Par6α Interacts with the Dynactin Subunit p150GLUED and Is a Critical Regulator of Centrosomal Protein Recruitment

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Submitted May 13, 2010; Revised July 28, 2010; Accepted August 5, 2010
Monitoring Editor: Stephen Doxsey

The centrosome contains proteins that control the organization of the microtubule cytoskeleton in interphase and mitosis. Its protein composition is tightly regulated through selective and cell cycle–dependent recruitment, retention, and removal of components. However, the mechanisms underlying protein delivery to the centrosome are not completely understood. We describe a novel function for the polarity protein Par6α in protein transport to the centrosome. We detected Par6α at the centrosome and centriolar satellites where it interacted with the centriolar satellite protein PCM-1 and the dynactin subunit p150GLUED. Depletion of Par6α caused the mislocalization of p150GLUED and centrosomal components that are critical for microtubule anchoring at the centrosome. As a consequence, there were severe alterations in the organization of the microtubule cytoskeleton in the absence of Par6α and cell division was blocked. We propose a model in which Par6α controls centrosome organization through its association with the dynactin subunit p150GLUED.

INTRODUCTION

The composition of the centrosome and its function in microtubule organization in interphase and mitosis are critical for cell homeostasis. A newly formed daughter cell contains two orthogonally arranged centrioles, which are characterized by a unique set of proteins at their proximal and distal ends (Strnad and Gonczy, 2008). The two centrioles are surrounded by electron-dense pericentriolar material (PCM), which contains proteins necessary for microtubule nucleation and anchoring (Bornens et al., 1987; Bobiniec et al., 1998). After centrosome duplication in S-phase, the two centrosomes move to opposite sides of the nucleus to form the poles of the mitotic spindle. Each daughter cell inherits one spindle pole that then becomes its centrosome. Abnormalities in centrosome number and organization promote chromosome segregation errors and aneuploidy and may contribute to the development of cancer (Nigg, 2006; Ganem et al., 2009).

An interphase centrosome contains at least 100 proteins that are recruited by different mechanisms (Andersen et al., 2003). AKAP450 and pericentrin contain specific localization motifs that are sufficient to target these large scaffold proteins to the centrosome without a requirement for intact microtubules (Gillingham and Munro, 2000). BBs6 and Poc5 also reach the centrosome in a dynein–microtubule-independent manner, although specific localization domains within these centrosomal proteins have not yet been identified (Kim et al., 2005; Azimzadeh et al., 2009). In contrast, PCM-1, centrin, and ninein are transported along microtubules to the centrosome by the dynein–dynactin motor complex (Dammermann and Merdes, 2002). These centrosomal proteins are mislocalized if microtubules are depolymerized or if dynein function is disrupted (Balczon et al., 1999; Kubo et al., 1999; Dammermann and Merdes, 2002; Hames et al., 2005). Studies with γ-tubulin have revealed a role for microtubules in the initial recruitment to the centrosome from the cytosol, but not in its retention at the centrosome (Khodjakov and Rieder, 1999; Young et al., 2000), indicating that there are differential microtubule requirements during centrosomal protein localization.

The dynein–dynactin complex is involved in the regulation of centrosome organization and function. Dynein is a minus end–directed microtubule motor that depends on the multisubunit complex dynactin for the regulation of its processivity and cargo interaction (King and Schroer, 2000; Holleran et al., 2001; Kardon and Vale, 2009). The dynein–dynactin complex is necessary for centrosomal delivery of proteins that maintain the radial organization of microtubules at the centrosome (Quintyne et al., 1999; Dammermann and Merdes, 2002). Intriguingly, there are differences in the association of dynein and dynactin with the centrosome during the cell cycle (Quintyne and Schroer, 2002). Dynactin is detected at the centrosome throughout the cell cycle, whereas dynein localizes to the centrosome in S-phase and G2, but not during mitosis (Quintyne and Schroer, 2002). Both complexes control microtubule anchoring and retention via a mechanism that is distinct from the role of the dynein–dynactin complex in the delivery of microtubule-organizing proteins to the centrosome (Quintyne et al., 1999; Quintyne and Schroer, 2002; Burakov et al., 2008).

