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Cdk1 Coordinates Timely Activation of MKlp2 Kinesin with Relocation of the Chromosome Passenger Complex for Cytokinesis

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SUMMARY

The chromosome passenger complex (CPC) must relocate from anaphase chromosomes to the cell equator for successful cytokinesis. Although this landmark event requires the mitotic kinesin MKlp2, the spatiotemporal mechanistic basis remains elusive. Here, we show that phosphoregulation of MKlp2 by the mitotic kinase Cdk1/cyclin B1 coordinates proper mitotic transition with CPC relocation. We identified multiple Cdk1/cyclin B1 phosphorylation sites within the stalk and C-terminal tail that inhibit microtubule binding and bundling, oligomerization/clustering, and chromosome targeting of MKlp2. Specifically, inhibition of these abilities by Cdk1/cyclin B1 phosphorylation is essential for proper early mitotic progression. Upon anaphase onset, however, reversal of Cdk1/cyclin B1 phosphorylation promotes MKlp2-CPC complex formation and relocates the CPC from anaphase chromosomes for successful cytokinesis. Thus, we propose that phosphoregulation of MKlp2 by Cdk1/cyclin B1 ensures that activation of MKlp2 kinesin and relocation of the CPC occur at the appropriate time and space for proper mitotic progression and genomic stability.

INTRODUCTION

For successful cell division, faithful segregation of duplicated sister chromatids to two daughter cells must be orchestrated with cytokinesis. A key component coordinating this process is the chromosome passenger complex (CPC), which consists of the enzymatic component Aurora B and three regulatory components: INCENP, survivin, and borealin (reviewed by Carmena et al., 2012). The CPC is essential for proper chromosome congression in early mitosis at the centromeres, while it relocates to the spindle midzone and equatorial cortex (hereby called the cell equator) in late mitosis (Cooke et al., 1987; Earnshaw and Cooke, 1991) to promote furrow ingression for cytokinesis.

The CPC relocation event starts at the metaphase-to-anaphase transition, a point of no return that is tightly regulated by maintaining Cdk1/cyclin B1 kinase activity (reviewed by Rhind and Russell, 2012). For this transition to occur, bipolar spindle attachment must be completed in order for the anaphase-promoting complex (APC) to degrade cyclin B1 that inactivates Cdk1. In budding yeast, Cdk1 and Cdc14p phosphatase antagonistically control Sli15/INCENP for Ipl1/Aurora B localization (Mirchenko and Uhlmann, 2010; Nakajima et al., 2011; Pereira and Schiebel, 2003). In mammalian cells, Cdk1/cyclin B1 phosphorylation of INCENP at the Thr-59 residue has an inhibitory effect on CPC relocation (Goto et al., 2006; Hümmer and Mayer, 2009). However, the mechanistic basis of the spatiotemporal redistribution of the CPC upon anaphase onset remains elusive, particularly in mammalian cells. Furthermore, despite the requirement of MKlp2 kinesin for CPC relocation and cytokinesis in mammalian cells (Gruneberg et al., 2004; Hill et al., 2000), the mechanism of spatiotemporal recognition between MKlp2 and the CPC at anaphase onset is unclear.

Here, we show a key regulatory mechanism that controls activation of MKlp2 at the appropriate time in the cell cycle via Cdk1/cyclin B1-mediated phosphoregulation. In brief, we demonstrate that Cdk1/cyclin B1-mediated inhibitory phosphorylation of MKlp2 is essential for proper early mitotic progression. Upon anaphase onset, however, reversing this phosphorylation is necessary for timely activation of MKlp2 kinesin to relocate the CPC from anaphase chromosomes for cytokinesis. Thus, Cdk1/cyclin B1-mediated phosphoregulation ensures that activation of MKlp2 kinesin and relocation of the CPC occur at the appropriate time and space for proper mitotic progression.

