Centrosomal CAP350 protein stabilises microtubules associated with the Golgi complex

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Summary

A comprehensive model of how the centrosome organises the microtubule network in animal cells has not yet been elucidated. Here we show that the centrosomal large CAP-Gly protein CAP350 is not only present at the centrosome, but is also present as numerous dots in the pericentrosomal area. Using in vitro and in vivo expression of partial constructs, we demonstrated that CAP350 binds microtubules through an N-terminal basic region rather than through its CAP-Gly domain. CAP-Gly-containing domains of CAP350 are targeted not only to the centrosome but also to a Golgi-like network. Interestingly, full-length GFP-tagged CAP350 bound preferentially to microtubules in the pericentrosomal area. These results indicate that the large CAP350 protein has a dual binding ability. Overexpression of CAP350 promoted an increase in the stability of the whole microtubule network, as judged by a significant decrease in the number of EB1 comets and by an enhanced microtubule resistance to Nocodazole treatment. In support of this, CAP350 depletion decreased microtubule stability. Moreover, both depletion and overexpression of CAP350 induced specific fragmentation of the Golgi complex while maintaining a juxtanuclear localisation. We propose that CAP350 specifically stabilises Golgi-associated microtubules and in this way participates in the maintenance of a continuous pericentrosomal Golgi ribbon.

Supplementary material available online at
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Key words: Golgi complex, Centrosome, Microtubules

Introduction

The temporal and spatial distribution of interphase and mitotic microtubule (MT) networks are determined largely by the activities of the centrosome in animal cells. This organelle thus plays crucial roles in the overall cytoplasmic organisation, cell polarity and in the fidelity of transmission of the genetic material during cell division. Centrosome organisation of MTs involves distinct processes such as nucleation, anchorage and release of MTs. In most undifferentiated animal cells, microtubule nucleation is closely associated with the centrosome, where proteins such as γ-tubulin (Stearns et al., 1991; Moudjou et al., 1996) and γ-tubulin-associated proteins are located, which constitute the nucleation complex γ-TuRC (γ-tubulin ring complex) (Murphy et al., 1998; Martin et al., 1998; Tassin et al., 1998; Murphy et al., 2001; Gunawardane et al., 2003; Haren et al., 2006). Both centrioles in the centrosome recruit γ-TuRCs and nucleate MTs, but only the mother centriole maintains an array of focused MTs (Piel et al., 2000), as it contains appendages, where ninein – a protein required for microtubule anchorage – is located (Mogensen et al., 2000; Delgehyr et al., 2005). Interestingly, some bona fide centrosomal components, such as AKAP450 (Witczak et al., 1999; Takahashi et al., 1999; Takahashi et al., 2002; Keryer et al., 2003), extend from the centrosome to the Golgi complex and form a Golgi-like network. It has been reported that γ-tubulin is also enriched in the Golgi complex (Chabin-Brion et al., 2001), where it is recruited by the *cis*-Golgi protein GMAP210 (Rios et al., 2004).

In this work, we studied the function of the large centrosomal protein CAP350, which possesses one CAP-Gly motif and two Ser-rich regions. This protein was previously shown to interact directly with FOP (FGFR1 oncogene partner) and EB1, and is proposed to be involved in MT-anchoring activity at the centrosome (Yan et al., 2006).

CAP-Gly proteins have been reported to bind MTs, tubulin oligomers or tubulin monomers through the CAP-Gly domain. This domain consists of about 70 a.a., and characteristically contains conserved hydrophobic and glycine residues (Pierre et al., 1994; Waterman-Storer et al., 1995; Hoogenraad et al., 2000). This motif is found in at least 10 human proteins, which mainly belong to the CLIP family (Pierre et al., 1994; Hoogenraad et al., 2000; Lallemand-Breitenbach et al., 2004), but also to motor-associated proteins (Waterman-Storer et al., 1995) and to tubulin co-factors (Tian et al., 1996). In CLIP proteins, a basic Ser-rich region is required in addition to the CAP-Gly domain for microtubule binding (Hoogenraad et al., 2000). However, the protein CYLD (cylindromatosis tumor suppressor), which possesses three CAP-Gly domains, does not bind MTs. Instead, the third CAP-Gly repeat was reported to bind the proline-rich region of NEMO (the regulatory subunit of the $I_{\kappa}B$ kinase), suggesting that the CAP-Gly domain could mediate interactions with proteins unrelated to tubulin (Saito et al., 2004).

We demonstrate here that CAP350 is a microtubule-binding protein, which binds MTs through an N-terminal basic region rather than via its CAP-Gly domain, whereas CAP-Gly-containing domains are targeted to the Golgi-like network. Full-length GFP-tagged CAP350 binds preferentially to MTs in the pericentrosomal area. We show that CAP350 influences MT stability and Golgi complex organisation and propose that CAP350 may specifically stabilise Golgi-associated MTs during interphase and in this way participates in the maintenance of a pericentrosomal Golgi ribbon.

Results

CAP350 associates with the centriole pair and within the pericentrosomal area

Using an antibody raised against a recombinant CAP350 fragment (1875-2055), we identified by western blotting a major high molecular mass band that was specifically enriched in the centrosomal fraction of KE37 lymphoblastic cells but also detectable in a total cell lysate of HeLa cells (Fig. 1A,B). Depletion of this protein after RNAi demonstrated the specificity of our antibody against CAP350 (Fig. 1B). Labelling of CAP350 with this antibody revealed a concentration of CAP350 at the centrosome throughout the cell cycle in agreement with Yan et al. (Yan et al., 2006) and in close association with the two centrioles (Fig. 1C-F). In addition, a cloud of numerous pericentrosomal dots (see arrows in Fig. 1C-F) was localised to the area of the Golgi complex using the Golgi-specific mAb CTR433 (Jasmin et al., 1989). CAP350 did not colocalise with MTs in interphase cells. However, the pericentrosomal dots were always very close to and possibly on MTs (Fig. 1C,D).

During mitosis, CAP350 was detected along some spindle MTs and at the spindle poles (Fig. 1G,H). Numerous distinct dots were also observed around the centrosome in telophase cells (Fig. 1H). These dots were concentrated in areas where the Golgi complex reforms after mitosis (see supplementary material Fig. S1).

When microtubule organisation was modified by treatment with drugs such as Nocodazole or Taxol, centrosomal localisation of CAP350 was not affected, indicating that CAP350 is a genuine centrosomal protein (Fig. I,J). Interestingly, the few Nocodazole-resistant MTs were covered with non-centrosomal CAP350. This would suggest – in agreement with the observation that overexpressed CAP350

