Bub1-the zombie protein that CRISPR cannot kill

MERALDI, Patrick


DOI: 10.15252/embj.2019101912
PMID: 30850387
Bub1—the zombie protein that CRISPR cannot kill

Patrick Meraldi

The conserved Bub1 kinase was proposed to be non-essential for the mitotic spindle assembly checkpoint based on recent findings that knocking-out Bub1 by CRISPR/Cas9 did not impair checkpoint function in human cells. New studies now demonstrate that CRISPR/Cas9 Bub1 knockout cells still express low levels of Bub1 protein and that only removal of this remaining Bub1 pool by RNA interference substantially weakens spindle assembly checkpoint signaling.

The EMBO Journal (2019) 38: e101912
See also: G Zhang et al (April 2019)

The spindle assembly checkpoint (SAC) is a highly sensitive mitotic surveillance mechanism, which delays anaphase onset in the presence of a single unattached chromosome until all chromosomes are bound by microtubules of the mitotic spindle (Musacchio, 2015). It is controlled by a conserved set of proteins that accumulate on unattached kinetochores, the microtubule-binding sites on chromosomes. One SAC component is the protein kinase Bub1, which is essential for the checkpoint in fungi or Drosophila (Hoyt et al., 1991; Bernard et al., 1998; Basu et al., 1999). In mammalian cells, the exact contribution of Bub1 to the checkpoint has been more controversial. First loss-of-function studies in human cells based on RNA interference (RNAi) yielded contradicting results as to whether Bub1 is essential for the SAC or not (Johnson et al., 2004; Meraldi & Sorger, 2005). A classical knockout of Bub1 in mouse embryonic fibroblasts and RNAi rescue experiments in human cells then led to the consensus that Bub1 is essential to sustain an SAC response in mammalian cells (Perera et al., 2007; Klebig et al., 2009). However, two recent studies employing present-day genome editing techniques reported that the SAC was still functional after a Bub1 knockout generated via CRISPR/Cas9-based short deletions or frame-shifts in the first few exons of the human BUB1 gene (Currie et al., 2018; Raaijmakers et al., 2018). The lack of a SAC impairment was explained by a second, Bub1-independent SAC pathway, which depends on the Rod-Zw10-Zwilch (RZZ) complex (Silio et al., 2015). The importance of Bub1 in the SAC has, however, now risen from the dead in two new studies, including work from Zhang and colleagues in this issue of The EMBO Journal (Rodriguez-Rodriguez et al., 2018; Zhang et al., 2019). These studies demonstrate that so-called Bub1−/− CRISPR/Cas9 “knockout” clones very often express alternatively spliced Bub1 mRNA (Rodriguez-Rodriguez et al., 2018), and that they express low levels of a Bub1 protein carrying a small deletion, as assessed by mass spectrometry (Zhang et al., 2019). Importantly, a combination of a Bub1 CRISPR/Cas9 knockout with a Bub1 siRNA treatment impaired the checkpoint response: In the absence of microtubules, cells delayed anaphase only for 1–2 h, as compared to over 10 h in control-treated cells (Zhang et al., 2019). This indicates that Bub1 plays a substantial role in SAC signaling, but that Bub1 must be very efficiently depleted for this to manifest in a strong effect, consistent with early RNAi observations (Meraldi & Sorger, 2005). In addition, Zhang and colleagues confirm the existence of two branches of the checkpoint, as they demonstrate that the small delay in anaphase onset in cells lacking detectable Bub1 depends on the RZZ complex.

Beyond the specific role of Bub1, this study highlights how for loss-of-function studies, even CRISPR/Cas9 “knockout” cells should be analyzed with caution. This is probably most relevant when studying near-essential genes, such as BUB1, whose loss causes massive chromosome segregation errors. Indeed, any mechanism that only partially rescues the function of such nearly essential gene, for example, alternative splicing or usage of an alternative start codon downstream of the original ATG, should offer a growth advantage that will be selected for when screening for “knockout” clones. Incomplete gene knockouts can be challenging to detect, as classical immunoblotting or immunofluorescence might not detect very low expression levels due to background noise. RT–PCR might help to detect alternative splicing events at the mRNA levels (Rodriguez-Rodriguez et al., 2018), but only highly sensitive mass spectrometry will detect any remaining protein pool at the protein level (Zhang et al., 2019). A potential way to address this issue, as shown in the Zhang et al study, is to test whether the depletion of the targeted gene by RNAi results in a more severe phenotype. This approach has, however, its own caveats, since an additional phenotype may be the result of an RNAi off-target effect. One alternative would be use of an inducible, acute CRISPR/Cas9 approach that in the short term will not select for suppression mechanisms (Rodriguez-Rodriguez et al., 2018). However, in the case of long-lived proteins, a sizable pool of the targeted protein might

1 Department of Cell Physiology and Metabolism, Faculty of Medicine, University of Geneva, Geneva, Switzerland. E-mail: patrick.meraldi@unige.ch
2 Faculty of Medicine, Translational Research Centre in Onco-hematology, University of Geneva, Geneva, Switzerland
DOI 10.15252/embj.2019101912 | Published online 8 March 2019
nevertheless remain; moreover, cells might still use alternative start codons, resulting in the expression of a mutant protein. Therefore, the best strategy for an unequivocal disruption of \textit{BUB1} or any other near-essential gene might the deletion of a large segment of the targeted gene.

The second important conclusion from the work of Zhang and colleagues is that the result of any study relying on a short deletion by CRISPR/Cas9 or RNAi-induced depletion should be particularly scrutinized in the case of enzymes or signaling cascades. Indeed, even very low amounts of a particular component can still result in overall strong activity/output of the respective signaling cascade: only a few Bub1 molecules per kinetochore suffice to elicit a full spindle assembly checkpoint response (Zhang \textit{et al}, 2019). This implies that, at the mechanistic level, Bub1 must have an enzymatic role in the checkpoint response that goes beyond mere stoichiometric binding and recruitment of the central SAC component Mad1 to kinetochores. This notion is also consistent with the observation that Bub1 does not need to accumulate on kinetochores to sustain a checkpoint response (Klebig \textit{et al}, 2009), and the fact that in an \textit{in vitro} reconstitution assay, Bub1 plays a catalytic role in building up a checkpoint response (Faesen \textit{et al}, 2017). One key aim of future investigation will therefore be to identify which key molecular step Bub1 catalyzes in the SAC signaling.

\textbf{References}


Silió V, McAinsh AD, Millar JBA (2015) KNL1-Bubs and RZZ provide two separable pathways for checkpoint activation at human kinetochores. \textit{Dev Cell} 35: 600 – 613