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Dynamics of microtubule bundle formation during prometaphase

The spindle during metaphase consists of evenly distributed and well-organized microtubule bundles. It is unknown how this recognizable architecture arises during prometaphase. Here we show that during prometaphase the spindle contains antiparallel PRC1 labelled bundles the number of which increases in time by longitudinal splitting of preexisting bundles and by de novo assembly. By using live cell imaging of HeLa cells expressing PRC1-GFP we observe that in prometaphase, the spindle has a small number (13+/-2 s.e.m.) of PRC1 labelled bundles of uneven size and irregular spatial distribution. The number of these bundles increases up to about 30, and the bundles become distributed evenly throughout the equatorial plane. We also show that siRNA of HAUS6 component of augmin complex causes a smaller number of bundles in metaphase (17+/-1 s.e.m.). The spatial distribution of these bundles is perturbed with more bundles being present around the perimeter of the spindle and fewer in the central part. Our results show how microtubule bundles form in prometaphase and provide evidence that microtubule nucleation by augmin is involved in this process.

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