

Stabilization of Cartwheel-less Centrioles for Duplication Requires CEP295-Mediated Centriole-to-Centrosome Conversion

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SUMMARY

Vertebrate centrioles lose their geometric scaffold, the cartwheel, during mitosis, concurrently with gaining the ability to recruit the pericentriolar material (PCM) and thereby function as the centrosome. Cartwheel removal has recently been implicated in centriole duplication, but whether “cartwheel-less” centrioles are intrinsically stable or must be maintained through other modifications remains unclear. Here, we identify a newborn centriole-enriched protein, KIAA1731/CEP295, specifically mediating centriole-to-centrosome conversion but dispensable for cartwheel removal. In the absence of CEP295, centrioles form in the S/G2 phase and lose their associated cartwheel in mitosis but cannot be converted to centrosomes, uncoupling the two events. Strikingly, centrioles devoid of both the PCM and the cartwheel progressively lose centriolar components, whereas centrioles associating with either the cartwheel or PCM alone can exist stably. Thus, cartwheel removal can have grave repercussions to centriole stability, and centriole-to-centrosome conversion mediated by CEP295 must occur in parallel to maintain cartwheel-less centrioles for duplication.

INTRODUCTION

Centrioles in cycling cells are carefully maintained. They duplicate exactly once in S phase, and they segregate equally through association with spindle poles during cell division. Centrioles can duplicate and associate with spindle poles only when they have been converted to centrosomes (Wang et al., 2011). The conversion of centrioles to centrosomes is a critical step of centriole modification in which newborn, unmodified centrioles acquire the competence to recruit the pericentriolar material (PCM) and thereby function as the microtubule-organizing center (MTOC)

or centrosome. Modification starts in early mitosis, depending on Plk1 activity, and completes at late mitosis, giving rise to one old/previously converted and one newly converted centriole that are both MTOC competent to start the new cell cycle. The converted centriole duplicates and then carries the newly formed, unconverted daughter centriole to which it is engaged through the segregation process, ensuring centriole homeostasis. While Plk1 plays an essential role in centriole-to-centrosome conversion, it is also required for many other cellular processes during mitosis (Petronczki et al., 2008), including centriole disengagement (Wang et al., 2011). No centriolar factors specifically involved in centriole-to-centrosome conversion have been identified.

Centrioles are stable structures. The integrity of a centriole is in part contributed by a geometric scaffold known as the cartwheel, which defines the nine-fold symmetry of a centriole (Anderson and Brenner, 1971). The cartwheel is located at the proximal lumen of a centriole, coincident with centriolar proteins SAS-6 (Nakazawa et al., 2007) and STIL/SAS-5 (Stevens et al., 2010). In particular, SAS-6 has been shown to form the primary backbone of the cartwheel (Kitagawa et al., 2011; van Breugel et al., 2011). Surprisingly, while the cartwheel is essential for centriole assembly, it is removed from newborn centrioles at the end of the cell cycle (Vorobjev and Chentsov, 1980; Vorobjev and Chentsov, 1982), when both SAS-6 and STIL are disassociated from centrioles during early mitosis (Arquint and Nigg, 2014), and degraded subsequently (Arquint and Nigg, 2014; Arquint et al., 2012; Strnad et al., 2007; Tang et al., 2011), generating “cartwheel-less” centrioles that appear to be stable.

The function of cartwheel removal is not clear. Recent studies showed that the empty proximal lumen of the cartwheel-less centriole can function as a template for SAS-6 assembly prior to daughter centriole formation, revealing a potential role of cartwheel elimination in centriole duplication (Fong et al., 2014). While the stability of centrioles can be maintained in the absence of the cartwheel, the underlying mechanism is unclear. Interestingly, cartwheel removal occurs during mitosis, when newborn centrioles are converted to centrosomes. When the conversion is blocked by Plk1 inhibition, cartwheel removal also fails to occur (Wang et al., 2011), revealing an intimate relationship between these two events.

