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 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 (K25Q). Using live-cell imaging, Choi et al found that whereas K25Q-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 ubiquitininated 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 down-stream of a parallel pathway controlled by the attachment of
microtubules to kinetochores? This can be addressed by testing whether BubR1 is acetylated in cells in which check-point 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.

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