Molecular Biology of the Cell
Vol. 17, 3423–3434, August 2006

CP110 Cooperates with Two Calcium-binding Proteins to Regulate Cytokinesis and Genome Stability

William Y. Tsang,* Alexander Spektor,* Daniel J. Luciano,* Vahan B. Indjeian,† Zhihong Chen,‡ Jeffery L. Salisbury,§ Irma Sánchez,* and Brian David Dynlacht*

*Department of Pathology, New York University School of Medicine, New York, NY 10016; †Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138; ‡Esai Research Institute, Andover, MA 01810; and §Tumor Biology Program, Mayo Clinic, Rochester, MN 55905

Submitted May 1, 2006; Revised May 18, 2006; Accepted May 25, 2006
Monitoring Editor: Trisha Davis

The centrosome is an integral component of the eukaryotic cell cycle machinery, yet very few centrosomal proteins have been fully characterized to date. We have undertaken a series of biochemical and RNA interference (RNAi) studies to elucidate a role for CP110 in the centrosome cycle. Using a combination of yeast two-hybrid screens and biochemical analyses, we report that CP110 interacts with two different Ca^{2+}-binding proteins, calmodulin (CaM) and centrin, in vivo. In vitro binding experiments reveal a direct, robust interaction between CP110 and CaM and the existence of multiple high-affinity CaM-binding domains in CP110. Native CP110 exists in large (~300 kDa to 3 MDa) complexes that contain both centrin and CaM. We investigated a role for CP110 in CaM-mediated events using RNAi and show that its depletion leads to a failure at a late stage of cytokinesis and the formation of binucleate cells, mirroring the defects resulting from ablation of either CaM or centrin function. Importantly, expression of a CP110 mutant unable to bind CaM also promotes cytokinesis failure and binucleate cell formation. Taken together, our data demonstrate a functional role for CaM binding to CP110 and suggest that CP110 cooperates with CaM and centrin to regulate progression through cytokinesis.

INTRODUCTION

The centrosome is the microtubule-nucleating center in most eukaryotic cells (Doxsey, 2001). It is composed of a pair of orthogonally arranged centrioles and surrounding pericentriolar material from which microtubules emanate and elongate. During cell cycle progression, centrosome duplication commences as cells enter S phase, coincident with the initiation of DNA replication. As a cell progresses through G_2 and enters mitosis, centrosomes separate and migrate to opposite poles to establish the mitotic spindle. The processes of centrosome duplication and separation, known collectively as the centrosome cycle, are precisely coordinated with the cell cycle to ensure proper chromosome segregation and cell division. Defects in the centrosome cycle often give rise to chromosome mis-segregation, genetic instability, aneuploidy, cancer, cell cycle arrest, or death (Lingle *et al.*, 1998; Pihan *et al.*, 1998; Sluder and Nordberg, 2004; Badano *et al.*, 2005).

In addition to its essential function in microtubule organization, the centrosome is thought to be crucial for cytokinesis. Acentriolar *Drosophila* cell lines exhibit incomplete cytokinesis and rapidly become binucleate and polyploid (Debec, 1978; Debec and Abbadie, 1989). In mammals, surgical removal of centrosomes results in cytokinesis failure without affecting spindle formation and chromosome segregation (Hinchcliffe *et al.*, 2001; Khodjakov and Rieder, 2001). Live cell imaging experiments have revealed a transient repositioning of the mother centriole to the intercellular bridge, called the midbody, and this translocation is presumably necessary for signaling the completion of cytokinesis (Piel *et al.*, 2001). Recent mass spectrometric analyses of purified centrosomes have identified many putative mammalian centrosomal proteins (Andersen *et al.*, 2003). Although a function has not yet been assigned to many of these proteins, a handful of centrosomal proteins have been shown to play a role in cytokinesis. Suppression of γ-tubulin, centrin, or centriolin using RNA interference (RNAi) results in persistent intercellular bridges between dividing cells or coalescence of emerging daughter cells, ultimately leading to the generation of syncytia, binucleate, or multinucleate cells (Shu *et al.*, 1995; Salisbury *et al.*, 2002; Gromley *et al.*, 2003; Fabbro *et al.*, 2005; Zou *et al.*, 2005). Likewise, displacement of a centrosomal protein, AKAP450, by overexpression of a dominant-negative form of the protein results in abnormal cytokinesis and induces polyploidy (Keryer *et al.*, 2003). Although these findings have unequivocally established a requirement of the centrosome in cytokinesis, the precise mechanisms by which centrosomal proteins regulate completion of this event remain largely elusive.

We previously identified CP110 as a centrosomal protein of 110 kDa in a screen for cyclin-dependent kinase (CDK) substrates (Chen *et al.*, 2002). Depletion of CP110 by RNAi abolishes centrosome reduplication in S-phase–arrested cells and induces premature centrosome separation, suggesting that this protein may positively regulate centrosome duplication and negatively control centrosome separation. Reduction of CP110 levels or expression of a CP110 mutant lacking most putative CDK phosphorylation sites promoted un-

This article was published online ahead of print in *MBC in Press* (http://www.molbiolcell.org/cgi/doi/10.1091/mbc.E06–04–0371) on June 7, 2006.

Address correspondence to: Brian David Dynlacht (brian.dynlacht@med.nyu.edu).

Abbreviations used: CaM, calmodulin; CDK, cyclin-dependent kinase; RNAi, RNA interference; siRNA, small interfering RNA.

scheduled centrosome separation, and cell lines stably expressing this phospho-acceptor mutant exhibit polyploid 4N or 8N DNA content. However, these studies did not address how polyploidy arises from a defect in CP110 and whether the mutant phenotypes result from abnormal cytokinesis.

To further explore a potential role for CP110 in cytokinesis and to elucidate components of pathways involving CP110 that are critical for its function, we initiated studies to identify CP110-interacting proteins. Using a combination of yeast two-hybrid screens and biochemical analysis, we demonstrate that CP110 interacts with two Ca^{2+}-binding proteins, calmodulin (CaM) and centrin, in vivo. In vitro experiments establish that the association between CP110 and CaM is direct and robust. In contrast, CP110 appears to interact indirectly with centrin, and these three proteins (CP110, CaM, and centrin) form high-molecular-weight native complexes in vivo. Ablation of CP110 by RNAi results in overt tetraploidy and binucleate cells, hallmarks of cytokinesis failure. These phenotypes are reminiscent of defects induced by abrogation of centrin or CaM function, consistent with the notion that both CaM and centrin are essential for the completion of cytokinesis. To more precisely delineate the defects caused by CP110 loss, we performed live cell imaging and showed that CP110 plays a role in cytokinesis. Furthermore, we generated a mutant form of CP110 that interacts with centrin but binds poorly to CaM. Expression of such a mutant induces the formation of binucleate cells. Our results reveal the functional significance of CaM binding to CP110 and suggest that CP110 regulates progression through cytokinesis by cooperating with CaM and centrin.

MATERIALS AND METHODS

Cell Culture and Plasmids

HeLa, 293T, T98G, U2OS, IMR90, and Saos2 cells were grown in DMEM supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO_2 atmosphere. Bacterial expression plasmids for GST and GST-centrin production were pGEXGP-1 (Amersham Pharmacia, Piscataway, NJ) and pRLS201 (Salisbury *et al.*, 2002), respectively. pCDNA3 and pCDNA3-GFP-CaM (generous gift from D. C. Chang) were used to express GFP and GFP-CaM, respectively, in mammalian cells. To generate Flag-tagged CP110 fusion proteins for in vitro translation studies, CP110 fragments encoding residues 1-991, 1-991(Δ67-82) (also named 1-991Δ1), 1-991(Δ67-82, Δ781-820, Δ914-933) (also named 1-991Δ123), 1-630, 1-565, 1-565(Δ67-82) (also named 1-565Δ1), 1-530, 1-223, 200-565, 350-991, 620-991, 620-991(Δ781-820, Δ914-933) (also named 620-991Δ23) were amplified by PCR using Pfu Turbo polymerase (Stratagene, La Jolla, CA) and subcloned into the BglII and NotI sites of pRSET-B vector. All constructs were verified by DNA sequencing. The fragments encoding residues 1-991 and 1-991Δ123 were also subcloned into a mammalian expression vector pCBF (generous gift from M. Cole).

Antibodies

Antibodies used in this study included polyclonal rabbit anti-CP110 (Chen *et al.*, 2002), anti-centrin (mouse monoclonal 20H5 for immunoprecipitation and immunoblotting; hCetn2.4 for immunofluorescence; Salisbury *et al.*, 2002), anti-CaM (Upstate Biotechnology, Lake Placid, NY), anti-GFP (Roche, Indianapolis, IN), anti-α-tubulin, anti-β-tubulin, and anti-γ-tubulin (all from Sigma-Aldrich, St. Louis, MO), anti-calnexin (BD Transduction Laboratories, Lexington, KY), anti-giantin (Covance, Madison, WI), and anti-kendrin and anti-CG-NAP (generous gifts from M. Takahaski and Y. Ono).

Yeast Two-Hybrid Screen

The yeast two-hybrid screen was performed exactly as described previously (Walhout and Vidal, 2001). The full-length cDNA of CP110 was cloned into the BglII and NotI of pPC97 bait vector. The resultant plasmid was transformed into the yeast reporter strain, MaV103, together with a human fetal brain cDNA library cloned into pPC86 prey vector (Invitrogen, Carlsbad, CA). A total of 380,000 independent colonies were screened, and a total of 149 positive colonies were picked and tested for the presence of inserts. Ninety-eight of 149 gave single PCR products and were subsequently sequenced. The identity of each clone was determined by performing BLAST searches against the human genome.

Cell Cycle Synchronization and FACS Analysis

T98G cells were synchronized by serum deprivation and restimulation as described (Chen *et al.*, 2002). FACS analysis was performed as reported previously (Woo *et al.*, 1997).

