether the self-association of NuMA into a matrix is cdc2-dependent is unclear; however, our data on cold-treated HeLa cells indicate that any NuMA structures at anaphase spindle poles are unstable and lost after microtubule depolymerization. We propose that phosphorylated NuMA interacts with dynein/dynactin and is transported towards microtubule minus-ends at the poles, whereas unphosphorylated NuMA can bind along microtubules but is no longer transported. In support of this, Compton & Luo (1995) reported that point mutations in

NuMA that prevent NuMA phosphorylation in mitosis abolish spindle pole localization but instead cause aberrant binding of NuMA along the plasma membrane. Interestingly, closer inspection of our images of anaphase cells revealed that part of NuMA is also visible along the plasma membrane at this stage (Fig 2A,B), although the molecular mechanisms for this are not understood.

We suggest that NuMA is needed in prometaphase for the correct assembly of spindle poles (Merdes *et al*, 1996). This requirement may not be absolute: inhibition of NuMA early in mitosis leads to spindle defects that can delay anaphase entry (Gordon *et al*, 2001), but chromosome separation does take place after a prolonged incubation period. We find that anaphase chromosome separation takes place despite spindle pole defects, supporting the concept that the force generation of chromosome movement is at or near the kinetochore (Nicklas, 1989). NuMA may therefore be a factor that contributes to early spindle pole formation, preventing mitotic defects that can otherwise develop in its absence. In support of this view, work by Kallajoki *et al*

Fig 5 | Completion of mitosis despite spindle pole disruption. (A–C) *Xenopus* A6 cells microinjected in prometaphase with control antibody, fixed and stained in (A) metaphase, (B) anaphase and (C) telophase. (D–L) *Xenopus* A6 cells microinjected in prometaphase with antibody against NuMA, fixed after various lengths of incubation. (D–F) Cells fixed and stained in prometaphase/metaphase. (G) Cell in anaphase A. (H–J) Cells in anaphase B. (K,L) Cells in telophase. Columns from left to right show tubulin, the localization of the injected antibody, and DNA, respectively. Arrowheads indicate centrosomal asters that are not properly connected to the rest of the spindle. Scale bar, 10 μ m.

(1993) showed that microinjection of antibody against NuMA in prometaphase causes the formation of micronuclei and defects in cytokinesis in part of the cells, but consistent with our data NuMA inhibition late in mitosis only leads to very few abnormalities. Additional support for the idea that NuMA and associated proteins lose their function in anaphase comes from Clark & Meyer (1999), who demonstrated that the overexpressed dynactin subunit Arp1 α induces the formation of NuMA-containing protein aggregates leading to spindle pole defects that slow down prometaphase, but allowing normal anaphase chromosome separation. These protein aggregates segregate NuMA transiently in prometaphase, but release NuMA later in mitosis, when spindle morphology recovers to a normal phenotype. Altogether, this shows that spindle poles are highly dynamic structures that are under the control of cyclin B/cdc2 kinase activity and that disassemble after cyclin B degradation.

METHODS

***Xenopus* egg extracts.** Metaphase-arrested *Xenopus laevis* egg extracts were prepared as described (Murray, 1991). Extracts were activated with 0.8 mM CaCl₂ to cycle through S-phase and re-enter mitosis. The formation of metaphase spindles was supported by the addition of 50 μ L CSF-arrested extract for every 100 μ L activated extract, at 90 min after activation. Spindle poles were inhibited by the simultaneous addition of rabbit anti-*Xenopus* NuMA tail II, or monoclonal anti-dynein 70.1 (Sigma), as described (Merdes *et al.*, 1996, 2000). To some extracts, a nondegradable form of cyclin B (Δ 90; Glotzer *et al.*, 1991) was added at the same time, exactly as described (Holloway *et al.*, 1993). Extracts were driven into anaphase by adding 0.8 mM

CaCl₂ at 90 min after CSF extract addition. Immunoprecipitation of NuMA and dynein was performed as described (Merdes *et al.*, 1996). Co-precipitated dynein intermediate chain and dynactin p150 glued were detected with monoclonal anti-dynein 70.1 (Sigma), or monoclonal anti-dynactin 150B from Dr T. Schroer (Quintyne *et al.*, 1999). To assay NuMA phosphorylation, 100 μ L of the extract was supplemented with 40 μ Ci of [γ -³²P]ATP after 60 min in S-phase. ³²P incorporation was detected on gels of NuMA immunoprecipitates, using a phosphorimager. Histone H1 kinase activity was assayed as described (Murray, 1991).

