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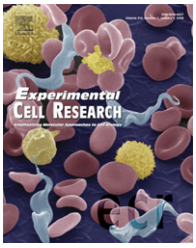

Research Article

A novel function of CEP135 as a platform protein of C-NAP1 for its centriolar localization

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ABSTRACT

A proteomic study predicted that about one hundred kinds of proteins constitute a basic structure of the centrosome. Most of the core centrosomal proteins contain extensive coiled-coil domains, suggesting that the protein–protein interaction is a critical force for the core centrosome configuration. In the present study, we investigated a novel interaction between CEP135 and C-NAP1, two core centriolar proteins. Depletion of CEP135 caused a premature centrosome splitting. Reduction of the centrosomal C-NAP1 level was accompanied in a specific manner. Ectopic expression of the CEP135 mutant proteins also caused centrosome splitting in association with the reduction of the centrosomal C-NAP1 levels. Based on these results, we propose that CEP135 acts as a platform protein for C-NAP1 at the centriole.

Introduction

The centrosome comprises a pair of centrioles embedded in an amorphous protein mesh called the pericentriolar material. Since the centrosome is the major microtubule-organizing center at which the minus ends of cellular microtubules are concentrated, it influences a number of microtubule-related processes such as intracellular trafficking and cell morphology and motility. In mitotic cells, centrosomes function as spindle poles for chromosome segregation. Further, the centrosome is involved in a process to release the central microtubules from the midbody for the completion of cell division [1]. The centrosome is also essential for the formation of the primary cilium. The flies without centrioles developed into morphologically normal adults, but had no cilium or flagellum and died shortly after birth [2].

It is believed that a number of proteins are recruited into or removed from the centrosome depending on the physiological status of the cell. Nonetheless, the core structure of the centrosome should be maintained for its integrity. A proteomic analysis identified over 500 proteins from the centrosome-enriched fraction of KE-37 human lymphoblastic cells, of which 114 were considered to be core components of the human centrosome [3]. Many of the core centrosomal proteins include extensive coiled-coil domains, suggesting that the protein–protein interaction is a critical force for the core centrosome configuration.

CEP135 is a core centrosome protein with coiled-coil domains. It is located around the centriolar surface as well as within the proximal lumen of the centrioles [4]. *CEP135* overexpression or knockdown was reported to result in abnormal organization of the microtubules in both the interphase and mitotic cells [5]. CEP135 was proposed to play a scaffolding role during centriole biogenesis [4], but exact functions of CEP135 remain to be elucidated.

C-NAP1 is another core centrosome protein with multiple coiled-coil domains. It is located at the proximal end of the

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centriole [6]. C-NAP1 suppression by a specific antibody injection or siRNA transfection induced premature centrosome splitting [7,8]. It was proposed that C-NAP1 holds an intercentriolar linker such as rootletin to maintain centrosome cohesion [8]. NEK2 has been considered a key regulator for centrosome separation since its overexpression induced the premature centrosome splitting and since its activity oscillates during the cell cycle [9,10]. C-NAP1 is the first known substrate of NEK2 for centrosome separation [6]. In addition, NEK2 also phosphorylates rootletin and β-catenin, another intercentrosomal linker protein [8,11]. However, exact mechanisms remain to be investigated further. For example, it is not clear whether all three proteins should be phosphorylated by NEK2 or not for completion of the centrosome separation.

We have generated a protein–protein interaction network among the core centrosome proteins, and observed a number of novel interactions within them. In the present study, we report a novel interaction of CEP135 with C-NAP1. Our results suggest that CEP135 functions as a platform for C-NAP1 at the centriole.

Materials and methods

Preparation of cDNAs

The centrosomal cDNA clones were cloned directly from a HeLa mRNA pool using a PCR-based method; purchased from German Resource Center for Genome Research (www.rzpd.de), Kazusa DNA Research Institute (www.kazusa.or.jp) and Korean UniGene Information (kugi.kribb.re.kr); or kindly gifted from Drs. A. Merdes (PCM-1), Y. Ono (CG-NAP), and J. B. Rattner (C-NAP1). The cDNAs were subcloned into *pGADT7* and *pGBKT7* (Clontech) for the yeast two-hybrid assays and into *pcDNA3.1* (Invitrogen) and *pCMV-Tag3* (Stratagene) for expression in mammalian cells. The cDNAs encoding proteins over 200 kDa in size were divided into 2–4 pieces for expression in yeast and mammalian cells.

Cell culture, transfection, and RNAi experiments

HeLa or 293T cells were cultured in DMEM supplemented with 10% FBS at 37 °C. We transfected the plasmid DNA and siRNA with the Lipofectamine Plus (Invitrogen) and Oligofectamine (Invitrogen) reagents, respectively. For the immunoprecipitation assay, the plasmids were transfected into 293T cells by using the polyethylenimine method. For RNAi experiments, we used *siCEP135* (5′-CAA GCA GAU UGA GCU AAG A-3′), *siC-NAP1* (5′-CUG GAA GAG CGU CUA ACU GAU-3′), and *siCTL* (control) (5′-GCA AUC GAA GCU CGG CUA C-3′).

Antibodies

The rabbit polyclonal antibodies were raised and affinity-purified against GST-CEP135$^{295-1141}$, GST-CPAP$^{970-1339}$, GST-CP110$^{1-334}$ or GST-PIBF1$^{1-757}$. The C-NAP1 antibody was described previously [12]. Several antibodies were kindly gifted from B. Edde (the polyglutamylated tubulin GT335), Y. Ono (CG-NAP), A. Merdes (PCM-1), and X. Zhu (NUDEL). We purchased antibodies against Myc (mouse 9B11, Cell Signaling), HA (mouse 16B12, Covance), γ-tubulin (mouse GTU-88, Sigma-Aldrich or Goat C-20, Santa Cruz), β-tubulin (rabbit T2200, Sigma-Aldrich), Nek2 (mouse, BD Transduction Laboratories) and GFP (mouse B-2, Santa Cruz).