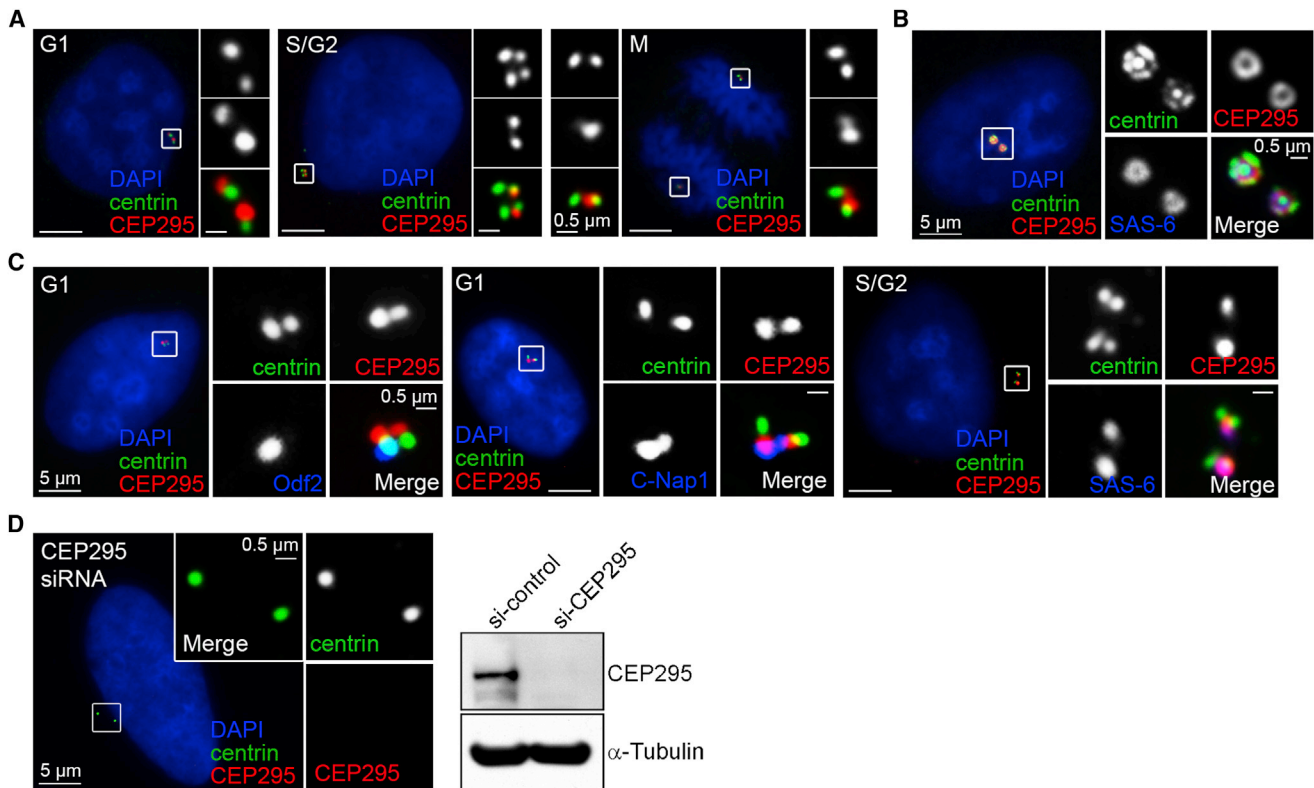


Figure 1. CEP295 Is a Daughter-Enriched Proximal-End Protein

(A) U2OS cells at different cell-cycle stages were stained with indicated antibodies. DAPI (blue) marks nuclei.
 (B) CEP295 localizes to the extra daughter centrioles of the rosette centrosome, inducibly formed by Plk4 overexpression in RPE1 cells.
 (C) G1 or S/G2 centrioles were visualized with indicated antibodies, including the proximal-end marker C-Nap1 or SAS-6 and the distal-end marker centrin or Odf2.
 (D) CEP295 signals were lost in CEP295-RNAi cells, revealed by both immunostaining and western blot.

RESULTS

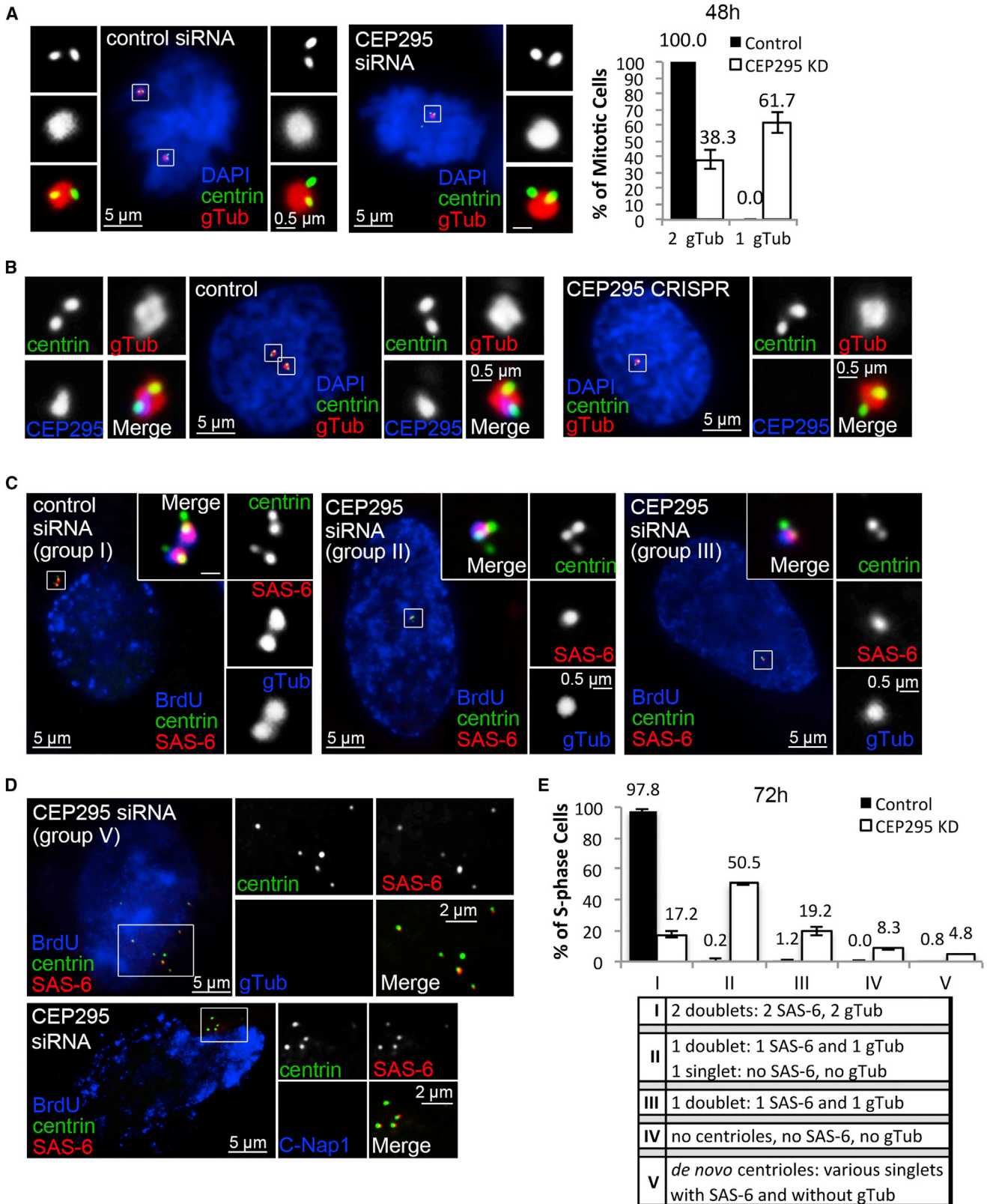
Identification of CEP295 as a Daughter Centriole-Enriched Proximal-End Protein

