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Microtubule-bundling activity of the centrosomal protein, Cep169, and its binding to microtubules

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ABSTRACT

CDK5RAP2 is a centrosomal protein that regulates the recruitment of a γ-tubulin ring complex (γ-TuRC) onto centrosomes and microtubules (MTs) dynamics as a member of MT plus-end-tracking proteins (+TIPs). In our previous report, we found mammalian Cep169 as a CDK5RAP2 binding partner, and Cep169 accumulates at the distal ends of MTs and centrosomes, and coincides with CDK5RAP2. Depletion of Cep169 induces MT depolymerization, indicating that Cep169 targets MT tips and regulates stability and dynamics of MTs. However, how Cep169 contributes to the stabilization of MT remains unclear. Here we show that Cep169 is able to stabilize MTs and induces formation of long MT bundles with intense acetylation of MTs with CDK5RAP2, when expressed at higher levels in U2OS cells. In addition, we demonstrated that Cep169 forms homodimers through its N-terminal domain and directly interacts with MTs through its C-terminal domain. Interestingly, Cep169 mutants, which lack each domains, completely abolished the activity, respectively. Therefore, Cep169 bundles MTs and induces solid structure of MTs by crosslinking each adjacent MTs as a homodimer.

1. Introduction

The centrosome consists of a pair centrioles surrounded by the pericentriolar matrix (PCM), and plays an important role in orchestrating the formation of interphase microtubule (MT) network and the mitotic spindle. During maturation at the G2/M transition, a number of proteins and complexes including γ-TuRC are recruited onto centrosomes to increase MT nucleating capacity. In the previous report, we showed that *Drosophila melanogaster* centrosomin (CNN) is required for centrosomal maturation and recruitment of γ-TuRC onto centrosomes through its amino-terminal CNN Motif 1 (CM1) [1]. CDK5RAP2, a functional human homolog of CNN required for a centrosomal maturation, is found to be mutated in primary microcephaly (MCPH), a neuro developmental disorder characterized by reduced brain size [2]. In addition, CDK5RAP2 regulates MT dynamics at MT plus-ends [3]. Depletion of CDK5RAP2 by RNA interference impacts the dynamic behaviors of MTs, indicating that CDK5RAP2–EB1 complex regulates MT dynamics and stability.

We have cloned a human Cep169 as a binding partner of CDK5RAP2 [4]. Cep169 interacts with CM1 of CDK5RAP2 through its N-terminal domain in vivo and in vitro. Cep169 has three SxIP motifs, and accumulates at the distal end of growing MTs and centrosomes with CDK5RAP2. Although Cep169 is not required for recruitment of γ-TuRC onto centrosomes, depletion of Cep169 promoted rapid depolymerization of interphase MTs, indicating that Cep169 regulates stability and dynamics of MTs. Consistent with these observations, overexpression of Cep169 induces MT assembly and bundle formation [4].

A number of MAPs such as MAP65/Ase1/PRC1 or CLIP-170 were reported to induce formation of MT bundle [5,6]. MAP65/Ase1/PRC1 is required for the central spindle composed of an anti-parallel MT bundles during cell division. In particular, Adenomatous polyposis coli (APC) tumor suppressor protein binds to MTs, leading to MT bundling and stabilization [7]. Although the physiological relevance is not well understood, one attractive model is that APC mediates neurite extension via MT bundling.

In order to analyze the Cep169 function in the MT stabilization, we generated a number of deletion and/or point mutation constructs and coupled with FLAG peptide or green fluorescent protein (GFP). Intact and deletion mutant forms of Cep169 were tested for the MT bundling activity in U2OS cells. Our observations indicate that Cep169 directly interacts with tubulin, and MT-binding is