Centriolar satellites have been implicated in microtubule-dependent protein transport to the centrosome, but their exact role in this process remains unclear. These spherical cytosolic granules are often found in the vicinity of the centrosome and are characterized by the presence of PCM-1,
BB54, and Cep290 (Balczi et al., 1994; Kubo et al., 1999; Kim et al., 2004; Kim et al., 2008). PCM-1 is proposed to serve as a scaffold for centrosomal cargo proteins, including centrin, ninein, and Nek2 (Zimmerman and Doyxsey, 2000; Dammermann and Merdes, 2002; Hames et al., 2005). BB54 and Cep290 may have roles in linking PCM-1 to the dynein motor complex through binding to the dynactin subunits p150Glued and p50 dynamin (Kim et al., 2004; Kim et al., 2008; Chang et al., 2006). Thus, centriolar satellite components and the dynein–dynactin motor complex appear to form an interaction network that is important for protein delivery to the centrosome. In support of this model, inactivation of any one of these three centriolar satellite proteins causes defects in centrosomal protein trafficking and aberrant microtubule organization (Dammermann and Merdes, 2002; Kim et al., 2004; Kim et al., 2008). As centriolar satellites move toward the centrosome in a dynein- and microtubule-dependent mechanism (Kubo et al., 1999; Zimmerman and Doyxsey, 2000), it is possible that they promote the association of cargo with the dynein motor complex for efficient transport to the centrosome.

Par6, a member of the Par6 family of polarity proteins, plays an important role in the control of centrosome organization and function (Solecki et al., 2004). Par6 was identified in Caenorhabditis elegans zygotes as a critical regulator of asymmetric cell division (Watts et al., 1996; Hung and Kemphues, 1999; Lin et al., 2000), and homologues have been found in other species. There is a single Par6 gene in C. elegans and in Drosophila melanogaster, but mammalian cells contain four isoforms that are called Par6A–D (Joberty et al., 2000; Gao and Macara, 2004). Only Par6B (Par6β) and Par6C (Par6α) are expressed in HeLa cells (Joberty et al., 2000). It is well established that Par6 proteins regulate cell polarity, cytoskeletal rearrangement, and the assembly of tight junctions by forming a complex with the activated form of Cdc42, atypical protein kinase C (PKCζ) and the polarity protein Par3 (Lin et al., 2000; Gao et al., 2002; Etienne-Manneville et al., 2005). Solecki and colleagues reported an additional role for Par6α in centrosome organization by showing that its depletion in neurons caused mislocalization of centrosomal proteins, alterations in the perinuclear tubulin cage and defects in centrosome movement during cell migration (Solecki et al., 2004).

In this study, we present our results on the role of Par6α at the centrosome of epithelial cells. Par6α localized to the centrosome and centriolar satellites by a mechanism that involved microtubules and the dynein motor complex. RNAi-mediated depletion of this protein caused the mislocalization of specific centrosomal proteins, including regulators of microtubule anchoring. Thus, in the absence of Par6α, there were defects in microtubule organization in interphase, but also in mitosis, which affected cell cycle progression. Par6α associated with the dynactin subunit p150Glued and was necessary for its localization to the centrosome. We propose that Par6α-mediated centrosome regulation involves the binding of Par6α to p150Glued.

MATERIALS AND METHODS

Antibodies

The following antibodies were used in this study: anti-Par6α (Dr. R. Lammers, University of Tubingen, Tübingen, Germany, and T-20 Lot L0406, Santa Cruz Biotechnology, Santa Cruz, CA), anti-Par6/β (Santa Cruz Biotechnology), anti-PCM-1 (Dr. A. Merdes CNRS Toulouse, Toulouse, France, and H-262, Santa Cruz Biotechnology), anti-centrin (Dr. J. Salisbury, Mayo Clinic, Rochester, MN), CPAP (Dr. P. Gonczy, EPFL, Lausanne, Switzerland), anti-Cep170 (Dr. G. Guarguagli, University of Rome, Rome, Italy), anti-Cep192 (Dr. D. Sharp, Albert Einstein College of Medicine, New York, NY), anti-kendrin/peri-}

To the centrosome.

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Par6α staining in the centrosomal region was seen in HeLa cells and in other human cell lines, including U2-OS and hTERT-RPE-1 (data not shown). To verify the specificity of this staining pattern, we depleted Par6α by RNA interference (RNAi). This treatment led to the absence of a 37-kDa protein by Western blotting (Figure 1B) and the loss of centrosomal staining by immunofluorescence microscopy (Figure 1C). Because depletion of Par6α had no effect on the levels of the 45-kDa Par6α isoform (Figure 1B, right), we conclude that our antibody specifically recognizes Par6α.

