Polo-Like Kinase 1: Target and Regulator of Anaphase-Promoting Complex/Cyclosome–Dependent Proteolysis

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Abstract

Polo-like kinase 1 (Plk1) is a key regulator of progression through mitosis. Although Plk1 seems to be dispensable for entry into mitosis, its role in spindle formation and exit from mitosis is crucial. Recent evidence suggests that a major role of Plk1 in exit from mitosis is the regulation of inhibitors of the anaphase-promoting complex/cyclosome (APC/C), such as the early mitotic inhibitor 1 (Emi1) and spindle checkpoint proteins. Thus, Plk1 and the APC/C control mitotic regulators by both phosphorylation and targeted ubiquitylation to ensure the fidelity of chromosome separation at the metaphase to anaphase transition. The mechanisms underlying the control of genomic stability by Plk1 are discussed in this review. (Cancer Res 2006; 66(14): 6895-8)

Anaphase-Promoting Complex/Cyclosome Activity: Ordered Proteolysis of Mitotic Proteins, Including Polo-Like Kinase 1

A failure in passing on the duplicated genetic material to both daughter cells contributes to cellular transformation, which in turn might lead to cancer. To ensure the proper segregation of chromosomes, mammalian cells undergo mitosis in a tightly controlled manner. Polo-like kinase 1 (Plk1) fulfills a crucial role in this process, and deregulation of Plk1 activity is tightly linked to tumorigenesis in humans (1). Plk1 is exclusively expressed in proliferating cells regulating a diverse set of processes, including centrosome maturation, bipolar spindle formation, activation of the cyclin B/cyclin-dependent kinase 1 (Cdk1) complex, and cytokinesis. Furthermore, Plk1 is a target of the DNA damage checkpoint and is involved in regulating the spindle assembly checkpoint. RNA interference (RNAi)-mediated depletion of Plk1 in unsynchronized HeLa cells results in stabilization of cyclin B and elevated Cdk1 activity. Moreover, Plk1-depleted cells enter mitosis with normal kinetics, implying that Plk1 is dispensable for the G_2-M transition in human cells (2). By contrast, the leading role of Plk1 during late stages of mitosis is well established. Detailed research revealed that the involvement of Plk1 is crucial for the metaphase-anaphase transition. Most of these functions are linked to the regulation of the APC/C, an E3 ubiquitin ligase that is responsible for the timely destruction of various mitotic proteins, thereby regulating chromosome segregation, exit from mitosis, and a stable subsequent G_1 phase (3). The APC/C is first activated by the ancillary protein Cdc20, targeting proteins containing a destruction box (D box), like securin. Once APC/C^{Cdc20} has initiated mitotic exit, Cdc20 itself is degraded and is replaced by Cdh1, allowing the degradation of a wider spectrum of substrates. The APC/C^{Cdh1} complex targets not only D box–containing proteins but also proteins exhibiting a KEN box and/or an A box (e.g., cyclin B1 and Aurora A). Plk1 itself is an APC/C^{Cdh1} target. However, the destruction of a mutated securin, which is degraded exclusively by APC/C^{Cdh1}, precedes that of Plk1 by several minutes, indicating that activation of the APC/C^{Cdh1} alone is not sufficient to initiate Plk1 destruction (4). Moreover, degradation of hyperactive $Plk1^{T210D}$ is impaired, suggesting that Plk1 inactivation during anaphase may be required for its timely degradation. Taken together, these results indicate that Plk1 is a substrate of APC/C^{Cdh1}, and its degradation seems to be tied to specific events during mitotic exit.

Liberating APC/C from Its Inhibitors

The activation of the ubiquitin ligase APC/C requires the phosphorylation of multiple subunits. Because depletion or inactivation of the *Xenopus* Polo-like kinase 1 (Plx1) in meiotically arrested egg extracts blocks APC/C-dependent degradation of cyclin B (5), many investigators have tried to directly link the activities of Plk1 and APC/C. Although Plk1 is able to phosphorylate subunits of the APC/C *in vitro*, this phosphorylation contributes only marginally to its activation (6). In contrast, cyclin B/Cdk1 seems to have a major role in the phosphorylation and activation of the APC/C, thereby triggering its own inactivation at the end of mitosis (7).