Immunoprecipitation, Immunoblotting, and Immunofluorescence Microscopy

Cells were lysed with buffer containing 50 mM HEPES, pH 7, 250 mM NaCl, 5 mM EDTA, pH 8, 0.1% NP-40, 1 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), 2 μg/ml leupeptin, 2 μg aprotinin, 10 mM NaF, 50 mM β-glycerophosphate, and 10% glycerol at 4°C for 30 min. In experiments involving calcium, 2.5 mM $CaCl_2$ ($+Ca^{2+}$) or 5 mM EGTA ($-Ca^{2+}$) was also added to the lysis buffer as indicated. After centrifugation, 2 mg of the resulting supernatant was incubated with an appropriate antibody at 4°C for 1 h and collected using protein A- or G-Sepharose. The resin was washed with lysis buffer, and the bound polypeptides were analyzed by SDS-PAGE and immunoblotting. Typically, 50–100 μg of lysate was loaded into the input (IN) lane. Indirect immunofluorescence detection was performed as described (Chen *et al.*, 2002), using a Nikon Eclipse E800 microscope (100× objective lens; Melville, NY) equipped with a Photometrics Coolsnap HQ CCD camera (Tucson, AZ).

Differential Interference Contrast and Real-Time Videomicroscopy

HeLa cells were plated on glass-bottom dishes (MatTek, Ashland, MA) and transfected with either rhodamine-labeled and CP110 small interfering RNA (siRNA) oligonucleotides, or rhodamine-labeled and nonspecific control siRNA oligonucleotides 12 h later at 30–50% confluency. Seventy-two hours after transfection, cells were transferred to a live cell-imaging chamber on a Zeiss Axiovert 200 epifluorescence microscope (Thornwood, NY) equipped with Nomarski optics. Cells were imaged every 5 min for 12 h using a 40× phase-contrast lens on an inverted microscope. Images were captured using attached Retiga EX CCD (QImaging, Burnaby, BC, Canada) and OpenLab 4.0.1 software (Improvision, Lexington, MA).

Centrin and CaM Interaction Assays

Bacterially expressed GST or GST-tagged centrin was incubated with glutathione-agarose beads (Sigma) in lysis buffer at 4°C for 1 h. The beads were washed with lysis buffer and were incubated with cell extract either at room temperature for 1 h or at 4°C for 2 h. After washing beads with lysis buffer, bound proteins were analyzed by SDS-PAGE and immunoblotting. For CaM interaction assays, CaM agarose (Sigma) was incubated at 4°C with cell extracts for 2 h, washed, and subjected to SDS-PAGE and immunoblotting. Alternatively, CaM agarose was incubated at room temperature for 1 h with CP110 that was in vitro translated and labeled with ^{35}S-methonine. CP110 was in vitro translated according to the manufacturer's protocol using the TNT Quick Coupled Transcription/Translation System (Promega, Madison, WI). Proteins were resolved by SDS-PAGE and visualized by autoradiography.

RNAi

Synthetic siRNA oligonucleotides were obtained from Dharmacon (Boulder, CO). Transfection of siRNAs using Oligofectamine (Invitrogen) was performed according to the manufacturer's instructions. The 21-nucleotide siRNA sequence for the nonspecific control was 5′-AATTCTCCGAACGTGTCACGT-3′. The 21-nucleotide siRNA sequence for CP110 was 5′-AAGCAGCATGAGTATGCCAGT-3′. The siRNA for centrin silencing was described previously (Salisbury *et al.*, 2002). For long-term depletion of CP110, we used a plasmid (pBS/U6) expressing shRNA as described (Sui *et al.*, 2002). Briefly, the sense sequence (with DNA oligos 5′-GGGATCATCAACTAGTGGCTA-3′ and 5′-AGCTTAGCCACTAGTTGATGATCCC-3′) was inserted into ApaI and HindIII sites of pBS/U6. The inverted sequence (with DNA oligos 5′-AGCTTAGCCACTAGTTGATGATCCCTTTTTTG-3′ and 5′-AATTCAAAAAGGGGATCATCAACTAGTGGCTA-3′) was then inserted into HindIII and EcoRI sites of the intermediate plasmid to generate the final product, pBS/U6-CP110. U2OS cells were transfected with either pBS/U6 or pBS/U6-CP110 and a plasmid carrying a G418-resistant marker using calcium phosphate. After 48 h, the cells were split and treated with G418 for 1 mo. Stable transfectants were cloned, and single-cell colonies were selected and confirmed by Western blot assay.

Superose 6 Gel Filtration Analysis

Two milligrams of extract were chromatographed over a Superose 6 gel filtration column (Amersham Pharmacia) in lysis buffer (50 mM HEPES, pH 7, 250 mM NaCl, 5 mM EDTA, pH 8, 0.1% NP-40, 0.5 mM PMSF, and 10% glycerol). Equal volumes of each fraction were precipitated with TCA and analyzed by SDS-PAGE and immunoblotting. In immunoprecipitation experiments involving Superose 6 fractions, an equivalent of 10 mg of cell extract was loaded onto the column to collect the fractions.

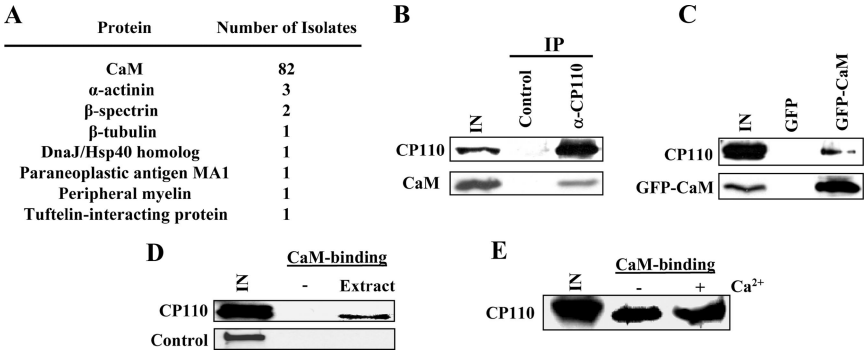

Figure 1. CaM interacts with CP110 in vivo. (A) The number of instances that each annotated protein was identified in the yeast two-hybrid screen is shown. (B) Western blotting of endogenous CP110 and CaM after immunoprecipitation with anti-CP110 antibody or control (anti-calnexin) antibody from 293T cell extracts. IN, input. (C) Western blotting of endogenous CP110 and recombinant GFP-CaM after immunoprecipitation with anti-GFP antibody using extracts from GFP-CaM- (GFP-CaM) and GFP-expressing cells (GFP). (D) Western blot detection of endogenous CP110 and control (giantin) after binding of CaM agarose with 293 extracts (extract) or with lysis buffer (−). (E) Western blot detection of endogenous CP110 after binding of CaM agarose with 293 extracts supplemented with either EGTA ($-Ca^{2+}$) or calcium ($+Ca^{2+}$).

RESULTS

Biochemical Interaction between CP110 and CaM

To understand how CP110 functions at the centrosome and to identify potential upstream and downstream regulators, we performed a stringent yeast two-hybrid screen using full-length CP110 as bait. From ~380,000 transformants, 98 positive clones were identified, and their inserts were sequenced. Remarkably, 82 clones carried cDNAs that encoded CaM (Figure 1A). Several other well- or partially characterized proteins were also identified, but each was isolated once or in some cases a few times (Figure 1A). We focused on CaM because this protein has been previously implicated in centrosome function (Matsumoto and Maller, 2002).

CaM belongs to the ubiquitous, EF-hand family of small Ca^{2+}-binding proteins that modulate a wide spectrum of cellular processes such as cell growth, division, proliferation, motility, and differentiation, in addition to centrosome function (Takuwa *et al.*, 1995; Li *et al.*, 1999; Chin and Means, 2000). To begin exploring whether the observed interaction between CP110 and CaM is physiologically relevant in mammalian cells, we examined the interaction of endogenous proteins by immunoprecipitation of crude cell extract with an antibody specific for CP110 (Chen *et al.*, 2002). Immunoprecipitation of CP110 from 293T cell extract and subsequent immunoblot analysis suggested that CP110 and CaM association is robust and specific (Figure 1B). Comparable results were also obtained using other immortal (HeLa, U2OS, T98G) and normal diploid (IMR90) human cell lines. Furthermore, we generated a U2OS cell line stably expressing a GFP-CaM fusion protein to examine whether immunoprecipitates of the epitope-tagged protein contain endogenous CP110. We observed that endogenous CP110 specifically coimmunoprecipitates with recombinant GFP-CaM (Figure 1C). In addition, endogenous CP110 from 293T cell extracts binds specifically to a CaM agarose conjugate under low calcium (Figure 1D). Furthermore, we showed that CP110 and CaM interact in the presence or absence of calcium from extracts supplemented with either EGTA ($-Ca^{2+}$) or calcium ($+Ca^{2+}$), suggesting that CP110 may contain both Ca^{2+}-dependent and Ca^{2+}-independent CaM-binding domains (Figure 1E).

CP110 and CaM Associate throughout the Cell Cycle

We investigated the CP110-CaM association using immunofluorescence to determine if this interaction is restricted to a specific portion of the cell cycle. During interphase, CaM is widely distributed throughout the cell, but during mitosis, the protein dramatically localizes to the spindle poles and the spindle microtubules, consistent with previous reports (Li *et al.*, 1999; Figure 2A). Because CP110 is a centrosomal protein, we examined whether these proteins colocalize during mitosis. We found that CP110 and a portion of CaM colocalized, substantially overlapping during metaphase, anaphase, and telophase (Figure 2A). To determine whether CP110 and CaM interact before mitosis, we synchronized cells by serum deprivation and immunoprecipitated CP110 from extracts of cells synchronously traversing the cell cycle. CP110 and CaM were coimmunoprecipitated from extracts of cells in G_0, G_1, S, and G_2 (Figure 2B). Interestingly, although CP110 levels vary during the cell cycle, the amount of immunoprecipitated CaM remains relatively unchanged (Figure 2B), suggesting that the levels of centrosomal CaM associated with CP110 may be constant throughout the cell cycle. Thus, we conclude that CP110 and CaM associate at the centrosome throughout the cell cycle, although the interaction is most easily visualized at the cellular level during mitosis.

CP110 Has Multiple CaM-binding Domains

To determine whether the interaction between CP110 and CaM is direct or is mediated by other proteins, we incubated in vitro-translated, radiolabeled CP110 with CaM agarose, and we observed robust binding (Figure 3A). No interaction was detected between CaM agarose and control proteins, strongly suggesting that the binding between CaM and CP110 is direct and specific (Figure 3A).