To corroborate Par6α as a centrosomal component, we examined centrosome-enriched fractions for the presence of Par6α by Western blot analysis. These fractions were isolated by gradient centrifugation of total HeLa cell lysates and contained Par6α and the centrosomal marker proteins, centrin, γ-tubulin, and PCM-1 (Figure 1D). Par6α was also found in cytosolic fractions on top of the gradient, indicating that this protein also exists in the cytosol.

We next examined the association of Par6α with the centrosome and centriolar satellites during the cell cycle. We costained a nonsynchronous population of HeLa cells with antibodies to Par6α and PCM-1, marking the position of the centrosome by staining for γ-tubulin. Par6α colocalized with PCM-1 at the centrosome and at centriolar satellites during interphase and at spindle poles during prometaphase, followed by dissociation from spindle poles between metaphase and telophase (Figure 1E). These results suggest that the association of Par6α with the centrosome and centriolar satellites is regulated during the cell cycle in a manner similar to PCM-1 (Figure 1E, middle panel; Dammermann and Merdes, 2002).

**Par6α Controls the Centrosome during the Cell Cycle**

We analyzed the function of Par6α at the centrosome. As a measure of centrosome organization, we monitored the localization of known centrosomal proteins in cells depleted of Par6α by RNAi. We first looked at proteins that reach the centrosome by a microtubule-dependent mechanism (pericentrin, PCM-1, centrin, and ninein). The centrosomal levels of these proteins were substantially reduced in Par6α-depleted cells when compared with control cells transfected with scrambled RNAi (Figure 2, A and B). In contrast, the
localization of γ-tubulin, whose retention at the centrosome is reported to be independent of microtubules (Khodjakov and Rieder, 1999), was not affected by the absence of Par6α (Figure 2, A and B). We also examined the effects of Par6α depletion on proteins of proximal (Cep192, hSAS6) and subdistal (Cep170) regions within centrioles. In addition, we analyzed the distribution of CPAP, which is likely to localize to the proximal and subdistal region of the centriole through

Figure 2. Par6α-depleted cells contain disorganized, nonfunctional centrosomes. (A) Control and Par6α-depleted cells were stained with antibodies to Par6α (red, 6) to confirm protein depletion, to γ-tubulin (γ, cyan) and to PCM-1 (P), centrin (C), CPAP (C), ninein (N), and Cep170 (7; all in green) to monitor centrosomal protein organization. Scale bar, 5 μm. (B) Quantifications of the fluorescence intensity at the centrosome are shown. The control was defined as 100% and represents the fluorescent signal detected for each marker protein at the centrosome of control siRNA-transfected cells. Fifteen cells were analyzed per experiment, and three independent experiments were performed. The p values for each experiment were <0.005 (paired t test). (C) Microtubule organization was examined by staining control and Par6α-depleted cells with antibodies to Par6α and γ-tubulin. Scale bar, 10 μm. (D) Staining with a specific antibody to phospho-Histone H3 was used to determine the percentage of cells in mitosis (n = 3), *p < 0.001, paired t test. (E) Mitotic control and Par6α-depleted cells were stained with antibodies to phospho-Histone H3, and to α-tubulin and the DNA dye, Hoechst, to visualize spindle organization or DNA alignment, respectively. The percentage of mitotic cells with aberrant spindles is shown (n = 3), *p < 0.001, paired t test. Scale bar, 5 μm.
an association with the subdistal centriole protein Protein 4.1R (Hung et al., 2000; Krauss et al., 2008; Kohlmaier et al., 2009). Cep192 and hSAS6 levels were unaffected by Par6 depletion (data not shown), but the centrosomal levels of Cep170 and CPAP were significantly reduced in the absence of Par6 (Figure 2, A and B). These results indicate that Par6 controls protein recruitment to the centrosome, although its regulatory effects appear limited to specific subsets of proteins.

