Centromere Capture by Microtubules In Silico and In Vivo

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In many cell types, chromosomes in the nucleus have “Rabl orientation” and centromeres are located at the nuclear periphery. Using high-throughput microscopy, automated image analysis and computer simulation, however, we found that centromeres are, on average, clustered near the center of the nucleus and are proximal to the nucleolus in Drosophila S2 cells. Long-term live cell imaging revealed that the disruption of Rabl orientation occurs ~ 5 hours after anaphase. Upon nuclear envelope breakdown in prometaphase, these centromeres were captured by microtubules and congressed to the metaphase plate within 20 min in S2 cells. However, our computer simulation suggested that the ‘search-and-capture’ mechanism of centrosomal microtubules alone cannot account for the rapid capture of multiple centromeres of S2 cells. We therefore investigated additional mechanisms for the centromere capture, using both computer simulations and experiments. We considered three experimentally verified phenomena as possible mechanisms that might impact the capture time: chromosome-proximal microtubule nucleation, microtubule amplification in the spindle and pre-mitotic centromere clustering. Our simulation indicated that each mechanism indeed accelerates centromere capture in silico. Interestingly, using high-throughput, live cell analysis in S2 cells, we found that RNAi of a class of Dgt proteins, which has been implicated to play a role in microtubule nucleation inside the spindle [1], delays chromosome congression. This class of Dgt proteins may contribute to microtubule amplification in the spindle in order to facilitate centromere capture during prometaphase.

1. Goshima G, Wollman R, Goodwin SS, Zhang N, Scholey JM, Vale RD, Stuurman N (2007) Genes required for mitotic spindle assembly in Drosophila S2 cells. Science, 20, 417-21