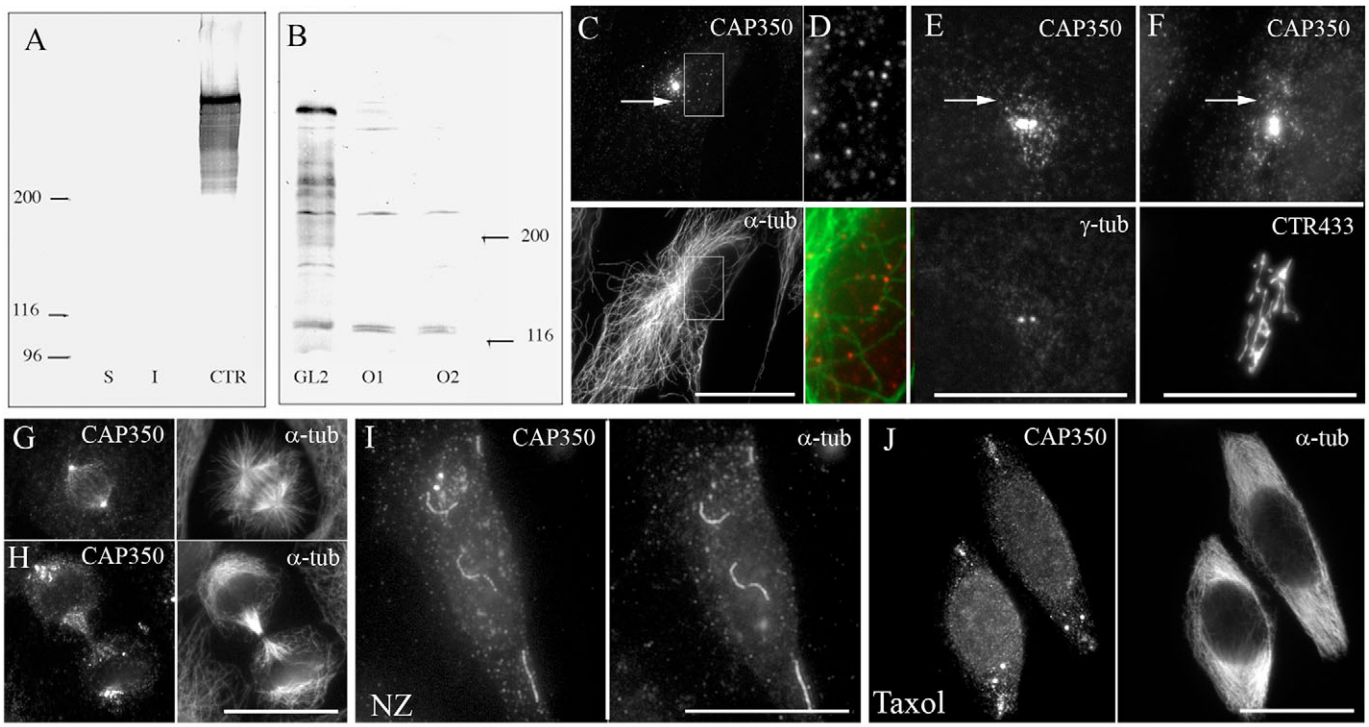

Fig. 1. CAP350 localisation during the cell cycle and after microtubule drug treatment. (A) Western blot analysis of Triton X-100-soluble (S) and insoluble (I) protein fractions from KE37 cells and a highly enriched centrosomal fraction (CTR). CAP350 is only detectable in the centrosomal fraction. (B) Western Blot analysis of total cell lysate obtained from HeLa cells after CAP350 SiRNA. The high molecular mass band, revealed by CAP350 antibody, is detectable in the control siRNA sample (GL2 lane), and absent after CAP350 RNAi (O1 and O2 lanes) indicating that our antibody is specific. (C-F) Double-labelling experiments on interphase cells using the CAP350 antibody and either α-tubulin (C-D), γ-tubulin (E) or CTR433 a Golgi complex antibody (F). Note that a cloud of dots is evident around the centrosome with the CAP350 antibody (arrow). (D) 3× magnification of the boxed area in C, showing that the pericentrosomal dots are aligned with MTs. These dots are located in the Golgi complex area (F). (G) Metaphase cell double stained with anti-CAP350 and anti-α-tubulin antibodies. During metaphase, CAP350 still localised to the centrosome, but was also found along the spindle microtubules. (H) Cell in telophase, double stained with anti-CAP350 and anti-α-tubulin antibodies. At this stage, the CAP350 antibodies labelled the centrosome but also some dots around it, as well as the central spindle. (I) Nocodazole (NZ)-treated cells double labelled with CAP350 and α-tubulin antibodies during interphase. The CAP350 antibody decorated the centrosome and the remaining microtubules. (J) Taxol-treated cells double labelled with CAP350 and α-tubulin antibodies. CAP350 redistributed to the ends of the microtubule bundles. Bars, 20 μm.

decorates MTs unevenly (see below) – that when CAP350 is abundant with respect to the MT lattice, as occurs when MTs are depolymerised, the binding involves some cooperativity. This then results in a higher concentration of the protein on regions along the remaining microtubules (Fig. 1I). In Taxol-treated cells, CAP350 redistributed to form clouds of dots localised at the minus end of MT bundles (Fig. 1J) as identified by a double-labelling experiment with AKAP450, which redistributes at minus ends (data not shown) (Gosti-Testu et al., 1986). These results suggest that CAP350 is able to bind directly or indirectly to MTs or to a subset of MTs, and preferentially accumulates at the MT minus ends.

CAP350 co-sediments with microtubules in cell lysate

Since CAP350 contains a CAP-Gly motif and colocalised with MTs, we analysed the ability of CAP350 to bind to MTs by studying its sedimentation behaviour in a high-speed centrifugation HeLa cell lysate in the presence of MT drugs. Addition of Nocodazole to the cell lysate resulted in complete solubilisation of tubulin and CAP350. Addition of Taxol led to full polymerisation of tubulin into MTs with a small fraction of CAP350 co-sedimenting with the MT pellet (Fig. 2A). As expected for a MT-binding protein, increasing the tubulin pool by adding purified tubulin to the lysate before drug treatment, led to an increased sedimentation of CAP350 although it was never complete (Fig. 2A). This was also observed with p150^{Glued}, a well-known microtubule-binding protein that possesses a CAP-Gly domain, which was used as a control. These results confirmed the MT-binding properties of CAP350.

To pinpoint the domain responsible for CAP350 co-sedimentation with MTs, we made large partial constructs of CAP350 corresponding to the N-terminal domain (a.a. 1-983), the Ser-rich plus CAP-Gly region (a.a. 983-2589) and the C-terminal domain (a.a. 2590-3117). Each fragment was amplified by PCR for transcription and translation in a reticulocyte lysate. Each translation product was incubated with pre-assembled Taxol-stabilised MTs. Our results showed that the N-terminal domain of CAP350 co-sedimented with MTs whereas the Ser-rich plus CAP-Gly domain and the C-terminal domain remained in the supernatant (Fig. 2B). To ensure that the CAP-Gly domain was not involved in the MT binding, we restricted this fragment by sequentially deleting the Ser-rich regions, which led to three new partial constructs (see Materials and Methods). None of these was able to co-sediment with MTs (data not shown). To determine the exact N-terminal domain responsible for MT co-sedimentation, we shortened and cut it in two parts (a.a. 1-402 and a.a. 403-725). Each domain was further analysed for its association with the MT fraction as before. We found that both were able to co-sediment with MTs (Fig. 2C) suggesting that two independent domains within the N-terminus of CAP350 are able to bind MTs independently.

The N-terminal domain of CAP350 binds microtubules directly

To determine whether the N-terminal domains were able to bind MTs directly, we tagged each fragment with GST and generated the peptides in bacteria. Unfortunately, fragment 1-402 was insoluble. Fragment 403-725 was soluble and purified on a GST column and analysed for its MT binding capability. The fusion

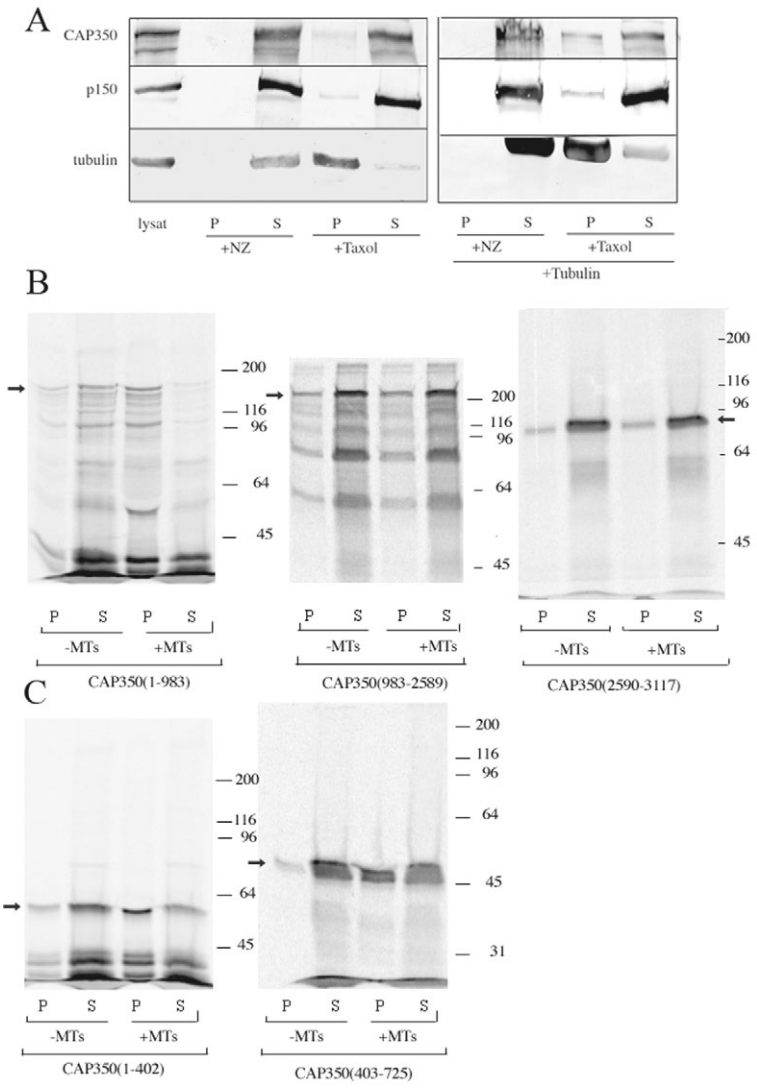

Fig. 2. CAP350 binds microtubules through an N-terminal domain and not through the CAP-Gly domain. (A) HeLa cell lysate in which tubulin was added or not (see Materials and Methods) and treated with either Nocodazole (NZ) to prevent tubulin polymerisation or with Taxol to stabilise the microtubules. After a 1-hour incubation the cell lysate was centrifuged through a glycerol cushion. Proteins from the supernatants (S) and pellets (P) were analysed by SDS-PAGE and western blotting using anti-tubulin, anti-p150 and anti-CAP350 antibodies. Note that a small amount of CAP350 and p150 specifically co-precipitated with microtubules in the absence of exogenous tubulin, which significantly increased after addition of tubulin in the lysate. (B) Different partial constructs of CAP350 as indicated on the figure were transcribed in reticulocyte lysate and analysed for their binding to pre-formed microtubules (+MTs). Control experiments were performed without pre-formed microtubules (–MTs). As in A, proteins from the supernatants (S) and pellets (P) were analysed by SDS-PAGE and exposed for different times using a phosphorimager. Only the N-terminal construct (1-983) sedimented with microtubules. This ability was observed with the two N-terminal constructs (C). Arrows indicate the mobility of the in vitro labelled CAP350 constructs.

protein clearly co-sedimented with preassembled Taxol-stabilised MTs from purified tubulin whereas purified GST remained in the supernatant (Fig. 3A,B). A higher molecular mass protein that co-purified with the fusion protein remained in the supernatant serving as an internal control (Fig. 3A).