We next investigated whether Par6 is necessary for centrosome function by visualizing microtubule organization in the absence of Par6. Control cells in interphase contained a radial microtubule array that was nucleated at the centrosome (Figure 2C, top panel). In contrast, microtubules in Par6-depleted cells were randomly arranged in the cytosol or on top of the nucleus (Figure 2C, bottom panel). We also assayed centrosome function in mitosis by examining the ability of Par6-depleted cells to form bipolar spindles. Intriguingly, the percentage of mitotic cells, as detected by staining with an antibody to phospho-Histone H3, increased from 3% in control cells to 14% in Par6-depleted cells, which is indicative of a cell cycle arrest in mitosis (Figure 2D). Of these mitotic Par6-depleted cells, 95% contained multipolar spindles that were unable to align DNA in the metaphase plate (Figure 2, E and F). As a consequence of these spindle abnormalities and the mitotic arrest, Par6-depleted cells eventually died (data not shown). We conclude that Par6 is critical for normal microtubule organization in interphase and mitosis, which are key functions of the centrosome.

**Par6 and PCM-1 Associate with the Centrosome as a Complex**

Because Par6 colocalized with PCM-1 throughout the cell cycle and controlled the localization of PCM-1 in the peri-centriolar region, we tested if these proteins interact. We detected a specific association between Par6 and PCM-1 in reciprocal coimmunoprecipitation experiments (Figure 3A). To test the significance of this interaction for protein localization, we mislocalized Par6 to mitochondria and measured the effect on the intracellular distribution of PCM-1. We targeted Par6 to the mitochondria by fusing the trans-membrane targeting motif of the mitochondrial protein, Tom20 (Kanaji et al., 2000) to GFP-tagged Par6 (Mito-GFP-Par6) or to GFP as a negative control (Mito-GFP; Figure 3B). We verified that each fusion protein associated with mitochondria by colocalization with the mitochondrial dye MitoTracker (Figure 3C, left panel). Intriguingly, PCM-1 was recruited to mitochondria by colocalization with the mitochondrial dye MitoTracker (Figure 3C, left panel). Under these conditions, Mito-GFP-Par6, leading to the partial colocalization of these two proteins at mitochondria. Expression of Mito-GFP, in contrast, had no effect on PCM-1 localization (Figure 3C, right panel, top). Under these conditions, Mito-GFP-Par6 and PCM-1 interacted as shown in parallel coimmunoprecipitation experiments (Figure 3D). Our data indicate that the binding of PCM-1 to Mito-GFP-Par6 is sufficient to direct PCM-1 to mitochondria.

In a reciprocal experiment, we measured the effects of PCM-1 mislocalization on Par6 distribution. Instead of targeting PCM-1 to mitochondria, we expressed a truncated form of PCM-1, GFP-PCM-1AC (amino acids 1-1468), which is reported to induce the formation of large, PCM-1-containing aggregates in the cytosol (Dammermann and Merdes, 2002). Par6 colocalized with PCM-1 at these aggregates (Figure 4A), demonstrating that PCM-1 can also modify the intracellular localization of Par6.

Because these results indicate that a complex of Par6 and PCM-1 is critical for the intracellular localization of these
proteins, we tested the effect of PCM-1 depletion on the localization of Par6α to the pericentriolar region. In cells in which PCM-1 was depleted to 5% of control levels (Figure 4B), there was a significant reduction in the Par6α signal at the centrosome and centriolar satellites (Figure 4C). However, loss of PCM-1 had no obvious effects on centrosomal γ-tubulin or centrin levels (Figure 4C). In addition, microtubule organization and cell cycle progression were normal in the absence of PCM-1 (data not shown). Taken together, these results indicate that Par6α and PCM-1 associate with the centrosome as a complex, but that only Par6α is necessary for the regulation of centrosomal protein composition and function.

Localization of Par6α to the Pericentriolar Region Depends on Intact Microtubules and the Dynein–Dynactin Motor Complex

We examined the role of microtubules and the dynein–dynactin motor complex in the centrosomal localization of Par6α because these factors have been implicated in the delivery of centrosomal proteins via centriolar satellite (Dammermann and Merdes, 2002; Kim et al., 2004; Kim et al., 2008). We first depolymerized microtubules with nocodazole, which leads to the formation of cytoplasmic PCM-1-positive aggregates (Dammermann and Merdes, 2002). Under these conditions, Par6α was mislocalized from the pericentriolar region to these prominent cytosolic structures (Figure 5A). To test the involvement of the dynein–dynactin complex in Par6α localization, we blocked dynein function by overexpressing a tagged form of the dynactin subunit p150Glued (HA-p150Glued). This treatment is reported to disrupt centrosome cohesion, producing two or more γ-tubulin–positive foci (Quintyne et al., 1999). Interestingly, Par6α did not associate with any of these γ-tubulin foci (Figure 5B, left panel). We obtained similar results when we antagonized dynactin function by overexpressing myc-tagged p50-dynamitin (Figure 5B, right panel). In both experiments, cellular Par6α levels were unaffected (Figure 5C), which demonstrates that blocking dynein–dynactin motor function does not cause Par6α degradation but induces its redistribution into the cytosol. These results demonstrate that Par6α is recruited to the centrosome by a microtubule and dynein–dynactin-dependent mechanism.

