THE EMBO JOURNAL

Have you seen . . . ?

The EMBO Journal (2009) 28, 1991–1993 | © 2009 European Molecular Biology Organization | Some Rights Reserved 0261-4189/09
www.embojournal.org

Escaping the firing squad: acetylation of BubR1 protects it from degradation in checkpoint cells

Mona Yekezare and Jonathon Pines*

The Gurdon Institute, Tennis Court Road, Cambridge, UK.
*Correspondence to: j.pines@gurdon.cam.ac.uk

The EMBO Journal (2009) **28,** 1991–1993. doi:10.1038/emboj.2009.149

The spindle assembly checkpoint (SAC) is responsible for blocking cells in mitosis in the presence of unattached kinetochores. A substantial body of work has identified many of the players in this checkpoint and shown that they target the Cdc20 protein to prevent the ubiquitylation of cyclin B and securin by the anaphase promoting complex/cyclosome (APC/C). The exact mechanism by which the SAC restrains Cdc20, however, remains elusive but some evidence indicates that it may target Cdc20 for destruction by the APC/C. An interesting study in this issue of *The EMBO Journal* offers a new insight into this by showing that BubR1, a crucial effector, of the SAC, is acetylated in checkpoint-arrested cells and this modification is crucial for preventing BubR1 itself from destruction and the inactivation of the SAC.

The proper transition from one stage to the next in mitosis relies on the ubiquitin-mediated degradation of the right proteins at the right time (Pines, 2005). Crucially, the anaphase and mitotic-exit inhibitors, securin and cyclin B, respectively, must not be degraded until all chromosomes have achieved bipolar attachment to the spindle. This is achieved by unattached kinetochores activating the spindle assembly checkpoint (SAC) (Rieder *et al*, 1995). Once activated, the SAC prevents the APC/C ubiquitin ligase from ubiquitylating cyclin B and securin, and targeting them for degradation. The main target of the SAC is Cdc20 (Hwang *et al*, 1998; Kim *et al*, 1998), which is an essential activator of the APC/C (Peters, 2006). Mad2 and BubR1, two proteins crucial for SAC function, bind directly to Cc20 and it has been suggested that Cdc20 binds to both Mad2 and BubR1–Bub3 to form the inhibitory mitotic checkpoint complex (Musacchio and Salmon, 2007). Other studies, however, have indicated that this complex is transient and Cdc20 mostly ends up in complex with BubR1–Bub3 (Musacchio and Salmon, 2007; Nilsson *et al*, 2008; Kulukian *et al*, 2009) that binds to the APC/C and thereby targets Cdc20 for degradation (Pan and Chen, 2004; Nilsson *et al*, 2008). Yet BubR1 itself has an APC/C-recognition motif, a 'KEN' box and is degraded as cells exit mitosis, therefore it was unclear how BubR1 escaped ubiquitylation when it was bound to Cdc20 during the checkpoint. In this issue, Choi *et al* shed light on this issue. They show that BubR1 is acetylated on a specific lysine residue in checkpoint-arrested cells and this blocks its ubiquitylation and degradation.