Although Plk1 can contribute synergistically to the cyclin B/Cdk1–mediated activation of the APC/C (6), this observation is not sufficient to explain the crucial role of Plk1/Plx1 in the activation of the APC/C. Intriguing insights have come from studies of the cytostatic factor (CSF) in *Xenopus* oocytes, where CSF activity prevents parthogenetic development by blocking exit from metaphase of meiosis II until fertilization (8). CSF activity has not been completely characterized, but it is known to involve APC/C inhibition. Because APC/C^{Cdc20} activation is essential for the release from CSF arrest, the inhibition of APC/C^{Cdc20} seems to be the ultimate function of the CSF. Recently, the APC/C inhibitors early mitotic inhibitor 1 and Emi2 (previously named XErp1) have emerged as candidate components of CSF activity. Emi2 seems to be the key element for CSF arrest in the oocyte, as specific inhibition of Emi2 leads to activation of the APC/C^{Cdc20} (9). Importantly, this inhibition of Emi2 is mediated by the *Xenopus* Polo-like kinase Plx1 (10). In response to fertilization, Ca^{2+}/

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calmodulin-dependent protein kinase II (CaMKII) phosphorylates Emi2, thereby acting as a priming kinase for Plx1, which in turn promotes its degradation by phosphorylating Emi2 in a polo-box domain–dependent manner (10, 11). These findings finally provide an explanation for the essential role of Plx1 for APC/C activation and subsequent CSF release (5). Unless an Emi2 homologue is identified in somatic cells, it seems that Emi1 is the major APC/C inhibitor in mitotic cells, where a strikingly similar mechanism can be observed. Emi1 regulates mitotic entry by inhibiting the APC/C, thereby allowing cyclin B accumulation. In mammalian cells, Plk1 also triggers the degradation of Emi1, suggesting that Plks have a conserved role in liberating the APC/C from its inhibitors (12, 13). Prior phosphorylation of Emi1 by cyclin B/Cdk1 potentiates the action of Plk1, suggesting that cyclin B/Cdk1 is a priming kinase for Plk1. These results are in agreement with our previous studies, providing the first evidence for the requirement of a priming kinase to ensure efficient phosphorylation of proteins by Plk1 (14). Phosphorylation of serine residues within the DSGxxS sequence of Emi1 by Plk1 generates a phospho-degron, which increases binding of Emi1 to the Skp1-Cullin-F-box$^{\beta\text{-TRCP}}$ ($SCF^{\beta\text{-TRCP}}$) ubiquitin ligase complex, leading to the formation of Emi1-ubiquitin conjugates (12, 13). Emi1 destruction activates the APC/C, thereby enabling the cell to degrade early APC/C targets like cyclin A. However, Plk1 activity might not be essential for cyclin A destruction because Plk1-depleted cells are still able to degrade cyclin A (2, 7). Possibly, this cyclin A degradation is due to incomplete Plk1 depletion as suggested by a novel study reporting a G_2 arrest in synchronized HeLa cells when Plk1 is completely depleted (15). This idea is in line with the observation that cyclin B/Cdk1 serves as a priming kinase for Plk1, suggesting that in the presence of high cyclin B/Cdk1 activity, very low amounts of Plk1 might be sufficient to initiate Emi1 degradation. Still, increased Plk1/Plx1 activity accelerates mitotic progression in human cells and is sufficient for CSF release in *Xenopus* egg extracts in the absence of calcium (1, 10). Because Plk1 is overexpressed in a large variety of human cancers (1), it is likely that increased Plk1 activity might lead to premature activation of the APC/C, which in turn leads to genomic instability and thereby contributes to oncogenesis.

Role of Plk1 in the Spindle Checkpoint-Regulated Activity of the APC/C

Another mechanism controlling APC/C activity is exerted by the spindle checkpoint, which ensures the accuracy of chromosome segregation in mitosis by blocking the activity of the APC/C until all chromosomes are properly orientated on the metaphase spindle (16). Down-regulation of Plk1 interferes with the formation of a bipolar spindle and leads to prometaphase arrest (2). Interestingly, cells depleted of Plk1 fail to arrest if survivin is codepleted. Because survivin is required for checkpoint maintenance in response to lack of tension, these observations imply that Plk1 activity is required for the generation of tension on kinetochores and thus is involved in spindle checkpoint signaling. This view was supported by recent studies investigating the effect of hyperactive Plk1 on mitotic progression. Cells expressing Plk1^{S137D} or Plk1$^{S137D/T210D}$ could not be properly blocked in mitosis by nocodazole probably due to a failure in establishing checkpoint mediated arrest (17). Under these conditions, the premature degradation of cyclin B1 was observed. These cells could be arrested in mitosis again after expression of nondegradable cyclin B1, suggesting that expression of Plk1^{S137D} induces a spindle checkpoint failure, leading to untimely activation of the APC/C. Taken together, the precise control of Plk1 activity is crucial to maintain APC/C activation under the control of the spindle assembly checkpoint.

