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Tracking dynein-mediated transport of spindle assembly checkpoint proteins from kinetochore to spindle pole

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Accurate chromosome segregation relies on the activity of the spindle assembly checkpoint (SAC), a surveillance mechanism that monitors the status of kinetochore-microtubule attachment and prevents anaphase onset until all chromosomes are bipolarly attached to the mitotic spindle and aligned at the metaphase plate. Once all kinetochore-microtubule attachments are efficiently established, SAC must be extinguished in order to allow for mitotic progression, a process known as SAC silencing. A predominant mechanism of SAC silencing is dynein-mediated transport of certain kinetochore proteins along microtubules (a phenomenon named stripping or streaming). However, there are still conflicting data as to which SAC proteins are dynein cargoes. Using two ATP reduction assays and in vitro fluorescence microscopy, we found that the core SAC proteins Mad1, Mad2, Bub1, BubR1, and Bub3 redistributed from attached kinetochores to spindle poles (Figure 1), in a dynein-dependent manner, suggesting that their removal from the kinetochores is needed for SAC silencing. This redistribution still occurred at late metaphase (Figure 2), suggesting that cells are still able to activate the SAC even after complete bi-polar chromosome alignment. Live-cell imaging experiments, and taking advantage of the HeLa cell line stably expressing EGFP-Bub3, essentially recapitulated these results (Figure 3). Unexpectedly, we found that a pool of Hec1 and Mis12 also relocates to spindle poles suggesting a role for the KMN network in SAC silencing.

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