Published in final edited form as:

Biochem Biophys Res Commun. 2017 March 18; 484(4): 864–870. doi:10.1016/j.bbrc.2017.02.004.

Recruitment of PP1 to the centrosomal scaffold protein CEP192

Isha Nasa[a], **Laura Trinkle-Mulcahy**[b], **P. Douglas**[c], **Sibapriya Chaudhuri**[a], **S.P. Lees-Miller**[c], **Kyung S. Lee**[d], **Greg B. Moorhead**[a,*]

[a]Department of Biological Sciences, University of Calgary, Calgary, Alberta T2N 1N4, Canada

[b]Department of Cellular & Molecular Biology and Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, ON, Canada

[c]Departments of Biochemistry & Molecular Biology and Oncology, Robson DNA Science Centre, Southern Alberta Cancer Research Institute, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

[d]Laboratory of Metabolism, National Cancer Institute, Bethesda, MD 20892, USA

Abstract

Centrosomal protein of 192 kDa (CEP192) is a scaffolding protein that recruits the mitotic protein kinases Aurora A and PLK1 to the centrosome. Here we demonstrate that CEP192 also recruits the type one protein phosphatase (PP1) via a highly conserved KHVTF docking motif. The threonine of the KHVTF motif is phosphorylated during mitosis and protein kinase inhibition studies suggest this to be a PLK1-dependent process.

Keywords

CEP192; Protein phosphatase one; Polo-like kinase one; Mitosis; Centrosome

1. Introduction

Centrosomes form the major microtubule-organizing centers in mammalian cells and are critical for the processes of spindle formation, spindle organization, and chromosome segregation. Centrosome duplication and maturation have been shown to be regulated by serine/threonine protein kinases including Aurora A, PLK1 and Nek2A [1–5]. Major players involved in centrosomal function include the protein pericentrin, which forms the pericentriolar matrix (PCM) inner layer. The PCM outer matrix is composed of other crucial centrosomal proteins including the γ-tubulin ring complex, centrosomal protein of 192 kDa (CEP192) and centrosomal protein of 215 kDa (CEP215), among others [6]. All of these components couple the centrosomal cycle with the cell cycle which is crucial for accurate cell division.

*Corresponding author. moorhead@ucalgary.ca (G.B. Moorhead).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2017.02.004.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2017.02.004.

CEP192 has a critical function in centrosome maturation and centriole duplication as well as bipolar spindle formation [7,8]. This protein not only determines the centrosome size, but is also essential for the recruitment of other PCM components, including the γ-tubulin ring complex, through its interaction with multiple centrosomal proteins [9]. CEP192 has been proposed to act as a scaffold for nucleation of microtubules and other regulatory factors during mitosis. CEP192 is also a critical player in the protein kinase activation cascade of Aurora A and PLK1 at the centrosomes and acts as a scaffold to recruit and activate centrosomal Aurora A via autophosphorylation at the T-loop Thr288 residue [10–12]. Once activated, Aurora A can trigger the activation of PLK1 by phosphorylating its T-loop Thr210 residue. This active PLK1 can then dock phospho-Thr44 of CEP192 and phosphorylate the protein on several additional serine residues [11]. These phosphorylated residues act as a recruiting platform for other PCM proteins, such as the γ-tubulin ring complex, which controls microtubule nucleation. Loss of CEP192 in mammalian cells leads to numerous centrosomal abnormalities due to incorrect centrosome segregation caused by impaired centrosome separation and centrosome maturation, highlighting the importance of CEP192 as a critical regulator of centrosome function.

Most protein phosphorylation occurs on serine and threonine residues, and the protein phosphatases PP1 and PP2A are thought to account for majority of the dephosphorylation events on these amino acids [13–15]. Humans have three highly related PP1 isoforms that achieve substrate specificity through a vast array of docking proteins. Greater than 90% of these docking proteins bind PP1 in a hydrophobic cleft distant from the active site via a short sequence known as the RVxF motif. The RVxF motif on PP1 binding partners is slightly degenerate and conforms to the sequence [KR] [KR][VI]x[FW].

CEP192 is a heavily phosphorylated protein with 67 phosphorylated serine, threonine or tyrosine residues identified in different phospho-proteomic data sets collected and catalogued at PhosphoSitePlus [10,11]. Given the dynamic nature of phosphorylation, it can be presumed that these sites need to be dephosphorylated during the centrosomal cycle. To this end, very little is known about protein phosphatases working to dephosphorylate CEP192. Here we show that CEP192 recruits PP1 via its RVxF motif (‘KHVTF’) and propose that this interaction is critical for the dephosphorylation and function of CEP192 at the centrosome.

2. Materials and methods

2.1. Conservation of a potential PP1 binding motif among mammalian CEP192

The mammalian protein orthologs of CEP192 were extracted from the sequences of organisms with a full genome sequence available using EggNOG[4.5] [16]. Smith-Waterman algorithm along with composition based score adjustment was used to calculate sequence similarities. The sequences from all orthologous proteins identified were aligned using Clustal Omega to determine the conservation of the potential PP1 binding RVxF motif among these organisms [17]. A potential PP1 binding RVxF motif (‘KHVTF’) sits at amino acid positions 948 to 952 in human CEP192 and was used to generate a trimmed alignment containing the ‘KHVTF’ motif and this inturn was used to create a weblogo [18,19].

