+TIP protein EB1 interacts with Ska1: implication for its role in spindle - kinetochore attachment

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Abstract

Chromosomes segregation into the daughter cells during mitosis requires stable and bi-oriented attachment of the chromosomes to the spindle microtubules. The process involves regulated interaction of the spindle microtubules with kinetochore. However, considering that the spindle microtubules are highly dynamic in nature stochastically switching between phases of growth and shortening, the mechanism of how chromosome movement is coupled with the dynamic microtubules is yet to be fully understood. Recent studies have identified spindle and kinetochore associated protein 1 (Ska1) as a factor to be involved in this process. However, details of the molecular mechanism underlying Ska1 mediated spindle-KT attachment are not fully understood. Microtubules associate with a specialized class of proteins, called as the plus Tip bindIng Proteins (+TIPs), that localize onto the growing plus ends of microtubules and regulate numerous plus end mediated processes. Recent studies have emphasized the central role of EB1 among the +TIPs. It is highly conserved and is the core component of the multi-protein complexes at the plus ends. Previous studies have shown EB1 to be associated with the kinetochores in cells in the late pro-metaphase till metaphase in mitosis through attached and polymerizing microtubules. However, molecular details of EB1-kinetochore interaction are not clearly understood.

In this work, we provide evidence that EB1 interacts with Ska1 and is part of a larger complex in HeLa cells. Detailed analyses using purified recombinant proteins using gel filtration, immunoprecipitation and far western analyses showed that EB1 directly binds to Ska1. Microtubule pulldown assay using purified proteins showed that EB1 regulate Ska1 recruitment onto the purified microtubules. Detailed microscopic analysis using purified proteins also showed EB1 mediated enhancement of Ska1 recruitment onto the microtubules. The results implicate that EB1-Ska1 interaction may play a critical role in the interaction of KT with the spindle microtubules in their polymerizing state.