Immunoblot, immunoprecipitation, and immunocytochemistry

These experiments were carried out as described previously [12]. In brief, a single detergent lysis buffer (50 mM Tris–HCl at pH 8.0, 150 mM NaCl, 0.02% sodium azide, and 1% Triton X-100) or the RIPA buffer (50 mM Tris–HCl at pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, and 0.5% sodium deoxycholate) with protease inhibitors was used for lysis of the 293T cells. The lysates were centrifuged at 12,000 rpm for 20 min at 4 °C, and the supernatants were incubated with an antibody for 5 h, followed by incubation with protein A sepharose (Amersham Pharmacia) for 1 h at 4 °C. The beads were then washed 3 times with the same lysis buffer and suspended in SDS-PAGE sample buffer for immunoblot analysis, as described previously [13].

For immunocytochemistry, HeLa cells were cultured on a 12-mm coverslip and transfected. At an indicated time after transfection, the cells were fixed with cold methanol for 10 min, permeabilized and blocked with phosphate-buffered saline with 0.5% triton X-100 (0.5% PBST) plus 3% BSA for 20 min, incubated with primary antibodies diluted in 0.1% PBST with 3% BSA for 1 h, washed 3 times with 0.1% PBST, incubated with either an FITC- or a TRITC-conjugated secondary antibody (Jackson Immunoresearch) for 30 min, and washed 3 times with 0.1% PBST. DAPI solution was used at the final step for DNA staining. The cells were mounted on a slide glass and observed using a fluorescence microscope (Olympus IX51) equipped with a CCD (Qicam fast 1394, Qimaging) camera. The images were analyzed using ImagePro 5.0 (Media Cybernetics, Inc.) and statistically analyzed with SigmaPlot (Systat Software, Inc.).

Cell synchronization and flow cytometry

HeLa cells were synchronized at G1/S boundary by double thymidine block with 2 mM thymidine, and released for 6 h to accumulate S/G2 population. For flow cytometry, HeLa cells were harvested by trypsinization and stained with 10 μg/ml propidium iodide. Stained cells were loaded to FACScalibur (Beckton Dickinson) and DNA contents were analyzed using Cell Quest Pro (BD Biosciences).

Determination of the centrosome intensity

After immunostaining, fluorescent images of the centrosome were measured using ImagePro 5.0 (Media Cybernetics, Inc.). The centrosome intensity of a specific protein was determined by the sum fluorescent intensity subtracted by background intensity. For normalization, the centrosome intensity was divided by the intensity of polyglutamylated tubulin (GT335) measured in the same cell. In each experiment, the mean intensity of unsplit centrosome group in *siCTL*-transfected cells was normalized to 1.0. Measurements were carried out in 10 cells and repeated 3 times.

Results

Specific interaction of CEP135 with C-NAP1

We carried out yeast two-hybrid interaction assays with CEP135 as bait and identified 10 centrosomal proteins (data not shown).

Among the proteins interacting with CEP135, C-NAP1 revealed the strongest interaction. When C-NAP1 was used for yeast two-hybrid interaction assays among the core centrosome proteins, it exhibited many interactions in addition to known ones such as rootletin and NEK2; thus, it may have additional functions that have not been reported to date (unpublished data). Therefore, we decided to characterize the CEP135 interaction with C-NAP1 in detail, in the hope of uncovering novel functions of these proteins.

