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Regulating PLK1 dynamics by Cullin3/KLHL22-mediated ubiquitylation

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Polo-like kinase 1 (PLK1) is a critical regulator of mitosis, controlling chromosome alignment, centrosome function, and spindle assembly. PLK1 localizes to centrosomes and kinetochores in early mitosis and relocalizes to microtubules of the spindle midzone after anaphase onset. However, there is no comprehensive understanding of the mechanisms governing this dynamic localization. Recent evidence suggests that ubiquitylation of PLK1 by the ubiquitin ligase Cullin 3 (CUL3) with the substrate adaptor Kelch-like 22 (KLHL22) is necessary to specifically remove PLK1 from kinetochores upon chromosome biorientation (**Fig. 1A**).[1] Preventing CUL3/KLHL22-mediated PLK1 ubiquitylation results in chromosome congression defects, which are likely caused by PLK1 accumulation at kinetochores and consequent local hyperphosphorylation of its substrate BubR1 at S676.[1] Functionally, BubR1 hyperphosphorylation by PLK1 was proposed to signal stabilization of initial kinetochore–microtubule attachments by recruiting the phosphatase PP2A-B56 to counteract the destabilizing activity of Aurora B kinase during prometaphase.[2,3] Since proper chromosome biorientation requires kinetochore–microtubule attachments to be sufficiently unstable to allow correction of incorrect attachments, we propose that sustained PLK1 activity at kinetochores leads to artificial stabilization of kinetochore–microtubule attachment, thereby preventing efficient chromosome congression. Consistent with this notion, Lampson and coworkers recently showed that artificial tethering of PLK1 to kinetochores leads to sustained BubR1 hyperphosphorylation, checkpoint-dependent mitotic arrest and increased occurrence of merotelic attachments.[4] Together, these data support the hypothesis that high levels of PLK1 at metaphase kinetochores prevents efficient chromosome congression by over-stabilizing kinetochore–microtubule attachments.

However, this interpretation may not be the only plausible explanation for the defects observed. Indeed, PLK1 was shown to reduce microtubule plus-end dynamics, possibly by regulating the activities of the microtubule-depolymerizing proteins MCAK as well as the recruitment and activity of the motor protein Kif2b.[5,6] It is, therefore, difficult to determine the primary cause for the observed chromosome alignment defects. While the described functions of BubR1 phosphorylation by PLK1, the phenotypes of kinetochore-anchored PLK1, and the observation that KLHL22-depleted cells form cold-stable mitotic spindles (unpublished results) favor the notion that preventing PLK1 ubiquitylation by CUL3/KLHL22 leads to hyperstable kinetochore–microtubule connections, the high frequency of Mad2-positive kinetochores and the failure to localize RanGAP1 in KLHL22-depleted cells argue for unstable kinetochore–microtubule interactions under these conditions.[2,4] Thus, additional experiments will be required to distinguish between these 2 possible defects.

It should also be noted that PLK1 may not be the only target of KHL22 that regulates mitotic progression. Although expression of the non-ubiquitylatable PLK1-K492R mutant mimics KLHL22 depletion, the observed mitotic defects were less pronounced. This may be explained by incomplete downregulation of endogenous PLK1, by acceptor site switching of CUL3/KLHL22 in the absence of the primary ubiquitin acceptor site, or by additional CUL3/KLHL22 substrates. PLK1 was initially identified as a CUL3/KLHL22 substrate through protein microarrays, in which we tested which proteins bind KLHL22 and are ubiquitylated by CUL3/KLHL22. These screens suggested that in addition to PLK1, KLHL22 binds several other kinases with known mitotic functions, including Cdk1, casein kinases, and Nek2. KLHL22 may thus regulate multiple kinases, which influence kinetochore–microtubule attachment and other mitotic processes at multiple levels. However, these in vitro results have yet to be verified in vivo.

Even without the detailed mechanism, it is clear that CUL3/KLHL22-mediated accumulation of PLK1 at kinetochores severely impacts chromosome congression. We propose that CUL3/KLHL22 ubiquitylates PLK1 predominantly upon chromosome biorientation, leading to PLK1 removal from kinetochores by blocking PDB-dependent interactions (**Fig. 1A**). As PLK1 turnover at kinetochores is very high (FRAP t½ ~1s, unpublished results), its depletion from kinetochores could be caused by PLK1 ubiquitylation either at kinetochores to promote PLK1 release or outside kinetochores to prevent PLK1 re-association with kinetochores. We could not observe accumulation of KLHL22 at kinetochores, but KLHL22 associates with the mitotic spindle just when most chromosomes become bioriented. This interesting observation may suggest that