To screen for centriolar factors specifically involved in centriole-to-centrosome conversion, we focused on proteins that were enriched in newborn (unconverted) centrioles, using the “proteomic map” of the centrosome generated recently (Tanos et al., 2013; Wang et al., 2013). The study of one protein, KIAA1731, hereafter named CEP295, is presented. KIAA1731/CEP295 has been previously characterized as an essential factor for centriole assembly or stability (Knorz et al., 2010), but its exact role in centriole biogenesis and maintenance remains unclear. Our immunofluorescence studies showed that CEP295 associates with both of the (converted) centrioles during G1 (Figure 1A) but becomes more enriched at the newly formed daughter (or unconverted) centrioles during S, G2, and early M phases (Figures 1A and 1C), a pattern distinct from other known daughter centriole proteins. Consistently, CEP295 was found to label the extra daughter centrioles induced by overexpression of Plk4 (Figure 1B, centriole rosettes). Detailed examination further revealed that CEP295 colocalizes with the proximal-end markers C-Nap1 or SAS-6 (Figure 1C), but not the distal-end proteins

centrin or ODF2 (Figure 1C). Note that the signal recognized by CEP295 antibodies is specific, as it disappeared in cells depleted of CEP295 by RNAi (Figure 1D) or by clustered regularly interspaced short palindromic repeats (CRISPR)-mediated gene targeting (Figure 2B). Together, our data demonstrate that CEP295 localizes to all centrioles at the proximal end, with a strong preference at newly formed daughter centrioles.

Loss of CEP295 Has No Effect on Initial Centriole Assembly but Leads to Centrosome Reduction

Consistent with the previous report (Knorz et al., 2010), knock-down of CEP295 by small interfering RNA (siRNA) quickly resulted in centrosome number reduction (Figure 2A). The same defect was also seen by CRISPR/Cas9-mediated gene targeting (Figure 2B) (Mali et al., 2013). Specifically, only one γ -tubulin focus was seen in most M phase cells depleted of CEP295 (Figures 2A and 2B), suggesting a potential defect in centriole duplication. Surprisingly, however, a pair of centrioles (or a centrin doublet), instead of a singlet, was always observed within the remaining γ -tubulin focus (Figures 2A and 2B), indicating that while the number of centrosomes (γ -tubulin foci) reduces, loss of CEP295 does not seem to abolish centriole assembly. To more carefully examine centriole duplication, S phase cells treated



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with control or CEP295 siRNA for 72 hr were examined for centrin, γ -tubulin, and the cartwheel protein SAS-6. Control S phase cells uniformly carried two centriole doublets (or two duplicated centriole pairs), each of which was marked by γ -tubulin and SAS-6 (Figures 2C and 2E; group I). In contrast, most of the CEP295-depleted cells either had one doublet and one singlet (group II) or one doublet alone (group III), with SAS-6 and γ -tubulin present only on the doublet (Figures 2C and 2E). Other centriolar proteins such as CPAP, CP110, and centrin also properly localize to these centriole doublets formed in the absence of CEP295 (Figures S1A–S1C). Moreover, electron microscopy analyses also revealed the duplication of centrioles in the absence of CEP295 (Figure S1D). Interestingly, in a small percentage of CEP295-depleted cells, no γ -tubulin foci could be detected (Figure 2E; group IV and V); instead, de novo centrioles appeared to form in some of these acentrosomal cells (Figures 2D and 2E; group V), as indicated by the presence of variable number of centrin singlets marked with SAS-6 (Figure 2D) but lacking C-Nap1 or PCM-associated γ -tubulin (Figure 2D) (Wang et al., 2011). Note that these de novo centrioles are distinct from the centriole singlet described above in group II (Figures 2C and 2E), which was devoid of SAS-6 (Figure 2C). Together, these findings suggest that CEP295 is not critically required for initial centriole assembly per se. Instead, the reduction of the centrosome number throughout the cell cycle suggests a potential role of CEP295 in centriole-to-centrosome conversion.