2.2. Molecular cloning and site-directed mutagenesis of CEP192

Residues 507–1065 (containing the 'KHVTF' motif) of human CEP192 were amplified from a pEGFP-C1-CEP192 construct by PCR using Gateway compatible primers and inserted into pDONR201 via BP ligation reaction (Life Technologies). This construct was subcloned into bacterial expression vector pDEST42 to create a C-terminal V5 and 6XHis tagged fusion construct (CEP192-WT). The putative PP1 binding motif 'KHVTF' in this construct was mutated to 'KHATA' (CEP192-RARA) by site-directed mutagenesis following the manufacturers' protocol. The constructs were sequence verified by Eurofin MWG Operon LLC sequencing.

2.3. Expression and purification of human CEP192 and its RARA mutant

CEP192-WT and CEP192-RARA constructs were transformed into BL-21 (DE3) *E. coli* cells and were grown at 37 °C until they reached an O.D. 600 of 0.4. The cells were induced with 0.1 mM IPTG at 22 °C for 16 h. Bacteria were pelleted at 4000 rpm for 20 min, re-suspended in 1X extraction buffer (50 mM Hepes-NaOH pH 7.5, 150 mM NaCl, 5% (v/v) glycerol and 10 mM imidazole) and frozen at −80 °C until further use. For protein purification, cells were thawed with the addition of 1% (v/v) Tween-20, 20 mM imidazole, 1 mM phenylmethanesulfonylfluoride (PMSF), 1 mM benzamidine, 2 μg mL^{-1} leupeptin, 5 μg mL^{-1} pepstatin and lysed using sonication. Crude lysates were clarified at 20,000 rpm for 30 min at 4 °C. Supernatants were removed, and an additional 1 mM PMSF and 1 mM benzamidine were added prior to incubation with Ni-NTA agarose (Qiagen) end-over-end at 4 °C for 1.5 h. Matrix was captured in a column and washed with 500 column volumes of Buffer A (1 × extraction buffer with 1 M NaCl, 1% (v/v) Tween-20, and 20 mM imidazole) followed by 100 column volumes of Buffer B (1 × extraction buffer). Ni-NTA bound proteins were eluted in 1 × extraction buffer containing 300 mM imidazole, pH 7.5. Protein eluates were concentrated using a 30,000 Da molecular weight cutoff Amicon concentrator (EMD Millipore).

2.4. In-vitro PP1 binding assay

Human PP1 isoforms were purified as described in Ref. [20]. PP1 (α/β/γ) (6 μg) was pre-incubated with either CEP192-WT or CEP192-RARA in 500 μl binding buffer (25 mM Tris, pH 7.5, 5% glycerol, 150 mM NaCl and 10 mM imidazole). To prevent non-specific binding, Ni-NTA beads were blocked with 1 mg mL^{-1} BSA in binding buffer for 1 h prior to incubation with PP1 and CEP192 for 2 h at 4 °C. The beads were cleaned with wash buffer (25 mM Tris, pH 7.5, 5% glycerol, 1 M NaCl and 10 mM imidazole) with 1% (v/ v) Tween-20 followed by a wash with the binding buffer. The proteins were eluted off the beads using 2X SDS-PAGE cocktail and run on SDS-PAGE for further analysis.

2.5. GFP-TRAP with U2OS-GFP-PP1 and HeLa-GFP-CEP192 cells

Stable cell lines integrated with a bacteria artificial chromosome containing the murine homolog of CEP192 tagged with GFP at the C-terminus (CEP192-GFP) were cultured in DMEM supplemented with 10% FBS, 100 U ml^{-1} penicillin-streptomycin and 400 mμ mL^{-1} geneticin. Asynchronous lysates were prepared by scraping the cells in lysis buffer (50 mM Tris, pH 7.5, 1 mM EDTA, 1 mM EGTA, 150 mM KCl) with protease inhibitor cocktail

(Sigma) and phosphatase inhibitors (25 mM NaF, 0.5 μM microcystin-LR), followed by sonication. The lysates were clarified by centrifugation at 14,000 rpm for 10 min. The protein concentration in the supernatant was determined by Bradford reagent using BSA as standard. To obtain mitotic cells, the cells were synchronized with 100 ng mL^{-1} nocodazole for 16 h followed by mitotic shake-off and release for 30 min. The cell pellet was suspended in lysis buffer and lysates prepared as described for asynchronous cells. Equal amount of cell lysate was incubated with GFP-TRAP beads (Chromotek) for 1.5 h at 4 °C followed by washing the beads three times with wash buffer (25 mM Tris, pH 7.5, 5% glycerol, 500 mM NaCl, 0.5% NP-40). The bound proteins were eluted using 2X SDS-PAGE cocktail and run on SDS-PAGE to analyze for the presence of bound PP1. GFP-only or one of the three isoforms of GFP-tagged human PP1 (GFP-Control, GFP-PP1α, GFP-PP1β and GFP-PP1γ) were expressed in asynchronous or mitotic U2OS cells and purified using GFP-TRAP as described in Ref. [21]. Immunoprecipitation of endogenous CEP192 was performed as in Ref. [22] using the antibody from Ref. [23].

2.6. Generation and validation of phospho 'KHVTF' CEP192 antibody

The phospho and dephospho versions of CEP192 peptide containing the potential PP1 binding motif SE**KHVTF**ENHK were synthesized with 98% purity at GL Biochem (Shanghai) Ltd., China. The phospho-specific antibody was generated by injecting keyhole limpet haemocyanin (KLH) (Imject Maleimide-activated KLH kit, Pierce) and bovine serum albumin (BSA)-coupled phospho-CEP192 peptide into rabbits [24]. The antibody was affinity purified using phospho-CEP192 peptide conjugated column. The purified antibody (called p-CEP192 hereafter) was validated with dot blots using phospho and dephospho CEP192 peptides and western blots (Fig. S1). To confirm the phospho-specificity, the antibody was quenched with 5 μg mL^{-1} of the antigen (SE**KHVpTF**ENHK) before incubating the membrane with the antibody.