The Calmodulin Target Database (Yap *et al.*, 2000) predicts the existence of three putative CaM-binding domains within CP110 (residues 64–82, red box 1; residues 781–821, red box 2; and residues 909–924, red box 3; Figures 3B and 4A). To identify the region(s) that confer(s) binding to CaM, we generated a series of epitope-tagged truncation mutants and examined their ability to bind CaM in vitro. To examine calcium-dependent binding, each reaction was supplemented with either EGTA ($-Ca^{2+}$) or calcium ($+Ca^{2+}$). These experiments demonstrated that CP110 contains independent amino-terminal (1–223) and carboxy-terminal CaM-binding domains (620–991) that are Ca^{2+}-dependent (Figure 3, B and C, lanes 11, 12 and 20, 21), consistent with database predictions. In contrast, the region spanning residues 200–565 does not associate with CaM irrespective of calcium addition (Figure 3, B and C, lanes 14 and 15). We attempted to refine the identification of CaM-binding domains by deleting smaller regions of CP110. Interestingly, when the predicted CaM-binding domains were deleted singly or in

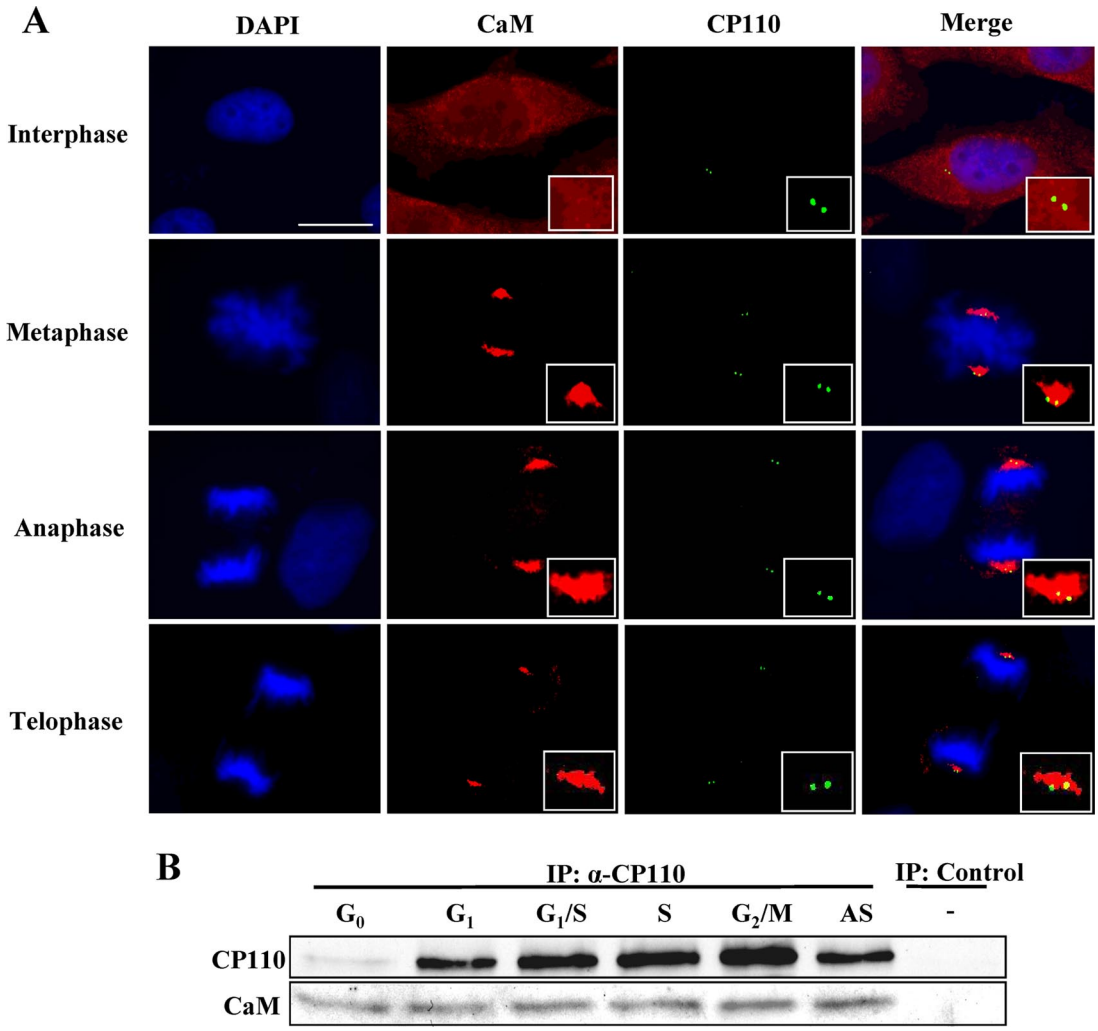

Figure 2. Colocalization and coimmunoprecipitation of CP110 and CaM across the cell cycle. (A) U2OS cells were processed for immunofluorescence with anti-CaM antibody (red) and anti-CP110 antibody (green). DNA was stained with DAPI (blue). A representative interphase, metaphase, anaphase, and telophase cell are illustrated. Yellow color in merged images indicates substantial colocalization of CP110 and CaM. Insets show magnified view of centrosomes. Bar, 10 μm; insets, 3 μm. (B) T98G cells were synchronized by serum starvation and restimulated with serum to initiate cell cycle reentry. Cell lysates from different cell cycle stages were collected. Western blots of endogenous CP110 and CaM after immunoprecipitation with anti-CP110 antibody or control (anti-calnexin) antibody.

combination, calcium-dependent binding to CaM was essentially abolished (Figure 4, A and B, compare lane 2 to lanes 5 and 8; compare lane 11 to lane 14). We detected a weak, residual interaction between CaM and full-length CP110 or carboxy-terminal fragments when calcium is absent (Figure 3, B and C, lanes 3, 18, and 21, and Figure 4, A and 4, lanes 3 and 18), and this interaction was observed even when the putative CaM-binding domains were deleted (Figure 4, A and B, lanes 9 and 21), suggesting that additional Ca^{2+}-independent CaM binding sites, not revealed by the database, may be present at the carboxy terminus. Thus, our data show that CP110 can bind directly to CaM via Ca^{2+}-dependent and -independent domains, in accordance with experiments in which CaM was shown to interact with endogenous CP110 in cell extracts in the absence or presence of calcium (Figure 1E). To determine the overall impact of ablating all three CP110 CaM-binding sites in vivo, we expressed this CP110 mutant in cells and determined whether it could associate with endogenous CaM. Remarkably, removal of all three sites nearly abolished its interaction with CaM in vivo (see Figure 9D), indicating that this assay may be somewhat more specific than the in vitro binding assay and prompting us to test the functional impact of abridging the CP110-CaM interaction in vivo (see below).

Native CP110 Associates with CaM and Centrin in High-Molecular-Weight Complexes

Our experiments identified CaM as a physiologically relevant interacting protein. We asked whether CaM was the major protein associated with CP110 or if additional proteins could interact with this centriolar protein. We determined the native molecular weight of CP110–containing complexes by fractionating whole cell extracts using size-exclusion chromatography. Interestingly, a substantial portion of CP110 reproducibly migrated as a high-molecular-mass complex, ranging from 300 kDa to 3 MDa (Figure 5A, fractions 15–24). Certain high molecular weight fractions (fractions 18–19) contained CP110-CaM complexes (Figure 5B), but given the mass of CaM, we inves-

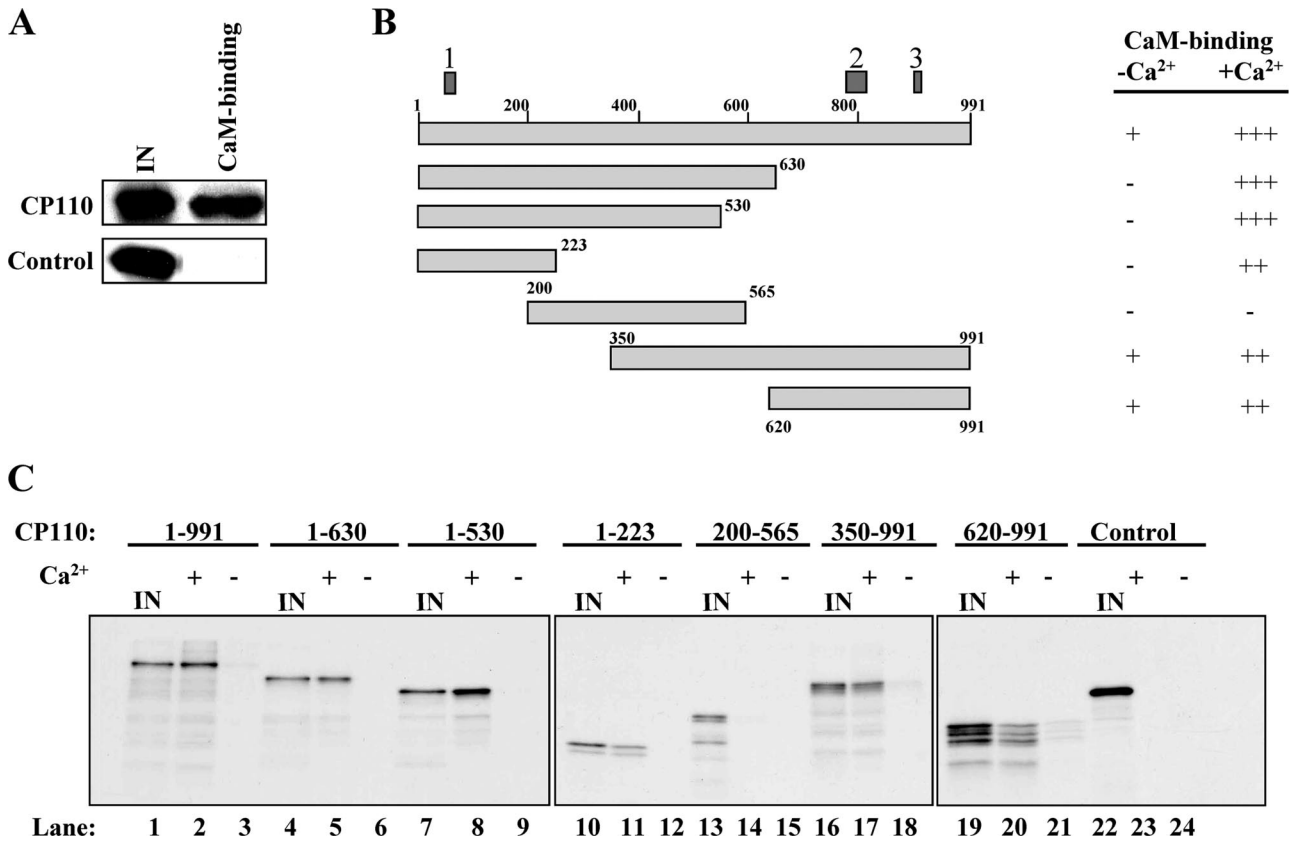

Figure 3. CP110 interacts with CaM through multiple domains in vitro. (A) Binding of in vitro–translated ^{35}S-labeled CP110 or ^{35}S-labeled control (luciferase) with CaM agarose beads. The binding was carried out in a lysis buffer without the addition of EGTA or calcium. (B) Schematic representation of CP110 truncation mutants used to map CaM-binding domains. The three boxes (1, 2, and 3) denote the three CaM-binding domains predicted by the Calmodulin Target Database. The strength of CaM-binding was quantitated with a densitometer and is categorized as strong (+++), intermediate (++), weak (+), or none (−). (C) In vitro binding assays. The results were summarized in B.

tigated whether CP110 could associate with additional proteins.

