Regulation of the Final Stage of Mitosis by Components of the Pre-Replicative Complex and a Polo Kinase

Extra View

Regulation of the final stage of mitosis by components of the pre-replicative complex and a polo kinase

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ABBREVIATIONS

Plk1, Polo-like kinase 1; Cdc6, Cell division cycle 6; Cdk1, Cyclin-dependent kinase 1; Orc, Origin recongnition complex; Mcm, Mini-chromosomal maintenance.

RUNNING TITLE

Components of pre-RC in mitosis

ABSTRACT

The accurate division of duplicated DNA is essential for maintenance of genomic stability in proliferating eukaryotic cells. Errors in DNA replication and chromosomal segregation may lead to cell death or genomic mutations that lead to oncogenic properties. Thus, tight regulation of DNA replication and mitosis is essential for maintaining genomic integrity. Cell division cycle 6 (Cdc6) is an essential factor for initiating DNA replication. Recent work shows that phosphorylation of Cdc6 by polo-like kinase 1 (Plk1), one of the essential mitotic kinases, regulates mitotic exit mediated by Cdk1 and separase. Here we discuss how pre-replicative complex factors are connected with Plk1 and affect mitotic exit.

Coupling Plk1 and Pre-RC proteins

Polo-like kinase 1 (Plk1), a mammalian ortholog of *Drosophila* Polo, is a serine/threonine kinase that is involved in several mitotic events including centrosome maturation, bipolar spindle formation, chromatin segregation and cytokinesis.[1-3] Polo kinases are highly conserved and include Plx1 in *Xenopus*, Polo in *Drosophila*, and Cdc5 in budding yeast. Plk1 is highly expressed in proliferating cells. Expression of Plk1 increases in S phase and peaks during M phase.[1, 3] Its specific activity increases at the G2/M boundary as the result of phosphorylation.[4] The multiple roles of Plk1 during the mitotic process depend on the interaction of the polo-box domain of Plk1 with its substrates for phosphorylation at different subcellular locations.[5-7] Plk1 localizes to centrosomes in prophase, then is enriched in the kinetochores after nuclear envelope breakdown. Plk1 is recruited to the spindle pole in metaphase, the central spindle in anaphase, and mid-body in telophase.[1, 5, 8]

To investigate the function of Plk1 at other points in the cell cycle, several groups have studied the role of Plk1 in cellular events such as DNA damage repair and DNA replication. Starting in late mitosis, a DNA pre-replicative complex (pre-RC) begins to assemble on an origin recognition complex (ORC). Initiation factors Cdc6 and Cdt1 are recruited to the ORC after which the Mcm2-7 hexamer (MCM complex) is loaded. [9-11] The critical event in pre-RC formation is the activation of the MCM complex helicase at the origin of replication. Although pre-RCs form in late mitosis and G1 phase, MCM complexes remain inactive at replication origins and unwound DNA is not detected.[11] Two protein kinases, the Dbf4-Cdc7 kinase (DDK) and cyclin-dependent kinase (CDK), trigger the initiation of DNA synthesis by phosphorylating the MCM complexes.

It is plausible that mitotic Plk1 and pre-RC components work together in mitosis or S phase when the Plk1 level increases. This suggestion is supported by studies that show Plk1 interacts with and phosphorylates several pre-RC proteins. Mcm2 and Mcm7 are shown to bind Plk1 through its polo-box domain in co-immunoprecipitation assays and by MALDI-mass spectrometry or yeast two-hybrid studies.[12, 13] Plk1 binds to Mcm7 in response to adriamycin, a topoisomerase inhibitor, that causes DNA breaks, suggesting that Plk1 may regulate the phosphorylation of Mcm7 following disruption of DNA replication.[13] Immunofluorescence studies using GFP-tagged Mcm subunits showed that the MCM complex proteins weakly localize to the centrosome. The depletion of Mcm3 induces multinucleated cells and abnormal organization of microtubules.[13] These data suggest that apart from the role in pre-RC formation Mcm subunits have an additional role in mitotic progression.