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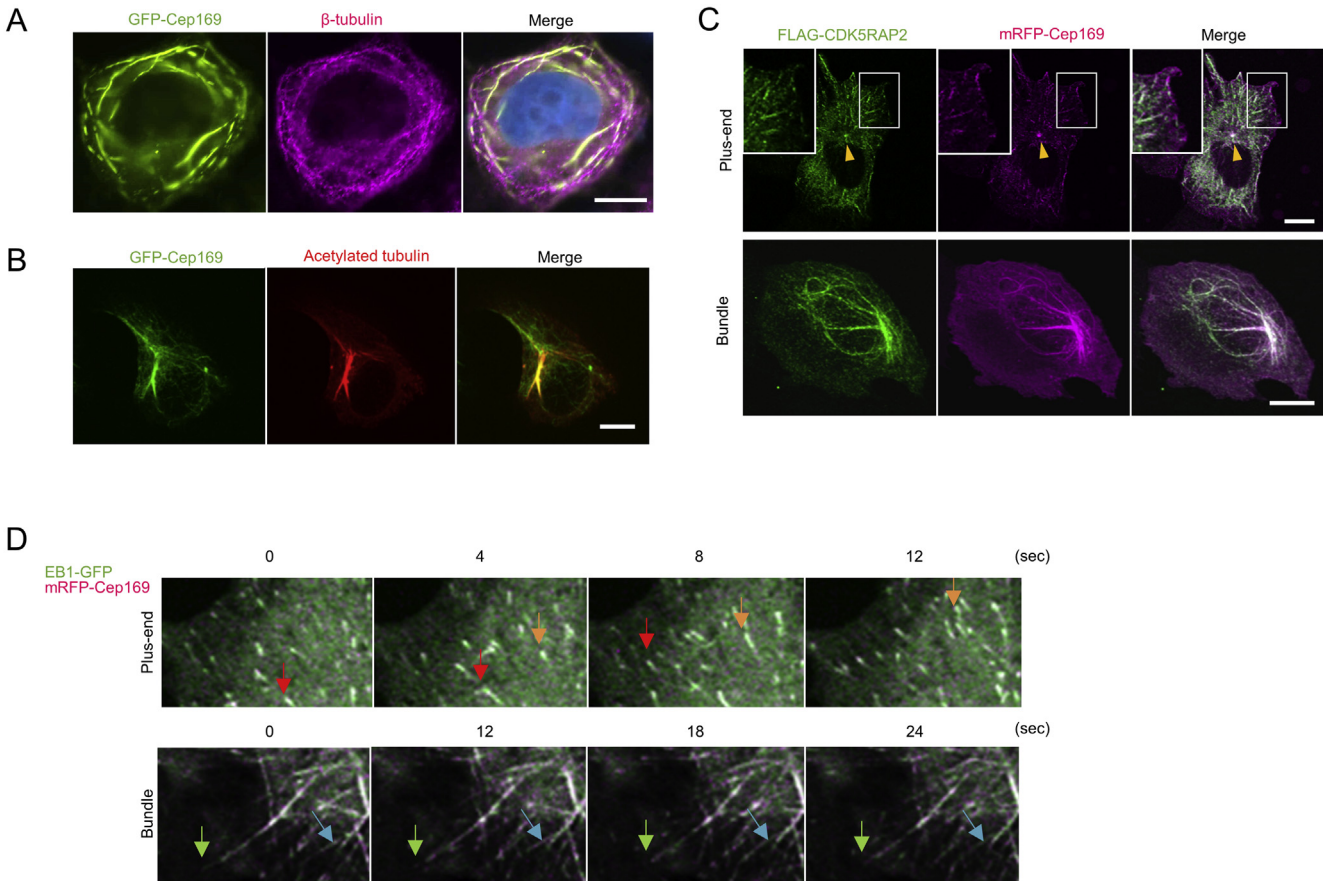

Fig. 1. Cep169 induces MT bundling and acetylation. A and B: U2OS cells were transfected with GFP-Cep169, and stained for β-tubulin (A) and acetylated tubulin antibodies (B). Bars, 10 μm. C: U2OS cells were co-transfected with mRFP-Cep169 and FLAG-CDK5RAP2, and immunostained for CDK5RAP2 (anti-FLAG antibody). Arrowheads indicate centrosomes. Bars, 10 μm. D: Time-laps images from U2OS cells expressing EB1-GFP. At low level expression of mRFP-Cep169, Cep169 displayed dynamic comet-like fluorescence patterns that highlighted the growing MT tips with EB1 and moved toward the cell periphery (Upper panel: orange and red arrows). At higher level expression of mRFP-Cep169, Cep169 associated with the entire lateral MTs (Lower panel: green and blue arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