Centrin, like CaM, is a member of the EF-hand family of small Ca^{2+}-binding proteins, shares significant sequence identity (45%) with CaM at the amino acid level, and is a centrosomal component concentrated within the distal lumen of centrioles (Salisbury, 1995, 2002; Paoletti *et al.*, 1996). We have previously reported colocalization of CP110 with centrin, suggesting that CP110 might localize to centrioles (Chen *et al.*, 2002). In light of the similarity of centrin to CaM, we tested the possibility that centrin could also interact with CP110 in vivo. We demonstrated that endogenous CP110 and centrin interact in cells by showing that both proteins coimmunoprecipated from 293T cell extracts with anti-centrin antibody but not with an irrelevant antibody (Figure 5C). This in vivo interaction was also observed using both normal diploid and transformed cells (HeLa, T98G, Saos2, and IMR90). Moreover, bacterially expressed GST-centrin incubated with 293T extracts associated with endogenous CP110, but not with a noncentrosomal protein, both in the absence ($-Ca^{2+}$) and presence of calcium ($+Ca^{2+}$; Figure 5D).

We performed experiments to determine whether CP110 fractionated with the bulk of centriolar or pericentriolar proteins in an initial attempt to more finely resolve the network of proteins that interact with CP110. Immunoblotting indicated that a percentage of the total cellular pool of centrin was present in a monomeric form with a molecular mass of ~20 kDa (Figure 5A, fractions 30–32). Interestingly, a substantial portion of centrin reproducibly migrated as a high-molecular-mass complex, ranging from 300 kDa to 3 MDa (Figure 5A, fractions 15–26), that cofractionated with CP110 (Figure 5A, fractions 15–24). To rule out the possibility that this observation resulted from trapping of centrosomal fragments in microtubule networks (rather than high-molecular-weight complexes consisting of partially disassembled centrosome components), we treated extracts with nocodazole to depolymerize microtubules and performed chromatography in the presence of this drug. Interestingly, the chromatographic behavior of CP110 and centrin was not altered, suggesting that the observed molecular weight estimates are not simply a result of CP110 or centrin association with microtubules (unpublished data). We have also examined the distribution of a number of unrelated proteins under identical conditions and found that they were present in a distinct set of fractions, rendering unlikely the possibility that the observed fractionation was a result of nonspecific aggregation. Interestingly, the distribution of CP110 more closely resembled that of centrin than pericentriolar matrix proteins CG-NAP (Figure 5A, fractions 14–19) and kendrin (Figure 5A, fractions 15–19). The cofractionation of CG-NAP and kendrin is also consistent with previous findings, indicating that CG-NAP and kendrin interact with one another (Takahashi *et al.*, 2002). Next, we asked whether centrin associated with CP110 in the very high-molecular-weight range fractions. Immunoprecipitation experiments indicated

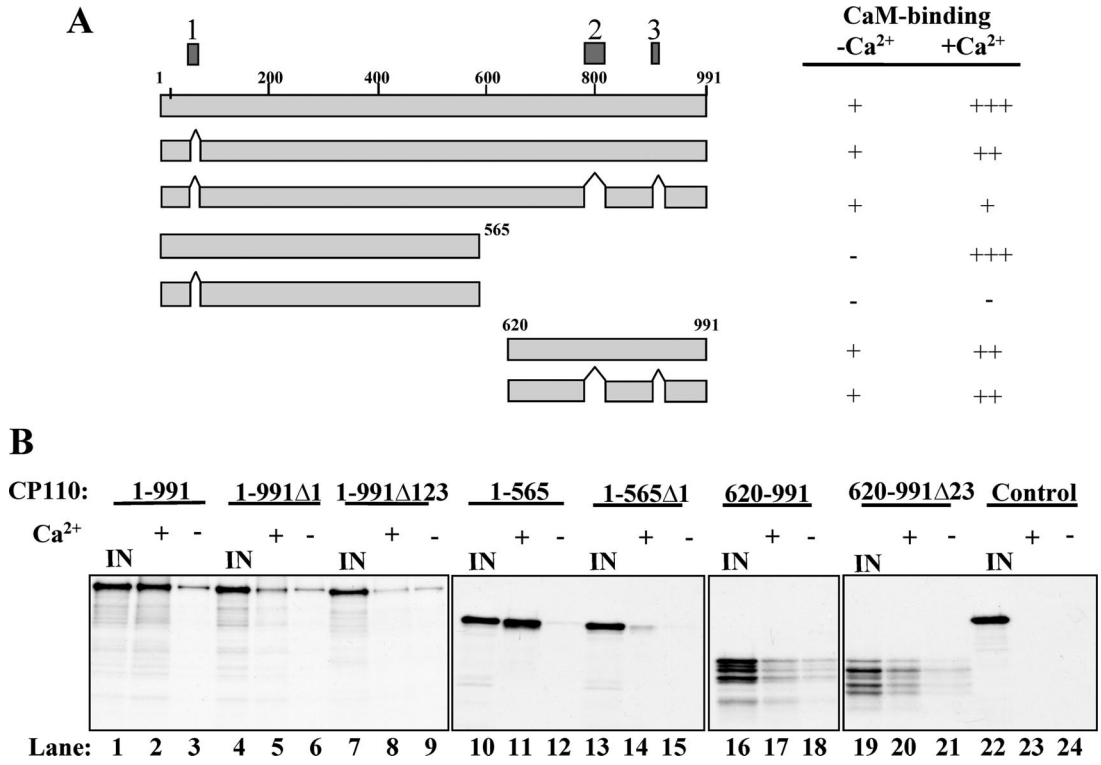

Figure 4. CP110 CaM-binding mutants 1-565Δ1 and 1-991Δ123 interact poorly with CaM in vitro. (A) Schematic representation of CP110 truncation mutants and CaM-binding domains. The three boxes (1, 2, and 3) denote the three CaM-binding domains predicted by the Calmodulin Target Database. The strength of CaM-binding was quantitated with a densitometer and is categorized as strong (+++), intermediate (++), weak (+), or none (−). (B) In vitro binding assays. The results were summarized in A.

that CP110 indeed associated with centrin in a subset of the high-molecular-weight fractions in which the proteins coeluted (Figure 5E, fractions 16–18). Thus, we believe that CP110 forms very large complexes with centrin, CaM, and a cohort of uncharacterized proteins.

Because CP110 and centrin associate under physiological conditions, we tested whether this interaction was direct or mediated through additional proteins. We performed binding assays by incubating in vitro–translated CP110 with either GST-tagged or in vitro–translated centrin. These studies failed to reveal significant binding between CP110 and centrin, indicating that the association is most likely mediated through additional, unknown protein(s). Thus, CP110 interacts with two calcium-binding proteins, CaM and centrin, in a fundamentally different manner.

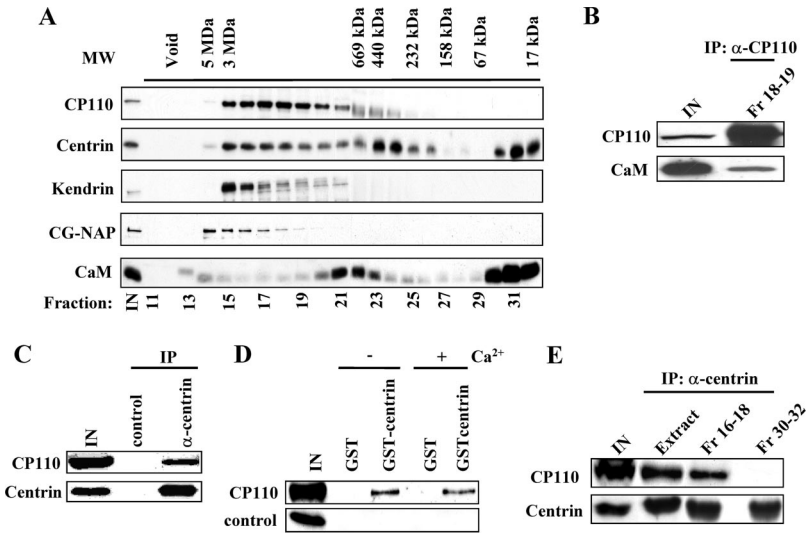

Figure 5. Centrin interacts with CP110 in vivo and cofractionates with CP110 and CaM in high-molecular-weight complexes. (A) Cell extract was chromatographed on a Superose 6 gel filtration column, and the resulting fractions were Western blotted with antibodies against CP110, centrin, kendrin, CG-NAP, or CaM. Estimated molecular weights are indicated at the top of the panel. (B) Western blotting of endogenous CP110 and CaM after immunoprecipitation with anti-CP110 antibody using fractions 18–19 (Fr 18–19) from the Superose 6 column. (C) Western blot of endogenous CP110 and centrin after immunoprecipitation with anti-centrin antibody or control (anti-calnexin) antibody using extracts from 293T cells. (D) Western blotting of endogenous CP110 and control (giantin) after binding of either GST or GST-centrin prebound to glutathione agarose beads with 293T extracts. The buffer used for prebinding and the 293T extracts were supplemented with either EGTA ($-Ca^{2+}$) or calcium ($+Ca^{2+}$). (E) Western blotting of endogenous CP110 and centrin after immunoprecipitation with anti-centrin antibody using 293T cell extract (Extract), fractions 16–18 (Fr 16–18), or fractions 30–32 (Fr 30–32) from the Superose 6 column.