CEP295 Is Required for Centriole-to-Centrosome Conversion but Dispensable for Cartwheel Removal or Centriole Disengagement

To test if CEP295 depletion blocks centriole-to-centrosome conversion that normally occurs at the end of mitosis, G1 centrioles exiting from mitosis were examined for γ -tubulin or C-Nap1, two markers of MTOC-competent centrioles (Wang et al., 2011). Control G1 cells displayed two γ -tubulin or C-Nap1 foci, one at each centriole, indicating that both centrioles are MTOC competent (Figure 3A). In CEP295-depleted cells, however, only the mother centriole that had previously acquired the MTOC competency, as marked by Odf2, carried the γ -tubulin or C-Nap1 signal (Figure 3A), whereas the majority of daughter centrioles born in the previous S phase remained devoid of C-Nap1 or γ -tubulin (Figure 3A). These results indicate that CEP295 is essential for the conversion of newborn centrioles to centrosomes but is dispensable for centrioles that have already converted, similar

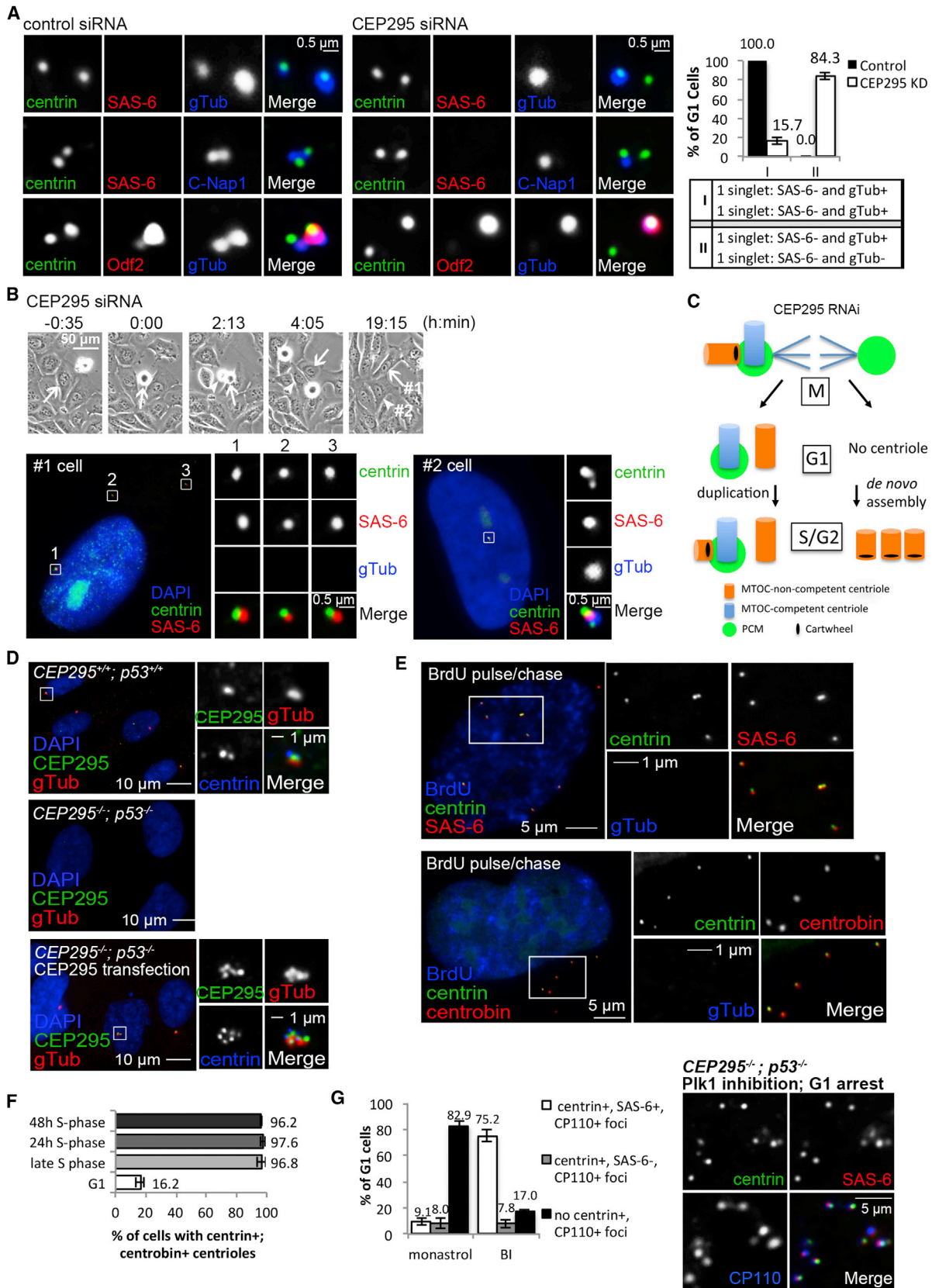
to the function of Plk1 (Wang et al., 2011). Interestingly, however, while centriole disengagement and cartwheel removal at mitosis both depend on Plk1 (Wang et al., 2011), they occur normally in the absence of CEP295, as revealed by the extensive separation of the mother and daughter centrioles and the loss of the SAS-6 signal from the daughter centriole (Figure 3A). Together, we demonstrate that the conversion of centrioles to centrosomes is specifically mediated by CEP295 and can be uncoupled from cartwheel removal or centriole disengagement.