To test whether this N-terminal fragment (a.a. 403-725) was able to bind MTs in vivo, it was overexpressed in fusion with a Myc tag and it was targeted to the nucleus (data not shown). To abolish this nuclear targeting, we mutagenised the two NLS present in this fragment. In these conditions, as expected for a microtubule-binding domain, all MTs were decorated in a double labelling experiment with anti-tubulin antibody (Fig. 3C).

CAP-Gly containing domains of CAP350 are targeted to a Golgi-like network

We then investigated the targeting of different fragments of CAP350 that excluded the MT binding N-terminal domain (see Fig. 4D). The shortest C-terminal domain (a.a. 2590-3117) was found in the cytosol (data not shown). Two CAP-Gly containing domains (a.a. 984-2589 and a.a. 1896-3117) were produced. At low expression levels the 984-2589 domain, which contains the Ser-rich and CAP-Gly domains, was strictly targeted to the centrosome (Fig. 4A). When the expression level was increased, a Triton-resistant Golgi-like staining was observed, in addition to the centrosome staining (Fig. 4B). Interestingly, the 1896-3117 domain, which contains one Ser-rich and the CAP-Gly domain decorated only the Golgi-like network and not the centrosome (Fig. 4C). These results suggested that a domain containing the CAP-Gly targeted the protein to a Golgi-like network. To verify this hypothesis, we further analysed the localisation of two additional constructs: the Ser-rich domain without the CAP-Gly (a.a. 984-2495) and the CAP-Gly domain alone (a.a. 2400-2566). The Ser-rich domain, when overexpressed in cells, was strictly targeted to the centrosome, whereas the CAP-Gly domain localised in the cytosol, suggesting that the CAP-Gly is necessary but not sufficient for the pericentrosomal network targeting (data not shown).

Altogether, these results suggest that the centrosome localisation domain is located between a.a. 983 and 2495 (see also Yan et al., 2006) and that the pericentrosomal network localisation domain contains the CAP-Gly but is not restricted to it.

Overexpressed CAP350 redistributes to juxtanuclear microtubules and stabilises them

To investigate the function of CAP350, we analysed the localisation of full-length CAP350 tagged with GFP. At low expression levels, the protein localised similar to the endogenous protein, with staining observed at the centrosome and dotted around it (Fig. 5A). When expression levels increased, some MTs localised within the juxtanuclear area became associated with CAP350 (Fig. 5B) and extended further into the cell (Fig. 5C). This localisation was confirmed using full-length CAP350 in RPE1 GFP- α-tubulin cells (supplementary material Fig. S2). However, CAP350 seemed to be unevenly distributed along the MTs (arrows in Fig. 5D).

Thus, the targeting of full-length CAP350 contrasts with the targeting of the N-terminal construct (a.a. 403-725) (see Fig. 3C), which associates with all MTs. We suggest that full-length CAP350 can bind to MTs that are localised in the juxtanuclear area, where the protein can also interact with the Golgi-like network through its CAP-Gly containing domain.

To determine the function of CAP350, we investigated whether CAP350 overexpression affects microtubule stability. For this purpose, we overexpressed the full-length CAP350 tagged with GFP in cells, and subsequently depolymerised the MTs with Nocodazole for 20 or 40 minutes before fixation. After treatment with Nocodazole for 40 minutes, more than 50% of the CAP350 overexpressing cells still possessed a MT network observed by labelling acetylated tubulin (n=200 cells from three experiments), whereas most of the nontransfected cells were devoid of MTs. Representative cells are shown in Fig. 6A. This result indicated that overexpression of CAP350-GFP stabilised the MTs.

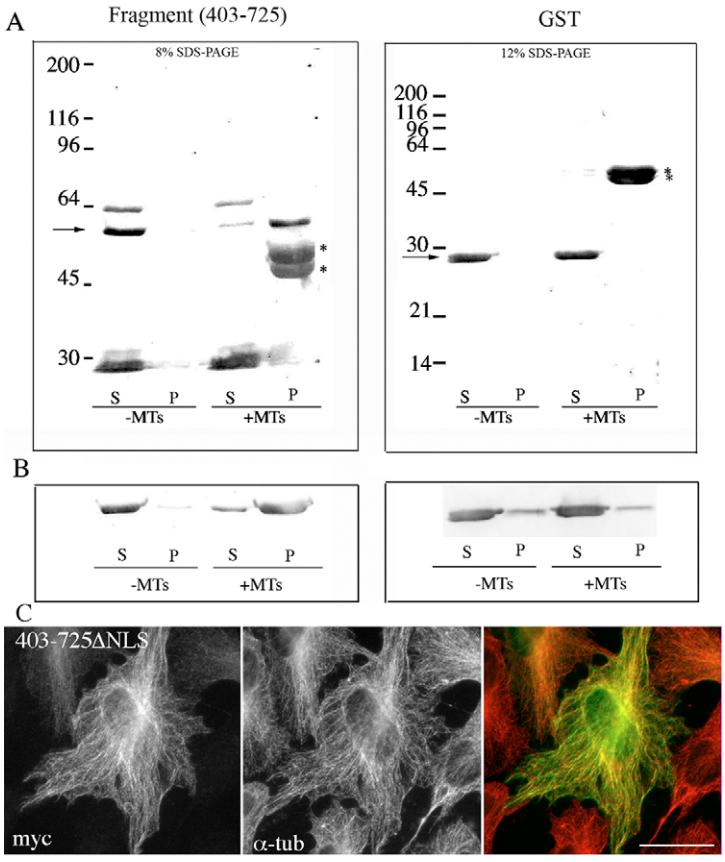

Fig. 3. The N-terminal domain of CAP350 binds microtubules directly. (A,B) The fragment (403-725) produced in bacteria as a GST fusion protein and purified using the GST tag, as well as GST, were incubated for 1 hour with preformed MTs. The MTs were then centrifuged on a glycerol cushion and the proteins from the supernatants (S) and pellets (P) were analysed by SDS-PAGE. (A) Coomassie Blue staining. The arrow indicates either purified GST fusion protein or GST. Asterisks indicate tubulin. (B) Western blotting using anti-GST antibody. (C) Double immunofluorescence of the microtubule-binding domain (403-725) mutated in the two NLS in fusion with the 6×Myc tag using anti-myc (left and green in merged image) or α-tubulin antibody (middle and red in merged image). As expected, it colocalised with the microtubule network. Bar, 20 μm.

This was confirmed by analysing the behaviour of EB1 in cells overexpressing CAP350-GFP. EB1 is known to be localised at the plus ends of dynamic MTs and its staining can be used to monitor the number of growing MTs (Piehl et al., 2004; Tirnauer et al., 2004). Cells overexpressing CAP350-GFP consistently had a greatly reduced number of EB1 spots compared with untransfected cells (Fig. 6B).

Finally, we checked whether MT regrowth after Nocodazole treatment was affected in CAP350-overexpressing cells. Our results indicated that microtubule regrowth was not modified in these cells compared with untransfected cells (not shown). Taken together, these results indicate that CAP350 overexpression significantly increases MT stability.

CAP350 siRNA decreases microtubule stability

We next studied the behaviour of MTs in CAP350-depleted cells. HeLa cells were transfected for 48 or 72 hours with either control (GL2) or one of two distinct CAP350-targeting siRNA oligonucleotide duplexes. As shown by western blotting of total HeLa cell lysates, each of the CAP350 siRNA duplexes caused a strong reduction in CAP350 protein levels (Fig. 1A). CAP350 depletion at the centrosome and in the pericentrosomal area could also be confirmed by immunofluorescence microscopy with the anti-CAP350 antibody (Figs 7, 8, CAP350 SiRNA). MT organisation in CAP350-depleted cells or GL2-treated cells appeared very similar (supplementary material Fig. S3). Centrosome integrity was analysed by staining control and CAP350-depleted cells with different antibodies recognising the centrosomal proteins including centrin, ninein, γ-tubulin, Nek2 and C-NAP1, AKAP450. No discernible differences in the deployment of these centrosomal proteins could be observed between control and CAP350-depleted cells, indicating that CAP350 is not essential for the structure of the centrosome or the recruitment of centrosomal protein (data not shown).

MT stability was determined after 5, 10 and 15 minutes of Nocodazole treatment. MT depolymerisation was almost complete in more than 80% of CAP350-depleted cells (n=400 cells from three experiments) after 10 minutes of Nocodazole treatment, whereas cells transfected with the control GL2 duplex still had a distinct MT network in the vast majority (90%) of cells (n=400 cells from three experiments). MT regrowth at the centrosome was similar in CAP350-depleted and control cells showing similar kinetics and centrosomally focused MTs following 5 minutes of regrowth (data not shown).

This suggested that depletion of CAP350 reduced MT stability and thus had the opposite effect to overexpression, which stabilised the MT network. These results strongly argue for a role of CAP350 in controlling MTs dynamics.

