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Bub1 is not essential for the checkpoint response to unattached kinetochores in diploid human cells

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Error-free chromosome segregation during mitosis depends on a functional spindle assembly checkpoint (SAC). The SAC is a multi-component signalling system that is recruited to unattached or incorrectly attached kinetochores to catalyse the formation of a soluble inhibitor, known as the Mitotic Checkpoint Complex (MCC), which binds and inhibits the anaphase promoting complex (APC/C) [1]. We have previously proposed that two separable pathways, composed of KNL1–Bub3–Bub1 (KBB) and Rod–Zw10–Zw11 (RZZ), recruit Mad1–Mad2 complexes to human kinetochores to activate the SAC [2]. Although Bub1 is absolutely required for checkpoint signalling in yeast (which lack RZZ), there is conflicting evidence as to whether this is the case in human cells based on siRNA studies [2–5]. Here we show that, while Bub1 is required for recruitment of BubR1, it is not strictly required for the checkpoint response to unattached kinetochores in diploid human cells.

We used CRISPR/Cas9 genome editing to target BUB1 in human diploid hTERT-RPE1 cells using small guide (sg)RNAs targeting exon 2. We initially recovered a mosaic clonal population which arrested in nocodazole and included cells in which Bub1 was undetectable at kinetochores (Figure S1A,B in Supplemental Information, published with this article online). These kinetochores could, however, still recruit Mad2 suggesting that Bub1 is dispensable for SAC activation. To confirm this, we isolated a second clonal cell line with no detectable Bub1 protein by immunoblotting and no detectable Bub1 at kinetochores by quantitative immunofluorescence, while Mad2 could still be recruited (Figures 1A–C and S1D). Genome sequencing revealed a frame shift in both BUB1 alleles that allows expression of only the first 23 amino acids of Bub1 (Figure S1C). Importantly, Rod and Zwilch, as well as KNL1, bound kinetochores to the same extent in parental and Bub1−/− cells (Figures 1C and S1D). By contrast, BubR1-kinetochores-binding was abolished and binding of CENP-F severely compromised (Figures 1C and S1D), as reported previously in studies using Bub1 knockdown by siRNA [6]. Importantly, steady state levels of Mad2 at pro-metaphase kinetochores were lower in Bub1−/− cells than in the parental control (Figures 1C and S1D) consistent with previous findings following siRNA-mediated knockdown of KNL1 or Bub1 [2].

We next used live cell imaging of chromosomes labelled with a cell permeable dye (SiR-DNA) to assess the effect of Bub1 knockout on timing of chromosome congression and anaphase onset in individual cells. Although the efficiency of chromosome congression was largely unaffected in Bub1−/− cells compared to control cells, the time from nuclear breakdown (NBD) to anaphase onset was extended by ~3 min (Figure 1D). To test directly whether Bub1 is required for the SAC response to unattached kinetochores we filmed cells from a nocodazole arrest and measured the time to completion of congression and anaphase onset. Both events were delayed in Bub1−/− cells compared to the control, revealing the role for Bub1 in error correction (Figure S2D). Consistently, Sgo1 binding is reduced by approximately 50% in Bub1−/− cells (Figure S2E), in line with previous experiments in HeLa cells [8]. Interestingly, the frequency of lagging chromosomes was unchanged in Bub1−/− compared to parental cells (10% vs. 19%, p = 0.184 Fisher’s exact test) (data not shown), suggesting that, while the efficiency of error correction is reduced, Bub1−/− cells are still able to successfully complete chromosome bi-orientation. Moreover, Bub1−/− cells with uncongressed chromosomes delay in mitosis, consistent with our conclusion that the SAC can operate without Bub1. We assume that error correction is completed in Bub1−/− cells by a pool of Aurora-B kinase associated with centromeric DNA via phosphorylation of histone H3 on threonine 3 (H3T3) by centromere-associated Haspin kinase [9].

The data in this paper support the notion that KBB and RZZ complexes can provide two separate receptors for the Mad1–Mad2 complex at human kinetochores [2]. Moreover, they are consistent with a recent report showing that Bub1 and RZZ are not essential for the SAC response to unattached kinetochores in near-haploid human
Figure 1. BUB1 is not essential for SAC activity in diploid human cells.
(A) Full immunoblots of whole-cell lysates from parental or Bub1<sup>−/−</sup> cells treated with 3.3 µM nocodazole using antibodies against Bub1 aa336–489 (left) or aa1–300 (right) and α-Tubulin as loading control. (B) Representative images of prometaphase parental or Bub1<sup>−/−</sup> cells stained with DAPI, CenpC, and Bub1 (aa1–130) antibodies. (C) Quantification of kinetochore-bound Bub1, BubR1, CenpF, KNL1, Rod, Zwilch and Mad2 signals relative to Crest or CenpC intensity (data normalised to respective Bub1+/+ median value; p-value from a two-sided Mann-Whitney U test; data from >2 independent experiments with >500 kinetochores per dataset). Representative images in Figure S1D. (D) Time spent in mitosis (from NEB to anaphase onset/mitotic exit) from 12 hr live-cell imaging of parental or Bub1<sup>−/−</sup> cells treated with SiR-DNA (n = 218 for Bub1<sup>−/−</sup> and n = 183 for parental). (E) Same experiment as (D) except cells treated with 330 nM nocodazole (n = 71 for Bub1<sup>−/−</sup> and n = 102 for parental).

HAP1 cells [10]. It is unclear why human cells have two receptors (KBB and RZZ) for the Mad1–Mad2 complex, whereas yeast only has one (KBB). One possibility is that the KBB and RZZ pathways monitor different attachment states [2]. Furthermore, the contribution of the KBB pathway to SAC signalling and error correction may vary between cell types or state of cellular transformation [2,7]. Future work will be needed to understand the relative roles of KBB and RZZ in these different contexts.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures, experimental procedures, author contributions and acknowledgments, and can be found with this article online at https://doi.org/10.1016/j.cub.2018.07.040.

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