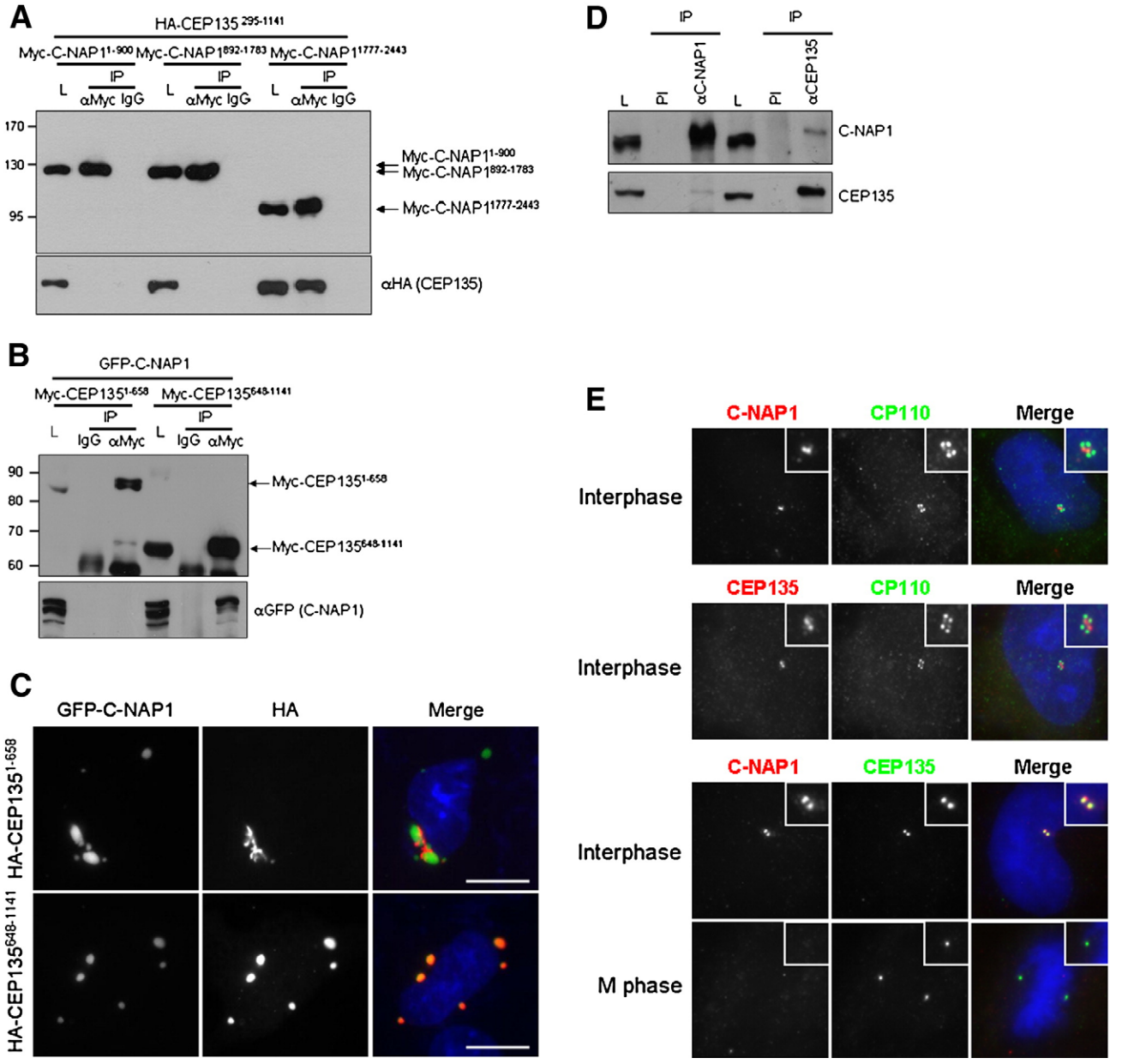

Fig. 1 – Specific interactions of CEP135 with C-NAP1. (A) HA-CEP135$^{295–1141}$ was coexpressed with the truncated mutants of C-NAP1 (Myc-C-NAP1$^{1–900}$, Myc-C-NAP1$^{892–1783}$, or Myc-C-NAP1$^{1777–2443}$) in 293T cells. The cell lysates (L) were subjected to an immunoprecipitation (IP) assay using the Myc antibody. The coimmunoprecipitated HA-CEP135$^{295–1141}$ was detected by using the HA antibody. (B) GFP-C-NAP1 was coexpressed with the truncated mutants of CEP135 (Myc-CEP135$^{1–658}$ or Myc-CEP135$^{648–1141}$) in 293T cells. The cell lysates (L) were subjected to an immunoprecipitation (IP) assay using the Myc antibody. Coimmunoprecipitated GFP-C-NAP1 was detected by using the GFP antibody. (C) HeLa cells were co-transfected with GFP-C-NAP1 (green) and the HA-CEP135 mutants (HA-CEP135$^{1–658}$ or HA-CEP135$^{648–1141}$; red). (D) The endogenous C-NAP1 and CEP135 proteins were immunoprecipitated with the corresponding specific antibodies. The coimmunoprecipitated proteins were detected by using the same set of antibodies. Preimmune (PI) sera were used as controls. (E) The endogenous C-NAP1 or CEP135 protein was coimmunostained with CP110, a marker for distal end of the centriole. Endogenous C-NAP1 and CEP135 were also coimmunostained in both interphase and M-phase HeLa cells.

We examined the physical interactions occurring between CEP135 and C-NAP1 by performing coimmunoprecipitation assays in the 293T cells. The results revealed that the C-terminal domain of C-NAP1 is responsible for its interaction with CEP135 (Fig. 1A). Likewise, the C-terminal domain of CEP135 was responsible for the interaction with C-NAP1 (Fig. 1B). The CEP135 truncated mutant proteins formed cytoplasmic aggregates when expressed in excess (Fig. 1C). Coexpressed GFP-C-NAP1 was colocalized with HA-CEP135$^{648–1141}$, but not with HA-CEP135$^{1–658}$ (Fig. 1C). Such colocalization pattern is consistent with the coimmunoprecipitation data showing that the C-terminal end of CEP135 is responsible for interaction with C-NAP1. Specific interactions among the endogenous proteins were confirmed by the coimmunoprecipitation assays (Fig. 1D). These results indicate that CEP135 interacts with C-NAP1 in vivo.

We coimmunostained the endogenous CEP135 and C-NAP1 along with CP110, a marker for distal end of the centriole. The results showed that the CP110 signals did not overlap with the C-NAP1 and CEP135 signals (Fig. 1E). On the other hand, the endogenous CEP135 and C-NAP1 were colocalized perfectly in the interphase cells (Fig. 1E). The C-NAP1 signals in the M phase cells were reduced significantly as reported previously [7]. These results indicate that both CEP135 and C-NAP1 are colocalized to the proximal end of the centriole.

CEP135 is critical for the centrosomal retention of C-NAP1

Centrosomal as well as total cellular levels of CEP135 and C-NAP1 were reduced efficiently 96 h after transfection of the specific siRNAs (*siCEP135* and *siC-NAP1*) in HeLa cells (Figs. 2A, B). A prominent phenotype in the *CEP135*-suppressed cells was the centrosome splitting (Fig. 2C). The centrosome splitting was observed in over 40% of the *CEP135*-suppressed cells, as comparable to the *C-NAP1*-suppressed cells, and it occurred prematurely beginning from the G1/S phase cells (Fig. 2C). The FACS analysis with the siRNA-tranfected cells revealed that the *CEP135*-suppressed cells were not accumulated at G2 phase (Supplementary Fig. 1). These results indicate *CEP135* suppression induced the centrosome splitting regardless of the cell cycle stages.

Recently, Graser et al. [14] screened a selected number of the centrosome proteins that are critical for the centrosome cohesion using the siRNA transfection method. CEP135 was included in this screen but not selected as a split-causing protein. One possibility in such discrepancy may reside in the *CEP135* suppression efficiency. In our hand, it took 96 h for the centrosomal CEP135 levels to be reduced sufficiently, and the centrosome splitting was accompanied in this time frame.