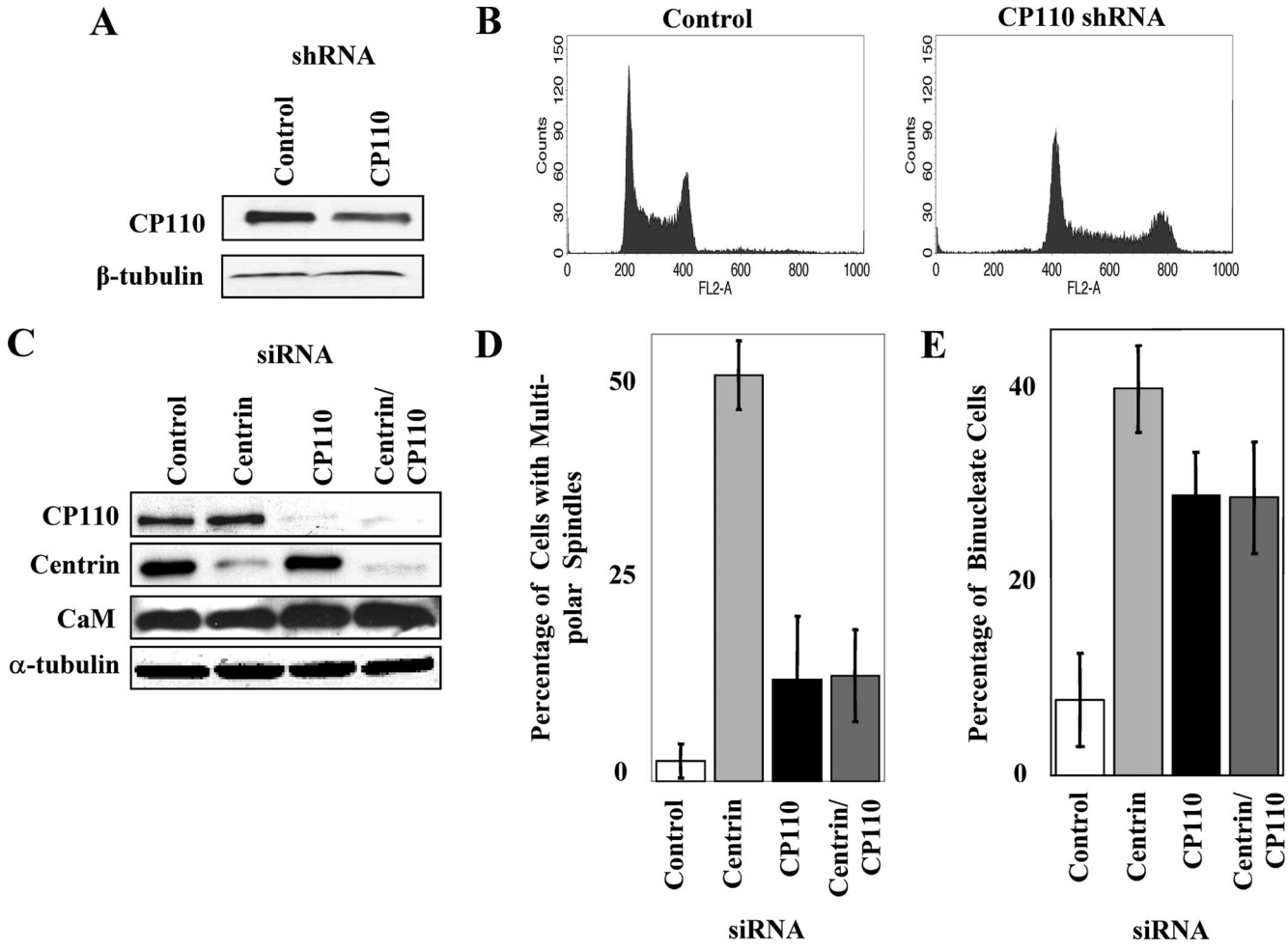

Figure 6. RNAi-mediated suppression of CP110 induces polyploidy and results in the formation of binucleate cells. (A) Western blot detection of CP110 proteins in U2OS cells transfected with a control or CP110 shRNA expression vector. β-tubulin was used as a loading control. (B) FACS analysis of control or CP110 shRNA-expressing cells. (C) Western blotting of CP110, centrin, and CaM in HeLa cells treated with control, centrin, CP110, or centrin and CP110 siRNAs. α-tubulin was used as a loading control. (D) The percentages of cells with multipolar spindles were determined. About 50 mitotic cells were scored for each condition, and the experiments were repeated at least twice. (E) The percentages of binucleate cells were determined. About 200 cells were scored for each condition, and the experiments were repeated at least three times.

Phenotypic Effect of CP110 Depletion and Induction of Binucleate Cell Formation

We have previously reported that cells stably expressing a mutant version of CP110 that lacks the majority of CDK phosphorylation sites exhibit marked tetraploidy (Chen *et al.*, 2002). To determine the effects of long-term depletion of CP110, we derived several independent, stable clones expressing short hairpin RNAs (shRNA) targeting the CP110 coding sequence. As a control, we generated several lines harboring only the integrated expression vector. Suppression of CP110 with the shRNA resulted in a ~50% reduction in CP110 protein levels when compared with the control (Figure 6A). Remarkably, clones expressing the CP110 shRNA displayed polyploidy and exhibited tetraploid 4N or 8N DNA content, in contrast to controls that were diploid (Figure 6B). We confirmed that these cells were in tetraploid G_1 and G_2 state using a nocodazole block (Chen *et al.*, 2002 and unpublished data). Thus, normal cell cycle progression appears to be exquisitely sensitive to the dosage of CP110 protein, because a twofold reduction in protein levels has dramatic effects on genomic stability.

Recent studies demonstrated a requirement for centrin in centriole duplication and cytokinesis in human cells (Salisbury *et al.*, 2002). The occurrence of tetraploidy in cells stably depleted of CP110 suggested that loss of this protein could provoke cytokinesis defects. To examine this possibility and to comprehensively describe the phenotypic consequences of CP110 depletion, we used RNAi to acutely deplete CP110 and centrin individually. We showed that treatment of cells with a centrin-specific siRNA resulted in a substantial reduction of centrin expression with no discernible effect on CP110 or CaM protein levels, and conversely, depletion of CP110 did not alter centrin or CaM expression (Figure 6C).

Microscopic examination of siRNA-treated cells led to several important conclusions. First, diminution of CP110 led to unscheduled centrosome separation, as expected (Chen *et al.*, 2002). Second, immunofluorescent detection of both centrin and CP110 indicated that depletion of CP110 had no apparent impact on the localization of at least three centrosomal markers, namely, centrin, γ-tubulin, and C-NAP (Figure 7 and unpublished data). Furthermore, sup-

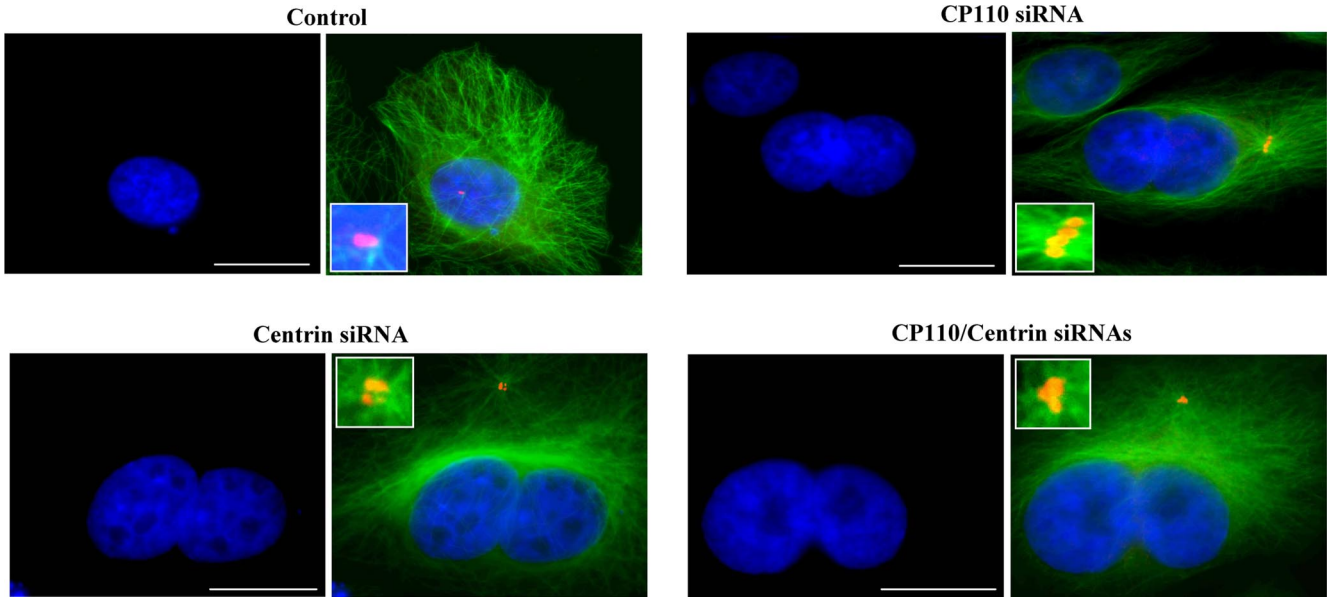

Figure 7. Ablation of CP110 and centrin results in the formation of binucleate cells. HeLa cells transiently transfected with control, CP110, centrin, or CP110 and centrin siRNAs stained with antibodies to α-tubulin (green), γ-tubulin (red), and with DAPI (blue). Only DAPI and merged images are shown. Bar, 10 μm; insets, 2 μm.

pression of centrin expression did not affect CP110 localization, suggesting that these proteins are independently recruited and anchored to the centrosome. Third, we frequently observed multipolar spindles in cells with diminished CP110 (Figure 6D). This result is strikingly reminiscent of centrin-depleted cells in which multipolar mitotic figures predominated (Salisbury *et al.*, 2002; Figure 6D). Remarkably, like centrin depletion, CP110 depletion also resulted in a significant increase in binucleate cells (Figure 6E). Importantly, this phenotype was also observed both with a second, distinct CP110 siRNA and in normal diploid IMR90 cells. Interestingly, we note that CP110-depleted binucleate cells often display centrosomal clustering, and these centrosomes appear to be functional microtubule-organizing centers (Figure 7). Indeed, loss of CP110 had no apparent effect on de novo microtubule nucleation (unpublished data). We also tested in parallel the impact of centrin depletion. In agreement with previous findings, depletion of centrin resulted in a fivefold increase in the incidence of binucleate cells (Figure 6E).