2.7. Protein kinase inhibitor assays

HeLa cells were treated with 100 ng mL^{-1} nocodazole for 15 h for inducing a prometaphase arrest. The respective inhibitors Aurora A inhibitor I (S1451, Selleckchem, 100 nM), hesperadin (S1529, Selleckchem, 100 nM), and BI2536 (S1109, Selleckchem, 100 nM) were added for 1 h before the nocodazole was washed off the cells. The cells were then released into media with inhibitors for 30 min or 90 min after nocodazole wash-off. For hesperadin treated cells, MG132 (10 μM, 474790, Millipore (Calbiochem)) was added along with the protein kinase inhibitor to prevent mitotic exit.

2.8. Immunofluorescence and microscopy

HeLa cells were grown on poly-lysine coated coverslips, fixed with 3.7% formaldehyde, permeabilized with 0.5% Triton X-100 and blocked in 1% BSA/PBS. The coverslips were then incubated with either CEP192 antibody (Bethyl, 1:1000), pericentrin antibody (Abcam 28144) or affinity purified phospho-CEP192 antibody (2 μg mL^{-1}) for 2 h followed by Alexa Fluor 488-conjugated goat anti-rabbit and Alexa Fluor 594-conjugated goat anti-mouse secondary antibodies (Molecular Probes, Thermo Fisher Scientific, 1:500) for 1 h. Nuclei were counterstained with DAPI (Sigma Aldrich, 1 μg mL^{-1}) and images acquired

using a Leica DMIRE2 microscope equipped with a digital charge-coupled device (CCD) camera (Hamamatsu, Photonics, K.K.). The co-localization images were acquired with Nikon A1R confocal microscope.

3. Results

3.1. Conservation of the putative PP1 binding 'KHVTF' motif in CEP192

Analysis of protein orthologs of human CEP192 showed that the potential PP1 binding 'KHVTF', located between amino acids 948–952 in the human protein, is highly conserved among mammals. Among the CEP192 proteins identified, all contained the 'KHVTF' motif. The residues critical for PP1 binding (valine and phenylalanine) are completely conserved among the 'KHVTF' containing CEP192 proteins (Fig. 1A). Another striking observation is high conservation of two 'SP' sites just C-terminal to the 'KHVTF' motif which might be important in regulating PP1 binding with CEP192 (Fig. 1B).

3.2. CEP192 co-localizes with PP1α

Centrosomal imaging of metaphase U2OS-GFP-PP1α cells showed staining of CEP192 as expected (Fig. 2A). This centrosomal signal co-localized with the GFP-PP1α signal suggesting that CEP192 and PP1α are in close vicinity, at least during metaphase (Fig. 2A).

3.3. CEP192 interacts with PP1

A GFP-TRAP experiment using GFP-tagged PP1 expressing U2OS cells showed an enrichment of CEP192 in both the asynchronous and mitotic eluates compared to GFP alone (Fig. 2B). This suggests CEP192 and PP1 may be a part of the same complex. This was confirmed by reciprocal GFP-TRAP where PP1 was enriched in the GFP-CEP192 TRAP eluates (Fig. 2C). This was further supported by co-immunoprecipitation of endogenous PP1 with CEP192 (Fig. 2D). To determine if the interaction between CEP192 and PP1 is direct, the eluates from the binding experiment using CEP192-WT and CEP192-RARA and PP1 were immunoblotted for PP1. This blot showed a dramatic enrichment of PP1 in CEP192-WT bound beads compared to the control beads, but not in CEP192-RARA bound beads, suggesting a direct interaction via the 'KHVTF' motif (Fig. 2E).

3.4. 'KHVTF' motif in CEP192 is phosphorylated during mitosis

CEP192 contains a threonine in its PP1 binding RVxF motif ('KHVTF'). Previous work has shown that phosphorylation within the RVxF motif abolishes PP1 binding, so we explored the possibility that the CEP192 'KHVTF' motif is phosphorylated during the cell cycle. To determine the phospho-status of 'KHVTF' motif in CEP192, phospho-specific 'KHVTF' antibody was generated and validated (Figs. S1A, B, and C). These results also indicated that the 'KHVTF' motif of CEP192 is only phosphorylated in mitosis, and not interphase. To explore this further, this antibody was used to analyze the phosphorylation within 'KHVTF' throughout the cell cycle. As shown by the immunoblot, the 'KHVTF' motif in CEP192 is phosphorylated specifically during mitosis (Fig. 3A and Figs. S1B and C). This result was also confirmed by immunofluorescence of cells stained with p-CEP192 antibody which revealed centrosomal staining (Fig. 3B) only during mitosis. The centrosomal location was confirmed by co-staining with pericentrin. Prior to using the p-CEP192 antibody in

immunofluorescence experiments, we established that CEP192 co-localizes with pericentrin throughout the cell cycle (Fig. S2).

3.5. PLK1 regulates the phosphorylation of the 'KHVTF' motif in CEP192

Phosphorylation within the 'KHVTF' motif in CEP192 might be a crucial regulator of its interaction with PP1. To uncover the protein kinase responsible, we monitored phosphorylation at this site in the presence of inhibitors for key mitotic protein kinases. Immunofluorescence studies with kinase-inhibited cells stained with CEP192 antibody confirmed that CEP192 co-localizes with pericentrin at centrosomes (Fig. 3C) in the presence of these inhibitors (although CEP192 did decease slightly in the presence of PLK1 inhibitor). Having established that CEP192 remains at centrosomes during kinase inhibition, we then determined that the p-CEP192 signal is only abolished when PLK1 is inhibited in mitotic cells (Fig. 3D).