Par6α Regulates the Centrosomal Localization of p150Glued

Because p150Glued was necessary for the localization of Par6α to the centrosome and centriolar satellites, we examined whether Par6α can bind to p150Glued. In reciprocal coimmunoprecipitation experiments, we detected an interaction between Par6α, p150Glued and PCM-1 (Figure 6A), suggesting that these three proteins form a trimeric complex. As Par6α and p150Glued coimmunoprecipitated in the absence of PCM-1 (Figure 6B), we conclude that PCM-1 is not necessary for Par6α•p150Glued binding and that Par6α is more closely linked to p150Glued than PCM-1. Thus, Par6α and p150Glued appear to form the core of a protein complex that is important for centrosomal protein delivery.

To determine the functional significance of Par6α binding to p150Glued, we tested the effects of Par6α depletion on p150Glued localization. Loss of Par6α caused a significant reduction in centrosomal p150Glued levels to 37.4 ± 1.2% of control levels (Figure 6C, left panel, and D). In contrast, centrosomal p150Glued levels were unaffected in PCM-1—depleted cells and p150Glued remained at 94.8 ± 1.1% of control levels (Figure 6C, right panel, and D). These experiments demonstrate that Par6α is critical for the centrosomal localization of p150Glued, which itself is important for microtubule anchoring at the centrosome (Quintyne et al., 1999).

DISCUSSION

In this study, we have identified a novel role for Par6α in the delivery of centrosomal proteins that function in microtubule organization during interphase and mitosis and ultimately control cell cycle progression. Our findings support a model in which Par6α binds to selected centrosomal cargo proteins, to the dynactin subunit p150Glued, and to PCM-1 at centriolar satellites (Figure 7). This complex is then delivered to the centrosome via microtubule-dependent transport. On arrival, cargo proteins dissociate from the complex, integrate into centrioles and the pericentriolar matrix, and function in the control of microtubule organization (Figure 7A). In the absence of Par6α, PCM-1 and cargo proteins cannot associate with dynactin (Figure 7B). As a consequence, proteins with critical roles in centrosome function are not delivered to the centrosome, leading to abnormalities...
in microtubule organization. In the absence of PCM-1, in contrast, Par6α depends on PCM-1 for its retention at the centrosome, Par6α is mislocalized to the cytosol under these conditions (Figure 7C). Our model implicates Par6α with a role in centrosomal protein delivery in contrast to PCM-1, which may function in the retention of Par6α at the centrosome.

Our study provides strong support for Par6α as a centrosomal component in epithelial cells. We detected this specific Par6 isoform at the centrosome and centriolar satellites in three human cell lines using an antibody against an internal peptide that is unique to Par6α and not present in other Par6 isoforms (Gao and Macara, 2004). In addition, we verified the association of this protein with the centrosome by four different approaches. First, we probed HeLa cells with an antibody against the two Par6 isoforms, Par6α and Par6β, that revealed immunofluorescence staining at the centrosome (data not shown). Next, RNAi-mediated depletion of Par6α resulted in the specific loss of the 37-kDa Par6 isoform Par6α and the absence of Par6α at the centrosome. Third, Par6α colocalized with known centrosomal proteins when we fractionated whole HeLa cell lysates on a sucrose density gradient. Finally, Par6α interacted with centrosomal proteins, such as PCM-1, in communoprecipitation experiments and in a mass spectrometry screen (Kodani, Huang, and Sütterlin, unpublished results). These findings are consistent with published reports on the role of this polarity protein in the regulation of centrosome function in neuronal and neutrophil-like cells (Solecki et al., 2004; Xu et al., 2007). However, our results differ from a study by Cline and Nelson (2007), who detected a Par6 isoform in the nucleus and cytosol of HeLa cells. Although Cline and Nelson named this Par6 isoform Par6α (or Par 6), it is likely that their analysis focused on the 45-kDa Par6 isoform Par6β, instead of the 37-kDa isoform Par6α.