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PLK1 is preferentially ubiquitylated at the mitotic spindle to prevent PLK1 re-recruitment to its kinetochore receptors. Consistent with this notion, it is unlikely that the CUL3/KLHL22 complex could access the PLK1 ubiquitylation site in the PBD while PLK1 is bound to its phosphorylated kinetochore targets. After removal from kinetochores and ubiquitylation at K492, PLK1 accumulates at centrosomes. This could be consequence of inhibited kinetochore recruitment, but it may also indicate PLK1 sequestration on centrosomes by an unknown ubiquitin binding protein (UBP, **Fig. 1B**). A similar mechanism has been proposed for the CUL3-regulated translocation of Aurora B to the central spindle (**Fig. 1B**).[7] Upon anaphase onset, PLK1 could be de-ubiquitylated by an unknown deubiquitylation enzyme (DUB). Several studies have identified specific UBPs and DUBs associated with centrosomes and/or the mitotic spindle, and some of these are differentially regulated in early and late mitosis by Cdk1/Cyclin B.[8] PLK1 de-ubiquitylation during anaphase would allow it to engage again in PDB-dependent interactions, now targeting PLK1 to the central spindle, because its kinetochore receptors have been removed from kinetochores (**Fig. 1B**). Indeed, several DUBs were already shown to influence mitotic progression, and it would thus be interesting to test whether they may target PLK1. Clearly, investigating the role of DUBs and UBPs in the regulation of mitosis will be an important and exciting future direction.

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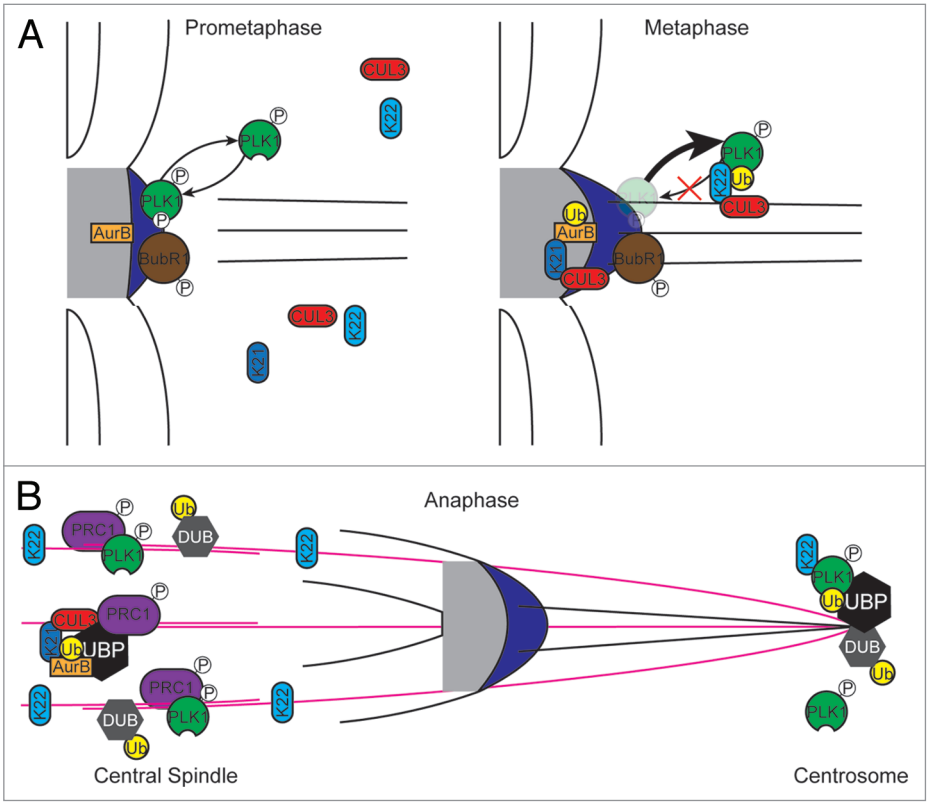

Figure 1. Model of PLK1 regulation by CUL3/KLHL22 mediated ubiquitylation (**A**) During prometaphase, PLK1 is recruited to kinetochores via PDB-mediated interactions with PLK1 kinetochore receptors, and there is highly dynamic exchange of kinetochore-bound PLK1 with the free cytoplasmic pool. Upon chromosome biorientation during metaphase, CUL3/KLHL22 accumulates at the mitotic spindle, where it potentially ubiquitylates the free pool of PLK1 in the PBD at K492, thus preventing its recruitment to kinetochore-associated interactors, resulting in PLK1 removal from kinetochores. Alternatively, the structural changes in centromere and kinetochore regions during metaphase may allow CUL3/KLHL22 to access kinetochore-bound PLK1, promoting its release and preventing its re-recruitment. (**B**) After removal of PLK1 from metaphase kinetochores, PLK1 may first be sequestered by a centrosome-associated UBP. At anaphase onset, PLK1 might be deubiquitylated by an unknown DUB, which could be activated by Cyclin B degradation. Without ubiquitin conjugated to its PBD, PLK1 can now establish new interactions, e.g., with PRC1, thus targeting PLK1 to the central spindle. Similarly, we propose that CUL3/KLHL21-ubiquitylated Aurora B is transferred from centromeres and anchored to the central spindle via the action of an ubiquitin-binding protein associated with central spindle microtubules.