Further evidence for a connection between components of the pre-RC and

mitosis was revealed when Orc2 was shown to be a binding partner and substrate of Plk1 in yeast two-hybrid studies and in vitro kinase assays.[13] Orc2 also colocalizes to the centrosome along with Plk1.[13-15] Although the physiological role of the phosphorylation of Orc2 by Plk1 remains to be elucidated, the depletion of Orc2 by siRNA affects chromosome segregation and results in multipolar spindle organization and multinucleated cells.[13-14] This suggests that Plk1-mediated phosphorylation of Orc2 may function in mitotic progression. Expression of mutant Orc2 or Orc5 in *Drosophila* leads to arrest in metaphase with abnormally condensed chromosomes.[16] As with Orc2, Orc6 also localizes to nuclear chromatin during interphase and is found at the kinetochore and centrosomes in prophase.[17, 18] Cells that lack Orc6 have cytokinetic defects and a multinucleated phenotype.[17] Along with the localization of Orc6, the phenotype of Orc6-depleted cells indicates that Orc6 may function in chromosomal segregation and cytokinesis before DNA replication is initiated.[17]

Further data indicating a role for pre-RC components in mitosis are reports showing that Dbf4/Cdc7, one of the major kinases involved in pre-RC initiation, interacts with Plk1 and regulates mitotic exit by blocking Plk1 in budding yeast.[19, 20] The depletion of Dbf4 leads to nuclear segregation defects and misorientated spindles.[20] The evidence that the deficiency of pre-RC components results in common mitotic defects suggests that pre-RC components are tightly connected with roles in mitotic progression or exit before pre-RCs form.

Cdc6-mediated mitotic exit through phosphorylation by Plk1

During pre-RC assembly ORC recruits Cdc6, which is required for loading the MCM complex, and is essential for S phase entry.[21] After replication is initiated, Cdc6

is phosphorylated by S-phase CDK, which facilitates the degradation of yeast Cdc6 or translocation of human Cdc6 from the nucleus to the cytosol. Both cases lead to down-regulation of Cdc6 activity to prevent re-initiation of replication.[21-23] However, there are reports that a high level of mammalian Cdc6 is still bound to chromatin in the nucleus after S phase,[24-28] implicating Cdc6 in another post-S phase function. Other evidence shows that Cdc6 may have a function in mitotic progression. Overexpression of Cdc6 causes a cell cycle delay and blocks the onset of mitosis, which is dependent on inhibition of mitotic CDK activity and the integrity of checkpoint pathways.[29-31] In addition to delaying mitotic entry, Cdk1 inhibition by Cdc6 has been implicated in accelerating timely exit from mitosis through APC/Cdc20 modulation.[32-35] Mouse Cdc6 has been reported to be associated with the mitotic apparatus,[36] showing that Cdc6 persists in mitotic cells as does Orc6, which is associated with the outer kinetochore during mitosis.[17]

Recently, we have reported that Cdc6 phosphorylation by Plk1 regulates chromosomal segregation through separase and cycin-dependent kinase 1 (Cdk1) in late mitosis.[37] Plk1 interacts with Cdc6 through its C-terminal polo-box domain. Cdc6 interacting with Plk1 is hyper-phosphorylated and binding is elevated in mitotic cells.[37] Perhaps the high levels of Cdc6 and Plk1 serve to promote their interaction. It is also possible that Cdc6 may be first phopshorylated by Cdk2 thus priming it for Plk1 phosphorylation. Immunochemistry studies reveal that Cdc6 and Plk1 colocalize to the spindle pole in metaphase, and the central spindle in anaphase.[37] In telophase, Cdc6 localizes in the newly formed nuclei whereas Plk1 is found at the mid-body. The colocalization of Cdc6 and Plk1 in the central spindle during anaphase may suggest an important function for p-Cdc6. Plk1 phosphorylates Cdc6 on T37 and expression of

T37V mutant induces multinucleated cells and incompletely separated nuclei.[37] However, cells co-expressing Cdc6-T37V and Cdc25C , which overcome mitotic delay, have a high percentage of mis-segregated chromosomes compared to cells expressing wild type Cdc6 and Cdc25C (unpublished data), suggesting that phosphorylation of Cdc6 on threonine 37 is important for mitotic exit.