4. Discussion

CEP192 is a crucial regulator of centriole duplication, maturation, and separation through its interaction with other centrosomal proteins [25]. CEP192 has been proposed to play a scaffolding role in the recruitment of PCM proteins at different times in the centrosomal cycle [8]. This has been shown to be true in case of the recruitment of protein kinases Aurora A and PLK1, which are the major regulators of centrosomal protein phosphorylation. CEP192 binds both Aurora A and PLK1 protein kinases and is essential for the sequential T-loop phosphorylation and therefore activation of both these protein kinases at the centrosomes [10,11]. Loss of interaction between CEP192 and Aurora A and/or PLK1 results in centrosome and spindle assembly defects highlighting the importance of this signaling pathway [11]. However, the reverse, or dephosphorylation of CEP192 and other centrosomal proteins, has not been studied in detail with much less known about any centrosomal protein phosphatases.

PP1 binding proteins mediate their interaction with the PP1 catalytic subunit through docking motifs, primarily the RVxF motif [13–15,22]. CEP192 contains the classic PP1 binding motif 'KHVTF' which is highly conserved among eukaryotes (Fig. 1A). The association of PP1 with its regulatory proteins has also been shown to be regulated by phosphorylation in or around the PP1 docking RVxF motif. This phosphorylation in some cases has been attributed to CDK1 phosphorylation of a 'S/TP' site near the RVxF motif as in case of the regulatory protein CDCA2 (Repoman) [26]. Interestingly, there are two conserved 'SP' sites C-terminal to the 'KHVTF' motif in CEP192 (Fig. 1B) which might regulate the binding of PP1 with CEP192 in a CDK1-dependent manner. These sequence-based observations make CEP192 an ideal candidate for PP1 binding and regulation by PP1.

PP1α localizes to the centrosomes through its interaction with Nek2 [27,28]. Apart from Nek2, no centrosomal targeting subunits for PP1 have been identified. Here the localization of PP1α was confirmed using GFP-tagged PP1 expressing U2OS cells. In addition, this places PP1 at centrosomes with CEP192 (Fig. 2A). With GFP-tagged CEP192 expressing HeLa cells and GFP-tagged PP1 expressing U2OS cells, we could show that CEP192 and PP1 are a part of the same complex (Fig. 2B and C). Additionally, this interaction was direct

as confirmed with the *in-vitro* binding assay and was abolished in the case of CEP192-RARA mutant (Fig. 2D), suggesting that the interaction occurs via the KHVTF motif and as expected, the valine and the phenylalanine residues are crucial for this interaction.

Phosphorylation within the RVxF motif in PP1 regulatory proteins plays a role in controlling the association of PP1 with its regulatory proteins [29–31]. Here, we show that the threonine within the 'KHVTF' motif of CEP192 is phosphorylated specifically during mitosis (Fig. 3A) and that PLK1 protein kinase regulates the phosphorylation of this site (Fig. 3C). Although the 'KHVTF' sequence does not align well with the classic PLK1 consensus sequence D/E-X-S/T-Φ-X-D/E (X, any amino acid; Φ, a hydrophobic amino acid), the phosphorylation of this site is directly or indirectly regulated by PLK1 as shown by the data presented here. PLK1 has a strong preference for hydrophobic amino acid (F) at +1 position, but it can phosphorylate sequences lacking acidic amino acids at −2, and have basic residues (K/R) at −1 or −2 position [32] supporting the notion that PLK1 can directly phosphorylate the CEP192 'KHVTF' sequence. PLK1 mediated phosphorylation of CEP192 is essential for the recruitment of other proteins like the γ-tubulin ring complex, which may regulate the phosphorylation of this motif through additional protein kinases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to extend their thanks to Dr. Laurence Pelletier for CEP192 antibody. Funding for this work was provided by the Cancer Research Society of Canada (to G.M.), Alberta Cancer Foundation (Project 26313), Eyes High International Doctoral Scholarship and Faculty of Graduate Studies Doctoral Scholarship from University of Calgary (to I.N.).

List of Abbreviations

PLK1	Polo-like kinase 1
Nek2A	NIMA-related kinase 2A
CEP192	centrosomal protein of 192 kDa
PCM	pericentriolar matrix
PP1	protein phosphatase 1

References

[1]. Archambault V, Glover DM, Polo-like kinases: conservation and divergence in their functions and regulation, Nat. Rev. Mol. Cell Biol 10 (2009) 265–275. [PubMed: 19305416]

[2]. Berdnik D, Knoblich JA, Drosophila aurora-A is required for centrosome maturation and actin-dependent asymmetric protein localization during mitosis, Curr. Biol 12 (2002) 640–647. [PubMed: 11967150]

[3]. Hannak E, Kirkham M, Hyman AA, Oegema K, Aurora-A kinase is required for centrosome maturation in Caenorhabditis elegans, J. Cell Biol 155 (2001) 1109–1115. [PubMed: 11748251]

[4]. Lee K, Rhee K, PLK1 phosphorylation of pericentrin initiates centrosome maturation at the onset of mitosis, J. Cell Biol 195 (2011) 1093–1101. [PubMed: 22184200]

[5]. Tsai MY, Zheng YX, Aurora A kinase-coated beads function as microtubule-organizing centers and enhance RanGTP-induced spindle assembly, Curr. Biol 15 (2005) 2156–2163. [PubMed: 16332542]