Loss of CP110 Results in Cytokinesis Failure

Next, we investigated in greater detail how binucleate cells could arise as a result of CP110 depletion. Depletion of at least four other centrosomal proteins (centriolin, γ-tubulin, CEP55, and centrobin) besides centrin results in cytokinesis defects (Shu *et al.*, 1995; Gromley *et al.*, 2003; Fabbro *et al.*, 2005; Zou *et al.*, 2005). Furthermore, given the fact that CaM plays a well-established role in cytokinesis (Moser *et al.*, 1997; Lippincott and Li, 1998; Osman and Cerione, 1998), we asked whether binucleate cells with a polyploid DNA content arose after CP110 depletion as a consequence of cytokinesis failure using real-time videomicroscopy. We monitored siRNA-transfected cells with rhodamine-labeled oligonucleotides and used differential interference contrast (DIC) to image live HeLa cells progressing through mitosis (Figure 8). Cells treated with a nonspecific control duplex progressed through mitosis and cleavage, after which daughter cells separated from one another, as expected. In striking contrast, cells treated with CP110 siRNAs progressed through mitosis but failed at a late stage in cytokinesis, leading to rapid fusion of emerging daughter cells and binucleate cell formation. We observed a similar block in cells treated with a centrin siRNA (unpublished data). We note that unlike the centriolin knockdown, which also gives rise to cytokinesis defects, we did not detect a frequent occurrence of long intercellular bridges.

Immunofluorescence experiments indicated that microtubules were not globally disrupted by CP110 knockdown, nor did we observe inappropriate localization of centrosomal markers (centrin, γ-tubulin, and C-NAP), suggesting that catastrophic disruption of centrosome assembly or microtubule arrays is not likely to underlie the cytokinesis defect (unpublished data). We conclude that CP110 plays a role in cytokinesis and that loss of this protein results in cytokinesis failure.

Functional Interactions between CP110 and Centrin

Given that CP110 and centrin depletion have profound effects on centrosome function and cytokinesis, we initiated a series of experiments to examine functional interactions between CP110 and centrin in vivo. We reasoned that if the two proteins function in concert in a linear manner, it might be expected that depletion of both proteins through RNAi would not have additive effects on binucleate cell formation. We compared the extent of induction of the binucleate phenotype in cells singly depleted of CP110 or centrin and cells depleted of both proteins. Western blotting confirmed that both CP110 and centrin protein levels were significantly reduced (Figure 6C). Simultaneous depletion resulted in a significant enrichment of binucleate cells (Figure 7), and interestingly, the percentage of binucleate cells closely approximated what we observed when either CP110 or centrin

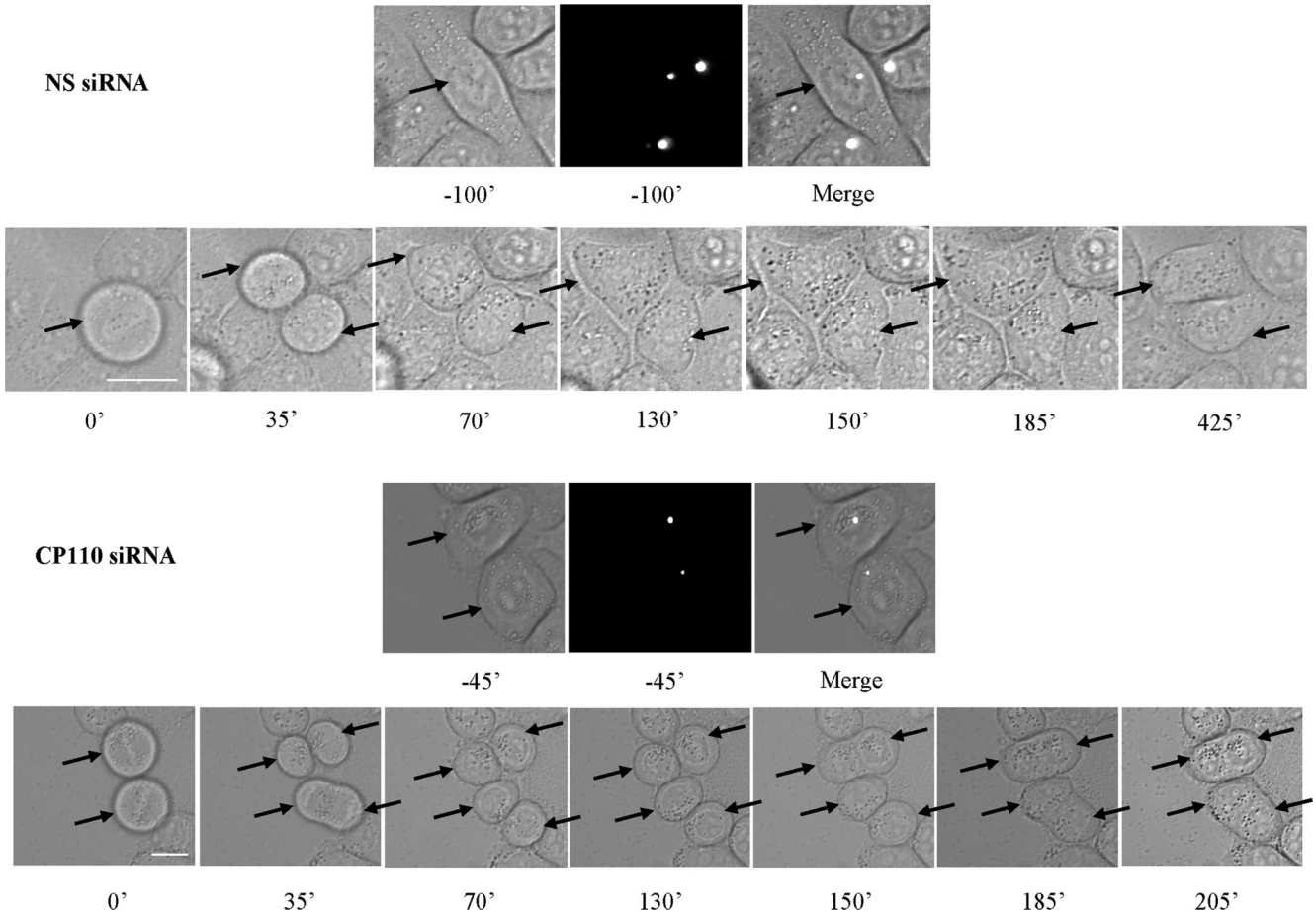

Figure 8. Cells depleted of CP110 exhibit a late cytokinesis defect. HeLa cells transfected with rhodamine-labeled and nonspecific control siRNA oligonucleotides or rhodamine-labeled and CP110 siRNA oligonucleotides were observed by DIC time-lapse videomicroscopy. At least 80 mitotic events were scored for each condition. A representative cell treated with control and two representative cells treated with CP110 siRNAs are illustrated. Times from the onset of metaphase are indicated. The fluorescent images were taken before mitosis. Arrows indicate transfected cells and their daughter cells. Bar, 10 μm.

was knocked down individually (Figure 6E). A similar observation was also made in IMR90 cells. As a control, we showed that the frequency of binucleate cell formation in cells cotransfected with both control and centrin siRNAs was comparable to cells treated with centrin siRNA alone. These observations indicate that CP110 and centrin interact functionally, consistent with the possibility that interference with CP110 or centrin deregulates a common step in cytokinesis.

Ectopic Expression of the CP110 CaM-binding Mutant Induces Binucleate Cell Formation

Our in vitro binding data suggested that removal of CaM-binding motifs dramatically reduced the ability of CP110 to interact with CaM (Figure 4). To address the functional significance of CaM-binding to CP110 in vivo, we ectopically expressed Flag-tagged CP110 CaM-binding mutants (Flag-1-565Δ1 and Flag-1-991Δ123) in human cells and investigated the impact on cell cycle progression and completion of cytokinesis. These two mutants were chosen because they exhibited the most dramatic decreases in affinity for CaM in vitro. Immunofluorescence microscopy revealed that only a single mutant, Flag-1-991Δ123 (in addition to Flag-1-991), properly localized to the centrosome (Figure 9A and unpublished data), and therefore, we studied this mutant in detail. Western blot analysis indicated a three- to fourfold increase in the levels of epitope-tagged CP110 relative to the endogenous protein (Figure 9B). Flag-1-991Δ123 exhibited significantly reduced affinity for CaM compared with the wild-type control in coimmunoprecipitation experiments (Figure 9D). Interestingly, both the wild type and CaM-binding mutant proteins interacted equally well with centrin (Figure 9C), rendering less likely the possibility that the mutant protein fails to interact with CaM owing to global misfolding. Remarkably, expression of the CaM-binding mutant did not grossly affect progression through G_1 or S phase (unpublished data), although it resulted in a significant ($p < 0.01$) and reproducible elevation in the number of binucleate cells when compared with vector and wild-type controls (Figure 9E). In conclusion, our studies strongly support the notion that disrupting the interaction between CP110 and CaM induces cytokinesis failure and binucleate cell formation and that CP110 interacts with CaM and centrin in fundamentally different ways. We have therefore for the first time established a clear and important functional link between CaM-binding to CP110 and cytokinesis.