The first hint that acetylation might be involved in the SAC came from the finding of Choi *et al*, which suggests that BubR1 interacts with the PCAF acetylase in SAC-arrested cells. Mass spectrometry analysis of BubR1 immunoprecipitated from interphase or nocodazole-arrested cells showed that BubR1 is acetylated on Lysine 250 specifically in the prometaphase cells. To elucidate the role of this acetylation, the endogenous BubR1 was substituted by mutant forms of BubR1 that either could not be acetylated (K25R) or had the lysine replaced by glutamine to mimic acetylation (K250Q). Using live-cell imaging, Choi *et al* found that whereas K250Q-BubR1 mutant was capable of mediating a SAC-dependent arrest, the K250R mutant could not. One intriguing explanation for this was that the mutation allowed BubR1 to be targeted for degradation, as acetylation has been suggested to interfere with ubiquitylation (Minucci and Pelicci, 2006). To test this, endogenous BubR1 was substituted by fluorescently tagged wild-type BubR1, or the K250R or K250Q mutants. Live-cell imaging, showed that wild-type BubR1 was degraded as cells exited mitosis and that its degradation began just before that of cyclin B when the SAC was inactivated. In contrast, the K250R mutant was very unstable and cells exited mitosis prematurely. Furthermore, the K250Q mutant was completely stable and cells were unable to exit mitosis. These findings are consistent with the hypothesis that acetylation of BubR1 allows it to bind and inhibit the APC/C by preventing BubR1 from being ubiquitinated. It is not yet clear, however, why cells with the K250R mutant override the checkpoint: is it simply because unacetylated BubR1 is unstable or that it might also be deficient in inhibiting Cdc20?

This study ties in very neatly with proposed models where BubR1 presents Cdc20 to the APC/C as a substrate in SAC-arrested cells but with the additional insight that the acetyl group on BubR1 protects it from collateral ubiquitination. Subsequently, when all the chromosomes have bound properly to the spindle and the checkpoint is turned off, it seems that BubR1 is deacetylated. The deacetylated BubR1 can be ubiquitinated by the APC/C and consequently degraded, thereby releasing Cc20 to activate the APC/C against its metaphase substrates, cyclin B and securin (Figure 1). Thus, it will be very interesting to determine how the acetylation and deacetylation of BubR1 is regulated. Is acetylation of BubR1 activated by the SAC itself or is it downstream of a parallel pathway controlled by the attachment of