[6]. Brito DA, Gouveia SM, Bettencourt-Dias M, Deconstructing the centriole: structure and number control, Curr. Opin. Cell Biol 24 (2012) 4–13. [PubMed: 22321829]

[7]. Gomez-Ferreria MA, Rath U, Buster DW, Chanda SK, Caldwell JS, Rines DR, Sharp DJ, Human cep192 is required for mitotic centrosome and spindle assembly, Curr. Biol 17 (2007) 1960–1966. [PubMed: 17980596]

[8]. Joukov V, De Nicolo A, Rodriguez A, Walter JC, Livingston DM, Centrosomal protein of 192 kDa (Cep192) promotes centrosome-driven spindle assembly by engaging in organelle-specific Aurora A activation, Proc. Natl. Acad. Sci. U. S. A 107 (2010) 21022–21027. [PubMed: 21097701]

[9]. O'Rourke BP, Gomez-Ferreria MA, Berk RH, Hackl AM, Nicholas MP, O'Rourke SC, Pelletier L, Sharp DJ, Cep192 controls the balance of centrosome and non-centrosomal microtubules during interphase, PLoS One 9 (2014) e101001. [PubMed: 24971877]

[10]. Asteriti IA, De Mattia F, Guarguaglini G, Cross-talk between AURKA and Plk1 in mitotic entry and spindle assembly, Front. Oncol 5 (2015) 283. [PubMed: 26779436]

[11]. Joukov V, Walter JC, De Nicolo A, The Cep192-organized aurora A-Plk1 cascade is essential for centrosome cycle and bipolar spindle assembly, Mol. Cell 55 (2014) 578–591. [PubMed: 25042804]

[12]. Yang R, Feldman JL, SPD-2/CEP192 and CDK are limiting for microtubule-organizing center function at the centrosome, Curr. Biol. CB 25 (2015) 1924–1931. [PubMed: 26119750]

[13]. Wakula P, Beullens M, Ceulemans H, Stalmans W, Bollen M, Degeneracy and function of the ubiquitous RVXF motif that mediates binding to protein phosphatase-1, J. Biol. Chem 278 (2003) 18817–18823. [PubMed: 12657641]

[14]. Heroes E, Lesage B, Gornemann J, Beullens M, Van Meervelt L, Bollen M, The PP1 binding code: a molecular-lego strategy that governs specificity, FEBS J 280 (2013) 584–595. [PubMed: 22360570]

[15]. Moorhead GB, Trinkle-Mulcahy L, Ulke-Lemee A, Emerging roles of nuclear protein phosphatases, Nat. Rev. Mol. Cell Biol 8 (2007) 234–244. [PubMed: 17318227]

[16]. Huerta-Cepas J, Szklarczyk D, Forslund K, Cook H, Heller D, Walter MC, Rattei T, Mende DR, Sunagawa S, Kuhn M, Jensen LJ, von Mering C, Bork P, EggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences, Nucleic acids Res 44 (2016) D286–D293. [PubMed: 26582926]

[17]. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG, Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega, Mol. Syst. Biol 7 (2011) 539. [PubMed: 21988835]

[18]. Crooks GE, Hon G, Chandonia JM, Brenner SE, WebLogo: a sequence logo generator, Genome Res 14 (2004) 1188–1190. [PubMed: 15173120]

[19]. Schneider TD, Stephens RM, Sequence logos: a new way to display consensus sequences, Nucleic acids Res 18 (1990) 6097–6100. [PubMed: 2172928]

[20]. Moorhead G, MacKintosh RW, Morrice N, Gallagher T, MacKintosh C, Purification of type 1 protein (serine/threonine) phosphatases by microcystin-Sepharose affinity chromatography, FEBS Lett 356 (1994) 46–50. [PubMed: 7988718]

[21]. Trinkle-Mulcahy L, Chusainow J, Lam YW, Swift S, Lamond A, Visualization of intracellular PP1 targeting through transiently and stably expressed fluorescent protein fusions, Methods Mol. Biol 365 (2007) 133–154. [PubMed: 17200560]

[22]. De Wever V, Nasa I, Chamousset D, Lloyd D, Nimick M, Xu H, Trinkle-Mulcahy L, Moorhead GB, The human mitotic kinesin KIF18A binds protein phosphatase 1 (PP1) through a highly conserved docking motif, Biochem. Biophys. Res. Commun 453 (2014) 432–437. [PubMed: 25281536]

[23]. Zhu F, Lawo S, Bird A, Pinchev D, Ralph A, Richter C, Mueller-Reichert T, Kittler R, Hyman AA, Pelletier L, The mammalian SPD-2 ortholog Cep192 regulates centrosome biogenesis, Curr. Biol 18 (2008) 136–141. [PubMed: 18207742]

[24]. Tran HT, Ulke A, Morrice N, Johannes CJ, Moorhead GB, Proteomic characterization of protein phosphatase complexes of the mammalian nucleus, Mol. Cell. proteomics MCP 3 (2004) 257–265. [PubMed: 14724321]

[25]. Kemp CA, Kopish KR, Zipperlen P, Ahringer J, O'Connell KF, Centrosome maturation and duplication in C. elegans require the coiled-coil protein SPD-2, Dev. Cell 6 (2004) 511–523. [PubMed: 15068791]

[26]. Qian J, Beullens M, Huang J, De Munter S, Lesage B, Bollen M, Cdk1 orders mitotic events through coordination of a chromosome-associated phosphatase switch, Nat. Commun 6 (2015) 10215. [PubMed: 26674376]

[27]. Mi J, Guo C, Brautigan DL, Larner JM, Protein phosphatase-1alpha regulates centrosome splitting through Nek2, Cancer Res 67 (2007) 1082–1089. [PubMed: 17283141]