Our findings implicate Par6α with the regulation of centrosome organization and function. Specific centrosomal proteins, including PCM-1, pericentrin, ninein, and Cep170, which have known roles in the organization and dynamics of centrosomal microtubules (Li et al., 2001; Dammermann and Merdes, 2002; Guarguaglini et al., 2005), were unable to associate with the centrosome in the absence of Par6α. Consistent with this mislocalization phenotype, Par6α-depleted cells contained severely disorganized interphase microtu-
bulles. We also observed dramatic effects of Par6 depletion on mitotic microtubule organization with abnormal spindles in more than 90% of mitotic cells. Because Par6 does not associate with mitotic spindle poles, it is likely that these spindle defects are the consequence of centrosome defects in interphase. Mislocalized centrosomal proteins in the cytosol may be sufficient to induce the formation of acentrosomal asters that may be able to form extra spindle poles upon entry into mitosis. In support of this model, we previously found that dysregulation of centrosome organization can produce spindle abnormalities (Kodani and Sutterlin, 2008; Kodani et al., 2009).

We propose that Par6 binding to p150Glued is important for protein recruitment to the centrosome. There are at least three different mechanisms to explain how this interaction could promote centrosomal protein delivery. First, Par6 binding to p150Glued may link centrosomal cargo proteins to the dynein–dynactin motor complex. Thus, Par6 would function as a cargo receptor, which is consistent with the observed mislocalization of specific centrosomal proteins in the absence of Par6. However, it is not known whether Par6 has direct interactions with specific centrosomally targeted proteins. Alternatively, Par6 may bind p150Glued to modify interactions with microtubules, dynein, or the centriolar satellite proteins BBS4 and Cep290. As each of these factors contributes to centrosomal protein delivery (Quintyne and Schroer, 2002; Kim et al., 2004; Burakov et al., 2008), it is possible that Par6-dependent regulation of these interactions may promote the assembly of proteins at the centrosome. Finally, Par6–p150Glued interaction may control the localization and retention of p150Glued and the entire dynactin complex at the centrosome, which is critical for centrosome organization and function (Quintyne et al., 1999; Quintyne and Schroer, 2002). Additional studies on Par6 and its binding partners will be necessary to define the specific role of this protein in centrosomal protein delivery.

Although we have identified a complex of Par6, PCM-1, and p150Glued, many features of this complex remain to be determined. For example, it is not known if Par6 binds PCM-1 and p150Glued directly or if this complex contains additional factors. Good candidates include the centriolar satellite proteins BBS4 and Cep290 (Kim et al., 2004; Kim et al., 2008). BBS4 binds to PCM-1 and p150Glued, is required for centrosome organization and function (Kim et al., 2004), and is mislocalized in Par6-depleted cells (data not shown). Similarly, Cep290 is reported to bind to PCM-1 and the dynactin subunits, p150Glued and p50 (Chang et al., 2006;
Kim et al., 2008). Other possible components include known Par6-interacting proteins, such as Par3 and aPKC. Although a role for these proteins in centrosome assembly has not been tested, a recent study reported an association between Par3 and dynein, which is important for the regulation of microtubule dynamics and centrosome orientation during cell migration (Schmoranzer et al., 2009).

Our data argue against a direct role of PCM-1 in Par6-dependent centrosome regulation. We found that Par6, but not PCM-1, binds to p150Glued. In addition, we showed that efficient depletion of PCM-1, for 48 h with siRNA against a published sequence in the PCM-1 cDNA (Kim et al., 2008), had no effect on centrosome organization and function and, thus, was not equivalent to Par6 depletion. Our findings, however, are in disagreement with reports by Dammermann and Merdes (2002), who detected defects in centrosome composition and microtubule organization when they used a different siRNA sequence to deplete PCM-1 for 96 h. A possible explanation for these discordant results is that prolonged knockdown of PCM-1 has an indirect effect on centrosome assembly by inducing the mislocalization of Par6.

In summary, our results support a role for Par6 in the regulation of centrosome assembly, which is important for centrosomal microtubule organization during the cell cycle.

We propose a model in which this novel function of Par6 depends on the interaction of Par6 with the dynactin subunit p150Glued. However, further analysis of the Par6 interaction network will be necessary to understand the precise role of this polarity protein in centrosomal protein delivery.

