

# NuMA phosphorylation dictates dynein-dependent spindle positioning

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What mechanisms ensure that temporal and spatial aspects of mitosis are properly coordinated? Part of the answer to this important question emerged from work published recently, which established that a connection between cell cycle progression and the large coiled-coil protein NuMA is key.<sup>1,2</sup>

The mitotic spindle must be positioned accurately during metaphase and anaphase to ensure that the resulting daughter cells are born with the correct relative sizes and in the proper locations within tissues. In animal cells, an evolutionary conserved ternary complex (NuMA/LGN/G $\alpha$ <sub>1-3</sub> in human cells) has been shown previously to anchor the minus-end-directed microtubule-dependent motor protein dynein at the cell cortex during metaphase.<sup>3-6</sup> There, dynein is thought to interact with the plus-end of astral microtubules and generate a pulling force on them owing to its motor activity and/or by virtue of its ability to retain interaction with depolymerizing microtubules.<sup>3-6</sup> However, whether components of the ternary complex also participate in anaphase spindle positioning in mammalian cells was not known.

In human cells, both NuMA and dynein are slightly enriched in the cortical regions located above the spindle poles in metaphase.<sup>4-6</sup> Furthermore, it has been established that the Polo-like kinase Plk1 negatively regulates the distribution of dynein at that time.<sup>5</sup> We reported previously that either decreasing or increasing the levels of ternary complex components at the cell cortex, and therefore, altering the levels of dynein, has a profound influence on metaphase spindle positioning.<sup>6</sup> Thus, lack of cortical dynein results in spindle positioning defects, presumably owing to

defective anchorage of astral microtubules to the cell cortex. Conversely, excess cortical dynein results in exaggerated spindle oscillations, presumably owing to abnormally high and unbalanced pulling forces.

Are similar mechanisms at play during anaphase? Intriguingly, it had been observed that levels of cortical dynein increase during mitotic progression.<sup>7</sup> What are the mechanisms underlying such an increase, and are they important for the proper execution of mitosis? Our recent study, together with work conducted by the laboratory of Iain Cheeseman, established that levels of cortical NuMA increase markedly as cells progress from metaphase into anaphase, explaining the concomitant augmentation of cortical dynein.<sup>1,2</sup> Interestingly, it was found that NuMA distribution is regulated by CDK1/cyclinB activity (referred to as CDK1 hereafter), as evidenced by the fact that chemical inactivation of CDK1 during metaphase results in excess NuMA and dynein at the cell cortex.<sup>1,2</sup> As expected from the prior work, this causes dynein-dependent spindle positioning defects during metaphase.<sup>1</sup> Therefore, CDK1 ensures proper spindle positioning during metaphase by maintaining low levels of cortical NuMA/dynein. Furthermore, we found that CDK1 phosphorylates NuMA at an evolutionary conserved T2055 residue.<sup>1</sup> By raising phospho-specific NuMA antibodies (p2055) and conducting immunofluorescence analysis, we found that the T2055-phosphorylated NuMA species is present during metaphase in the cytoplasm and at spindle poles, but not at the cell cortex. Moreover, we found that p2055 staining is substantially enriched in the nucleus during prophase and absent

in anaphase. Interestingly, these localizations mirror that of the active CDK1 species during mitosis.<sup>8</sup> Therefore, p2055 antibodies could provide a novel means to monitor CDK1 activity and thus reflect on the proliferation status of cells.