RESULTS

Cdk1/Cyclin B1-Dependent Phosphorylation of MKlp2 in Early Mitosis

It is unclear how MKlp2 kinesin function is controlled during mitotic progression. Notably, when HeLa cells arrested in mitosis

by the microtubule depolymerizer nocodazole (Noco) were briefly treated with the Cdk1 inhibitor purvalanol A (PurvA), endogenous MKlp2 showed band-shifting into a form of higher mobility in Phos-tag SDS-PAGE (Figure 1A), suggesting dephosphorylation. To evaluate the possibility of phosphoregulation of MKlp2, HeLa cell lines engineered to express Flag-tagged MKlp2 (Flag-MKlp2) at endogenous levels upon doxycycline (Dox) treatment (Kitagawa et al., 2013) were synchronized in prometaphase-like state by Noco treatment. After release, cells were harvested at different time points. Phos-tag SDS-PAGE revealed the mitotic mobility shift of the Flag-MKlp2^{WT} band gradually collapsing at anaphase onset (marked by a decrease in cyclin B1 levels) (Figure 1B). Although the amount of extracted Flag-MKlp2^{WT} also decreased after anaphase onset (Figure 1B, lanes 3 and 4), it was mainly due to Flag-MKlp2^{WT} becoming less soluble (Figures 2E, 2F, and 4C). Moreover, treating immunopurified Flag-MKlp2^{WT} with alkaline phosphatase (ALP) also caused band-shifting (Figure 1C, lanes 3 and 4), suggesting that MKlp2 is phosphorylated in early mitosis.

Human MKlp2 contains seven putative, evolutionarily conserved proline-directed Cdk1 phosphorylation sites (Figure 1D; Figure S1A). Indeed, Cdk1/cyclin B1 phosphorylated multiple proline-directed Ser/Thr residues in MKlp2 in vitro (Figure S1B). Thus, to determine whether the band-shifting of Flag-MKlp2^{WT} was due to phosphorylation of these residues, we substituted all putative phosphoresidues with alanine (MKlp2^{7A}; Figure 1D). Indeed, Flag-MKlp2^{7A} did not show a measurable band-shifting unlike Flag-MKlp2^{WT} (Figure 1E, lanes 2 and 4), even after ALP treatment (Figure 1C, lanes 7 and 8), suggesting that MKlp2 is phosphorylated on all or some proline-directed Ser/Thr residues. Thus, we conclude that MKlp2 is phosphorylated in early mitosis (at least in mitotic-arrested cells with spindle damages) in a Cdk1/cyclin B1-dependent manner.

Cdk1/Cyclin B1 Restrains MKlp2 in the Cytoplasm in Early Mitosis

Next, whether Cdk1/cyclin B1-dependent phosphorylation controls MKlp2 localization was tested. Notably, while endogenous MKlp2 localized in the cytoplasm of metaphase-arrested HeLa cells by the proteasome inhibitor MG132, a brief PurvA treatment for 2 min caused a dramatic redistribution of MKlp2 to the metaphase spindles, chromosomes, and the cell cortex without apparent chromosome separation (Figure 1F). Furthermore, in metaphase-arrested HeLa cells, Dox-induced Flag-MKlp2^{7A}, but not Flag-MKlp2^{WT}, constitutively localized to the metaphase spindles, chromosomes, and the cell cortex and induced misaligned chromosomes (Figure 1G, arrows; see also Figure 3H). Consistent with this localization pattern of MKlp2 determined by immunofluorescence staining, transiently expressed mCherry-tagged MKlp2^{7A}, but not MKlp2^{WT}, showed a similar localization pattern in HeLa cells arrested by inhibiting centrosome separation with the Eg5 inhibitor S-trityl-L-cysteine (STLC) (Figure 1H, subpanels a and b; Figure 1I). Thus, we conclude that Cdk1/cyclin B1 restrains MKlp2 in the cytoplasm in early mitosis.