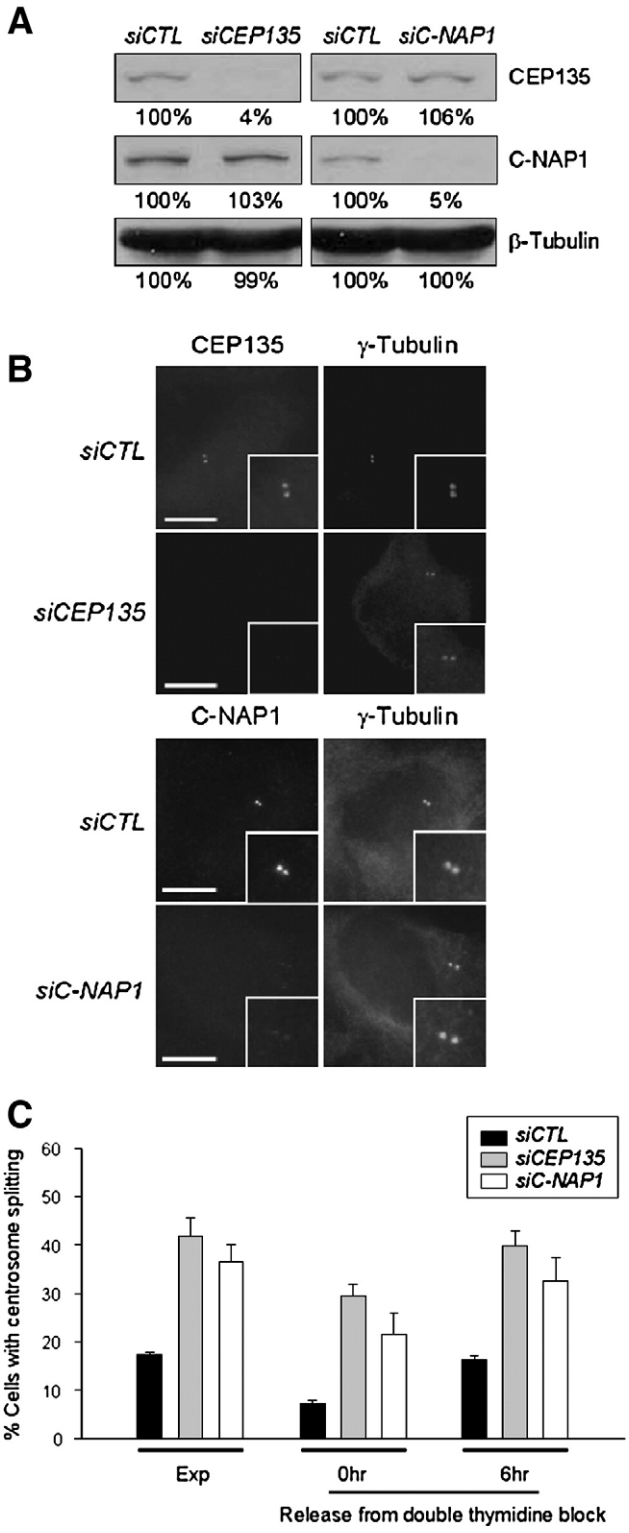

Fig. 2 – *CEP135* suppression induced the premature centrosome splitting. (A) HeLa cells were transfected with indicated siRNAs and cultured for 96 h. The cells were then subjected to immunoblot analysis with antibodies specific to CEP135, C-NAP1 and β-tubulin. Relative amounts of the indicated proteins were determined between the *siCTL*- and *siCEP135*- or *siC-NAP1*-transfected cell populations. (B) The *siCTL*-, *siCEP135*- or *siC-NAP1*-transfected cells were coimmunostained with antibodies specific to γ-tubulin and CEP135 or C-NAP1. The insets are magnified views of the centrosomes. Scale bars, 10 μm. (C) HeLa cells transfected with *siCTL*, *siCEP135* or *siC-NAP1* were cultured for 96 h and their cell cycle was synchronized by the double thymidine block. The G1/S and S/G2 populations were prepared by culturing 0 h and 6 h after release of the block, respectively. Exponentially growing cells (Exp) were used as a control. The centrosomes were visualized with the γ-tubulin antibody and the centrosome splitting was determined if they were separated over 2 μm apart [7]. For statistical analysis, this and following experiments were repeated 3 times. The graphs were expressed as means and standard errors.