ACKNOWLEDGMENTS

We thank Dr. Ming Tan for helpful comments on the manuscripts and Drs. Reiner Lammers, Andreas Merdes, Jeffrey Salisbury, Pierre Gণczy, Giulia Guarguaglini, David Sharp, Mikuho Takahashi, and Carla Koehler for generously providing reagents. This work was supported by a grant from the California Cancer Coordinating Committee (CRCC) and the NIH (R01GM089913) to C.S.

REFERENCES

Andersen, J. S., Wilkinson, C. J., Mayor, T., Mortensen, P., Nigg, E. A., and Mann, M. (2003). Proteomic characterization of the human centrosome by protein correlation profiling. Nature 426, 570–574.

Azimzadeh, J., Hergert, P., Delouvee, A., Euteneuer, U., Formstecher, E., Khodjakov, A., and Bornens, M. (2009). hPOC5 is a centrin-binding protein required for assembly of full-length centrioles. J. Cell Biol. 185, 101–114.

Balcizan, R., Bao, L., and Zimmer, W. E. (1994). PCM-1, a 228-kD centrosome autoantigen with a distinct cell cycle distribution. J. Cell Biol. 124, 783–793.
Balcon, R., Varden, C. E., and Schroer, T. A. (1999). Role for microtubules in centrosome doubling in Chinese hamster ovary cells. Cell Motil. Cytoskeleton. 42, 60–72.

Bobiniec, Y., Khodjakov, A., Mir, L. M., Rieder, C. L., Edde, B., and Bornens, M. (1998). Centriole disassembly in vivo and its effect on centrosome structure and function in vertebrate cells. J. Cell Biol. 143, 1575–1589.

Bornens, M., Paintrand, M., Berges, J., Marty, M. C., and Karsenti, E. (1987). Structural and chemical characterization of isolated centrosomes. Cell Motil. Cytoskeleton. 8, 238–249.

Burakov, A., Kovalenko, O., Semenova, I., Zhapparova, O., Nadezhzhina, E., and Rodionov, V. (2008). Cytoplasmic dynein is involved in the retention of microtubules at the centrosome in interphase cells. Traffic 9, 472–480.

Chang, B., et al. (2006). In-frame deletion in a novel centrosomal/ciliary protein CEP290/NPHP6 perturbs its interaction with RGC and results in early-onset retinal degeneration in the rd16 mouse. Hum. Mol. Genet. 15, 1847–1857.

Cline, E. G., and Nelson, W. J. (2007). Characterization of mammalian Par 6 as a dual-location protein. Mol. Cell. Biol. 27, 4431–4443.

Dammermann, A., and Merdes, A. (2002). Assembly of centrosomal proteins and microtubule organization depends on PCM-1. J. Cell Biol. 159, 255–266.

Etienne-Manneville, S., Manneville, J. B., Nicholls, S., Ferenczi, M. A., and Hall, A. (2003). Cdc42 and Par6-PKCzeta regulate the spatially localized association ofDlg1 and APC to control cell polarization. J. Cell Biol. 170, 895–901.

Frederick, I. A., Matthews, J. A., Jamieson, L., Justilien, V., Thompson, E. A., Radisky, D. C., and Fields, A. P. (2008). Matrix metalloproteinase-10 is a critical effector of protein kinase Cota-Par6alpha-mediated lung cancer. Oncogene 27, 4841–4853.

Ganem, N. J., Godinho, S. A., and Pelmán, D. (2009). A mechanism linking extra centrosomes to chromosomal instability. Nature 460, 278–282.

Gao, L., Joberty, G., and Macara, I. G. (2002). Assembly of epithelial tight junctions is negatively regulated by Par6. Curr. Biol. 12, 221–225.

Gill, S. R., and Macara, I. G. (2004). Isoforms of the polarity protein Par6 have distinct functions. J. Biol. Chem. 279, 41357–41362.

Gillingham, A. K., and Munro, S. (2000). The PACT domain, a conserved centrosomal targeting motif in the coiled-coil proteins AKAP450 and pericentriolar protein CPAP. Mol. Cell. Biol. 20, 1192–1200.

Gundersen, G. G. (2009). Par3 and dynein associate to regulate local microtubule dynamics and centrosome orientation during migration. Curr. Biol. 19, 1195–1200.

Hall, A. (2005). Cdc42 and Par6-PKCzeta regulate the spatially localized control of Cdc42 activity at the Golgi regulates centrosome organization. Mol. Biol. Cell 20, 1192–1200.