Notably, alanine substitutions at the Ser-532, Thr-857, Ser-867, and Ser-878 residues (MKlp2$^{C\text{-}4A}$) in the stalk and C-terminal tail domains were sufficient to induce MKlp2 redistribution (Figure 1H, subpanel c; Figure 1I), although slightly weaker than MKlp2^{7A} (Figures 3C, 3D, 5G, and 5H). In contrast, alanine substitutions at the Ser-7 and Ser-21 residues in the N-terminal tail (MKlp2$^{N\text{-}2A}$) weakly caused MKlp2 redistribution to chromosomes (Figure 1I). Alanine substitution at the Thr-198 residue did not cause measurable changes (M.K. and S.H.L. unpublished data). Furthermore, none of phosphomimetic mutants (generated by glutamate substitution) showed any measurable redistribution (Figure 1I). Interestingly, phosphoproteomic analysis (http://www.phosphosite.org) indicates that these 4 residues in the stalk and C-terminal tail domains are frequently phosphorylated. To determine the phosphorylation status of these residues in vivo, phosphospecific (pS-532, pS-867, and pS-878) antibodies were generated and validated for specificity (Figure S2). The phosphorylation status of the Thr-857 residue was determined by antibodies against a phospho-Thr-Pro motif (p-TP). Indeed, all phosphospecific antibodies strongly cross-reacted with Dox-induced Flag-MKlp2^{WT} in Noco-arrested HeLa cells, while PurvA treatment abolished such reactivity (Figure 1J, lanes 1 and 5). Notably, using these phosphospecific antibodies as indicators, these phosphoresidues were rapidly dephosphorylated upon anaphase onset (marked by a decrease in cyclin B1 levels) (Figure 1J). Thus, we conclude that Cdk1/cyclin B1 controls spatiotemporal localization of MKlp2 during mitotic progression via promoting phosphorylation of at least the stalk and C-terminal tail domains. This phosphorylation restrains MKlp2 in the cytoplasm in early mitosis.

Phosphoregulation of MKlp2 in Higher-Order Complex Formation, Direct Microtubule Binding, and Chromosome Targeting

Folding the tail and motor domains of kinesin together is a proposed mechanism for inhibiting kinesin motor (reviewed by Verhey and Hammond, 2009). Thus, whether C-terminal phosphorylation may maintain MKlp2 in a folded state was tested using recombinant MBP-tagged C-terminal tail (amino acids [aa] 710–890; MBP-MKlp2$^{710–890}$ and MBP-MKlp2$^{710–890/C\text{-}3E}$) and His-tagged N-terminal domain (aa 1–510; 6xHis-MKlp2$^{1–510}$) (Figure 2A). In contrast to our hypothesis, MBP-MKlp2$^{710–890}$ formed a complex with 6xHis-MKlp2$^{1–510}$ (Figure 2B, lanes 1–7) with patterns of sequential interaction (Figure 2C; K_D = 5.1 μM and K_D = 15.6 μM), while the phosphomimetic mutant MBP-MKlp2$^{710\text{-}890/C\text{-}3E}$ failed to do so in MBP-pull-down (Figure 2B, lanes 8–14) and isothermal titration calorimetry (ITC) assays (M.K. and S.H.L., unpublished data). Thus, Cdk1/cyclin B1-dependent phosphorylation may prevent the C-terminal tail from interacting with the motor domain.

Moreover, determined by sucrose density gradient centrifugation of cell lysates expressing mCherry-MKlp2, the majority of MKlp2^{WT} or the phosphoresistant MKlp2$^{C\text{-}4A}$ was found in the bottom fractions (Figure 2D). In contrast, the phosphomimetic MKlp2$^{C\text{-}4E}$ migrated much slower (Figure 2D), indicating that Cdk1/cyclin B1-dependent phosphorylation may inhibit higher-order complex formation (oligomerization/clustering or binding to large cellular components) of MKlp2. Previously characterized biochemical properties of the kinesin-6 family member MKlp1 becoming less soluble in low-ionic-strength solutions as well as during cytokinesis explains its tendency to assemble into