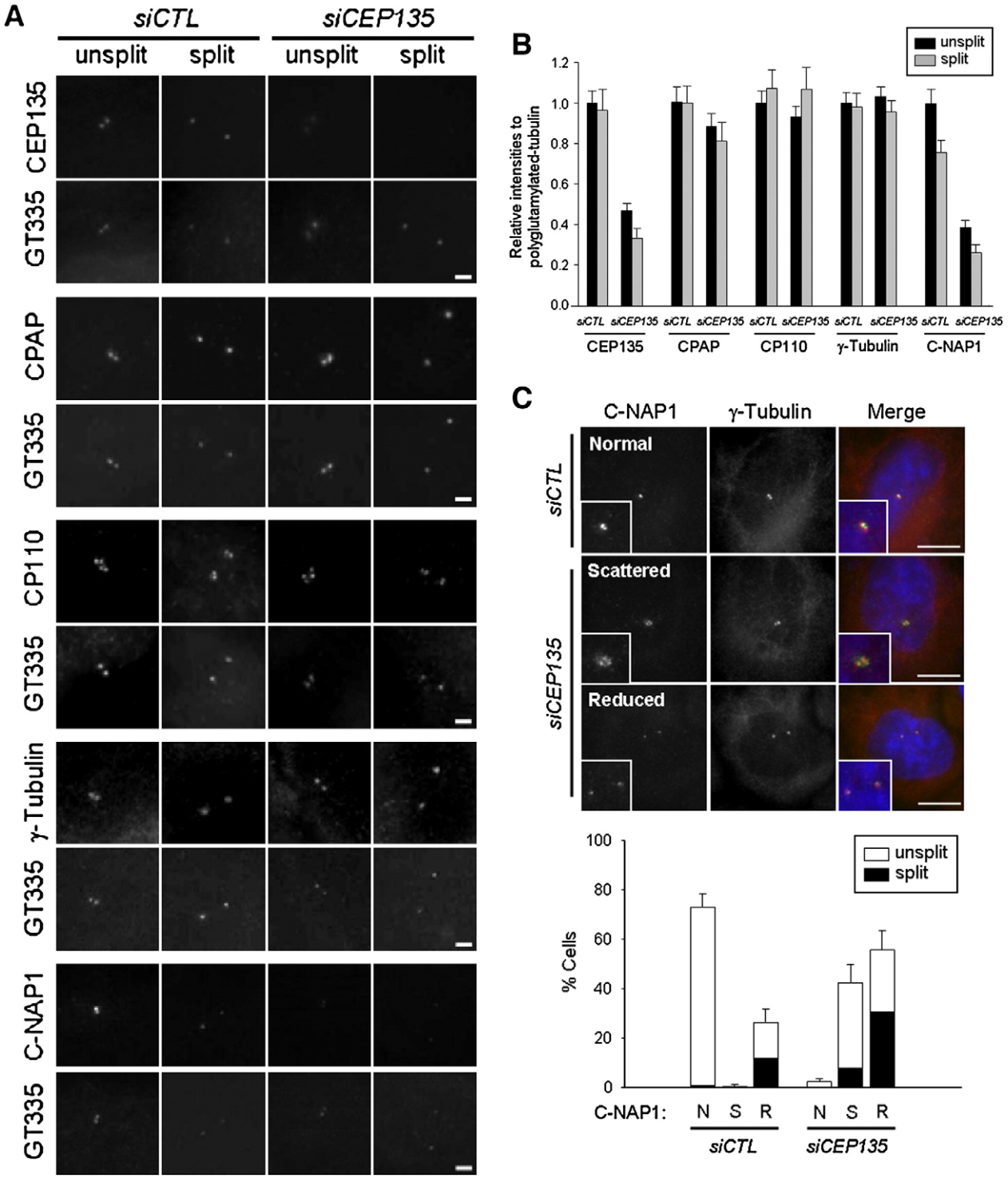

Fig. 3 – Effects of *CEP135* on the selected centrosomal components. (A) The *siCTL*- or *siCEP135*-transfected cells were immunostained with the antibodies specific to CEP135, CPAP, CP110, γ-tubulin or C-NAP1 along with polyglutamylated tubulin (GT335). Scale bars, 2 μm. (B) The centrosomal levels of the indicated proteins were determined as described in Materials and methods, and compared between the unsplit and split centrosome groups. (C) The *siCTL*- or *siCEP135*-transfected cells were coimmunostained with antibodies specific to C-NAP1 (green) and γ-tubulin (red). The centrosomal C-NAP1 was categorized as normal (N), scattered (S), or reduced (R). Quantification was carried out to determine the correlation between the centrosomal C-NAP1 protein level and centrosome splitting. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The integrity and amount of the centriolar components, such as CPAP and CP110, were not affected in the *CEP135*-suppressed cells (Fig. 3A). We did not observe a significant change in the centrosomal γ-tubulin level, either (Figs. 3A, B). However, the centrosomal C-NAP1 levels were reduced significantly in the *CEP135*-suppressed cells (Figs. 3A, B). In fact, C-NAP1 was the only protein whose centrosomal levels were reduced among the CEP135-interacting proteins tested, such as NEK2, CG-NAP, PCM1, NUDEL, and PIBF1 (Supplementary Fig. 2). These data suggest that the *CEP135* suppression induced premature centrosome splitting without disturbing the centrosomal components except C-NAP1.

Immunocytochemical analyses revealed that the C-NAP1 proteins in most of the *CEP135*-suppressed cells were either scattered around or reduced at the centrosomes (Fig. 3C). The C-NAP1 scattering was not due to centrosome fragmentation, because the centrosomal staining patterns of the centriolar proteins, such as GT335, CPAP and CP110, looked intact in the *CEP135*-suppressed cells (Fig. 3A). Over a half (54±6%) of the centrosomes with a reduced amount of C-NAP1 were split prematurely in the *CEP135*-suppressed cells (Fig. 3C). 17±4% of the C-NAP1-scattered centrosomes were also split (Fig. 3C). These results support the notion that the scattering or reduction of the centrosomal C-NAP1 caused premature centrosome splitting. In fact, 45±8% of the centrosomes in the control cells with reduced amounts of C-NAP1 were also separated (Fig. 3C).

The centrosomal CEP135 levels were determined in the *C-NAP1*-suppressed cells. As shown in Figs. 2A, B, siRNA transfection resulted in 95% suppression of the total cellular C-NAP1 and disappearance of the C-NAP1 signals at the centrosome. In such *C-NAP1*-suppressed cells, the centrosomal CEP135 levels were reduced to about 70% of the control (Supplementary Fig. 3). These results suggest that the centrosomal CEP135 levels are dependent on C-NAP1 to some extent, but not as much as centrosomal C-NAP1 dependence on CEP135.

Ectopic expression of the CEP135 mutants induced premature centrosome splitting

We ectopically expressed 2 types of CEP135-truncated mutants and observed their subcellular localizations. Immunoblot analysis revealed that the ectopic proteins were expressed over 50-fold excess of the endogenous CEP135 (Supplementary Fig. 4). Although cytoplasmic aggregates were formed when either of the mutant

