Kinetochore-microtubule attachment stability during congression and the Ska complex

**Abstract**

During mitosis, alignment of all chromosomes to the cell equator, a process named congression, is critical for error-free cell division. Congression is driven by the interaction of centromere-associated multi-protein machinery, the kinetochore, with the tips of spindle microtubules. The Ska complex (Ska1/Ska2/Ska3) functions to maintain the stability of kinetochore-microtubule attachments, particularly as kinetochores come under escalating force. The current model is that this functions as a mechanical self-checking process to ensure formation of mature kinetochore-microtubule attachments. We have also shown that part of this process involves the progressive recruitment Ska to kinetochores as they congress.

However, how the number of Ska molecules on kinetochores correlates with cycles of microtubule binding and unbinding remains unknown. The loading dynamics of Ska on moving kinetochores has been limited by our reliance on overexpressed transgenes or antibody staining in fixed cells. To test the mechanical self-checking model we have engineered human cells in which Ska1 is tagged at the amino-terminus with eGFP. We are combining this with lattice light sheet microscopy to probe the high spatio-temporal resolution of Ska loading and kinetochore dynamics. At the same time, we have also determined the nano-scale position of the Ska complex within the kinetochore. The overall aim is to establish how changes in Ska complex stoichiometry and conformation dynamics drive kinetochore function.