

TPX2 functions in microtubule organization are conserved in acentrosomal plant cells

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Abstract

TPX2 has been well studied in many systems for its role in microtubule organization and in spindle assembly. We focused on a role of TPX2 in higher plants where centrosomes are absent and microtubules are nucleated and organized from gamma-tubulin positive sites with dispersal localization mainly on nuclear membrane and pre-existing microtubules.

Arabidopsis homologue of TPX2 AtTPX2 has conserved Aurora binding sites as well as NLS and NES sequences, TPX_importin domain, TPX domain and MTs binding domains. We found that AtAurora1 colocalized in Arabidopsis cells with its activator AtTPX2 on mitotic microtubules from preprophase to early telophase together with gamma-tubulin. Interaction of AtTPX2 with AtAurora1 kinase is required for cell cycle specific localization with microtubules in late mitosis and stability of AtTPX2. Polar localization pattern observed for AtTPX2 on microtubules during late mitosis/telophase transition was disturbed for ΔNAtTPX2 protein that lacked two conserved Aurora kinase binding sites.

Importin was co-purified with AtTPX2-GFP; this finding together with colocalization analysis suggest active nuclear transport of AtTPX2/importin. We observed perinuclear and nuclear microtubular arrays in Arabidopsis cells overexpressing AtTPX2-GFP. The ectopic nuclear microtubules decorated by AtTPX2 were resistant to a microtubule depolymerizing drug but on the contrary to animal cells the arrays were not specific for plant cells undergoing apoptosis. The Ran cycle was shown to be involved in sequestration of AtTPX2 from importin that was required for assembly of the microtubular arrays. Altogether our data showed that presence of the conserved domains correlate with conserved functions of plant AtTPX2 protein with Aurora kinases and with RanGTPase/importin pathway.

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