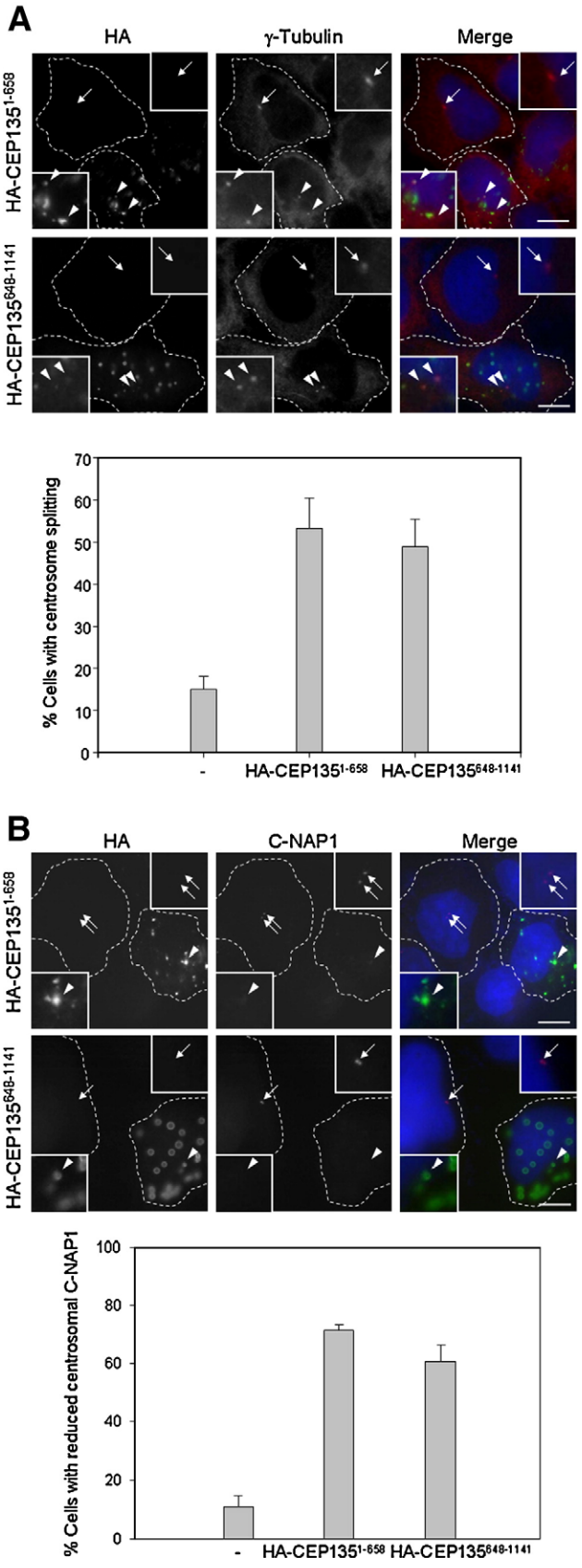

Fig. 4 – Induction of centrosome splitting by ectopic expression of the CEP135-truncated mutants. (A) HeLa cells were transfected with HA-CEP135$^{1-658}$ or HA-CEP135$^{648-1141}$ and coimmunostained with antibodies specific to HA (green) and γ-tubulin (red). The cell boundaries were outlined. The arrows and arrowheads mark the centrosomes of the untransfected and transfected cells, respectively. The insets are magnified views of the centrosomes. For statistical analysis of this and the following experiments, approximately 100 cells were counted per experimental group, and each experiment was repeated 3 times. The graphs were expressed as means and standard errors. (B) HeLa cells were transfected with HA-CEP135$^{1-658}$ or HA-CEP135$^{648-1141}$ and coimmunostained with antibodies specific to HA (green) and C-NAP1 (red). We counted the number of cells with reduced centrosomal C-NAP1 levels (less than 50% of the average centrosomal C-NAP1 levels in the untransfected cells). Scale bars, 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

