

## **Distinct clathrin nano-structures at the metaphase spindle are required for spindle bipolarity and integrity**

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A successful partition of chromosomes and intracellular contents during cell division requires a well organised and maintained bipolar spindle. The protein clathrin, which plays a key role in membrane trafficking during endocytosis and exocytosis, has an important role in the stabilisation of the mitotic spindle. This is thought to involve the formation of structural bridges connecting two microtubules within a kinetochore fibre by building a complex with TACC3 (transforming acidic coiled-coil-containing protein 3) and ch-TOG (colonic and hepatic tumor over-expressed protein). However, the nanoscale organisation of clathrin necessary for providing spindle integrity is unclear. Here, using superresolution microscopy techniques in combination with a chemical biology approach, we found that clathrin participates in spindle stabilisation also in a TACC3/ch-TOG-independent manner. We observed a pool of clathrin heavy chain (CHC) positive structures present at the mitotic spindle fibres are made up of distinct clusters, tightly packed and evenly distributed in the absence of TACC3 and ch-TOG. Our data revealed a periodicity within the localisations of those structures of  $157 \pm 28$  nm. RNA silencing experiments showed that CHC is needed for the loading of TACC3 onto the spindle and that TACC3 is not essential for CHC to be recruited to the mitotic spindle. We also observed that clathrin locates in a ring-shape around the spindle poles, suggesting a novel role in the assembly and maintenance of a bipolar spindle. Thus, our results elucidate the nano-organisation of clathrin at the mitotic spindle, and that clathrin can maintain spindle bipolarity and integrity in a TACC3/ch-TOG-independent manner.