CAP350 depletion and CAP350 overexpression induce Golgi complex fragmentation

Since partial constructs of CAP350 were targeted to the Golgi-like network and the full-length protein bound preferentially to MTs in

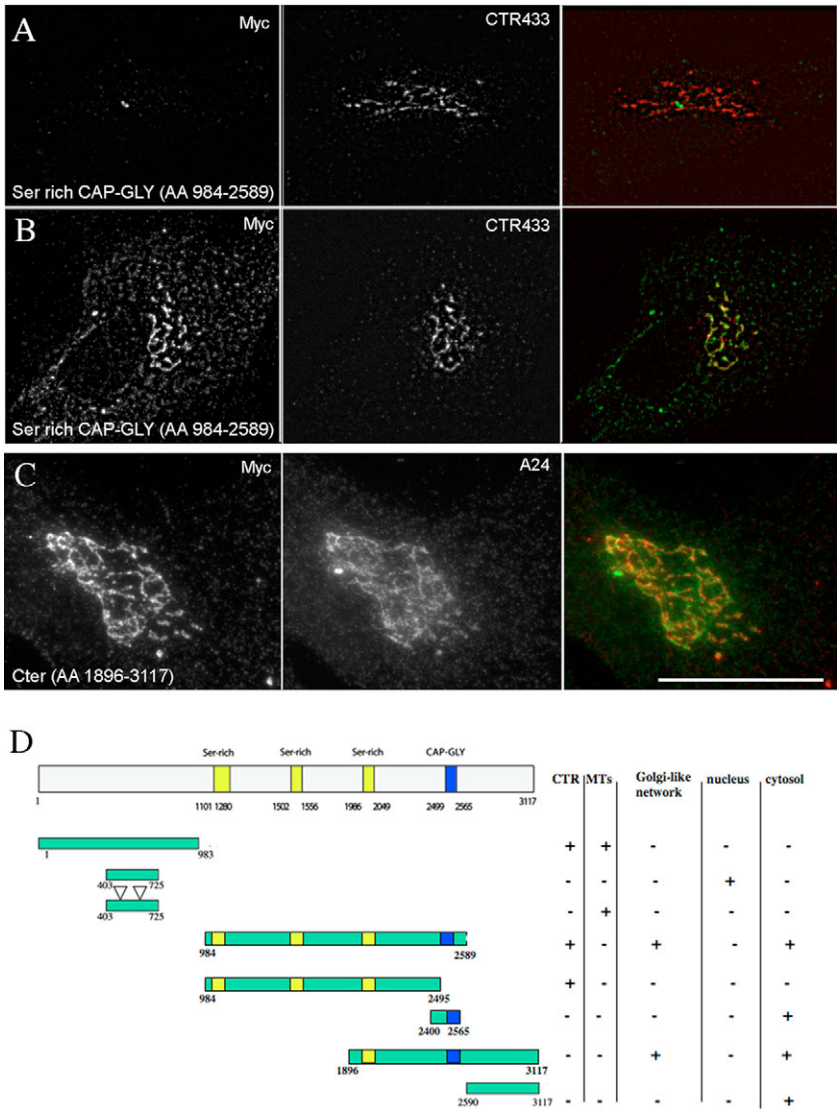

Fig. 4. CAP-Gly-containing domains are targeted to the Golgi-like network and CAP350 depletion induced Golgi complex scattering. Cells expressing either the Ser-rich CAP-Gly domain (984-2589) (A,B) or the C-terminus (1896-3117) (C) in fusion with a 6×Myc tag were fixed 24 hours after transfection with methanol after Triton X-100 extraction. Cells were subsequently stained with the anti-Myc antibody (green) and the Golgi antibody (red) CTR433, or A24 antibody which recognises the AKAP450 protein and stains the Golgi-like network. (A) The Ser-rich CAP-Gly domain is targeted to the centrosome when expressed at a low levels. (B) At higher expression levels, a Golgi-like network is decorated with the Myc antibody. (C) The C-terminal domain is also targeted to the Golgi-like network but no centrosome staining is observed. (D) Diagram representing the CAP350 protein with the Ser-rich regions in yellow and the CAP-Gly domain in blue. The different domains that were used for overexpression are shown below in green. The localisation of the overexpressed proteins are indicated for each construct in the table. Bar, 20 μm.

the same area, we investigated whether depletion of CAP350 also had a specific effect on the Golgi complex itself. Cells were treated for 48 hours with either GL2 or siRNA CAP350 duplexes and analysed by double immunofluorescence using CAP350 and CTR433, a monoclonal antibody reacting with the median Golgi complex (Jasmin et al., 1989). Cells treated with GL2 duplexes presented a classical Golgi complex close to the nucleus (Fig. 8A), whereas CAP350-depleted cells showed a fragmented Golgi complex, which was still localised close to the nucleus (Fig. 8B). This effect was different from the scattering observed after Nocodazole treatment, as Nocodazole treatment of these CAP350-depleted cells led to a further scattering of the Golgi (not shown). We also asked whether CAP350 overexpression had any effect on Golgi complex and Golgi-like network organisation. In the CAP350-overexpressing cells, Golgi scattering was observed in 60% of the cells whereas untransfected cells presented a Golgi ribbon around the centrosome (see Fig. 8C). The Golgi-like network that was revealed by AKAP450 staining as the Golgi complex, was also fragmented in CAP350-overexpressing cells (Fig. 8D). Surprisingly, the AKAP450 protein partially redistributed along with CAP350-GFP to juxtanuclear MTs, suggesting a direct or indirect interaction between these two proteins.

We conclude that the results obtained with CAP350 overexpression and depletion both support a role for CAP350 in Golgi organisation, either directly through CAP350 association with the Golgi-like network or indirectly through modulation of MT dynamics in the Golgi area.

CAP350 localisation in polarised epithelial cells

Our results do not support a role for CAP350 in MT minus-end anchorage at the centrosome (Yan et al., 2006) that is similar to the one described for ninein (Mogensen et al., 2000). Indeed, CAP350 appears to associate equally with both centrioles (see Fig. 1), unlike ninein, which is asymmetrically distributed and more abundant on the mother centriole (Mogensen et al., 2000). Furthermore, CAP350 depletion or overexpression did not affect MT organisation and anchorage at the centrosome. To gain further insight, we turned to differentiated polarised epithelial cells, such as inner-ear supporting cells and cultured polarised MDCK cells. These cells have proved ideal for the identification of proteins involved in MT anchorage because MT nucleation and anchorage are effected at spatially separate domains (Fig. 9A) (Mogensen et al., 1997; Mogensen et al., 2000; Mogensen et al., 2002; Mogensen, 1999). This provides a useful experimental system to determine whether CAP350 preferentially associates with MT nucleating or anchoring structures.

CAP350 was found to be concentrated at the centrosome in the pillar cells (Fig. 9B,C), but was absent from the apical anchoring sites, where the MT anchoring protein, ninein is known to accumulate (Fig. 9D,E) (Mogensen et al., 2000). Furthermore, apical views of the inner-pillar cells, which revealed distinct ring of MT minus ends showed no accumulation of CAP350 (Fig. 9B,C). Cytoplasmic CAP350 appeared to be evenly distributed throughout the cells with no accumulation at the cell bases where the plus ends of the MTs are anchored (data not shown) (see Mogensen et al., 2002). Similar results were obtained in polarised MDCK cells grown on filters. During MDCK cell polarisation, the radial array of MTs focused on the centrosome is lost or highly reduced and an apico-basal array anchored at non-centrosomal apical sites develops (Bacallao et al., 1989). In these cells, no distinct accumulation of CAP350 was observed within the apical peripheral region as found for ninein, whereas some CAP350 dots could be observed at the cell periphery in a few polarised MDCK cells (see supplementary material Fig. S4A-C).

These findings suggest that CAP350 does not play a major role in microtubule minus-end anchorage as has been established for ninein in both cochlear and polarised MDCK cells.