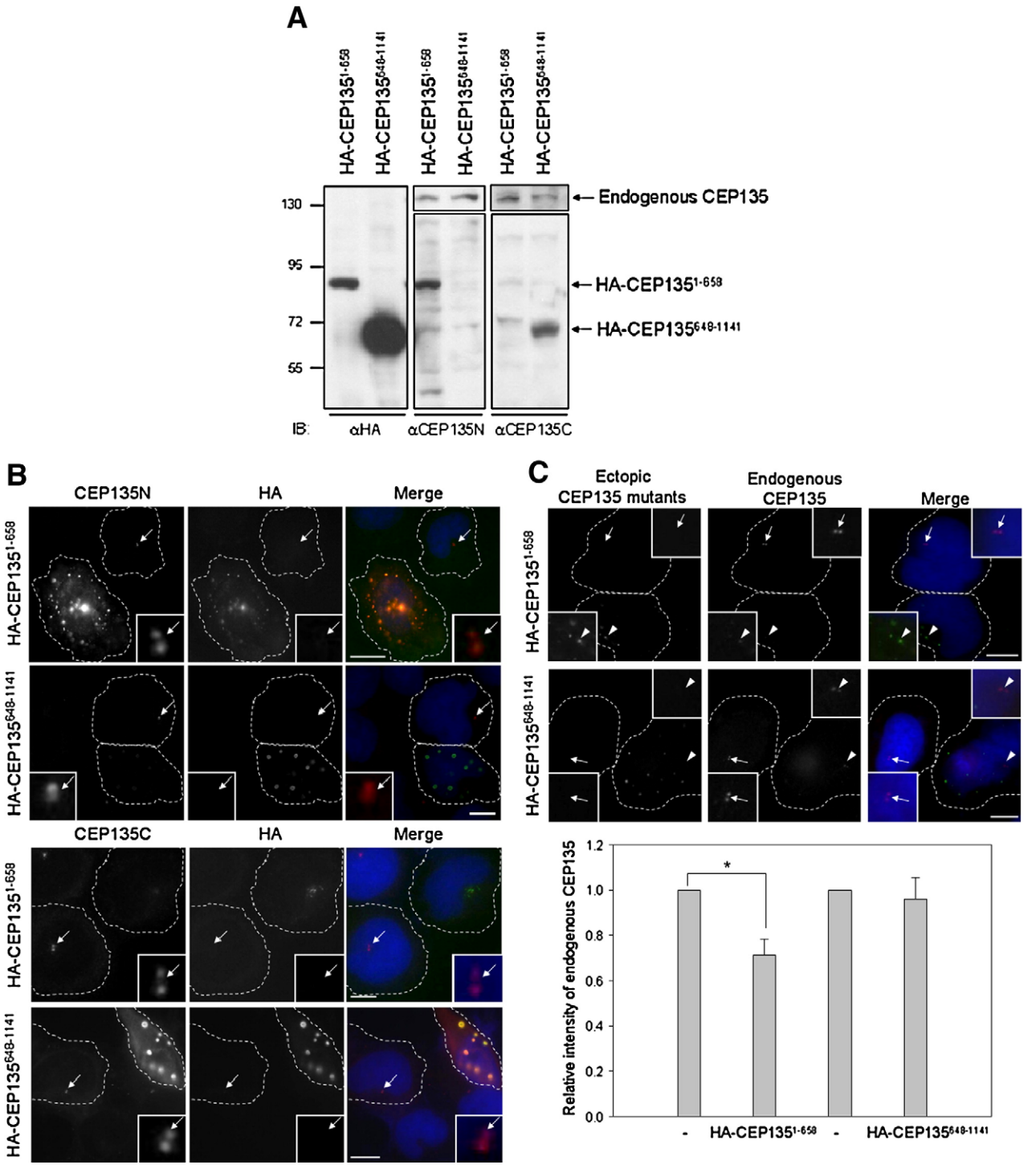

Fig. 5 – CEP135$^{1-658}$ withdrew endogenous CEP135 from the centrosome. (A, B) Preparation of the CEP135N and CEP135C antibodies. The CEP135 antibody was affinity purified with GST-CEP135$^{1-658}$ to enable detection of both endogenous CEP135 and HA-CEP135$^{1-658}$, but not of HA-CEP135$^{648-1141}$ (the CEP135N antibody). In the reverse cycle, the CEP135 antibody was affinity purified with GST-CEP135$^{648-1141}$ to enable detection of both endogenous CEP135 and HA-CEP135$^{648-1141}$, but not of HA-CEP135$^{1-658}$ (the CEP135C antibody). (A) Immunoblot and (B) immunocytochemical analyses revealed that both the CEP135N and CEP135C antibodies were able to detect the endogenous CEP135 protein as well as the corresponding truncated mutant proteins. The cells were outlined with dotted lines and the arrows marked centrosomal regions of untransfected cells. The insets are magnified views of the centrosomes of the untransfected cells. Both the CEP135N and CEP135C antibodies detected the endogenous CEP135 protein at the centrosome. (C) HeLa cells were transfected with HA-CEP135$^{1-658}$ or HA-CEP135$^{648-1141}$. Endogenous CEP135 (red) was detected along with the ectopically expressed mutant proteins (green) by using the CEP135 antibodies. The arrows and arrowheads mark the centrosomes of the untransfected and transfected cells, respectively. The endogenous centrosomal CEP135 levels of the transfected cells were compared with those of the untransfected cells. Asterisk, $P<0.05$ in a paired t test. Scale bars, 10 μm.

proteins was present in excess (Fig. 4A), centrosomal localization of HA-CEP135$^{1-658}$ was evident, whereas that of HA-CEP135$^{648-1141}$ was relatively limited (Fig. 4A, Supplementary Fig. 5). Nonetheless, premature centrosome splitting was induced in both HA-CEP135$^{1-658}$- and HA-CEP135$^{648-1141}$-overexpressed cells (Fig. 4A). These results suggest that both the CEP135 truncated mutants could function dominant-negatively for centrosome splitting. We observed that majority of the mutant-expressed cells had centrosomes with reduced C-NAP1 levels (Fig. 4B). These results suggest that the premature centrosome splitting by the CEP135 truncated mutants are linked to reduction in the centrosomal C-NAP1 levels.

We determined the endogenous CEP135 levels in the centrosomes of cells containing ectopic HA-CEP135$^{1-658}$ or HA-CEP135$^{648-1141}$ proteins. In order to distinguish endogenous CEP135 from the truncated mutants, we affinity purified the CEP135 antiserum with either the GST-CEP135$^{1-658}$ or GST-CEP135$^{648-1141}$ fusion protein. As expected, neither did the anti-CEP135$^{1-658}$ antibody detect HA-CEP135$^{648-1141}$ nor did the anti-CEP135$^{648-1141}$ antibody detect HA-CEP135$^{1-658}$ (Figs. 5A, B). However, both antibodies were able to detect the endogenous CEP135 protein in immunoblot and immunocytochemical analyses (Figs. 5A, B). Next, we determined the endogenous CEP135 levels in the centrosomes of the transfected cells. The intensity of endogenous CEP135 level in the HA-CEP135$^{1-658}$-transfected cells was reduced to 70% that in the untransfected cells (Fig. 5C). On the other hand, there was no significant reduction in the level of endogenous CEP135 in the HA-CEP135$^{648-1141}$-transfected cells (Fig. 5C). These results support the notion that CEP135$^{1-658}$ replaced endogenous CEP135 at the centrosome.

Discussion

In the present study, we report a novel interaction of CEP135 with C-NAP1. Knockdown of CEP135 caused a premature centrosome splitting. Reduction of the centrosomal C-NAP1 levels was accompanied. Based on these observations, we propose a model in which CEP135 acts as a platform for C-NAP1 at the proximal end of the centriole (Fig. 6). CEP135 suppression allowed release of the centrosomal C-NAP1, resulting in premature centrosome splitting.

