

Unique mitotic function of the kinesin-5 Kip1

L. Gheber

Ben-Gurion University of the Negev, Department of Chemistry, Beer-Sheva 84105, Israel,
lgheber@bgu.ac.il

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Abstract

The homotetrameric bipolar kinesin-5 motors perform essential functions in mitotic spindle dynamics by crosslinking and sliding apart antiparallel microtubules emanating from opposite poles. *S. cerevisiae* cells express two kinesin-5 homologues, Cin8 and Kip1, which partially overlap in function. Majority of studies thus far were performed on Cin8 which was the first N-terminal kinesin shown to move processively to the MTs minus-ends and to switch directionality *in vitro*. In contrast, less is known regarding *in vivo* functions and motile properties of Kip1.

Here we show that Kip1 localizes to the spindle-midzone at late-anaphase, when Cin8 has already detached from the spindle. This midzone attachment is essential to stabilize the plus-ends of inter-polar microtubules (iMTs). Detailed examination of iMT dynamics revealed that at the end of anaphase, iMTs depolymerize in two stages. During the first stage, one pair of anti-parallel iMTs depolymerizes while after ~90 s, during the second stage, the depolymerization of the remaining pair of iMTs occurs and coincides with spindle breakdown. Upon spindle breakdown, Kip1 follows the plus-ends of depolymerizing iMTs and translocates toward the spindle poles. This movement is independent of mitotic microtubule motor proteins or the major plus-end binding/tracking proteins. We also found that Kip1 processively tracks the plus-ends of growing and shrinking MTs, both inside and outside the nucleus. A rigor mutant of Kip1 did not exhibit this activity, indicating that motor activity is required for this plus-end tracking. *In vitro*, at high ionic strength, single Kip1 molecules move processively in the minus-end direction of the MTs. In a multi-motor gliding assay, Kip1 is plus-end directed, indicating that it is a bi-directional motor. Based on these results, we propose a model for the functions of Kip1 in mitosis.

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