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CELL DIVISION

A timely exit for PLK1

The dynamic localization of Polo-like kinase 1 (PLK1) orchestrates several aspects of cell division, with its removal from kinetochores being important for silencing the spindle assembly checkpoint (SAC) and ensuring normal anaphase. Sumara and colleagues find that the exit of PLK1 from kinetochores is triggered through its ubiquitylation by the E3 ligase complex containing cullin 3 (CUL3) and its adaptor protein Kelch-like protein 22 (KLHL22).

Both CUL3 and the BTB (bric-a-brac–tramtrack–broad complex) protein KLHL22 have been implicated in cell division. Here, the authors set out to characterize the substrates of KLHL22 that might be relevant for its role in mitosis. They first showed that depletion of KLHL22 from HeLa cells triggered defects in chromosome alignment and engagement of the SAC. Through microarray analysis of >8,000 human proteins, they saw that the CUL3–KLHL22 complex bound and ubiquitylated 30 proteins, including many kinases. One of these kinases, PLK1, interacted with KLHL22 *in vitro* and *in vivo*, indicating that PLK1 is directly ubiquitylated by CUL3–KLHL22. In cells depleted for CUL3–KLHL22, PLK1 levels were increased specifically at kinetochores, while its overall levels were unaffected. In the absence of KLHL22, PLK1 accumulated at kinetochores and the phosphorylation of its downstream target BUBR1 increased, with consequent defects in the maintenance of microtubule–kinetochore interactions.

It is known that the Polo-box domain (PBD) of PLK1 contributes to its dynamics at kinetochores, and also that Lys492 within this domain is a potential ubiquitin acceptor site *in vivo*. Through mass spectrometry analysis, the authors confirmed that Lys492 is a ubiquitin acceptor site and that expression of a non-ubiquitylatable version of PLK1 results in the persistent localization of PLK1 to kinetochores and a prometaphase delay, similar to that observed when KLHL22 was depleted. On the basis of this, the authors conclude that ubiquitylation of Lys492 in PLK1 by CUL3–KLHL22 triggers its release from kinetochores and that this exit is important for normal progression into anaphase.

> “Whether this complex can target other kinases to promote normal cell division is an interesting prospect”

What controls the onset of this interaction? The authors followed the dynamics of GFP–KLHL22 and saw that it associates with the mitotic spindle as chromosomes become bi-orientated. In addition, immunoprecipitation analysis showed an increase in the interaction between PLK1 and KLHL22 at this stage of mitosis. Thus, microtubule–kinetochore dynamics themselves may provide a mechanism that defines the timing of PLK1 and KLHL22 association and the subsequent control of PLK1 localization through Lys492 ubiquitylation.

As PLK1 ubiquitylation by CUL3–KLHL22 does not affect its overall levels, the authors conclude that this modification instead alters the subcellular localization of this kinase and may be rapidly reversed by a deubiquitinase. Whether this complex can target other kinases to promote normal cell division is an interesting prospect to be explored.

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