Contribution of non-centrosomal microtubules to mitotic spindle assembly in mammalian cells

Ustun Serdar Tulu, *University of Massachusetts Amherst*

Abstract

In mammalian cells, the formation of a bipolar spindle is an essential process as any mistake in the segregation of chromosomes can result in aneuploidy, an outcome that can be detrimental to the organism. Microtubules are the key structures required for the success of this operation, as they are the main constituents of mammalian bipolar spindles. Centrosomes also play an important role, being the primary source of microtubules. Although centrosomes are dispensable for the formation of bipolar spindles, the fidelity of this process decreases when they are experimentally removed.^ One way to explain how a bipolar spindle assembles has been the 'search and capture' model. According to this model, microtubules emanating from centrosomes search the cytoplasm for kinetochores, which capture microtubules laterally. Once captured, the chromosomes move towards the spindle equator, ultimately resulting in a bipolar spindle.^ In the research presented here, our aim is to understand the role of microtubule sources other than centrosomes in centrosome-containing mammalian cells. We use different techniques and manipulations to bypass the presence of the centrosomes in order to identify the origin of these sources and their importance. We mainly use LLCPK1, pig kidney epithelial, cells stably expressing GFP tagged proteins, such as alpha-tubulin, to follow microtubules and their associated proteins in live cells.^ Two main sources of microtubules other than centrosomes have been documented: peripheral microtubules and kinetochores. Peripheral, non-centrosome-associated microtubules were originally thought to disassemble at the beginning of mitosis. However, we found that they form bundles and contribute to the forming spindle. Our model for spindle assembly, which incorporates these peripheral microtubules, and the 'search and capture' model are not mutually exclusive; instead, they compliment each other in the formation of bipolar spindles. ^ Lastly, we develop a spindle assembly assay in which centrosomes and kinetochores can be observed separately in mammalian cells. The data documented here demonstrate that kinetochores also contribute to the formation of bipolar spindles by nucleating and organizing microtubules. In addition, TPX2, a microtubule associated protein, is revealed as one of the requirements for microtubule formation and organization at the kinetochores. ^

Subject Area

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