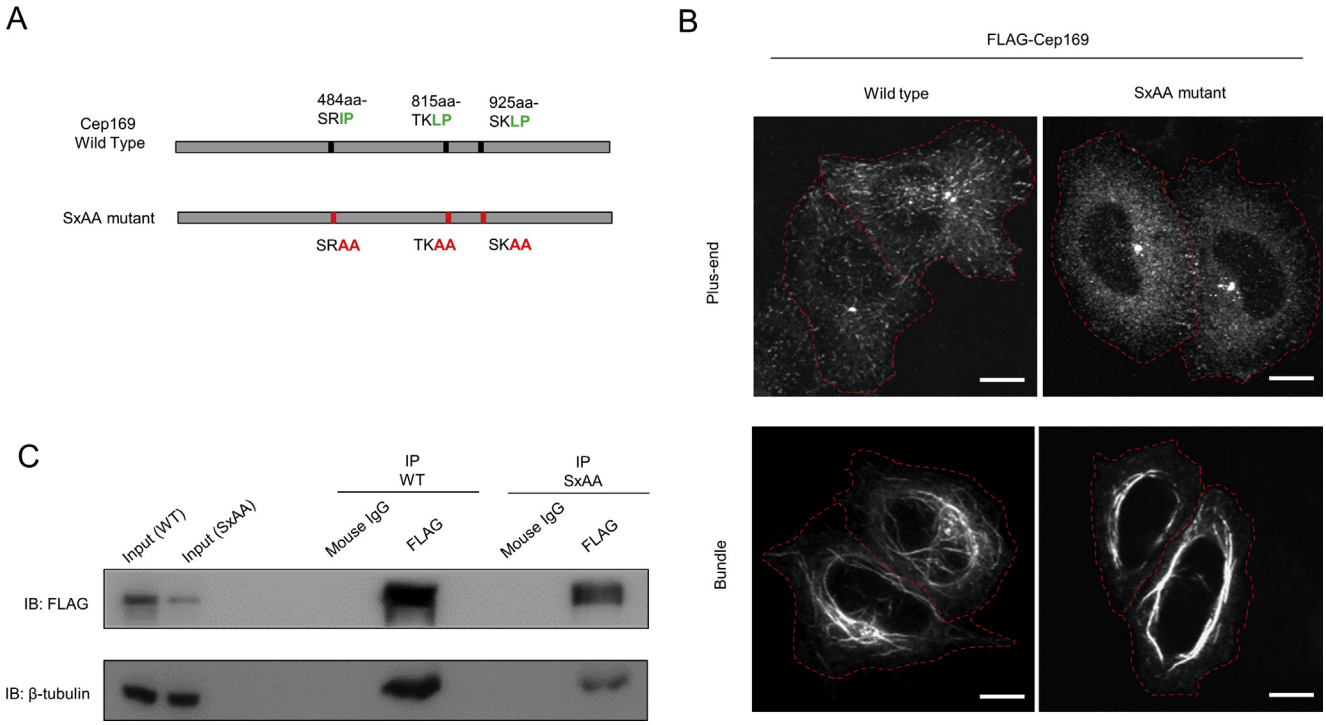

Fig. 2. Cep169 interacts with tubulin, and its EB1-binding domain is not required for Cep169-mediated bundle formation. A: Schema representation of the Cep169 SxIP motifs. Cep169 contains three short hydrophobic (S/T)x(I/L)P sequence motifs (SxIP) for EB1-binding. EB1-binding-deficient mutant, SxAA, contains three mutations, SRAA TKAA2, and SKAA. B: The U2OS cells were transfected with FLAG-Cep169 wild type or SxAA, and immunostained with FLAG-antibody. Bars, 10 μm. FLAG-Cep169 wild type, but not SxAA mutant, accumulates at growing MT plus-ends (Upper panel). Both Cep169 wild type and SxAA mutant induce MT bundle formation (lower panel). C: 293 T cells were transfected with FLAG-Cep169 wild type or SxAA. The tubulin-pull-down assay was performed by combining 293 T cell extracts with the purified tubulin. Complex formation was examined by immunoprecipitation with anti-FLAG antibody, followed by immunoblotting with anti-β-tubulin antibody.