[28]. Helps NR, Luo X, Barker HM, Cohen PT, NIMA-related kinase 2 (Nek2), a cell-cycle-regulated protein kinase localized to centrosomes, is complexed to protein phosphatase 1, Biochem. J 349 (2000) 509–518. [PubMed: 10880350]

[29]. Liu D, Vleugel M, Backer CB, Hori T, Fukagawa T, Cheeseman IM, Lampson MA, Regulated targeting of protein phosphatase 1 to the outer kinetochore by KNL1 opposes Aurora B kinase, J. Cell Biol 188 (2010) 809–820. [PubMed: 20231380]

[30]. Kim Y, Holland AJ, Lan W, Cleveland DW, Aurora kinases and protein phosphatase 1 mediate chromosome congression through regulation of CENP-E, Cell 142 (2010) 444–455. [PubMed: 20691903]

[31]. Dent P, Campbell DG, Caudwell FB, Cohen P, Identification of three in vivo phosphorylation sites on the glycogen-binding subunit of protein phosphatase 1 from rabbit skeletal muscle, and their response to adrenaline, FEBS Lett 259 (1990) 281–285. [PubMed: 2152882]

[32]. Kettenbach AN, Schweppe DK, Faherty BK, Pechenick D, Pletnev AA, Gerber SA, Quantitative phosphoproteomics identifies substrates and functional modules of Aurora and Polo-like kinase activities in mitotic cells, Sci. Signal 4 (2011) rs5. [PubMed: 21712546]

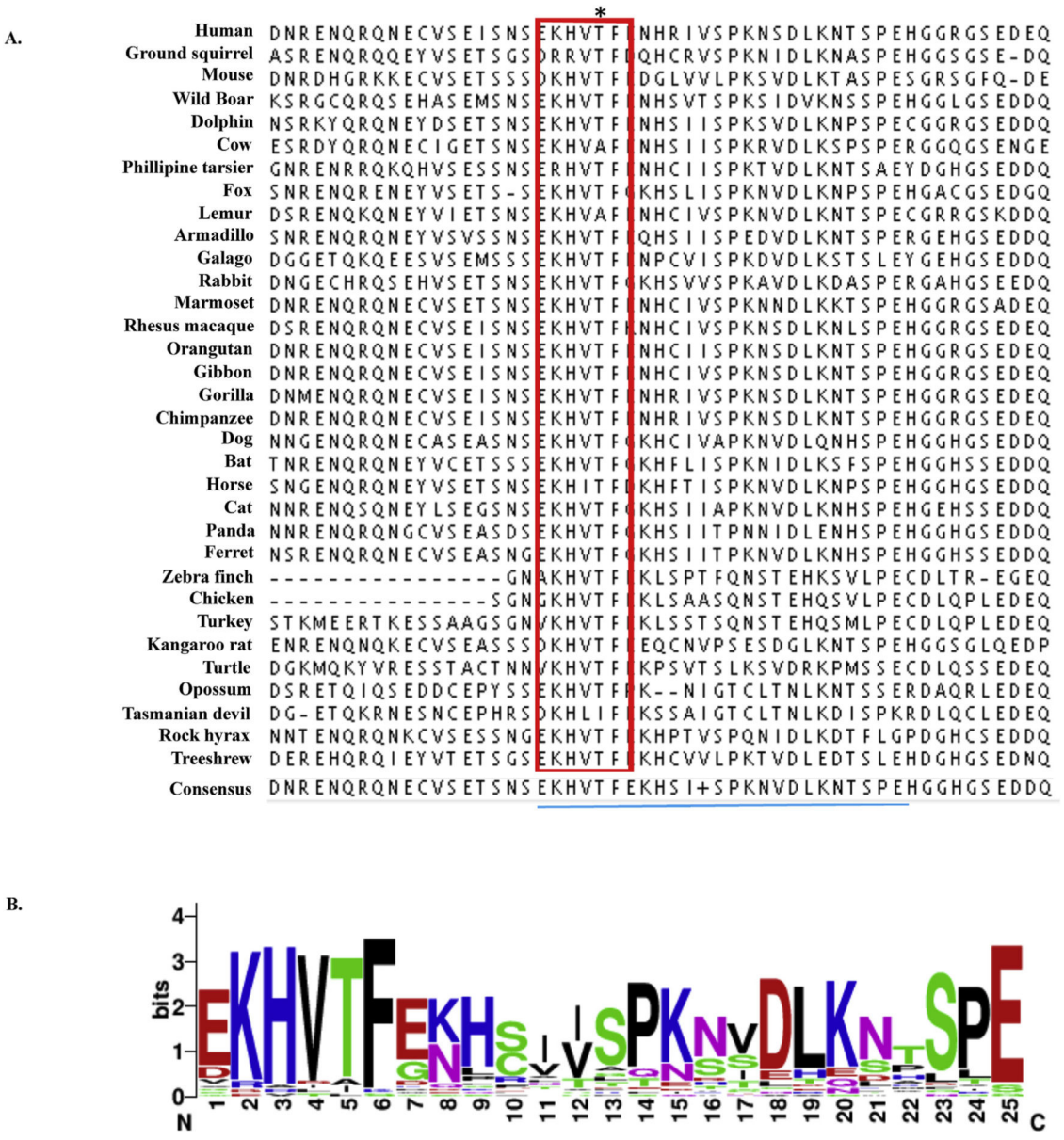

Fig. 1.

