Biochemical and Biophysical Research Communications 444 (2014) 644–650

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Asymmetric spindle pole formation in CPAP-depleted mitotic cells

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ARTICLE INFO

Article history:
Received 31 December 2013
Available online 1 February 2014

Keywords:
Spindle pole
CPAP
Centriole maturation
Asymmetric cell division

ABSTRACT

CPAP is an essential component for centriole formation. Here, we report that CPAP is also critical for symmetric spindle pole formation during mitosis. We observed that pericentriolar material between the mitotic spindle poles were asymmetrically distributed in CPAP-depleted cells even with intact numbers of centrioles. The length of procentrioles was slightly reduced by CPAP depletion, but the length of mother centrioles was not affected. Surprisingly, the young mother centrioles of the CPAP-depleted cells are not fully matured, as evidenced by the absence of distal and subdistal appendage proteins. We propose that the selective absence of centriolar appendages at the young mother centrioles may be responsible for asymmetric spindle pole formation in CPAP-depleted cells. Our results suggest that the neural stem cells with *CPAP* mutations might form asymmetric spindle poles, which results in premature initiation of differentiation.

1. Introduction

The centrosome is a major microtubule-organizing center that consists of a pair of centrioles surrounded by pericentriolar material (PCM). Centrioles duplicate and segregate in tight association with the cell cycle. Procentrioles begin to form next to mother centrioles at the G1/S phase. The procentrioles are elongated, and they eventually disengage from the mother centriole at the end of mitosis. Centriole disengagement is considered important for licensing a new round of centriole duplication [1]. The disengaged centriole becomes a mother centriole when a new procentriole is formed next to it. However, this young mother centriole is still structurally immature. For example, the young mother centriole lacks distal and subdistal appendages until the cell undergoes mitosis [2]. Therefore, it takes one and a half cell cycles for a procentriole to become a fully matured mother centriole.

Genetic analysis in *Caenorhabditis elegans* identified a number of centriolar proteins that are involved in centriole assembly, such as ZYG-1, a protein kinase, and SAS-4, SAS-5, SAS-6 and SPD-2, which contain coiled-coil domains [3,4]. During centriole biogenesis, these proteins are sequentially recruited to centrioles [5,6]. ZYG-1 recruits SAS-5 and SAS-6, which are required to SAS-4 incorporation [6,7]. It is proposed that the centriole duplication mechanism is evolutionally conserved from *C. elegans* to human [8]. CPAP, the human homolog of SAS-4, is essential for centriole formation in human cells [9–12].

Primary microcephaly is a rare, recessive genetic disease in which the prenatal brain growth is significantly reduced while the brain structure is left intact [13]. *CPAP* is one of the causal genes implicated in primary microcephaly [14]. However, it is not understood how the neural cell number is reduced in individuals with *CPAP* mutations. In this study, we revealed that CPAP depletion results in the asymmetry of spindle pole activity, which probably results in the premature initiation of asymmetric cell division.

2. Materials and methods

2.1. Antibodies

We generated antibodies against CPAP [9], CEP135 [15], CP110 [9], pericentrin [16], centrin-2 [17], CEP215 [18] and cenexin1 [19]. Antibodies against γ-tubulin (GTU-88, Sigma or C-20, Santa Cruz Biotechnology, Inc.) and CENP-B (H-65, Santa Cruz Biotechnology, Inc.) were purchased.

2.2. Cell culture and cell cycle synchronization

HeLa cells were grown at 37 °C and 5% CO_2 in high glucose DMEM supplemented with 10% fetal bovine serum. Mitotic HeLa

Abbreviation: PCM, pericentriolar material.

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http://dx.doi.org/10.1016/j.bbrc.2014.01.129
0006-291X/

cells were enriched with the double-thymidine block and release. In the rescue experiments, the cells were arrested at S phase with a single thymidine block and release followed by MG132 treatment.

2.3. Transfection and RNA interference

siCPAP (GGA CUG ACC UUG AAG AGA ATT), *siCTL* (scrambled sequence for control) (GCA AUC GAA GCU CGG CUA CTT), *sicenexin1* (AGA CUA AUG GAG CAA CAA G) were used for RNAi experiments. The siRNAs were transfected into HeLa cells using RNAi MAX reagents (Invitrogen). Plasmids were transfected with FuGENE HD (Roche). For rescue experiments, siRNAs and DNAs were sequentially transfected.

2.4. Immunoblot analysis

HeLa cells were lysed in the sample buffer (50 mM Tris–HCl, pH 6.8, 100 mM dithiothreitol, 2% SDS, 0.1% bromophenol blue, 10% glycerol). Samples were loaded in 8% polyacrylamide gels and then transferred into nitrocellulose membranes. The membranes were blocked in 5% skim milk in TBST (20 mM Tris, 150 mM NaCl, 0.3% Triton X-100) for 30 min, incubated with anti-CPAP (1:100), anti-β-tubulin (1:1000) antibodies for 3 h at room temperature. After washing three times with TBST for 5 min, the membranes were incubated with mouse and rabbit secondary antibodies (1:10,000) for 30 min. After the membranes were washed three times with TBST for 5 min, the peroxidase activity was detected using ECL solutions.