We next explored the fate of CEP295-depleted cells entering mitosis with one centrosome (Figures 2A and 2B). If these cells manage to divide, daughter cells inheriting no centrosome/centriole would arise, which in turn may trigger de novo centriole assembly as described above (Figure 2D) and previously (Khodjakov et al., 2002). A correlated time-lapse/immunofluorescence microscopy assay was performed to examine if de novo centrioles were indeed formed in this sequence of events. Cells grown on a gridded coverslip were depleted of CEP295 by RNAi and filmed for 72 hr before fixation. Cells carrying de novo centrioles were identified by immunofluorescence microscopy, and their sister cells were traced and located through the time-lapse movie. In all nine cells containing de novo centrioles whose sisters could be located, every sister was found to carry a duplicated centriole pair, i.e., a centrin doublet marked by SAS-6 (Figure 3B), with or without the extra centriole singlet (see below in Figure 4). This result supports the scenario (Figure 3C) that after cell division, one daughter cell inherits the only active centrosome in which the associated MTOC-competent centriole duplicates in the following S phase, whereas the sister cell that receives no centrosome undergoes de novo centriole assembly (Figure 3C).

To confirm that de novo centrioles form efficiently in the absence of CEP295, we generated a stable RPE1 cell line in which both *CEP295* and *p53* genes were mutated by CRISPR-mediated gene targeting (*CEP295*^{-/-}; *p53*^{-/-}), as cells devoid of centrosomes are not viable in the presence of p53 (Bazzi and Anderson, 2014). As expected, no centrosomes could be detected in proliferating *CEP295*^{-/-}; *p53*^{-/-} cells (Figure 3D; Figure S2), but strikingly, for cells that had entered S phase for at least 4 hr, nearly all (>90%, n > 100) carried some numbers of de novo centrioles marked by centrin, SAS-6, and centrin (Figure 3E), confirming that de novo centriole assembly initiates efficiently, but none are converted to centrosomes in the absence of CEP295. Note that the centrosome assembly defect in *CEP295*^{-/-}; *p53*^{-/-} cells can be rescued by putting back

Figure 2. Loss of CEP295 Does Not Block Centriole Assembly but Leads to Centrosome Reduction

- (A) U2OS cells transfected with control or CEP295 siRNA for 48 hr were stained with indicated antibodies. γ -Tubulin (gTub) marks active centrosomes. Only mitotic centrosomes were examined (boxed and magnified) and quantified (n > 50, from three independent experiments, right). Error bars represent SD.
- (B) RPE1 cells in which CEP295 gene was mutated by CRISPR/Cas9 gene targeting were analyzed and stained with the indicated antibodies. Control cells were nucleofected with Cas9 and empty gRNA vectors. Early prophase cells are shown. Approximately 15% of cells were effectively targeted by CRISPR (not shown).
- (C) Centriole duplication was analyzed in control or CEP295-RNAi cells in S phase 72 hr after transfection with the indicated antibodies. BrdU was added 30 min before fixation. Unlike control cells carrying two centriole doublets (group I), the majority of CEP295-RNAi cells contained only one centriole doublet, with or without an additional centrin singlet devoid of SAS-6 (group II and III).
- (D) De novo centrioles were found in a small percentage of CEP295-depleted cells lacking centrosomes (group V), labeled with centrin and SAS-6, but not with γ -tubulin or C-Nap1.
- (E) Quantification of centriole duplication (n > 160, from three independent experiments). Four groups of duplication defects were seen in CEP295-depleted cells as indicated (II–V). Error bars represent SD.
- See also Figure S1.



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wild-type CEP295 (Figure 3D), confirming the specificity. Together, we conclude that while CEP295 is not required for initial centriole assembly, it enables centriole-dependent centriole duplication by converting centrioles to centrosomes.

Centriole-to-Centrosome Conversion Stabilizes Cartwheel-less Centrioles

Vertebrate centrioles in cycling cells are associated with either the cartwheel or PCM, suggesting that neither plays a role in centriole stability, or alternatively, both elements function redundantly to stabilize centrioles. To differentiate between these two possibilities, centrioles devoid of both the cartwheel and PCM (or “DCP” centrioles for simplicity), which form at the end of mitosis in CEP295-depleted cells, were examined for their stability. We first examined if de novo centrioles formed in CEP295^{-/-}; p53^{-/-} cells are stable after mitosis (or cartwheel removal). Strikingly, while de novo centrioles were consistently present in nearly all late S phase or prolonged S phase-arrested cells (Figures 3E and 3F; Movie S1), they could not be detected in most postmitotic cells arrested in G0/G1 (Figure 3F), revealing an intriguing phenotype that de novo centrioles are repeatedly formed (in S phase) and lost (after mitosis) every cell cycle in CEP295^{-/-}; p53^{-/-} cells. To test if cartwheel removal underlies the loss of these de novo centrioles, CEP295^{-/-}; p53^{-/-} cells were allowed to pass through mitosis in the absence of Plk1 activity, a treatment known to retain the cartwheel in centrioles (Tsou et al., 2009; Wang et al., 2011). Strikingly, in the presence of the cartwheel, de novo centrioles became stable in most of postmitotic CEP295^{-/-}; p53^{-/-} cells arrested in G1 (Figure 3G).