Kadon, A., and Sutterlin, C. (2009). GM130-dependent control of Cdc42 activity at the Golgi regulates centrosome organization. Mol. Biol. Cell. 20, 1192–1200.

Kodani, A., and Sutterlin, C. (2008). The Golgi protein GM130 regulates centrosome morphology and function. Mol. Biol. Cell 19, 745–753.

Kohlmaier, G., Loncarek, J., Meng, X., McEwen, B. F., Mogensen, M. M., Spektor, A., Dynlacht, B. D., Khodjakov, A., and Gonyo, P. (2009). Over long centrosomes and defective cell division upon excess of the SAS-4-related protein CPAP. Curr. Biol. 19, 1012–1018.

Krauss, S. W., Spence, J. R., Bahmanyar, S., Barth, A. L. G., M. C., Czerwinski, D., and Meyer, A. J. (2008). Downregulation of protein 4.1R, a mature centriole protein, disrupts centrosomes, alters cell cycle progression, and perturbs mitotic spindles and anaphase. Mol. Biol. Cell. 28, 2283, 2294.

Kubo, A., Susaki, H., Yuba-Kubo, A., Tsukita, S., and Shina, N. (1999). Centriolar satellites: molecular characterization, ATP-dependent movement toward centrosoles and possible involvement in ciliogenesis. J. Cell Biol. 147, 969–980.

Li, Q., Hansen, D., Killilea, A., Joshii, H. C., Palazzo, R. E., and Balcon, R. (2001). Kdrin/pericentrin-B, a centrosome protein with homology to pericentrin that complexes with PCM-1. J. Cell Sci. 114, 797–809.

Lin, D., Edwards, A. S., Fawcett, J. P., Mambalgu, G., Scott, J. D., and Pawson, T. (2003). A mammalian PAR-3-PAR-6 complex implicated in Cdc42/Rac1 and aPKC signalling and cell polarity. Nat. Cell Biol. 5, 540–547.

Nigg, E. A. (2006). Origins and consequences of centrosome aberrations in human cancers. Int. J. Cancer 119, 2177–2173.

Quintyne, N. J., Gull, S. R., Eckley, D. M., Cregg, C. L., Compton, D. A., and Schroer, T. A. (1999). Dynactin is required for microtubule anchoring at centrosomes. J. Cell Biol. 147, 321–334.

Quintyne, N. J., and Schroer, T. A. (2002). Distinct cell cycle-dependent roles for dynactin and dynein at centrosomes. J. Cell Biol. 159, 245–254.

Schorrman, J., Fawcett, J. P., Segura, M., Tan, S., Alle, R. B., Pawson, T., and Gundersen, G. G. (2009). Par6 and dynein associate to regulate local microtubule dynamics and centrosome orientation during migration. Curr. Biol. 19, 1065–1074.

Solecki, D. J., Model, L., Gaetz, J., Kapoor, T. M., and Hatton, M. E. (2004). Par3 and par6 associate to regulate microtubule dynamics of the spermatid tail. J. Cell Sci. 117, 1195–1203.

Strood, P., and Gonyo, P. (2008). Mechanisms of procentriole formation. Trends Cell Biol. 18, 389–396.

Sutterlin, C., Polischuk, R., Pecot, M., and Malhotra, V. (2005). The Golgi-associated protein GRASP65 regulates spindle dynamics and is essential for cell division. Mol. Biol. Cell 16, 3211–3222.

Watts, J. L., Etemad-Moghadam, B., Guo, S., Boyd, L., Draper, B. W., Mello, C. C., Priess, J. R., and Kempkes, K. J. (1996). par-6, a gene involved in the establishment of asymmetry in early C. elegans embryos, mediates the asymmetric localization of PAR-5. Development 122, 3133–3140.

Xu, J., Van Keymeulen, A., Wada, K. M., Carlson, P., Berns, M. W., and Bourne, H. R. (2007). Polarity reveals intrinsic cell chirality. Proc. Natl. Acad. Sci. USA 104, 9296–9300.

Young, A., Dictenberg, J. B., Purohit, A., Tuft, R., and Dossey, S. J. (2000). Cytoplasmic dynein-mediated assembly of pericentrin and gamma tubulin onto centrosomes. Mol. Biol. Cell 11, 2047–2056.

Zimmerman, W., and Dossey, S. J. (2000). Construction of centrosomes and spindle poles by molecular motor-driven assembly of protein particles. Traffic 1, 927–934.