Potential PP1 binding ‘KHVTF’ motif in mammalian CEP192 proteins. (A) Clustal omega generated multiple sequence alignment with mammalian CEP192 orthologs shows a high degree of conservation of the potential PP1 binding ‘KHVTF’ motif. The ‘KHVTF’ motif is highlighted in a red box and the consensus below shows the most conserved residues at each position. Species names are as follows: Human, Homo sapiens; Ground squirrel, *Ictidomys tridecemlineatus*; Mouse, *Mus musculus*; Wild Boar, *Sus scrofa*; Dolphin, *Tursiops truncates*; Cow, *Bos Taurus*; Phillipine tarsier, *Tarsius syrichta*; Fox, *Pteropus vampyrus*; Lemur, *Microcebus murinus*; Armadillo, *Dasypus novemcinctus*; Galago, *Otolemur garnettii*; Rabbit, *Oryctolagus cuniculus*; Marmoset, *Callithrix jacchus*; Rhesus macaque, *Macaca mulatta*; Orangutan, *Pongo abelii*; Gibbon, *Nomascus leucogenys*; Gorilla, *Gorilla gorilla*; Chimpanzee, *Pan troglodytes*; Dog, *Canis lupus familiaris*; Bat, *Myotis lucifugus*;

Horse, *Equus caballus*; Cat, *Felis catus*; Panda, *Ailuropoda melanoleuca*; Ferret, *Mustela putorius furo*; Zebra finch, *Taeniopygia guttata*; Chicken, *Gallus gallus*; Turkey, *Meleagris gallopavo*; Kangaroo rat, *Dipodomys ordii*; Turtle, *Pelodiscus sinensis*; Opossum, *Monodelphis domestica*; Tasmanian devil, *Sarcophilus harrisii*; Rock hyrax, *Procavia capensis*; Treeshrew, *Tupaia belangeri*. (B) The sequence highlighted in cyan in (A) was obtained from all the protein sequences and was used as the input to generate a weblogo [18]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

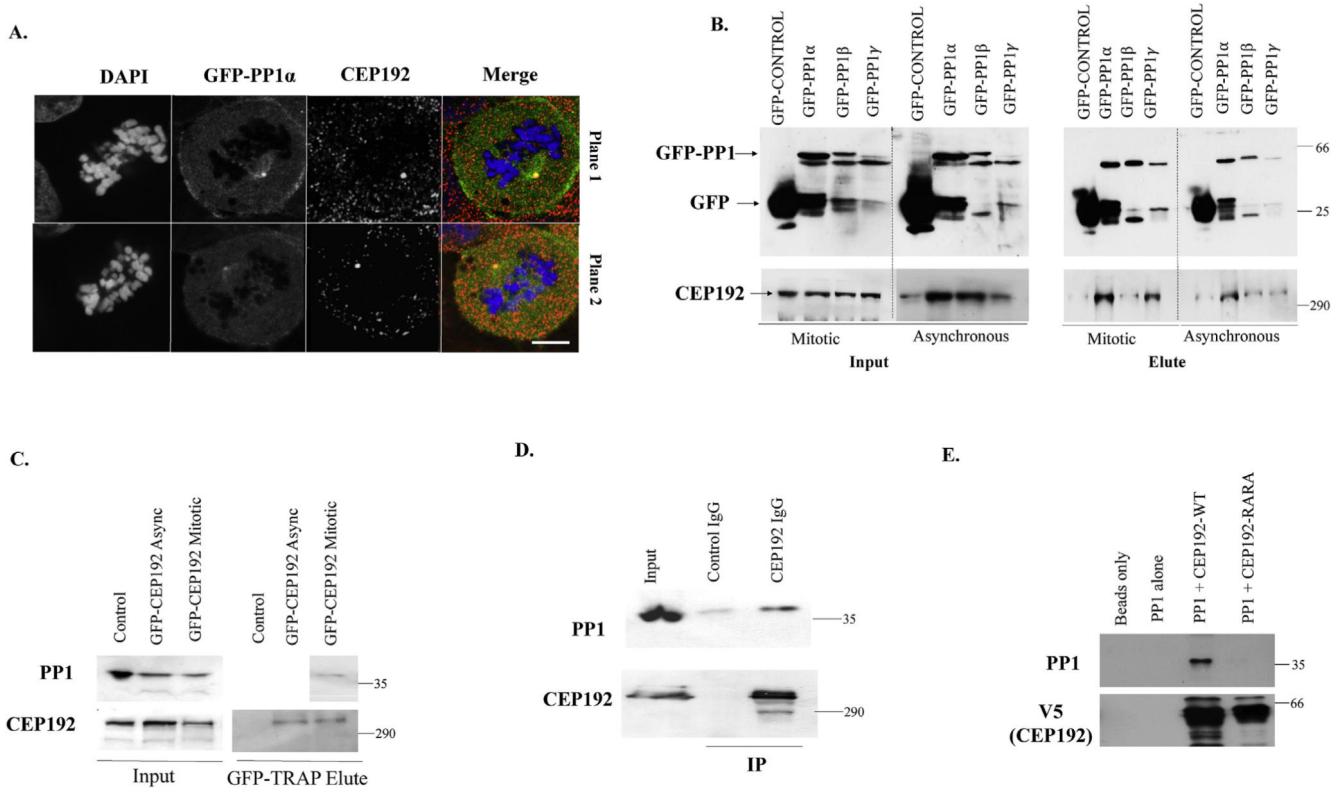

Fig. 2.