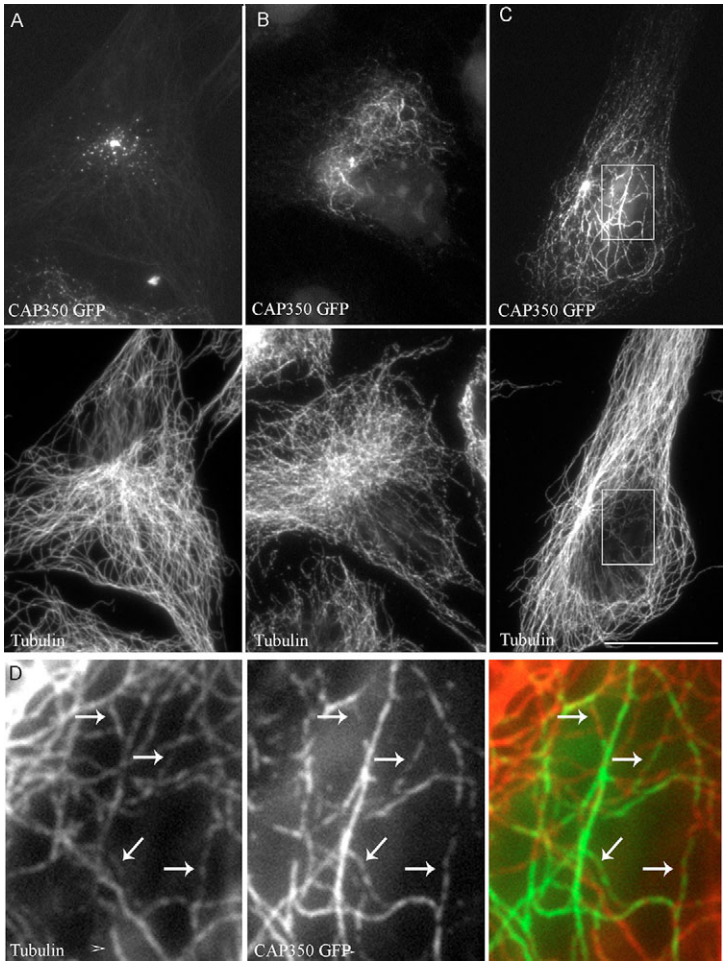

Fig. 5. Overexpressed full-length CAP350 GFP localises to the centrosome and to a subset of pericentrosomal microtubules. (A-C) Full-length CAP350 GFP overexpressed in cells and stained for α-tubulin. At low expression levels, GFP decorates the centrosome (A). When the expression level increases, a subset of microtubules close to the centrosome is covered with CAP350 (B) and extended further in the cell (C). (D) 2.5× magnification of the boxed area in C. Note that CAP350 GFP is not continuous on microtubules, and that where it is present the anti-tubulin antibody has no access to MTs (arrows). A microtubule well stained with the anti-tubulin is only partly decorated with the fusion protein (arrowhead). Bar, 20 μm.

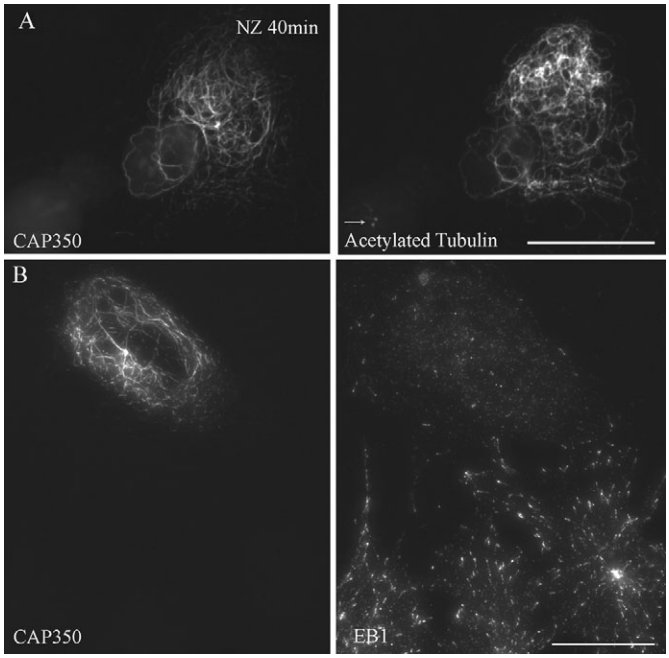

Fig. 6. CAP350 overexpression stabilises microtubules. (A) Representative cell expressing CAP350 GFP after 40 minutes of Nocodazole treatment, stained for acetylated tubulin. Note that in these cells the microtubule network is conspicuous whereas in the neighboring cell only the centrosome is observed (arrow). (B) Cell expressing CAP350 GFP stained for EB1. Cells expressing CAP350 GFP show a decrease of the number of EB1 spots compared with the non-transfected cells. Bars, 20 μm.

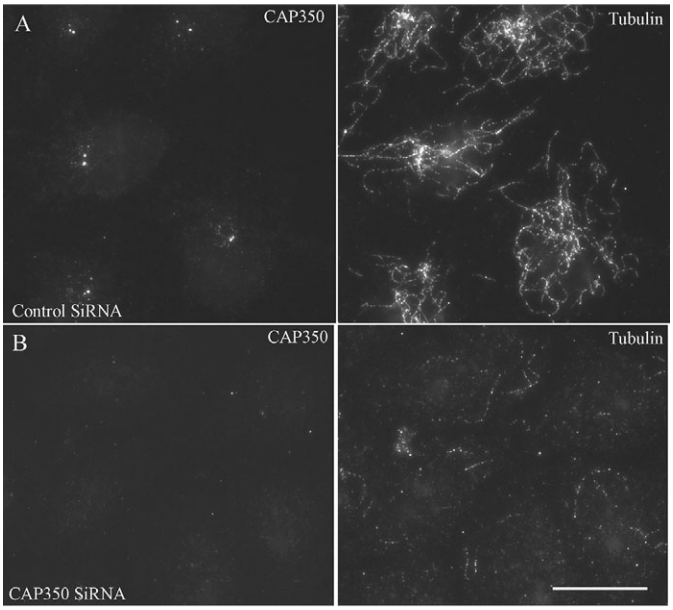

Fig. 7. Microtubules are less stable in CAP350-depleted cells. Cells were transfected with GL2 (A) or CAP350 (B) duplexes for 48 hours and further treated with 5 10^{-6} M Nocodazole for 10 minutes and stained for CAP350 and tubulin. Note the depletion of CAP350 at the centrosome and the decrease in the microtubule number after CAP350 siRNA treatment. All images have been scaled identically for fluorescence intensity using Metamorph software to directly compare the decrease in siRNA CAP350 cells at the centrosome and in the microtubule pattern. Bar, 20 μm.

Discussion

We showed that CAP350 is able to bind MTs through a basic N-terminal region, to the centriole pair by a domain containing the Ser-rich domains and to the Golgi-like network through a CAP-Gly-containing domain. We demonstrated that CAP350 levels can modulate both microtubule dynamics and Golgi complex ribbon organisation. Rather than a role for CAP350 in MT minus-end anchorage as previously proposed (Yan et al., 2006), our results seem to indicate that CAP350 concentrates within the pericentrosomal area where it could stabilise MTs enriched in the Golgi complex, and in this way help to maintain the Golgi in the vicinity of the centrosome.

Regulated association of CAP350 with MTs

Using a MT sedimentation assay, we identified an N-terminal domain distinct from the CAP-Gly, which is able to bind MTs. By contrast, we did not observe any microtubule-binding activity of the CAP-Gly domain, as described for the CLIP family member proteins and for the $p150^{Glued}$ protein. Five highly conserved amino acids (GKNDG) in the CAP-Gly domain, which have been shown to be crucial for MT binding (Pierre et al., 1994; Feierbach et al., 1999), are not conserved in CAP350. Accordingly, the protein CYLD, which possesses three CAP-Gly domains, that are highly divergent in these five amino acids, fails to bind MTs (Saito et al., 2004). Therefore, the CAP-Gly domain of CAP350 might be required for a function distinct from the microtubule binding, as reported for the protein CYLD (Saito et al., 2004).

However, it should be noted that the consensus sequence is not so divergent in CAP350 (GKNDG is replaced by GNNNG). Therefore, we cannot exclude that under specific regulations – for example, following phosphorylation – the CAP350 CAP-Gly could be functional in MT binding. Indeed, it has been shown that CAP350 is phosphorylated during mitosis (Yan et al., 2006), and we have observed that the mitotic MTs were decorated with the CAP350 antibody, whereas MTs were not stained during interphase. In support of this hypothesis, we note that CLIP-170 phosphorylation has been shown to regulate its MT binding (Rickard and Kreis, 1991; Choi et al., 2002). Therefore, CAP350 could bind MTs and regulate their dynamics through two domains, just like the p150 subunit of dynactin, which binds MTs through a highly basic domain increasing the dynein processivity and by the CAP-Gly inhibition of dynein motility (Culver-Hanlon et al., 2006).

A function for CAP350 in the pericentrosomal area

We have shown that constructs containing the CAP350 CAP-Gly domain were targeted to the Golgi-like network. The CAP-Gly domain appears to be necessary for this targeting because its deletion prevents Golgi-like network localisation. However, the CAP-Gly domain is not sufficient to enable targeting to the Golgi-like network, which suggests that other domains are also involved. Therefore, CAP350 binds MTs and the Golgi-like network by two different domains. This is further supported by data on the localisation of endogenous CAP350, which is present not only at the centrosome, but also in the

pericentrosomal area, where other centrosomal proteins, such as AKAP450 and centrin, localise.