A Cdc6 deficiency results in mitotic defects such as chromosomal misalignment, chromosomal lagging during segregation, and multinucleated cells. The location of Cdc6 and the phenotype in Cdc6-depleted cells are very similar to those of Mcm3-, Orc2-, or Orc6-depleted cells mentioned above.[13, 14, 17, 18] Orc6 shows mid-zone localization by late anaphase and begins to redistribute to the daughter nuclei by telophase,[17] similar to the localization of Cdc6. Depletion of Orc6 induces defects in chromosomal segregation and multinucleation. In addition, Mcm3 and Orc2 are located in the centrosome with Plk1 and the depletion of each protein induces multinucleated cells with insufficiently organized microtubules,[13] indicating that replication factors are important to regulate mitotic exit that is connected with pre-RC formation. It remains unclear how DNA replication factors can regulate chromosomal segregation and cytokinesis directly.

Inhibition of Cdk1 and activation of separase by pCdc6

Accurate control of chromosomal separation is critical for the faithful transfer of genetic material. Defects in chromosomal segregation in somatic cells often lead to aneuploidy associated with abnormal development and tumorigenesis.[38-40] Chromosomal segregation is controlled by separase activity, which cleaves cohesin, leading to sister chromosome separation.[41] Premature activation of separase and

chromosomal missegregation are prevented by multiple inhibitory mechanisms. One widely accepted model is that separase activity is regulated by securin through direct binding before securin is degraded by APC/C.[42-43] However, other mechanisms exist to regulate separase activity because cohesin is cleaved in an appropriate cell cycle phase in securin-deficient mice.[44] In addition to securin-dependent inhibition of separase, cyclin B1/Cdk1 associates with separase and inhibits its enzyme activity.[45-47] Yeast Cdc6 contributes to down-regulation of Cdk1 activity in mitosis and the N-terminus of Cdc6 is important for interaction with Cdk1[48] or cyclin B1[49]. The phosphorylation of Cdc6 on T37 directly promotes its interaction with Cdk1 and suppresses the activity of Cdk1. Separase is then activated and Rad21, a component of cohesin, is cleaved in cells expressing wild type Cdc6 but not mutant Cdc6, indicating that phosphorylation of Cdc6 on T37 leads to inhibition of Cdk1 through their direct interaction which in turn leads to activation of separase.[37] These results imply that phosphorylation of Cdc6 by Plk1 in mitosis regulates the activity of separase through association with Cdk1 (Figure 1). Plk1 phosphorylates Cdc6, which binds to Cdk1 and inhibits its activity. This sequential action leads to the release and activation of separase in a securin-independent manner, to promote chromosomal segregation. The importance of inhibition of Cdk1 and cohesin cleavage in anaphase progression is described in a recent work.[50] Microinjection studies reveal that cohesin cleavage and Cdk1 down-regulation are sufficient to form daughter nuclei in cells arrested in metaphase,[50] suggesting that cells pass from metaphase to anaphase when cohesin cleavage is combined with Cdk1 inhibition. Thus, Cdc6 could be a mediator of Cdk1 inhibition and cohesin cleavage resulting in mitotic progression to anaphase.

Another securin-independent mechanism for regulating separase activity

involves the phosphatase $PP2A^{Cdc55}$, which has been suggested to be an inhibitor of separase downstream of Shugoshin.[51] Interestingly, yeast Cdc6 interacts with $PP2A^{Cdc55}$ in mitosis and has an inhibitory effect because cells expressing Cdc6 show downregulation of $PP2A^{Cdc55}$ activity in anaphase.[32] These reports raise another possibility that Cdc6 binds with $PP2A^{Cdc55}$, disturbs the activity of $PP2A^{Cdc55}$, and consequently promotes separase activity in anaphase, leading to proper exit from mitosis[52]. These data imply that Cdc6 regulates separase activity to promote anaphase through two different ways: by inhibiting Cdk1 and/or by inhibiting $PP2A^{Cdc55}$.