Cell culture and microinjection experiments. *Xenopus* A6 cells were monitored by phase contrast microscopy and microinjected in prometaphase, when chromosome congression was visible, using a Narishige microinjection device (Nikon). Microinjected antibodies were affinity purified using sheep antibody against a hexa-histidine tagged fusion protein of the *Xenopus* NuMA head domain (Haren & Merdes, 2002), at 1 mg/ml in 0.1 M KCl, 10 mM potassium phosphate, pH 7.4, or purified FITC-labelled donkey-anti-sheep as a control. Cells were fixed in –20 °C cold methanol, and stained with monoclonal anti-tubulin DM1 α (Sigma), followed by TexasRed-labelled anti-mouse secondary antibody, and FITC-labelled donkey-anti-sheep, to detect the microinjected cells. DNA was stained with DAPI. HeLa cells were stained for immunofluorescence with monoclonal anti-dynein intermediate-chain MAB 1618 (Chemicon), rabbit anti-tubulin (Fant & Merdes, 2002), monoclonal anti-human NuMA NA09L (Calbiochem) or rabbit anti-NuMA provided by Dr D. Compton (Gaglio *et al.*, 1995).

Supplementary information is available at *EMBO reports* online (<http://www.emboreports.org>).

ACKNOWLEDGEMENTS

We thank our colleagues at the University of Edinburgh for advice and technical help. We thank Drs M. Glotzer (IMP Vienna), T. Schroer (Johns Hopkins University) and D. Compton (Dartmouth Medical School) for the gift of cyclin plasmids, and for antibodies against dynactin and NuMA, respectively. A.M. was supported by a Senior Research Fellowship from the Wellcome Trust.

REFERENCES

- Clark IB, Meyer DI (1999) Overexpression of normal and mutant Arp1alpha (centractin) differentially affects microtubule organization during mitosis and interphase. *J Cell Sci* **112**: 3507–3518
- Compton DA (1998) Focusing on spindle poles. *J Cell Sci* **111**: 1477–1481
- Compton DA, Luo C (1995) Mutations in the predicted p34cdc2 phosphorylation sites in NuMA impair the assembly of the mitotic spindle and block mitosis. *J Cell Sci* **108**: 621–633
- Dionne MA, Howard L, Compton DA (1999) NuMA is a component of an insoluble matrix at mitotic spindle poles. *Cell Motil Cytoskeleton* **42**: 189–203
- Fant X, Merdes A (2002) The carboxy-terminus of protein 4.1R resembles beta-tubulin. *Cell Biol Int* **26**: 371–377
- Gaglio T, Saredi A, Compton DA (1995) NuMA is required for the organization of microtubules into aster-like arrays. *J Cell Biol* **131**: 693–708
- Glotzer M, Murray AW, Kirschner MW (1991) Cyclin is degraded by the ubiquitin pathway. *Nature* **349**: 132–138
- Gordon MB, Howard L, Compton DA (2001) Chromosome movement in mitosis requires microtubule anchorage at spindle poles. *J Cell Biol* **152**: 425–434
- Haren L, Merdes A (2002) Direct binding of NuMA to tubulin is mediated by a novel sequence motif in the tail domain that bundles and stabilizes microtubules. *J Cell Sci* **115**: 1815–1824
- Heald R, Tournebise R, Blank T, Sandaltzopoulos R, Becker P, Hyman A, Karsenti E (1996) Self-organization of microtubules into bipolar spindles around artificial chromosomes in *Xenopus* extracts. *Nature* **382**: 420–425
- Holloway SL, Glotzer M, King RW, Murray AW (1993) Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor. *Cell* **73**: 1393–1402
- Kallajoki M, Harborth J, Weber K, Osborn M (1993) Microinjection of a monoclonal antibody against SPN antigen, now identified by peptide sequences as the NuMA protein, induces micronuclei in PtK2 cells. *J Cell Sci* **104**: 139–150
- Merdes A, Ramyar K, Vechio JD, Cleveland DW (1996) A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. *Cell* **87**: 447–458
- Merdes A, Heald R, Samejima K, Earnshaw WE, Cleveland DW (2000) Formation of spindle poles by dynein/dynactin-dependent transport of NuMA. *J Cell Biol* **149**: 851–861
- Murray AW (1991), In *Methods in Cell Biology*, Kay BK, Peng HB (eds) Vol 36, pp 581–605. San Diego, CA: Academic Press
- Murray AW, Desai AB, Salmon ED (1996) Real time observation of anaphase *in vitro*. *Proc Natl Acad Sci USA* **93**: 12327–12332
- Nicklas RB (1989) The motor for poleward chromosome movement in anaphase is in or near the kinetochore. *J Cell Biol* **109**: 2245–2255
- Quintyne NJ, Gill SR, Eckley DM, Crego CL, Compton DA, Schroer TA (1999) Dynactin is required for microtubule anchoring at centrosomes. *J Cell Biol* **147**: 321–334
- Saredi A, Howard L, Compton DA (1997) Phosphorylation regulates the assembly of NuMA in a mammalian mitotic extract. *J Cell Sci* **110**: 1287–1297
- Stemmann O, Zou H, Gerber SA, Gygi SP, Kirschner MW (2001) Dual inhibition of sister chromatid separation at metaphase. *Cell* **107**: 715–726
- Wheatley SP, Hinchcliffe EH, Glotzer M, Hyman AA, Sluder G, Wang Y (1997) CDK1 inactivation regulates anaphase spindle dynamics and cytokinesis *in vivo*. *J Cell Biol* **138**: 385–393