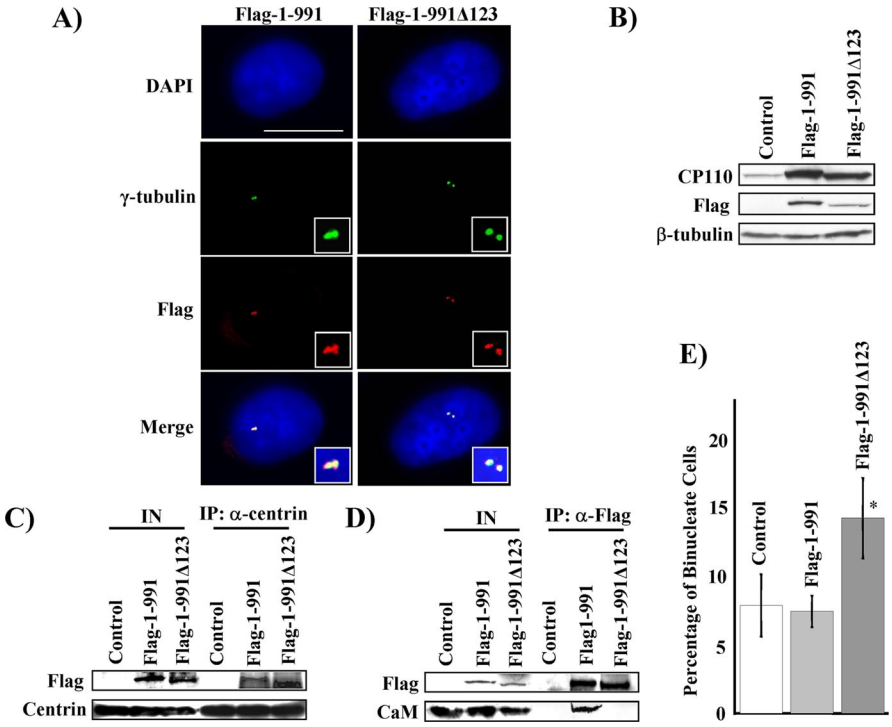

Figure 9. Ectopic expression of a CP110 CaM-binding mutant in vivo results in binucleate cell formation. (A) U2OS cells transiently transfected with a Flag-1-991 or Flag-1-991Δ123 expression vector stained with antibodies to γ-tubulin (green), Flag (red), and with DAPI (blue). Bar, 10 μm; insets: 2 μm. (B) Western blot detection of CP110 proteins in HeLa cells transfected with a control, Flag-1-991 or Flag-1-991Δ123 expression vector. The blots labeled CP110 and Flag were probed with anti-CP110 and anti-Flag antibodies, respectively. β-tubulin was used as a loading control. (C) Western blotting of Flag-CP110 and endogenous centrin after immunoprecipitation with anti-centrin antibody using 293T cell extract expressing Flag (control), Flag-1-991 or Flag-1-991Δ123 proteins. IN, input. (D) Western blotting of Flag-CP110 and endogenous CaM after immunoprecipitation with anti-Flag antibody using 293T cell extract expressing Flag (control), Flag-1-991 or Flag-1-991Δ123 proteins. IN, input. (E) HeLa cells were transfected with a G418-resistant marker along with a control, Flag-1-991 or Flag-1-991Δ123 expression vector. The percentages of binucleate cells were determined after 5–6 d of selection in the presence of G418. About 500 cells were scored for each condition, and the experiments were repeated at least twice. Asterisk denotes that the percentage of binucleate cells resulting from expression of the mutant (Flag-1-991Δ123) is significantly higher than that of control ($p < 0.01$) or wild-type ($p < 0.01$) based on a two-tailed Student's *t* test.

DISCUSSION

A detailed molecular description of the events involved in controlling the mammalian centrosome cycle has not yet been achieved. Beyond the events surrounding the duplication and function of this organelle, recent experiments involving surgical removal of this organelle and laser ablation also suggest that the centrosome plays a role in the initiation and completion of cytokinesis (Hinchcliffe *et al.*, 2001; Khodjakov and Rieder, 2001; Piel *et al.*, 2001). Interestingly, several centrosomal proteins participate in cytokinesis, including centrin, centriolin, γ-tubulin, CEP55, and centrobin (Shu *et al.*, 1995; Salisbury *et al.*, 2002; Gromley *et al.*, 2003; Keryer *et al.*, 2003; Fabbro *et al.*, 2005; Zou *et al.*, 2005). Clearly, elucidating the molecular architecture of the centrosome and deciphering the role of critical centrosomal proteins are essential for understanding not only centrosome function but also cytokinesis.

In an effort to begin identifying the proteins that play an important role in both processes, we have focused on CP110, which we have shown is important for centrosome reduplication and separation. Here, we have begun identifying CP110-interacting proteins and have found that two calcium-binding proteins, CaM and centrin, associate with high-molecular-weight, native CP110 complexes. CaM, a multifunctional Ca^{2+}-binding protein and an important cell cycle and cytokinesis regulator (Moser *et al.*, 1997; Lippincott and Li, 1998; Osman and Cerione, 1998), has also been implicated in centrosome function and has been shown to bind other centrosomal proteins (Flory *et al.*, 2000; Takahashi *et al.*, 2002). Centrin, like CaM, is a member of the EF-hand family of small Ca^{2+}-binding proteins. It shares significant sequence identity (45%) with CaM at the amino acid level and is a centrosomal component concentrated within the distal lumen of centrioles (Salisbury, 1995; Paoletti *et al.*, 1996; Salisbury *et al.*, 2002). Our studies show that although CP110 binds to CaM directly, the association between CP110 and centrin does not appear to be direct and therefore may be mediated through other proteins (Kilmartin, 2003). Further, we demonstrate that a CP110 mutant unable to bind CaM can still interact with centrin. Thus, two highly related calcium-binding proteins interact with CP110 in fundamentally different ways. Mass spectrometry should allow us to gain further insight into the molecular composition of the large, native CP110-CaM-centrin complexes and to identify additional proteins that connect CP110 to centrin.

Our observation that the association between CP110 and CaM is cell cycle–independent is especially interesting, because CaM does not appear to be concentrated at the centrosome during interphase. One likely explanation is that a small portion of total cellular CaM is always present at the centrosome. Indeed, CaM is an integral component of the central plaque of the yeast microtubule-nucleating center, the spindle pole body (Geiser *et al.*, 1993; Spang *et al.*, 1996; Sundberg *et al.*, 1996). Recent proteomic characterization has begun to shed light on the structure and composition of the human centrosome and has also detected the presence of CaM in this organelle during interphase (Andersen *et al.*, 2003). In addition, CaM has been shown to interact with two pericentriolar matrix proteins, kendrin and CG-NAP (Witczak *et al.*, 1999; Flory *et al.*, 2000). Further electron microscopic studies will be needed to define precisely the localization CaM at an ultrastructural level.

Several lines of evidence point toward a function for CP110 in cytokinesis, and the interaction between CP110, centrin, and CaM appears to play a pivotal role here. First, CP110 binds to CaM and centrin, both of which have been implicated in regulating this event (Li *et al.*, 1999; Salisbury *et al.*, 2002). Second, expression of a nonphosphorylated

CP110 mutant induces overt polyploidy, a common hallmark of cytokinesis failure (Chen *et al.*, 2002). In addition, long-term disruption of CP110 with shRNA leads to polyploidy and phenocopies the CP110 phospho-mutant, whereas short-term disruption results in a binucleate phenotype, another outcome of cytokinesis malfunction. The striking similarity between the phenotypes resulting from CP110, CaM, or centrin ablation indicates that these proteins are likely to be connected by a common biological function. Interestingly, when CP110 and centrin are disabled together, we do not observe additive increases in the percentage of binucleate cells. These experiments suggest functional interactions between these two proteins and raise the possibility that CP110, centrin, and CaM could function in a genetic pathway that regulates the centrosome cycle and progression through cytokinesis.

We have further demonstrated a functional link between CP110 and CaM and its relevance to cytokinesis by studying the effects of inactivating three high-affinity CaM-binding domains within CP110. This mutant, 1-991Δ123, localizes to the centrosome, binds poorly to CaM in vitro and in vivo, and induces binucleate cell formation in vivo. The phenotype resulting from expression of this mutant is less pronounced than that observed after depletion of CP110 with siRNAs. One possible explanation is that the effect of expressing this mutant is dampened by the presence of endogenous CP110. Another possibility is that suppression of CP110 expression disrupts its interaction with both CaM and centrin, whereas overexpression of the CaM-binding mutant strictly abolishes the CP110-CaM interaction. Indeed, the CP110 CaM-binding mutant is fully capable of interacting with centrin (Figure 9C). It will be interesting to map the centrin-binding domains within CP110 to create a CP110 mutant refractory to binding both CaM and centrin. Such a mutant could produce a more potent binucleate phenotype.

Our observation of binucleate cells subsequent to CP110 depletion is reminiscent of findings in which acentrosomal cells were choreographed through mitosis (Piel *et al.*, 2001). Acentriolar *Drosophila* cells exhibit hallmarks of incomplete cytokinesis, including intercellular bridges, binucleate formation, and polyploidy, arguing for a role for centrosomes in the process of cell abscission at the conclusion of cytokinesis (Debec, 1978; Debec and Abbadie, 1989). Recent reports have revealed several proteins whose depletion could result in cytokinesis defects, and it is intriguing that four additional centriolar proteins other than CP110 (centrin, centriolin, CEP55, and centrobin) appear to play a role in cytokinesis (Salisbury *et al.*, 2002; Gromley *et al.*, 2003; Fabbro *et al.*, 2005; Zou *et al.*, 2005). However, unlike centriolin or CEP55 silencing, which appears to result in long intercellular bridges, suppression of CP110 or centrobin expression leads to failed abscission without such bridges. This could suggest that centrosomes play multiple, distinct roles during cytokinesis. Although the basis for cytokinesis defects brought on by the loss of CP110 remains unknown, our findings suggest that abnormal cytokinesis does not result from an inability to nucleate or organize microtubules. Interestingly, recent evidence suggests that centriolin may play a role in anchoring vesicle-targeting and vesicle-fusion protein complexes at the midbody during abscission (Gromley *et al.*, 2005). On the basis of the conserved phenotypes resulting from depletion of CP110 and these centriolar proteins, it should be possible to begin dissecting the mechanisms that connect centrosome function and cytokinesis.