Thus, the functions of Cdc6 in two consecutive phases of the cell cycle converge. The first sequential role of Cdc6 along with Plk1 is to insure the fidelity of chromosome separation by promoting the regulation of separase leading to exit from mitosis. This leads to the second role of Cdc6 in assembly of pre-RC complexes in preparation for DNA replication.

Concluding Remarks

The function of many proteins is often changed by protein modification such as phosphorylation and dephosphorylation. As a result of these modifications, one protein can have several functions in a temporal- or spatial-dependent manner. Studies about the detailed modification of multiple factors will clarify the exact function of each molecule in different phases of the cell cycle.

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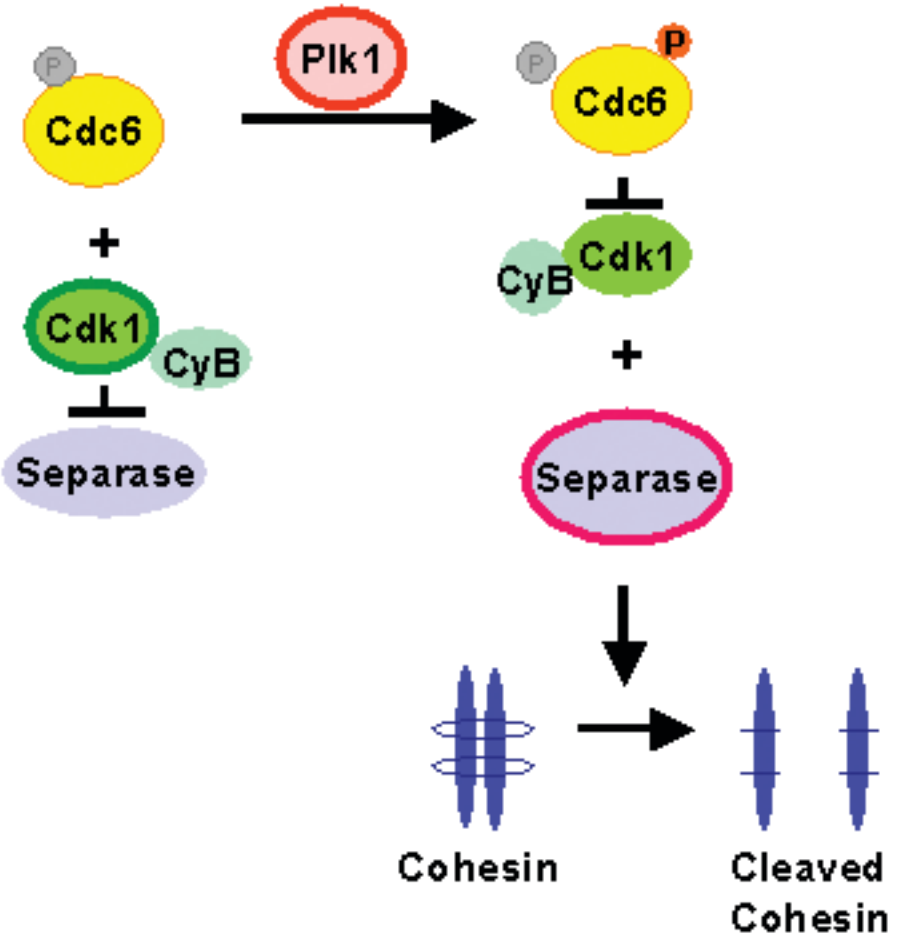
FIGURE LEGENDS

Figure 1. Possible role of phosphorylated Cdc6 in separase activation. Plk1-mediated phosphorylation of Cdc6 promotes its interaction with Cdk1, resulting in the inhibition of Cdk1 and the activation of separase. Consequently, separase accelerates chromosomal segregation and the progression of anaphase.

Figure 1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