The centrosome and the Golgi complex are intimately linked. The Golgi complex requires MTs for its location and organisation. Moreover, stable MTs have been observed in association with the Golgi complex (Thyberg and Mokalewki, 1999; Marsh et al., 2004). It has been proposed that stable MTs might be important for the maintenance of the Golgi structure and its localisation (Rios and Bornens, 2003). The Golgi protein GMAP210 binds MTs through its C-terminal domain and mediates interactions between Golgi membranes and stable MTs (Infante et al., 1999). GMAP210 has also been described to recruit γ-tubulin-containing complexes to Golgi membranes (Rios et al., 2004) and these γ-tubulin-enriched membranes have been shown to nucleate MTs in rat liver (Chabin-Brion et al., 2001). Therefore, CAP350 may specifically modulate the dynamics of a subpopulation of MTs associated with the Golgi. Indeed, we have shown in this study, that the CAP350 level functions as a regulator of MT dynamics. This was demonstrated by CAP350 overexpression, which induced MT stabilisation, and is further supported by the fact that the MTs appeared less stable after siRNA depletion of CAP350. How CAP350 modifies microtubule dynamics is not known and will require further study. Since CAP350 is localised on the microtubule spindle during mitosis, a mitotic phenotype might be expected. However, we have been unable to observe any obvious phenotype in our RNAi experiments as judged by immunofluorescence. However, a more detailed study using time-lapse imaging is required to analyse the effect of CAP350 depletion on mitotic and cytokinesis progression.

In support of a function for CAP350 in the Golgi area, we have shown that CAP350 siRNA treatment resulted in a specific fragmentation of the Golgi complex. Similarly, CAP350 overexpression caused a scattering of the Golgi complex. The fragmentation of the Golgi complex could be due to modifications in microtubule stability (Allan et al., 2002; Rios et al., 2004) following CAP350 overexpression or depletion. However, the fragmentation observed was different to the scattering observed after Nocodazole treatment, which is in agreement with the fact that after CAP350 depletion MTs are still present although they are less stable. The dual targeting of CAP350 to MTs by the N-terminus and to Golgi-like network by its CAP-Gly-containing domain could explain why endogenous CAP350 is concentrated in the pericentrosomal area. Once concentrated there, it could stabilise Golgi-associated MTs. After depletion of CAP350, these MTs would no longer be stabilised and consequently, the Golgi complex would fragment while still maintaining an association with the pericentrosomal area via other proteins such as GMAP210. When overexpressed, CAP350 is not only localised to the Golgi-associated MTs but also to MTs extending beyond it. In these conditions, other components of the Golgi-like network, such as AKAP450 partly redistributed along with CAP350 (see Fig. 8D). Owing to the intimate association of the Golgi with the pericentrosomal area, CAP350 overexpression could then trigger loss of the classical Golgi ribbon organisation by favouring the pulling of the Golgi membrane along the MTs.

In conclusion, we propose that CAP350, which possess two major binding properties, one for MTs and the other for the Golgi-like network, could specifically stabilise Golgi-associated MTs. In this way, CAP350 may play an important role in the maintenance and integrity of the Golgi complex in the vicinity of the centrosome.

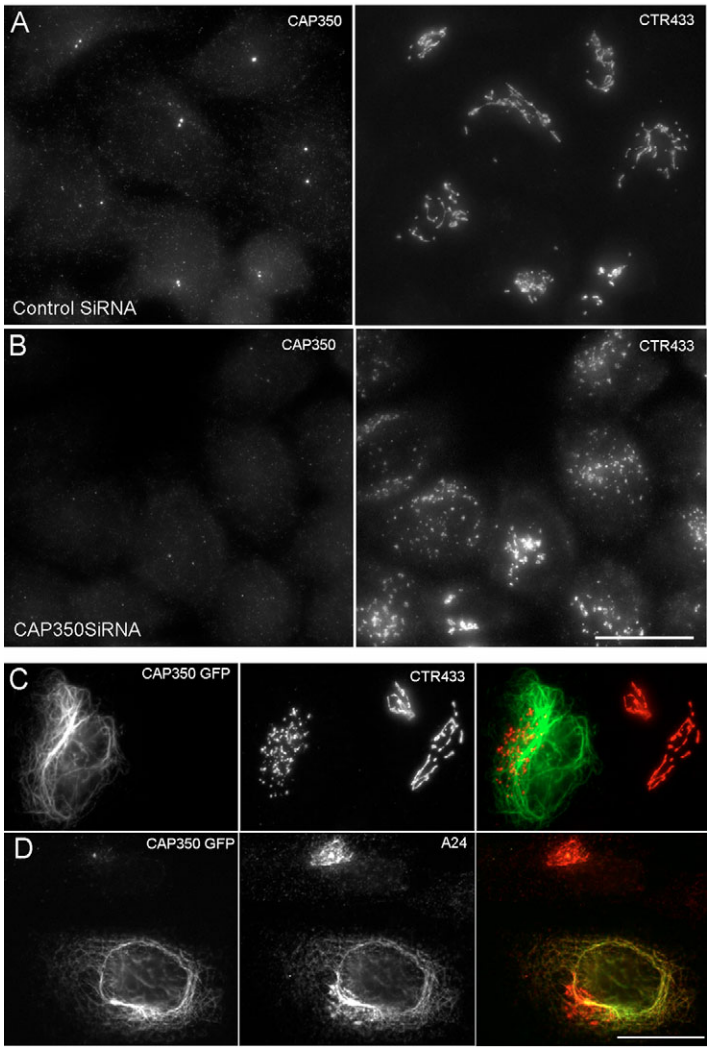

Fig. 8. Effect of CAP350 depletion or overexpression on the Golgi complex. Cells transfected with the GL2 (A) or CAP350 (B) duplexes for 48 hours were fixed with methanol and stained for CAP350 and CTR433 a median Golgi marker. Cells transfected with the GL2 duplex present a classical Golgi complex. After depletion of CAP350 with the siRNA CAP350, the Golgi complex appeared scattered. (C-D) Representative cell expressing CAP350-GFP and stained for CTR433 (C) or A24, an antibody directed against AKAP450 (D). Note that the cell overexpressing CAP350-GFP presented a scattered Golgi complex in contrast to the untransfected cells. Interestingly, the Golgi-like network marker AKAP450 partially redistributed and colocalised with CAP350. Bars, 20 μm

Materials and Methods

Cell culture

HeLa cells were grown in DME medium supplemented with 10% FCS. RPE1 cells were grown in DMEM-F12 supplemented with 10% FCS. Human lymphoblastic KE37 cells were grown in RPMI medium, supplemented with

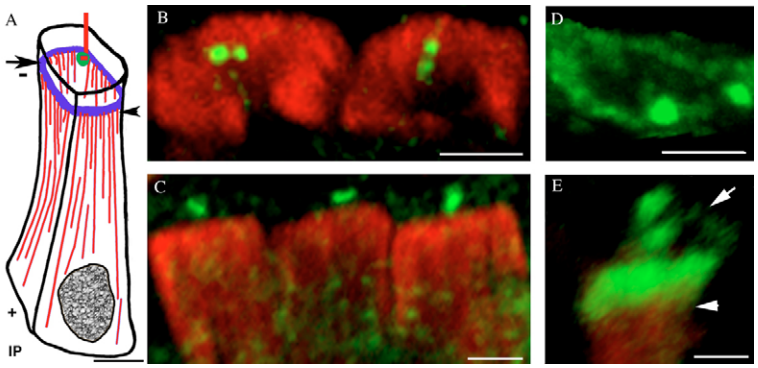

Fig. 9. Microtubule and CAP350 deployment in cochlear supporting cells. (A) Schematic diagram of the microtubule organisation (red lines) in a typical inner pillar cell at P5 (5 days postnatal) showing the apical centrosome (green with centrioles/primary cilium in red) and the microtubule minus-ends (arrowhead) anchored at apical sites (blue peripheral ring; arrow). (B) Apical view of a 3D reconstruction (based on confocal optical sections) of the apex of two inner pillar cells (P5) showing microtubules (red) organised in a tube and their minus ends forming an apical peripheral ring. CAP350 (green) is concentrated at the centrioles, which are located above the middle of the tube of microtubules (see A). Note there is no accumulation of CAP350 at the peripheral ring where some 3000 microtubule minus ends are located (arrow). (C) Lateral view of a 3D of the apical region of three inner pillar cells (P5) showing the apico-basally aligned microtubules with their minus-ends at the apex. CAP350 is concentrated at the centrosome (located above the microtubule array) and diffuse in the cytoplasm surrounding the microtubules. Note the cytoplasmic diffuse CAP350 is not evident at the minus ends of the microtubules and shows no apparent association with the microtubule array. (D) Apical view of a 3D reconstruction (based on confocal optical sections) of the apex of an inner pillar cell (P5) showing ninein at the centrosome and concentrated in a peripheral ring. (E) Lateral-to-oblique view of a 3D image of the apical region of an inner pillar cell (P5) with ninein accumulated at the minus ends of the apico-basal microtubules (red) (arrowhead). Part of the apex is also apparent, revealing part of the peripheral ninein ring (arrow) and ninein concentrated at the two centrioles. Bars, 5 μm.

7% FCS and MDCKII cells in DMEM supplemented with 10% FCS. MDCKII cells were seeded on 0.4 μm polycarbonate membrane filters (Nunc) (2×10^6 cells/filter) in DMEM and left to polarise for 4-5 days. Isolated pillar cells were obtained as previously described (Mogensen et al., 1997).

MT stabilisation experiments were carried out with HeLa cells incubated in 5 μM Taxol™ for 4 hours at 37°C. MT depolymerisation was carried out at 37°C with 5 μM Nocodazole for the indicated time.