Although the knowledge about the basic principles of spindle checkpoint control is improving, the exact mechanisms translating changes in tension and microtubule attachment at kinetochores into biochemical signals regulating checkpoint activity need further elucidation. Recent studies provide intriguing evidence for Plk1 playing a major role in this process, resulting in silencing of the spindle checkpoint and subsequent activation of the APC/C. Cdc20 is the target for inhibition of the APC/C by the spindle checkpoint whose signals originate at kinetochores. Importantly, kinetochores not under tension are specifically phosphorylated by Plk1 at an epitope recognized by the 3F3/2 antibody thereby creating the tension-sensing 3F3/2 phosphoepitope (18, 19). Moreover, Plk1 is required for the recruitment of the checkpoint protein Mad2 to kinetochores. Unattached kinetochores mediate checkpoint signals by activating the checkpoint kinase BubR1, which subsequently promotes the recruitment of a stable Mad1-Mad2 heterodimer leading to active Mad2 (16). Activated Mad2 and/or BubR1 associate with Cdc20, preventing it from activating the APC/C. Down-regulation of Plk1 by siRNA reduces the kinetochore-associated levels of both Mad2 and Cdc20 (18), further substantiating a role for Plk1 in the regulation of the spindle checkpoint. Still, the influence of Plk1 activity on the localization of Mad2 to kinetochores remains controversial. Although it was reported that Plk1 depletion leads to loss of Mad2 at kinetochores (18, 19), other groups observe an enrichment of Mad2 at kinetochores after Plk1 depletion (2, 20). Although this aspect requires further investigation, the involvement of Plk1 in the regulation of the spindle checkpoint seems established, because Plk1-depleted cells arrest in prometaphase with an activated spindle checkpoint, but these cells fail to arrest if Mad2 or BubR1 are codepleted (2). Taken together, these results suggest Plk1 as an antagonist for the inhibitory influence of the spindle checkpoint on APC/C^{Cdc20} activity.

Cells treated with microtubule drugs, like nocodazole or Taxol, accumulate Plk1 at kinetochores probably in response to a loss of tension (18). The underlying mechanism is poorly understood. However, in *Xenopus*, localization of Plx1 at the kinetochore is under the control of an essential checkpoint kinase, monopolar spindle 1 (Mps1) (19). These observations indicate that Plk1 is both target and regulator of the spindle checkpoint whose essential function is to inhibit APC/C^{Cdc20} and to block anaphase entry until chromosomes are properly attached to the mitotic spindle. This is of special interest because mutations in checkpoint genes were found in many chromosomal instability (CIN) cancer cell lines, indicating that mitotic checkpoint defects contribute to tumorigenesis likely by causing aneuploidy (reviewed in ref. 21).

Deregulated Proteolysis and Genomic Instability

Deregulation of cyclins and other cell cycle regulators, like Plk1, can lead to aberrant proliferation and illegitimate segregation of the genetic material. APC/C governs the timely proteolysis of such regulators, including mitotic cyclins A and B.