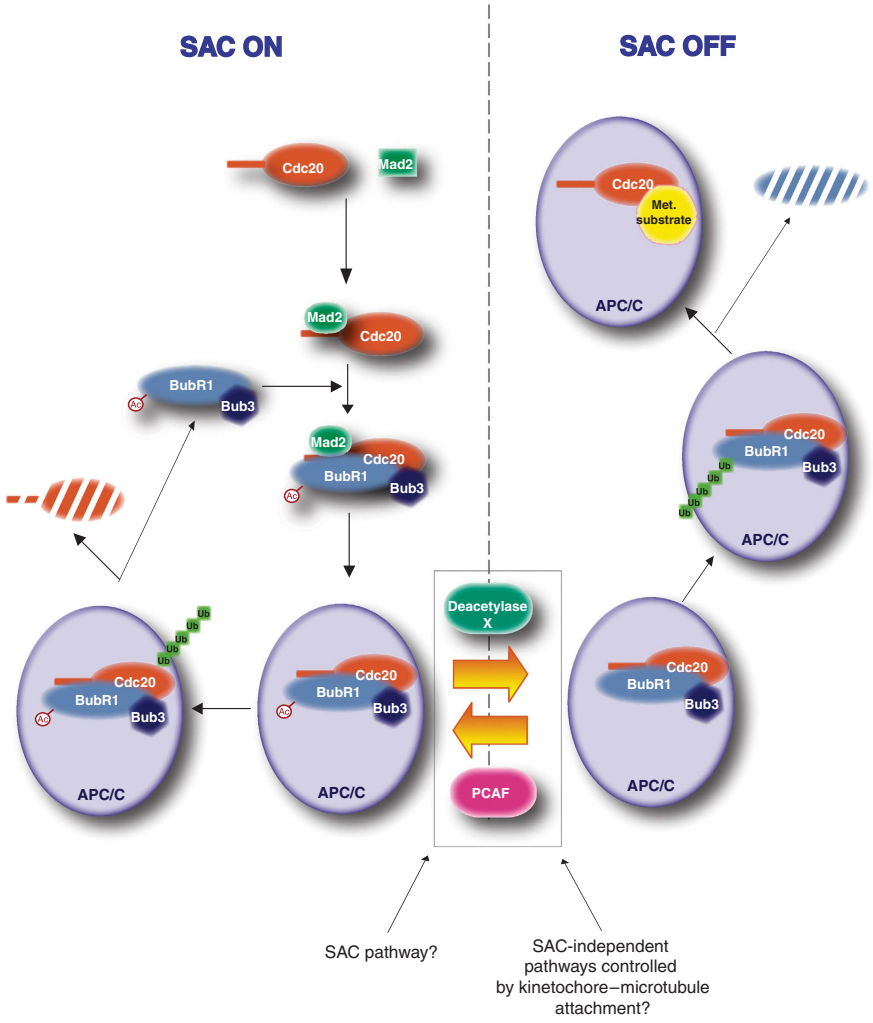

Figure 1 A model for the spindle assembly checkpoint (SAC). In checkpoint-arrested cells, Mad2 binds to Cdc20 and mediates Cdc20 binding to BubR1–Bub3. When bound to BubR1 and Bub3, Cdc20 is presented as a substrate to the APC/C and BubR1 is protected from ubiquitylation by the APC/C through acetylation by PCAF. When the SAC is turned off, BubR1 is deacetylated and can no longer escape ubiquitylation by APC/C and is targeted for degradation. This leaves Cdc20 free to activate the APC/C against its metaphase substrates, securin and cyclin B. Currently it is unclear what signals control the acetylation and deacetylation of BubR1. They might respond to the SAC itself or to a SAC-independent pathway controlled by attachment of kinetochore to the microtubules.

microtubules to kinetochores? This can be addressed by testing whether BubR1 is acetylated in cells in which checkpoint is compromised by Mad2 depletion but the cells are kept in mitosis with unattached kinetochores. It will also be important to determine whether BubR1 is protected from deacetylation when the checkpoint is active, and if so, how. Alternatively, a specific deacetylase may be activated only when the checkpoint is extinguished. Answers to these questions may give further insights into the SAC and its coordination with the behaviour of the kinetochores.

References

Choi E, Choe H, Min J, Choi JY, Kim J, Lee H (2009) BubR1 acetylation at prometaphase is required for modulating APC/C activity and timing of mitosis. *EMBO J* **28:** 2077–2089

Hwang LH, Lau LF, Smith DL, Mistrot CA, Hardwick KG, Hwang ES, Amon A, Murray AW (1998) Budding yeast Cdc20: a target of the spindle checkpoint. *Science* **279:** 1041–1044

Kim SH, Lin DP, Matsumoto S, Kitazono A, Matsumoto T (1998) Fission yeast Slp1: an effector of the Mad2-dependent spindle checkpoint. *Science* **279:** 1045–1047

Kulukian A, Han JS, Cleveland DW (2009) Unattached kinetochores catalyze production of an anaphase inhibitor that requires a Mad2 template to prime Cdc20 for BubR1 binding. *Dev Cell* **16:** 105–117

Minucci S, Pelicci PG (2006) Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer* **6:** 38–51

Musacchio A, Salmon ED (2007) The spindle assembly checkpoint in space and time. *Nature Rev Mol Cell Biol* **8:** 379–393

Nilsson J, Yekezare M, Minshul J, Pines J (2008) The APC/C maintains the spindle assembly checkpoint by targeting Cdc20 for destruction. *Nat Cell Biol* **10:** 1411–1420

Pan J, Chen RH (2004) Spindle checkpoint regulates Cdc20p stability in Saccharomyces cerevisiae. *Genes Dev* **18:** 1439–1451

Peters JM (2006) The anaphase promoting complex/cyclosome: a machine designed to destroy. *Nature Rev Mol Cell Biol* **7:** 644–656

Pines J (2005) Mitosis: a matter of getting rid of the right protein at the right time. *Trends Cell Biol* **16:** 55–63

Rieder CL, Cole RW, Khodjakov A, Sluder G (1995) The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J Cell Biol* **130:** 941–948