Although we have not yet fully defined the pathway(s) that link the function of centrosomal proteins such as CP110 or centrin with faithful completion of cytokinesis, the direct and robust interaction between CP110 and CaM offers several potential avenues for further exploration. Another interesting possibility involves interactions with a cytoskeletal network, and the identification of β-spectrin and α-actinin as CP110-interacting proteins in our two-hybrid screen may be particularly revealing in this regard. Furthermore, we have begun to biochemically fractionate centrosomal components, suggesting possible proteomic approaches for the identification of centrosome subassemblies. Future studies that combine genetic and proteomic approaches will allow us to unravel the complex functional relationships between different centrosomal components and provide important clues for elucidating their role in cytokinesis.

ACKNOWLEDGMENTS

We are grateful to M. Vidal for help with the yeast two-hybrid screen. We thank M. Takahaski and Y. Ono for the gift of anti-kendrin and anti-CG-NAP antibodies, M. Cole for the gift of pCBF plasmid DNA, and D. C. Chang for the gift of pCDNA3-GFP-CaM plasmid DNA. We are especially grateful to P. Asp in our laboratory for invaluable assistance with fast-performance liquid chromatography purification and to members of M. Philips' laboratory for assistance with real-time videomicroscopy. We thank all members of the Dynlacht laboratory for constructive advice and encouragement. This work was supported in part by an Irma T. Hirschl Career Scientist Award to B.D.D., for which he is grateful. W.Y.T. was supported by a Natural Sciences and Engineering Research Council of Canada postdoctoral fellowship and an Alberta Heritage Foundation for Medical Research full-time postdoctoral fellowship.

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.

Badano, J. L., Teslovich, T. M., and Katsanis, N. (2005). The centrosome in human genetic disease. Nat. Rev. Genet. *6*, 194–205.

Chen, Z., Indjeian, V. B., McManus, M., Wang, L., and Dynlacht, B. D. (2002). CP110, a cell cycle-dependent CDK substrate, regulates centrosome duplication in human cells. Dev. Cell *3*, 339–350.

Chin, D., and Means, A. R. (2000). Calmodulin: a prototypical calcium sensor. Trends Cell Biol. *10*, 322–328.

Debec, A. (1978). Haploid cell cultures of *Drosophila melanogaster*. Nature *274*, 255–256.

Debec, A., and Abbadie, C. (1989). The acentriolar state of the *Drosophila* cell lines 1182. Biol. Cell *67*, 307–311.

Doxsey, S. (2001). Re-evaluating centrosome function. Nat. Rev. Mol. Cell Biol. *2*, 688–698.

Fabbro, M. *et al.* (2005). Cdk1/Erk2- and Plk1-dependent phosphorylation of a centrosome protein, Cep55, is required for its recruitment to midbody and cytokinesis. Dev. Cell *9*, 477–488.

Flory, M. R., Moser, M. J., Monnat, R. J., Jr., and Davis, T. N. (2000). Identification of a human centrosomal calmodulin-binding protein that shares homology with pericentrin. Proc. Natl. Acad. Sci. USA *97*, 5919–5923.

Geiser, J. R., Sundberg, H. A., Chang, B. H., Muller, E. G., and Davis, T. N. (1993). The essential mitotic target of calmodulin is the 110-kilodalton component of the spindle pole body in *Saccharomyces cerevisiae*. Mol. Cell. Biol. *13*, 7913–7924.

Gromley, A., Jurczyk, A., Sillibourne, J., Halilovic, E., Mogensen, M., Groisman, I., Blomberg, M., and Doxsey, S. (2003). A novel human protein of the maternal centriole is required for the final stages of cytokinesis and entry into S phase. J. Cell Biol. *161*, 535–545.

Gromley, A., Yeaman, C., Rosa, J., Redick, S., Chen, C. T., Mirabelle, S., Guha, M., Sillibourne, J., and Doxsey, S. J. (2005). Centriolin anchoring of exocyst and SNARE complexes at the midbody is required for secretory-vesicle-mediated abscission. Cell *123*, 75–87.

Hinchcliffe, E. H., Miller, F. J., Cham, M., Khodjakov, A., and Sluder, G. (2001). Requirement of a centrosomal activity for cell cycle progression through G1 into S phase. Science *291*, 1547–1550.

Keryer, G., Witczak, O., Delouvee, A., Kemmner, W. A., Rouillard, D., Tasken, K., and Bornens, M. (2003). Dissociating the centrosomal matrix protein

AKAP450 from centrioles impairs centriole duplication and cell cycle progression. Mol. Biol. Cell *14*, 2436–2446.

Khodjakov, A., and Rieder, C. L. (2001). Centrosomes enhance the fidelity of cytokinesis in vertebrates and are required for cell cycle progression. J. Cell Biol. *153*, 237–242.

Kilmartin, J. V. (2003). Sfi1p has conserved centrin-binding sites and an essential function in budding yeast spindle pole body duplication. J. Cell Biol. *162*, 1211–1221.

Li, C. J., Heim, R., Lu, P., Pu, Y., Tsien, R. Y., and Chang, D. C. (1999). Dynamic redistribution of calmodulin in HeLa cells during cell division as revealed by a GFP-calmodulin fusion protein technique. J. Cell Sci. *112*(Pt 10), 1567–1577.

Lingle, W. L., Lutz, W. H., Ingle, J. N., Maihle, N. J., and Salisbury, J. L. (1998). Centrosome hypertrophy in human breast tumors: implications for genomic stability and cell polarity. Proc. Natl. Acad. Sci. USA *95*, 2950–2955.

Lippincott, J., and Li, R. (1998). Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis. J. Cell Biol. *140*, 355–366.

Matsumoto, Y., and Maller, J. L. (2002). Calcium, calmodulin, and CaMKII requirement for initiation of centrosome duplication in *Xenopus* egg extracts. Science *295*, 499–502.

Moser, M. J., Flory, M. R., and Davis, T. N. (1997). Calmodulin localizes to the spindle pole body of *Schizosaccharomyces pombe* and performs an essential function in chromosome segregation. J. Cell Sci. *110*(Pt 15), 1805–1812.

Osman, M. A., and Cerione, R. A. (1998). Iqg1p, a yeast homologue of the mammalian IQGAPs, mediates cdc42p effects on the actin cytoskeleton. J. Cell Biol. *142*, 443–455.

Paoletti, A., Moudjou, M., Paintrand, M., Salisbury, J. L., and Bornens, M. (1996). Most of centrin in animal cells is not centrosome-associated and centrosomal centrin is confined to the distal lumen of centrioles. J. Cell Sci. *109*(Pt 13), 3089–3102.

Piel, M., Nordberg, J., Euteneuer, U., and Bornens, M. (2001). Centrosome-dependent exit of cytokinesis in animal cells. Science *291*, 1550–1553.

Pihan, G. A., Purohit, A., Wallace, J., Knecht, H., Woda, B., Quesenberry, P., and Doxsey, S. J. (1998). Centrosome defects and genetic instability in malignant tumors. Cancer Res. *58*, 3974–3985.

Salisbury, J. L. (1995). Centrin, centrosomes, and mitotic spindle poles. Curr. Opin. Cell Biol. *7*, 39–45.

Salisbury, J. L., Suino, K. M., Busby, R., and Springett, M. (2002). Centrin-2 is required for centriole duplication in mammalian cells. Curr. Biol. *12*, 1287–1292.

Shu, H. B., Li, Z., Palacios, M. J., Li, Q., and Joshi, H. C. (1995). A transient association of gamma-tubulin at the midbody is required for the completion of cytokinesis during the mammalian cell division. J. Cell Sci. *108*(Pt 9), 2955–2962.

Sluder, G., and Nordberg, J. J. (2004). The good, the bad and the ugly: the practical consequences of centrosome amplification. Curr. Opin. Cell Biol. *16*, 49–54.

Spang, A., Grein, K., and Schiebel, E. (1996). The spacer protein Spc110p targets calmodulin to the central plaque of the yeast spindle pole body. J. Cell Sci. *109*(Pt 9), 2229–2237.

Sui, G., Soohoo, C., Affar el, B., Gay, F., Shi, Y., Forrester, W. C., and Shi, Y. (2002). A DNA vector-based RNAi technology to suppress gene expression in mammalian cells. Proc. Natl. Acad. Sci. USA *99*, 5515–5520.

Sundberg, H. A., Goetsch, L., Byers, B., and Davis, T. N. (1996). Role of calmodulin and Spc110p interaction in the proper assembly of spindle pole body components. J. Cell Biol. *133*, 111–124.

Takahashi, M., Yamagiwa, A., Nishimura, T., Mukai, H., and Ono, Y. (2002). Centrosomal proteins CG-NAP and kendrin provide microtubule nucleation sites by anchoring gamma-tubulin ring complex. Mol. Biol. Cell *13*, 3235–3245.

Takuwa, N., Zhou, W., and Takuwa, Y. (1995). Calcium, calmodulin and cell cycle progression. Cell Signal. *7*, 93–104.

Walhout, A. J., and Vidal, M. (2001). High-throughput yeast two-hybrid assays for large-scale protein interaction mapping. Methods *24*, 297–306.

Witczak, O., Skalhegg, B. S., Keryer, G., Bornens, M., Tasken, K., Jahnsen, T., and Orstavik, S. (1999). Cloning and characterization of a cDNA encoding an A-kinase anchoring protein located in the centrosome, AKAP450. EMBO J. *18*, 1858–1868.

Woo, M. S., Sanchez, I., and Dynlacht, B. D. (1997). p130 and p107 use a conserved domain to inhibit cellular cyclin-dependent kinase activity. Mol. Cell. Biol. *17*, 3566–3579.

Yap, K. L., Kim, J., Truong, K., Sherman, M., Yuan, T., and Ikura, M. (2000). Calmodulin target database. J. Struct. Funct. Genom. *1*, 8–14.

Zou, C., Li, J., Bai, Y., Gunning, W. T., Wazer, D. E., Band, V., and Gao, Q. (2005). Centrobin: a novel daughter centriole-associated protein that is required for centriole duplication. J. Cell Biol. *171*, 437–445.