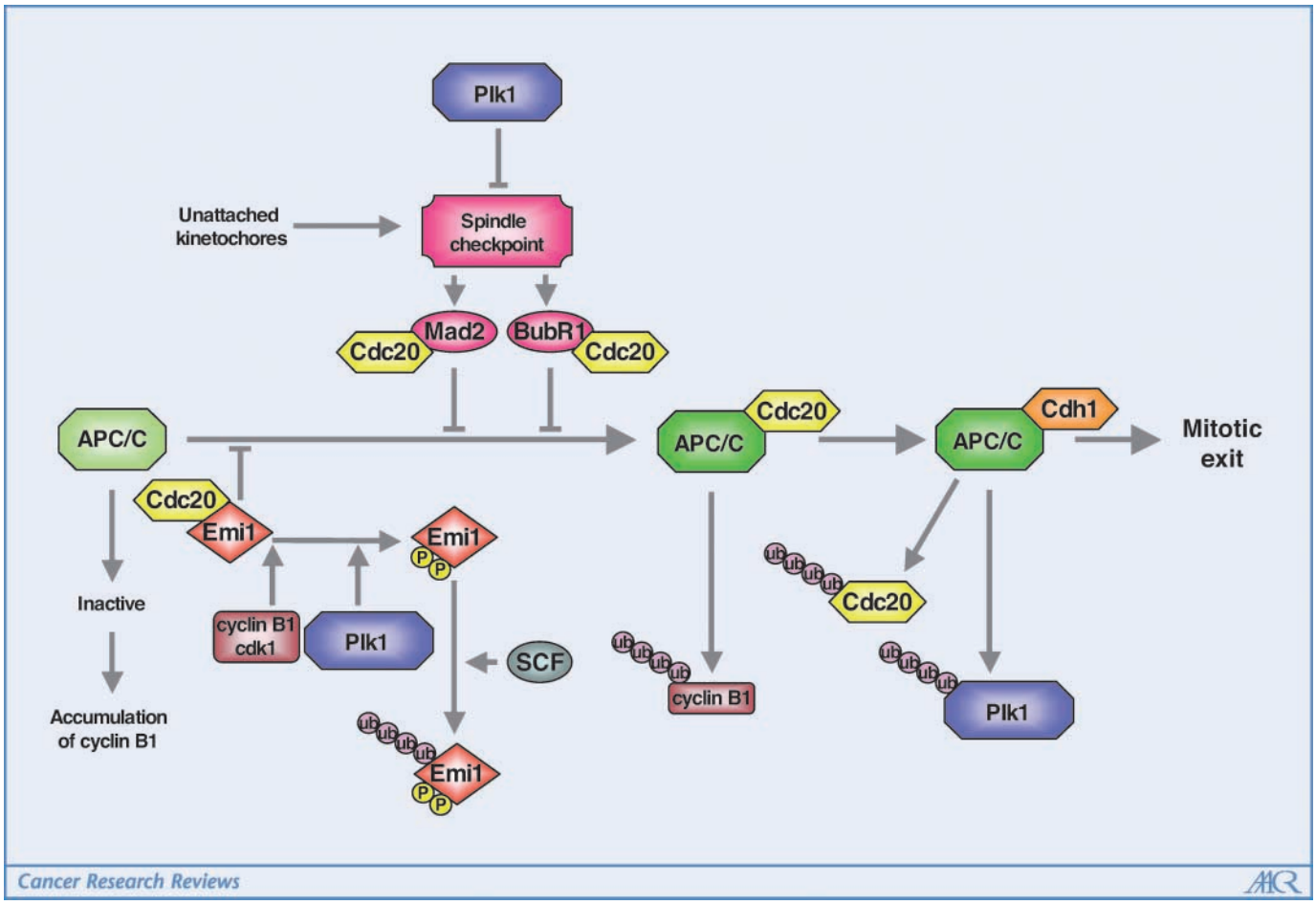

Figure 1. Plk1 activity regulates APC/C in multiple ways to promote mitotic progression. In early mitosis, Plk1 activity triggers the degradation of Emi1 via $SCF^{\beta\text{-}TRCP}$, a prerequisite for the generation of an active APC/C^{Cdc20} complex. However, in case of an activated spindle assembly checkpoint, Mad2 and/or BubR1 bind to Cdc20, thereby inhibiting the APC/C. Plk1 acts antagonistically to the spindle checkpoint and regulates the kinetochore localization of Mad2. Silencing of the spindle checkpoint enables the formation of active APC/C^{Cdc20} and the degradation of cyclin B1. When Cdc20 is replaced by Cdh1, additional substrates, like Cdc20 itself and Plk1, are degraded. Finally, active APC/C^{Cdh1} allows mitotic exit and entry into the subsequent G_1 phase.

Whereas the degradation of early APC/C targets like cyclin A depends on the timely destruction of Emi1, the destruction of later APC/C targets, like cyclin B and securin, is under the control of the spindle checkpoint. By targeting securin for degradation, the APC/C regulates sister chromatid separation. Although Plk1 is involved indirectly in all these scenarios by regulating the APC/C via Emi1 and the spindle checkpoint, it also directly regulates sister chromatid separation by targeting cohesin. Thus, the concerted action of Plk1 and APC/C ensures the proper division of the genetic material. Failure in this process will lead to aneuploidy, a characteristic feature of most tumor cells. Later in G_1 phase, APC/C^{Cdh1} targets the licensing factor cdc6 for destruction, an important step because overexpression of cdc6 results in re-replication and genetic alterations. Thus, regulation of APC/C activity controls the ordered proteolysis of cell cycle regulators, and deregulation of APC/C-mediated proteolysis likely leads to genomic instability (reviewed in ref. 3).

In summary, Plk1 triggers the activity of the APC/C-mediated proteolytic machinery in multiple ways (Fig. 1). In addition, Plk1 also directly regulates the stability of specific mitotic key components. While destruction of the Cdk1-inhibitory kinase Wee1 is orchestrated by different mitotic kinases, including Plk1, we found that Plk1 has also the ability to stabilize the peptidyl-prolyl *cis/trans*-isomerase Pin1 by phosphorylating Ser^{65} within its catalytic domain (22). Pin1 is known to regulate a broad spectum of biological processes and pathologic conditions such as cancer. Inhibition of Plk1 activity enhances the ubiquitylation of Pin1 and subsequently reduces the amount of Pin1 in human cancer cells.

Given the striking role of Plk1 in the regulation of mitosis via the spindle checkpoint, the APC/C, and other mitotic regulators, like Wee1, cohesin, and Pin1, Plk1 activity is involved in a variety of pathways, all acting in concert to ensure correct progression of mitosis in particular segregation of the genetic material. Because attacking the spindle apparatus has already proven to be a very successful chemotherapeutic strategy to fight neoplastic malignancies (16), accumulating evidence suggest Plk1 as a promising target for anticancer therapies.

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