Plasmid construction

Different fragments of CAP350 cDNA corresponding to a.a. 1-893, 1-983, 403-725, 403-725 ΔNLS, 984-2589, 1896-3117, 2590-3117, 984-2589, 2400-2565, 1-3117 introducing *Eco*R1 and *Xba*1 sites were obtained by PCR and subsequently subcloned in pCS2$^+$ with a 6×myc tag into *Eco*R1-*Xba*1 sites (pMT plasmid). All constructs were sequenced to verify the absence of mutations. The two NLSs in the a.a. 403-725 domain were treated with two rounds of mutagenesis using the quick-XL site mutagenesis kit from Stratagene. R571 and R588 were replaced by Thr. The oligonucleotides used were: oligo5′ R571T, 5′-GAGTCACAGCCCAGTAAAAA-CAAAACCTGACAAAATAACAG-3′; oligo3′R571T, 5′-CTGTTATTTTGTC-AGGTTTTGTTTTTACTGGGCTGTGACTC-3′; oligo5′R588T, 5′-CCCTGT-TATTTCCAAAACGCGCCACTATGACACAG-3′; oligo3′R588T, 5′-CTGTGT-CATAGTGGCGCGTTTTGGAAATAACAGGG-3′. The full-length protein was subcloned into a GFP vector (GFP-C3 from Clontech).

Antibodies

An antibody directed against CAP350 was obtained by immunising rabbits against the a.a. 1875-2055 domain of CAP350. Two antibodies directed against γ-tubulin were used: a polyclonal (Tassin et al., 1998) or a mAb (GTU88 from Sigma). A mAb CTR453 (Bailly et al., 1989), which recognises a sequence on exon 28 in the central domain of AKAP450 labels only the centrosome. The polyclonal antibody, a24 is directed against exons 24-27 of AKAP 450 and labels the centrosome, like mAb CTR453, but in addition reveals an extensive pericentrosomal Golgi-like network (Keryer et al., 2003). The monoclonal antibody CTR433 (Jasmin et al., 1989), which recognises the median Golgi compartment was used to label the Golgi complex. Anti-tubulin antibody was provided by Sigma (clone B5-1-2) and DAPI (4′,6-diamidino-2-phenyindole, dilactate) (Sigma) was used for DNA labelling.

Immunofluorescence microscopy and data processing

Cells were fixed with methanol for 6 minutes at –20°C or permeabilised for 30 seconds in PHEM buffer containing 0.25% Triton X-100 before fixation and processed for immunofluorescence as described previously (Tassin et al., 1998). After immunostaining, 3D video microscopy was performed using an upright motorised microscope (DM RXA2; Leica Microsystems, Mannheim, Germany) equipped with an oil-immersion 100×/NA 1.4 Plan-Apochromat objective lens and a cooled interline charge-coupled device detector (Roper Coolsnap HQ). *Z* positioning was accomplished using a piezoelectric driver (LVDT; Physik Instruments, Waldbronn, Germany) mounted underneath the objective lens. The whole system operated by using Metamorph software (Universal Imaging Corp., Downingtown, PA). 3D reconstruction was performed using the Metamorph PSF-based iterative constrained algorithm.

Polarised MDCKII, cochlear cells and ARPE-19 cells were fixed with 90% methanol with 10% MES (100 mM MES, 1 mM EGTA, 1 mM $MgSO_4$, pH 6.9) for 5 minutes. The cells were then permeabilised for 3 minutes with 1% NP40. Fluorescent images were recorded using either a Zeiss Axiovert 200M with a cooled monochrome CCD camera (Zeiss AxioCam) and Axiovision software or a Zeiss LSM 510 META confocal microscope with associated software. *Z*-stacks were generated from optical sections taken at 0.3 μm intervals and the Volocity (Improvision) software program was used for 3D reconstruction.

Cell fractionation

Centrosomes were isolated from KE37 cells as described previously (Moudjou and Bornens, 1994) and soluble or insoluble fractions in 1% Triton X-100 were obtained from KE37 cells as described (Tassin et al., 1998).

MT binding in cell extract

HeLa cells were washed twice with PBS and detached. Cell pellets were resuspended in 2 mM EGTA and 1 mM $MgCl_2$ on ice. Cells lysis was performed with a Dounce homogeniser and KPIPES pH 6.9 was then added to obtain a final concentration of 80 mM. The lysate was centrifuged for 1 hour at 100,000 ***g*** at 4°C. GTP (1 mM) and DTT (1 mM) were added to the supernatant and it was then divided into six aliquots of 100 μl. Nocodazole (10 μM) was added to aliquots 1 and 4 and Taxol (10 μM) to aliquots 2, 3, 5 and 6. 5 μg of purified tubulin (Cytoskeleton) was added to tubes 4-6, to increase tubulin concentration. All the aliquots were incubated at 37°C for 1 hour. Samples were then centrifuged at 100,000 ***g*** on a 40% glycerol cushion containing either Nocodazole or Taxol. Each supernatant was then precipitated with methanol and the protein pellet resuspended in 100 μl Laemmli buffer (Laemmli, 1970) and boiled; each microtubule pellet was resuspended in 80 mM KPIPES, pH 6.9, 1 mM EGTA, 2 mM $MgCl_2$ (KHM) and centrifuged again over a new glycerol cushion to wash the pellet. Finally, MT pellets were then resuspended in 100 μl Laemmli buffer and boiled.

In vitro transcription translation and microtubule binding

The cDNAs encoding different fragments of CAP350 were amplified by PCR after the 5′ introduction of a T7 promoter and a Kozak sequence, and addition of a poly(A) 3′ sequence. pfu Turbo (Stratagene) was used for the amplification. The presence of the PCR product at the right molecular weight was verified. The PCR products were then transcribed and translated in vitro using the TnT T7 Quick for PCR DNA kit (Promega) in the presence of 10 μCi [^{35}S]methionine for 90 minutes. Translation products were centrifuged for 30 minutes at 100,000 ***g*** and mixed for 1 hour with ready-assembled MTs. MTs were assembled by incubating 25 μg purified tubulin (Cytoskeleton) in 40 μM Taxol for 30 minutes at 30°C. MTs were then centrifuged through a 40% glycerol cushion made in KHM buffer. The translation-MT mix was then centrifuged on a 40% glycerol cushion at 100,000 ***g***. Proteins from the supernatant were precipitated using methanol, centrifuged and resuspended in Laemmli buffer as the microtubule pellet.

Production and purification of GST-CAP350 (403-725)

The cDNA of CAP350 corresponding to a.a. 403-725 was amplified by PCR introducing a 5′ *Bam*H1 site and 3′ *Eco*R1 site. The PCR product was introduced in pGEX4T3 and sequenced. GST-fusion polypeptides were expressed and isolated from bacterial lysates by affinity chromatography with glutathione-agarose beads.

The fusion protein was analysed by Coomassie Blue staining, concentrated and buffer exchanged for MT buffer (KHM) using a Microcons column (Millipore, 10,000 cut-off). MTs were then added to similar amounts of GST alone, or GST-CAP350. After incubation for 1 hour at room temperature, samples were centrifuged over a 40% glycerol cushion in KHM buffer at 100,000 ***g*** for 30 minutes. Proteins from supernatants were precipitated and resuspended in Laemmli buffer as MT pellets.

Transfection of CAP350 domains

Exponentially growing RPE1 cells were transfected by electroporation and seeded on collagen-fibronectin-coated coverslips for immunofluorescence analyses. Cells were fixed after transfection for 24 hours and processed as described above. Alternatively, cells were transfected using Fugene 6 (Roche) according to the manufacturer's protocol.

siRNA experiments

RNA interference was performed on HeLa cells. Transfections with duplex RNA were carried out with oligofectamine (Invitrogen) for 48 and 72 hours according to the manufacturer's protocol. The following siRNA duplex oligonucleotides were used for CAP350: 5′-CGACGTTTAGATGCAGAAGAA-3′ and 5′-ATGAACG-ATATCAGTGCTATA-3′. As a control, one duplex oligonucleotide described by Yan et al. (Yan et al., 2006) for CAP350 was also used. The duplex GL2 (Elbashir et al., 2001) was used for control. All the duplexes were from Qiagen.

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References

Allan, V. J., Thompson, H. M. and McNiven, M. A. (2002). Motoring around the Golgi. *Nat. Cell Biol.* **4**, E236-E242.

Bacallao, R., Antony, C., Dotti, C., Karsenti, E., Stelzer, E. H. and Simons, K. (1989). The subcellular organization of Madin-Darby canine kidney cells during the formation of a polarized epithelium. *J. Cell Biol.* **109**, 2817-2832.

Bailly, E., Doree, M., Nurse, P. and Bornens, M. (1989). p34cdc2 is located in both nucleus and cytoplasm; part is centrosomally associated at G2/M and enters vesicles at anaphase. *EMBO J.* **8**, 3985-3995.

Chabin-Brion, K., Marceiller, J., Perez, F., Settegrana, C., Drechou, A., Durand, G. and Pous, C. (2001). The Golgi complex is a microtubule-organizing organelle. *Mol. Biol. Cell* **12**, 2047-2060.

Choi, J. H., Bertram, P. G., Drenan, R., Carvalho, J., Zhou, H. H. and Zheng, X. F. (2002). The FKBP12-rapamycin-associated protein (FRAP) is a CLIP-170 kinase. *EMBO Rep.* **3**, 988-994.