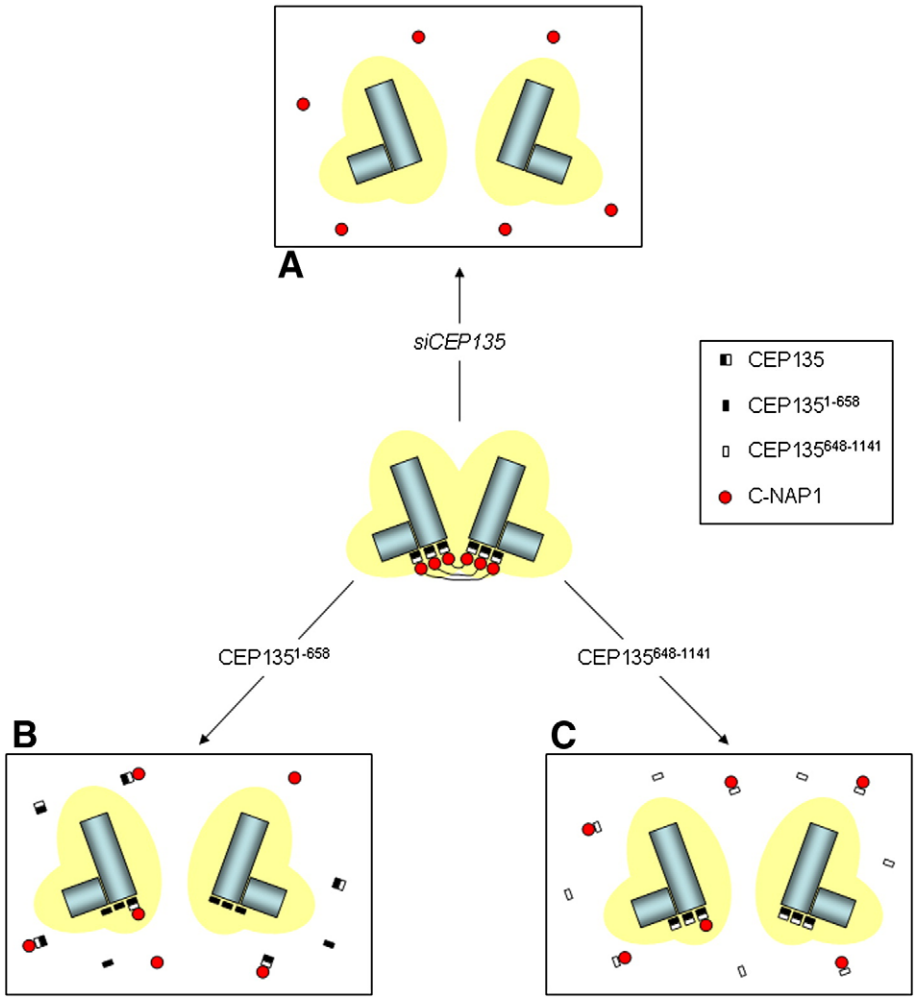

Fig. 6 – Model. CEP135 is distributed in the centrioles as well as in the PCM of the centrosome. A fraction of the CEP135 protein physically interacts with C-NAP1 at the proximal end of the centrioles. (A) Depletion of CEP135 eliminates a docking site for C-NAP1 at the centriole. (B) Excess CEP135$^{1-658}$ replaces endogenous CEP135 in the centriole. Since C-NAP1 cannot bind to CEP135$^{1-658}$, the former is released from the centrosome. (C) Excess CEP135$^{648-1141}$ draws C-NAP1 out of the centrosome. In all 3 cases, the reduction in the centrosomal C-NAP1 levels results in premature centrosome splitting.

We observed that the CEP135 truncated mutant proteins also induced the premature centrosome splitting. Since HA-CEP135$^{1-658}$ is localized within the centrosome, it may replace endogenous CEP135 complexed with C-NAP1 therein (Fig. 6). In accordance to this prediction, the intensity of endogenous CEP135 level in the HA-CEP135$^{1-658}$-transfected cells was reduced to 70% that in the untransfected cells, while there was no significant reduction in the level of endogenous CEP135 in the HA-CEP135$^{648-1141}$-transfected cells (Fig. 5C). In contrast, since HA-CEP135$^{648-1141}$ exhibits a binding affinity for C-NAP1, but is localized outside the centrosome mostly, it may pull C-NAP1 out of the centrosome (Fig. 6). If C-NAP1 was targeted to the cytosolic excess of CEP135$^{648-1141}$, it should have co-localized at the cytoplasmic aggregates. However, we were not able to detect such C-NAP1 signals in Fig. 4B. One possibility may be that the amount of the endogenous C-NAP1 proteins was too small to be detected immunocytochemically. In fact, overexpressed C-NAP1 co-localized with HA-CEP135$^{648-1141}$ perfectly, but not with HA-CEP135$^{1-658}$ (Fig. 1C). These observations suggest that C-NAP1 formed a complex with HA-CEP135$^{648-1141}$ in the cytoplasm, resulting in withdrawal of C-NAP1 out of the centrosome (Fig. 6).

Several centrosomal proteins have been reported to be critical for centrosome splitting. C-NAP1 is the first known centrosomal protein that is placed at the proximal end for mediating centriole-centriole cohesion [7,10]. Rootletin is a component of the linking fiber and CEP68 may function to stabilize the intercentriolar cohesion [8,14,15]. There are another group of the centrosomal proteins whose depletion also results in centrosome splitting, but which do not have a direct connection with the intercentriolar linker. It was suggested that the centrosomal proteins such as pericentrin, CEP215, and dynamin2, rather affect on cytoskeletal dynamics, eventually resulting in centrosome splitting indirectly [14,16]. We believe that CEP135 depletion did not affect on the centriolar configuration, as all examined CEP135-interacting proteins were intact except C-NAP1 (Supplementary Fig. 2). We rather believe that CEP135 participate in the intercentriolar linking structure by docking C-NAP1 at the proximal end of the centriole. Therefore, our model predicts that CEP68 and rootletin are also affected in the *CEP135*-suppressed cells.

Scaffold function of CEP135 was initially proposed during the centriole biogenesis [4]. CEP135 and CPAP were required to form a core structure within the proximal lumen of the centrioles [4]. In the current study, we propose that CEP135 also functions as a scaffold protein to hold C-NAP1 at the proximal end of the centriole. We currently investigate whether C-NAP1-CEP135 interaction is controlled during the cell cycle or not.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yexcr.2008.09.016.

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