CEP192 interacts with PP1. (A) Immunofluorescence using CEP192 antibody shows co-localization in GFP tagged PP1α expressing cells. The images of the two centrosomes were taken in two different planes and DNA is stained with DAPI. Scale bar, 20 mm. (B) Input extracts and GFP-TRAP eluates from control cells or GFP-PP1α or β or γ expressing cells were probed with GFP (top panel) or CEP192 (bottom panel) antibodies. (C) Input extracts and GFP-TRAP eluates from either control cells or GFP-CEP192 expressing cells were probed with PP1 (top panel) or CEP192 (bottom panel) antibodies. (D) Endogenous CEP192 was immunoprecipitated from extracts and probed with PP1 (top panel) or CEP192 (bottom panel) antibodies. (E) The eluates from *in-vitro* binding assay using PP1 α/β/γ with either CEP192-WT or CEP192-RARA were probed with either PP1 (top panel) or V5 (for CEP192; bottom panel) antibodies. Beads alone and PP1 alone act as a negative control for the assay.

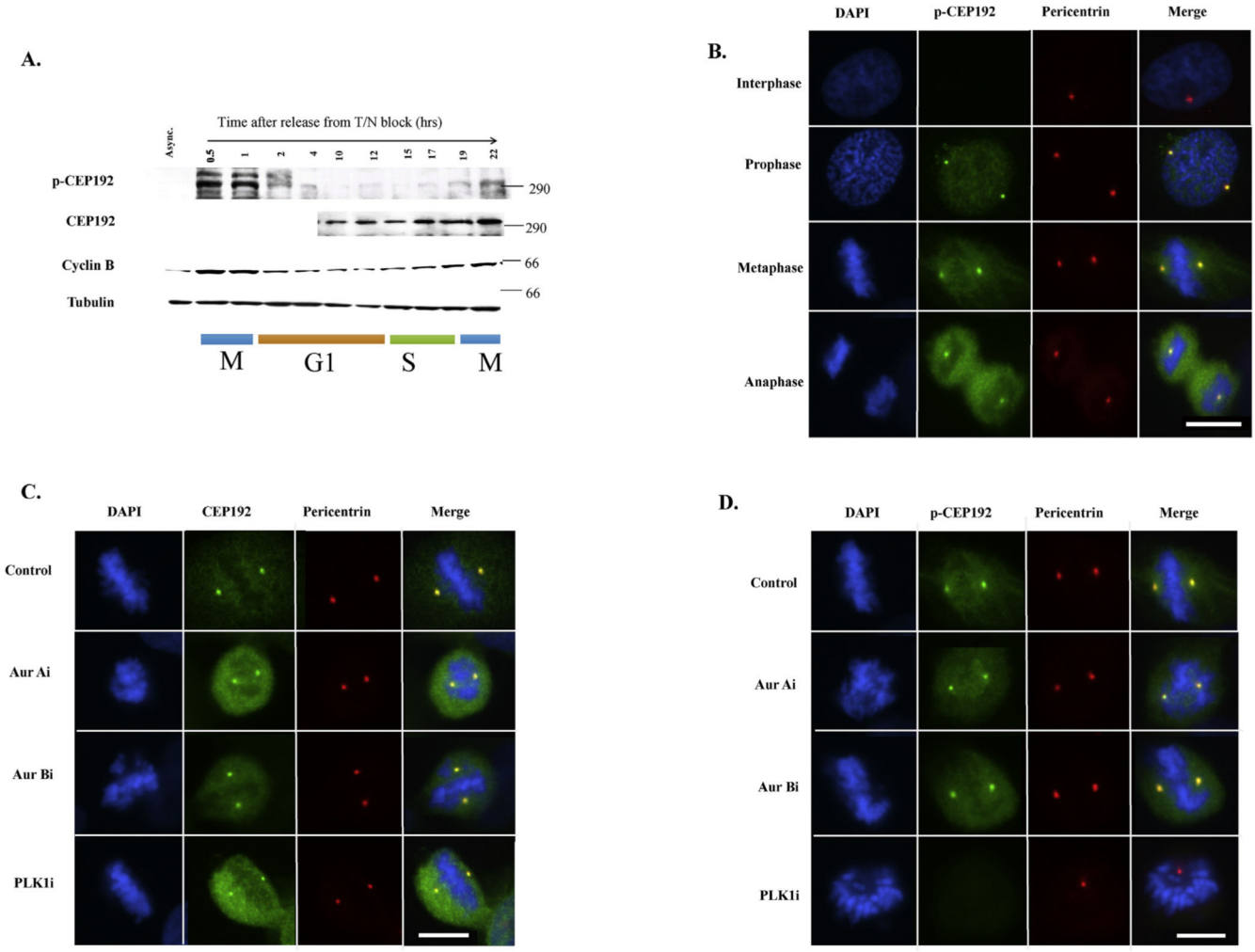

Fig. 3.

Phosphorylation of the ‘KHVTF’ motif in CEP192. (A) HeLa cells were synchronized in mitosis with thymidine-nocodazole block and released thereafter to collect cells at indicated times. The cell lysates were probed with the indicated antibodies including mitotic marker cyclin B and loading control tubulin (p-CEP192 is a phosphospecific antibody to ‘KHVpTF’ and is validated in Fig. S1). (B) HeLa cells were stained with DAPI and the indicated antibodies and imaged at different phases of the cell cycle as indicated. Scale bar, 20 μm. (C) HeLa cells were stained with DAPI and with the indicated antibodies to ensure CEP192 remained at centrosomes in the presence of the indicated protein kinase inhibitors. (D) HeLa cells were treated with indicated protein kinase inhibitors and stained with DAPI and with the indicated antibodies to monitor the status of CEP192 ‘KHVTF’ phosphorylation. Scale bar, 20 μm.