To ensure that loss of stability is not just for de novo centrioles, we tracked the fate of the engaged, newly duplicated daughter centriole in CEP295-RNAi cells. In early mitosis, before being converted to DCP centrioles, newborn daughter centrioles devoid of CEP295 were positively stained for acetylated α -tubulin (100%, n = 139) (Figure 4A, right), a marker for stabilized microtubules at the core of centrioles. Intriguingly, after mitosis, unlike converted mother centrioles that continued to be stable, DCP centrioles formed at the end of mitosis progressively lost centriolar components in the following interphase (Figures 4B and 4C). Specifically, in G1 phase, unlike control

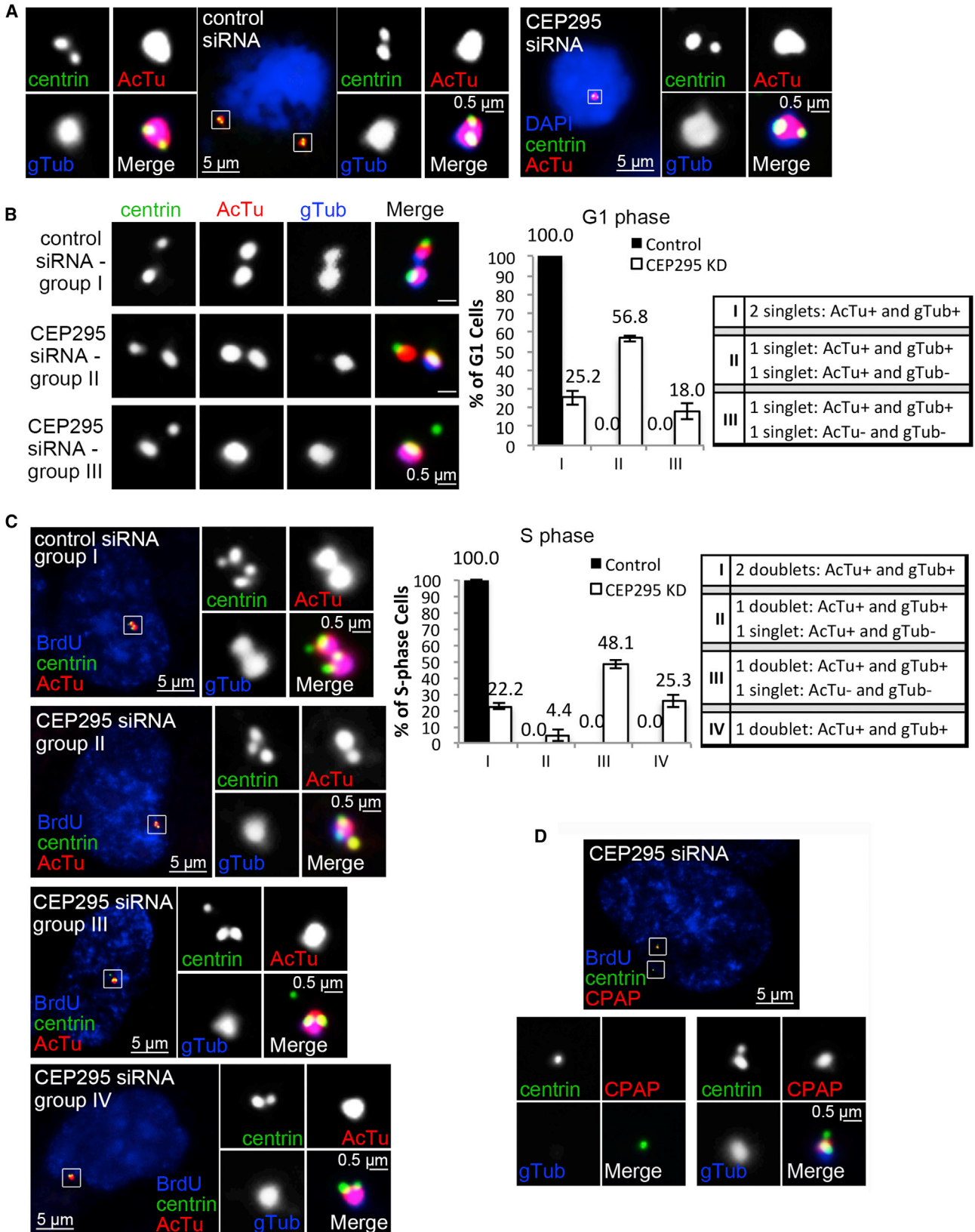
centrioles, which were all converted and associated with acetylated α -tubulin (Figure 4B; gTub⁺ and AcTu⁺ singlets in group I), DCP centrioles in 18% of RNAi-treated cells had lost the acetylated α -tubulin staining (Figure 4B; gTub⁻ and AcTu⁻ singlets in group III). More strikingly, by S phase, in contrast to converted centrioles seen both in control and CEP295-RNAi cells, which all stably duplicated (Figure 4C; doublets in group I-IV), DCP centriole singlets that remain positively stained for both centrin and acetylated α -tubulin were no longer seen in >90% of RNAi-affected cells (Figure 4C; group III and IV). Similarly, these DCP centrioles also lost the centriolar protein CPAP (Figure 4D) known to associate with centriolar microtubules (Hung et al., 2004). These results together indicate that while converted centrioles can exist stably in the absence of both the cartwheel and CEP295, unconverted centrioles formed in the absence of CEP295 become unstable after cartwheel removal.