Culver-Hanlon, T. L., Lex, S. A., Stephens, A. D., Quintyne, N. J. and King, S. J. (2006). A microtubule-binding domain in dynactin increases dynein processivity by skating along microtubules. *Nat. Cell Biol.* **8**, 264-270.

Delgehyr, N., Sillibourne, J. and Bornens, M. (2005). Microtubule nucleation and anchoring at the centrosome are independent processes linked by ninein function. *J. Cell Sci.* **118**, 1565-1575.

Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K. and Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* **411**, 494-498.

Feierbach, B., Nogales, E., Downing, K. H. and Stearns, T. (1999). Alf1p, a CLIP-170 domain-containing protein, is functionally and physically associated with alpha-tubulin. *J. Cell Biol.* **144**, 113-124.

Gosti-Testu, F., Marty, M. C., Berges, J., Maunoury, R. and Bornens, M. (1986). Identification of centrosomal proteins in a human lymphoblastic cell line. *EMBO J.* **5**, 2545-2550.

Gunawardane, R. N., Martin, O. C. and Zheng, Y. (2003). Characterization of a new gammaTuRC subunit with WD repeats. *Mol. Biol. Cell* **14**, 1017-1026.

Haren, L., Remy, M. H., Bazin, I., Callebaut, I., Wright, M. and Merdes, A. (2006). NEDD1-dependent recruitment of the gamma-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. *J. Cell Biol.* **172**, 505-515.

Hoogenraad, C. C., Akhmanova, A., Grosveld, F., De Zeeuw, C. I. and Galjart, N. (2000). Functional analysis of CLIP-115 and its binding to microtubules. *J. Cell Sci.* **113**, 2285-2297.

Infante, C., Ramos-Morales, F., Fedriani, C., Bornens, M. and Rios, R. M. (1999). GMAP-210, A cis-Golgi network-associated protein, is a minus end microtubule-binding protein. *J. Cell Biol.* **145**, 83-98.

Jasmin, B. J., Cartaud, J., Bornens, M. and Changeux, J. P. (1989). Golgi apparatus in chick skeletal muscle: changes in its distribution during end plate development and after denervation. *Proc. Natl. Acad. Sci. USA* **86**, 7218-7222.

Keryer, G., Di Fiore, B., Celati, C., Lechtreck, K. F., Mogensen, M., Delouvee, A., Lavia, P., Bornens, M. and Tassin, A. M. (2003). Part of Ran is associated with AKAP450 at the centrosome: involvement in microtubule-organizing activity. *Mol. Biol. Cell* **14**, 4260-4271.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lallemand-Breitenbach, V., Quesnoit, M., Braun, V., El Marjou, A., Pous, C., Goud, B. and Perez, F. (2004). CLIPR-59 is a lipid raft-associated protein containing a cytoskeleton-associated protein glycine-rich domain (CAP-Gly) that perturbs microtubule dynamics. *J. Biol. Chem.* **279**, 41168-41178.

Marsh, B. J., Volkmann, N., McIntosh, J. R. and Howell, K. E. (2004). Direct continuities between cisternae at different levels of the Golgi complex in glucose-stimulated mouse islet beta cells. *Proc. Natl. Acad. Sci. USA* **101**, 5565-5570.

Martin, O. C., Gunawardane, R. N., Iwamatsu, A. and Zheng, Y. (1998). Xgrip109: a gamma tubulin-associated protein with an essential role in gamma tubulin ring complex (gammaTuRC) assembly and centrosome function. *J. Cell Biol.* **141**, 675-687.

Mogensen, M. M. (1999). Microtubule release and capture in epithelial cells. *Biol. Cell* **91**, 331-341.

Mogensen, M. M., Mackie, J. B., Doxsey, S. J., Stearns, T. and Tucker, J. B. (1997). Centrosomal deployment of gamma-tubulin and pericentrin: evidence for a microtubule-nucleating domain and a minus-end docking domain in certain mouse epithelial cells. *Cell Motil. Cytoskeleton* **36**, 276-290.

Mogensen, M. M., Malik, A., Piel, M., Bouckson-Castaing, V. and Bornens, M. (2000). Microtubule minus-end anchorage at centrosomal and non-centrosomal sites: the role of ninein. *J. Cell Sci.* **113**, 3013-3023.

Mogensen, M. M., Tucker, J. B., Mackie, J. B., Prescott, A. R. and Nathke, I. S. (2002). The adenomatous polyposis coli protein unambiguously localizes to microtubule plus ends and is involved in establishing parallel arrays of microtubule bundles in highly polarized epithelial cells. *J. Cell Biol.* **157**, 1041-1048.

Moudjou, M. and Bornens, M. (1994). Isolation of centrosomes from cultured animal cells. In *Cell Biology: A Laboratory Handbook* (ed. J. E. Celis), pp. 595-604. New York: Academic Press.

Moudjou, M., Bordes, N., Paintrand, M. and Bornens, M. (1996). gamma-Tubulin in mammalian cells: the centrosomal and the cytosolic forms. *J. Cell Sci.* **109**, 875-887.

Murphy, S. M., Urbani, L. and Stearns, T. (1998). The mammalian gamma-tubulin complex contains homologues of the yeast spindle pole body components spc97p and spc98p. *J. Cell Biol.* **141**, 663-674.

Murphy, S. M., Preble, A. M., Patel, U. K., O'Connell, K. L., Dias, D. P., Moritz, M., Agard, D., Stults, J. T. and Stearns, T. (2001). GCP5 and GCP6: two new members of the human gamma-tubulin complex. *Mol. Biol. Cell* **12**, 3340-3352.

Piehl, M., Tulu, U. S., Wadsworth, P. and Cassimeris, L. (2004). Centrosome maturation: measurement of microtubule nucleation throughout the cell cycle by using GFP-tagged EB1. *Proc. Natl. Acad. Sci. USA* **101**, 1584-1588.

Piel, M., Meyer, P., Khodjakov, A., Rieder, C. L. and Bornens, M. (2000). The respective contributions of the mother and daughter centrioles to centrosome activity and behavior in vertebrate cells. *J. Cell Biol.* **149**, 317-330.

Pierre, P., Pepperkok, R. and Kreis, T. E. (1994). Molecular characterization of two functional domains of CLIP-170 in vivo. *J. Cell Sci.* **107**, 1909-1920.

Rickard, J. E. and Kreis, T. E. (1991). Binding of pp170 to microtubules is regulated by phosphorylation. *J. Biol. Chem.* **266**, 17597-17605.

Rios, R. M. and Bornens, M. (2003). The Golgi apparatus at the cell centre. *Curr. Opin. Cell Biol.* **15**, 60-66.

Rios, R. M., Sanchis, A., Tassin, A. M., Fedriani, C. and Bornens, M. (2004). GMAP-210 recruits gamma-tubulin complexes to cis-Golgi membranes and is required for Golgi ribbon formation. *Cell* **118**, 323-335.

Saito, K., Kigawa, T., Koshiba, S., Sato, K., Matsuo, Y., Sakamoto, A., Takagi, T., Shirouzu, M., Yabuki, T., Nunokawa, E. et al. (2004). The CAP-Gly domain of CYLD associates with the proline-rich sequence in NEMO/IKKgamma. *Structure* **12**, 1719-1728.

Stearns, T., Evans, L. and Kirschner, M. (1991). Gamma-tubulin is a highly conserved component of the centrosome. *Cell* **65**, 825-836.

Takahashi, M., Shibata, H., Shimakawa, M., Miyamoto, M., Mukai, H. and Ono, Y. (1999). Characterization of a novel giant scaffolding protein, CG- NAP, that anchors multiple signaling enzymes to centrosome and the Golgi apparatus. *J. Biol. Chem.* **274**, 17267-17274.

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.

Tassin, A. M., Celati, C., Moudjou, M. and Bornens, M. (1998). Characterization of the human homologue of the yeast spc98p and its association with gamma-tubulin. *J. Cell Biol.* **141**, 689-701.

Thyberg, J. and Moskalewski, S. (1999). Role of microtubules in the organization of the Golgi complex. *Exp. Cell Res.* **246**, 263-279.

Tian, G., Huang, Y., Rommelaere, H., Vandekerckhove, J., Ampe, C. and Cowan, N. J. (1996). Pathway leading to correctly folded beta-tubulin. *Cell* **86**, 287-296.

Tirnauer, J. S., Salmon, E. D. and Mitchison, T. J. (2004). Microtubule plus-end dynamics in Xenopus egg extract spindles. *Mol. Biol. Cell* **15**, 1776-1784.

Waterman-Storer, C. M., Karki, S. and Holzbaur, E. L. (1995). The p150Glued component of the dynactin complex binds to both microtubules and the actin-related protein centractin (Arp-1). *Proc. Natl. Acad. Sci. USA* **92**, 1634-1638.

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.

Yan, X., Habedanck, R. and Nigg, E. A. (2006). A complex of two centrosomal proteins, CAP350 and FOP, cooperates with EB1 in microtubule anchoring. *Mol. Biol. Cell* **17**, 634-644.