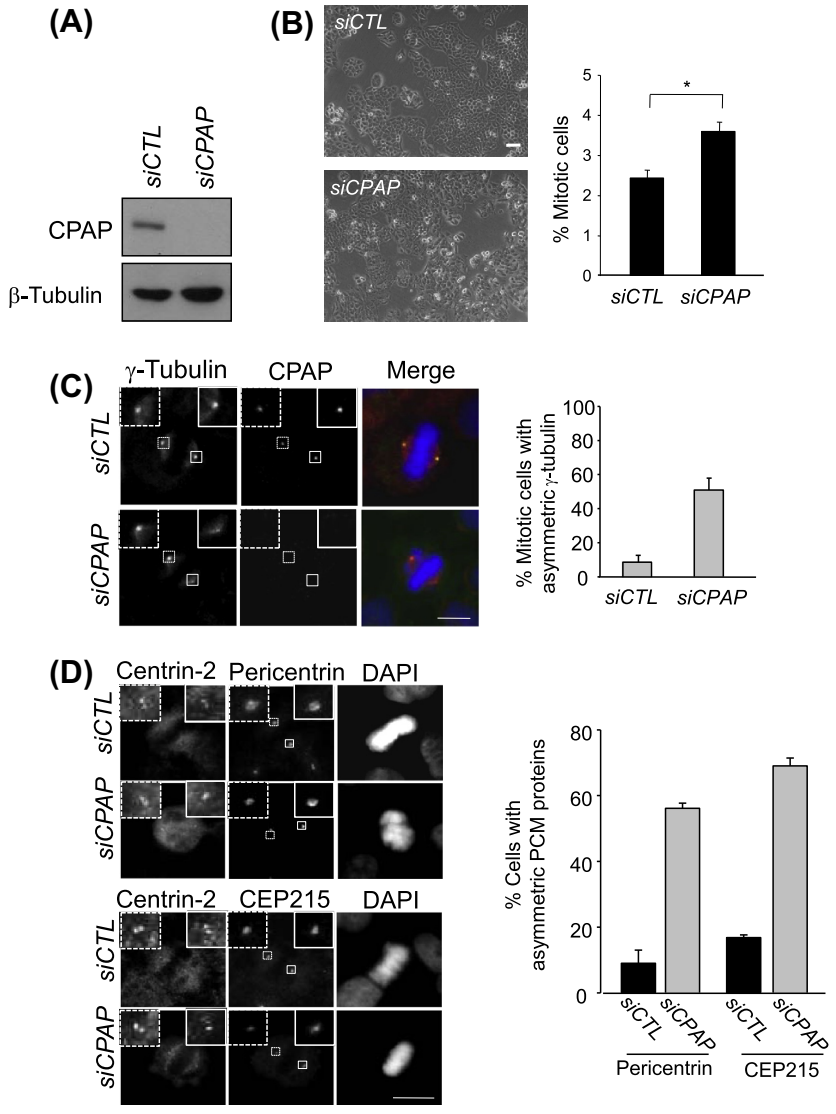

Fig. 1. Asymmetric spindle poles in CPAP-depleted mitotic cells. (A) HeLa cells were transfected with the control (*siCTL*) or CPAP (*siCPAP*) siRNAs and cultured for 48 h. The cells were subjected to immunoblot analysis with the CPAP and β-tubulin antibodies. (B) HeLa cells were transfected with control (*siCTL*) or CPAP (*siCPAP*) siRNAs. Forty-eight hours later, the cells were observed with a phase-contrast microscope. The scale bar represents 100 μm. The mitotic index was determined with more than 3000 cells per experimental group from three independent experiments. The results were presented as means and standard errors $^{*}P < 0.05$. (C) The CPAP-depleted HeLa cells were coimmunostained with the CPAP (green) and γ-tubulin (red) antibodies. The scale bar represents 10 μm. The relative γ-tubulin intensity between a pair of spindle poles was determined. A difference in intensity greater than 1.5 was defined as an asymmetric distribution. Over 300 cells per group were analyzed from three independent experiments, and the results are presented as means and standard errors. (D) The CPAP-depleted mitotic cells were immunostained with the centrin-2 antibody along with the pericentrin or CEP215 antibody. The scale bar represents 10 μm. Relative intensities of pericentrin and CEP215 were measured in spindle pole pairs with an intact number of centrioles. A difference in intensity greater than 1.5 was defined as an asymmetric distribution. For statistical analysis, over 180 mitotic cells per group were analyzed from two independent experiments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)