Cartwheel Is Essential for the Maintenance of Newborn Centrioles

To further test if the cartwheel is also essential for the stability of newborn centrioles formed in the presence of CEP295, we examined the effect of cartwheel removal in wild-type, S phase cells in which centriole duplication had already occurred prior to SAS-6 depletion (Figure S3). In brief, cells transfected with control or SAS-6 siRNA were pulsed with bromodeoxyuridine (BrdU) at 32 hr after the transfection (32 hr post-RNAi), followed immediately by S phase arrest (Figure S3A). These BrdU-labeled, S phase-arrested cells were then fixed at 36 hr, 48 hr, 60 hr, or 72 hr post-RNAi and examined for centrin, SAS-6, and C-Nap1. We found that at 36 hr, 48 hr, and 60 hr after SAS-6 RNAi, the majority of BrdU-positive cells (~70%) still contained two duplicated centriole pairs (doublets), each of which is associated with SAS-6 (Figure S3B; group I, see quantifications). However, at the 72 hr time point, the same population of cells that still carried normal centriole pairs dropped abruptly, with a significant increase in the number of cells in which the SAS-6 signal (or cartwheel) was lost. More importantly, most of these cells losing the SAS-6 signal carried only two centriole singlets, each of which was marked with C-Nap1 (group II; see Figure S3B quantifications), indicating that previously existing

Figure 3. Loss of CEP295 Blocks Centriole-to-Centrosome Conversion but Has No Effect on Centriole Disengagement and Cartwheel Removal

- (A) Control and CEP295 siRNA-treated cells were transiently labeled with BrdU before fixation. G1 cells, BrdU negative (not shown) and carrying two centrin singlets, were examined with indicated antibodies. Quantifications (n > 100, from three independent experiments) are shown (right). Error bars represent SD.
- (B) Cells grown on a gridded coverslip were treated with CEP295 siRNA and filmed for 72 hr before fixation. A cell carrying de novo centrioles was identified (arrow; #1 cell), and its sister cell from the previous cell division was traced through the time-lapse movie (arrowhead; #2 cell) and examined with indicated antibodies.
- (C) Schematic summary of the phenotypes seen in CEP295-depleted cells.
- (D) Unsynchronized wild-type or CEP295^{-/-}; p53^{-/-} RPE1 cells, and CEP295^{-/-}; p53^{-/-} cells transiently expressing full-length CEP295 were stained with the indicated antibodies.
- (E) Unsynchronized CEP295^{-/-}; p53^{-/-} cells pulsed with BrdU for 30 min and chased for 4 hr were stained with the indicated antibodies.
- (F) CEP295^{-/-}; p53^{-/-} cells in late S phase as described in (E) or arrested in S or G1 phase for 24–48 hr as indicated were examined with centrin and centrobilin antibodies. Quantifications are shown (n > 100, from three independent experiments). Error bars represent SD.
- (G) CEP295^{-/-}; p53^{-/-} cells were allowed to enter mitosis in the presence of the Plk1 inhibitor (BI-2536) or Eg5 inhibitor (monastrol) as a control and release to and arrest at G1 by Cdk inhibition with roscovitine for 16 hr before fixation and staining with the indicated antibodies. Cells displaying donut-shaped, multilobed, or multiple small nuclei (Tsou et al., 2009) were identified for analyses. In Plk1-inhibited cells arrested in G1, centrioles were stably present and retained the cartwheel and other markers, as shown in the panel on the right. Quantifications are shown (n > 50, from four independent experiments). Error bars represent SD. See also Figure S2 and Movie S1.



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newborn centrioles either are detached or become unstable upon SAS-6/cartwheel removal. Note that we also observed what appears to be an intermediate phenotype in a small percentage of cells (group IV), where newborn centrioles were seen nearby the mother centriole despite losing the SAS-6 signal (Figure S3B; group IV). To determine if the “lost” newborn centrioles were present somewhere in the cell, we examined centriole distal-end markers centrin, CEP162, and CP110, which are known to associate with all centrioles in S phase (Figure S3C) (Tsang et al., 2008; Wang et al., 2013). Intriguingly, in contrast to mother centrioles that could be reliably detected, no detached newborn centrioles marked with centrin and CEP162 or CP110 signals could be found in the majority of SAS-6 depleted cells, as revealed by the maximum projection of z stack images (Figure S3C). Thus, the SAS-6-based cartwheel is essential for the proper maintenance of newborn centrioles, even when they are born with CEP295. These results together support the idea that vertebrate centrioles associate with either the cartwheel or PCM to maintain their stable composition and that cartwheel removal can be detrimental if cartwheel-less centrioles are not converted to centrosomes.