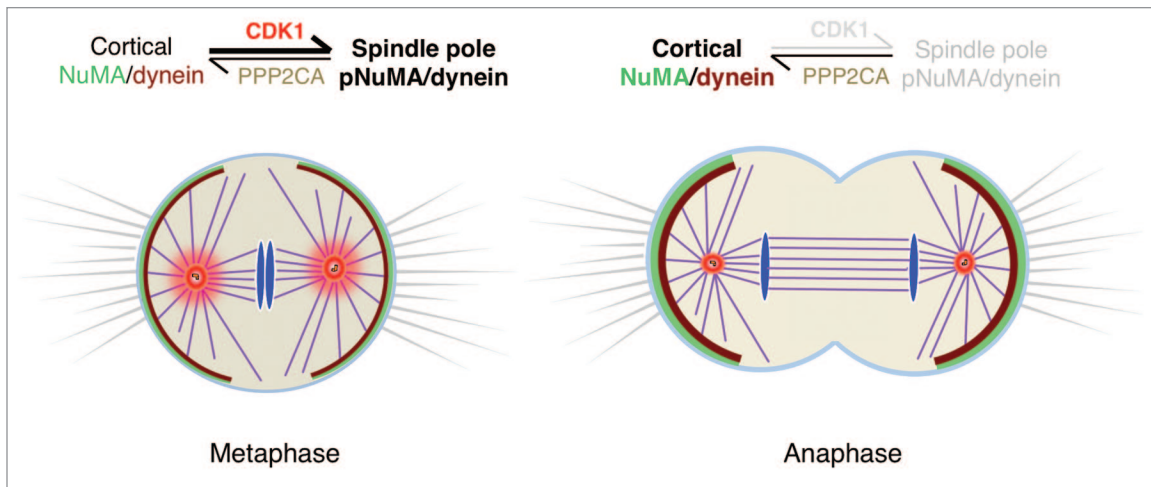
How can low levels of non-phosphorylated NuMA be present at the cell cortex during metaphase despite CDK1 being active? We discovered that low levels of cortical NuMA/dynein are achieved through a tug-of-war between CDK1 and PPP2CA phosphatase activity.<sup>1</sup> We found that chemical inactivation or siRNA-mediated depletion of PPP2CA causes loss of cortical NuMA during metaphase. Since active CDK1 is enriched at spindle poles, we propose that a gradient of CDK1 activity, being highest at the spindle poles and lowest at the cell cortex, together with uniform PPP2CA phosphatase activity, results in low levels of NuMA/dynein at the cell cortex during metaphase (Fig. 1, left). The nature of the regulatory and targeting subunits that associate with the PPP2CA catalytic subunit of the PP2A trimeric holoenzyme remain to be determined. Additionally, it is plausible that CDK1 phosphorylates and thereby negatively regulates PPP2CA, thus forming a feedback loop during metaphase, which may contribute to the fact that there is only a slight enrichment of cortical NuMA/dynein above the spindle poles. Regardless, and importantly, upon CDK1 inactivation at the onset of anaphase, PPP2CA activity causes substantial accumulation of cortical NuMA/dynein, which drives robust dynein dependent spindle elongation (Fig. 1, right).<sup>1</sup> Moreover, asymmetric membrane elongation in response to defective spindle positioning acts as a

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**Figure 1.** CDK1 and PPP2CA orchestrate levels of NuMA/dynein at the cell cortex during mitosis. Working model for NuMA/dynein cortical localization during metaphase and anaphase. During metaphase (left), the antagonistic activities of CDK1 (red), which is maximal at spindle poles, and PPP2CA phosphatase (light brown), which we postulate is active throughout the cell, on T2055 of NuMA, result in the presence of moderate levels of non-phosphorylated NuMA and, thus, of dynein, at the cell cortex (green and dark brown, respectively). Such moderate levels are critical for proper spindle positioning during metaphase. During anaphase (right), CDK1 inactivation and continuous uniform activity of PPP2CA results in higher levels of cortical non-phosphorylated NuMA, and thus of dynein, which is critical for robust spindle elongation during anaphase (see text for details). Microtubules are depicted in purple, DNA in blue, retraction fibers in gray.

correction mechanism during anaphase, eventually resulting in the generation of daughter cells with the appropriate relative sizes.<sup>2</sup>

In summary, the work published recently revealed a mechanism that helps coordinate temporal and spatial aspects of spindle positioning during mitosis, whereby a careful balance of CDK1 and PPP2CA activities ensures that balanced levels of cortical NuMA/dynein are present at the cell cortex throughout mitosis of tissue culture cells (Fig. 1). Levels are low during metaphase, which is essential for proper spindle positioning, and high

during anaphase, which is critical for proper spindle elongation. Since a rise in cortical NuMA during mitotic progression is observed in various cell types,<sup>1</sup> it is likely that similar mechanisms are at play also during development and in stem cells in an intact organism.

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