DISCUSSION

Centriole-to-centrosome conversion at late mitosis, which promotes the centriole to acquire the PCM, is essential for cycling cells to maintain centriole homeostasis, as both centriole duplication and segregation fully depend on it (Wang et al., 2011). Here, we identify CEP295 as a newborn centriole-enriched factor mediating this process and surprisingly discover that centriole-to-centrosome conversion also has an essential role in stabilizing cartwheel-less centrioles. In particular, we found that (1) when centrioles are devoid of both the PCM and cartwheel, they become unstable, even in the presence of CEP295; and (2) when centrioles are associated with either the PCM or cartwheel, they are perfectly stable, even in the absence of CEP295. These results together suggest that cartwheel removal can be harmful to centrioles and that centriole-to-centrosome conversion mediated by CEP295 is required to neutralize such negative impacts.

Our findings, however, raise a puzzling question as to why centrioles risk their stability to discard the cartwheel in the first place. The cartwheel is a geometric scaffold upon which all centrioles are assembled, but vertebrate centrioles naturally lose it before they support duplication in the next S phase. Both centriole disengagement and centriole-to-centrosome

conversion occurring at the late mitosis are known to enable centriole duplication, raising a possibility that cartwheel removal may function in the same process. Our previous studies showed that unconverted (or unmodified) centrioles, which carry the cartwheel, cannot support duplication even when they are disengaged (Wang et al., 2011). This is consistent with the idea that perhaps cartwheel removal and centriole-to-centrosome conversion work together to enable newborn centrioles for duplication, whereas centriole disengagement merely “licenses” the reduplication of mother centrioles that are otherwise competent to duplicate. Interestingly, recent studies showed that the empty lumen of the cartwheel-less centriole can function as a geometric template to shape SAS-6 assembly prior to centriole duplication (Fong et al., 2014), a process that may underlie the preservation of centriole number and structure in cycling cells. We thus speculate that loss of the cartwheel is specifically programmed in cycling cells to control centriole-dependent centriole duplication, while its repercussion, i.e., centriole destabilization, is shielded by centriole-to-centrosome conversion. More experiments are required to test these ideas.

EXPERIMENTAL PROCEDURES

CRISPR and $p53^{-/-}$; $CEP295^{-/-}$ Cell Lines

RNA-guided targeting of CEP295 in human RPE1 cells was achieved through coexpression of the Cas9 protein with guide RNAs (gRNAs) as described by the Church group (Mali et al., 2013). RPE1 cells nucleofected with Cas9 plasmid and gRNA were examined for the loss of CEP295 at 5, 6, or 7 days after nucleofection. We consistently see loss of the CEP295 signal in ~15% of transfected cells. $CEP295^{-/-}$ cells can divide but eventually die/arrest depending on p53 (Bazzi and Anderson, 2014). To obtain stable cell lines lacking CEP295, the p53 gene (*TP53*) was mutated by the same CRISPR method prior to targeting of CEP295, generating $p53^{-/-}$; $CEP295^{-/-}$ cells. $CEP295^{-/-}$; $p53^{-/-}$ cells proliferate actively (Figure S2), although mitosis is slightly lengthened due to lack of centrosomes.

Details of experimental procedures are provided in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, one figure, and one movie and can be found with this article online at <http://dx.doi.org/10.1016/j.celrep.2014.07.022>.

AUTHOR CONTRIBUTIONS

D.I. and M.-F.B.T. designed experiments. D.I. performed most of the experiments. W.-J.W. and K.U. performed electron microscopy analyses. W.-J.W.

Figure 4. Centriole-to-Centrosome Conversion Stabilizes Centrioles Devoid of the Cartwheel

(A) Mother centrioles and their engaged daughter centrioles in cells treated with control (left) or CEP295 siRNA (right) for 72 hr were examined at early mitosis with antibodies against centrin, γ -tubulin (gTub), and acetylated α -tubulin (AcTu).
 (B) Cells treated with control or CEP295 siRNA for 72 hr were labeled with BrdU before fixation. G1 cells, BrdU negative (not shown) and inheriting a pair of disengaged centrioles, were examined with indicated antibodies. Quantifications are shown (right) ($n > 100$, from three independent experiments). Error bars represent SD. Note that in CEP295 siRNA-treated cells, the majority of daughter centrioles failed to convert to centrosomes (group II and III; gTub⁻), some of which also lost the AcTu staining (group III) (gTub⁻ and AcTu⁻).
 (C) Cells were treated as described in (B). BrdU-positive cells inheriting active centrosomes (gTub foci) were examined with indicated antibodies. Cells inheriting no centrosomes were excluded. Quantifications are shown (right) ($n > 100$, from three independent experiments). Error bars represent SD. Note that in the majority of CEP295 RNAi cells, the inherited DCP/daughter centrioles either became nondetectable (group IV) or had lost the AcTu staining (group III) (gTub⁻; AcTu⁻).
 (D) DCP centrioles in S phase cells were examined for the localization of CPAP with the indicated antibodies.
 See also Figure S3.

helped perform CRISPR/Cas9 gene targeting and time-lapse fluorescence microscopy. D.I. and M.-F.B.T. wrote the manuscript.

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