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Phosphorylated β-catenin localizes to centrosomes of neuronal progenitors and is required for cell polarity and neurogenesis in developing midbrain

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ABSTRACT

β-catenin has well-established functions in cell growth and differentiation as part of the Wnt signaling pathway and in regulation of cellular adhesion with E-cadherin. Here we studied its significance in midbrain development by temporally controlled deletion of *β-catenin* allowing simultaneous analysis of complete (β-cat-null) and partial (β-cat-low) loss-of-function phenotypes in progenitor cells. β-cat-null cells did not contain centrosomes or a microtubule network and were unpolarized forming delaminated bulges. β-cat-low cells displayed defects in the orientation of the mitotic spindle, increased asymmetric cell divisions and premature differentiation in absence of alterations in polarity or adhesion. The spindle defect was associated with decreased centrosomal S33/S34/T41 phosphorylated β-catenin (p-β-cat) and centrosomal and microtubule defects. Interestingly, neural progenitor cells in mice expressing only unphosphorylatable β-catenin share several phenotypes with β-catenin loss-of-function mice with defects in microtubules and polarity. The results demonstrate a novel function for p-β-cat in maintaining neuroepithelial integrity and suggest that centrosomal p-ß-cat is required to maintain symmetric cleavages and polarity in neural progenitors.

Introduction

The Wnt/β-catenin (β-cat) signaling pathway is important for embryogenesis via the regulation of patterning, cell fate decisions, and cell polarity. A well-characterized part of the pathway involves regulation of the amount of β-cat available to act as a co-activator in transcription through β-cat phosphorylation and regulation of its stability. In the absence of Wnt ligands, cytoplasmic β-cat is phosphorylated on Ser-33, Ser-37, and Thr-41 by glycogen synthase kinase 3β (GSK3β) in a destruction complex with APC, axin1, and axin2/conductin leading to ubiquitination and degradation of the cytoplasmic phosphorylated β-cat (p-β-cat) in the proteasome. Binding of Wnts to cell surface receptors releases β-cat from the destruction complex and results in accumulation and nuclear translocation of unphosphorylated β-cat, which acts as a co-activator for TCF/LEF to regulate transcription and promote proliferation during organogenesis (reviewed in (Grigoryan et al., 2008; Huang and He, 2008)). In neuronal development transcriptional regulation by Wnt/β-cat is important in the spinal cord and forebrain (Chenn and Walsh, 2002; Machon et al., 2003, 2007; Megason and McMahon, 2002; Woodhead et al., 2006; Zechner et al., 2003, 2007) and for patterning, cell proliferation and neuron differentiation also in the developing midbrain (Andersson et al., 2008; Castelo-Branco et al., 2004, 2005; Chilov et al., 2010; Panhuysen et al., 2004; Prakash et al., 2006; Tang et al., 2009).

β-catenin also plays a pivotal role in regulating cell adhesion as a component of adherens junctions (AJ) suggested to be important in neuronal development. Conditional deletion of β-cat early in cortical development causes disruption of the neuroepithelium, loss of cell polarity, and apoptosis (Brault et al., 2001; Junghans et al., 2005; Machon et al., 2003). These effects were primarily attributed to the breakdown of cadherin-based cell adhesion (Junghans et al., 2005; Tang et al., 2009) although a direct effect of β-cat on neuronal nuclear translocation through influencing cytoskeleton dynamics was also considered (Machon et al., 2003).

More recently, β-cat has also been identified at centrosomes and implicated in both anchoring microtubules (MTs) to the centrosome (Huang et al., 2007) and in regulation of centrosome cohesion in cultured cell lines (Bahmanyar et al., 2008; Hadjihannas et al., 2010).

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Centrosomal β-cat is also phosphorylated by GSK3β and this phosphorylation is inhibited following Wnt signals. However, the centrosomal p-β-cat appears to be less susceptible to degradation as it is readily detectable in the absence of Wnt signals (Hadjihannas et al., 2010) suggesting a specific role for p-β-cat in centrosomes.

For neuronal development, it is crucial to establish apico-basal polarity in the ventricular zone progenitor cells and precisely regulate the plane of their division. Symmetrical vertical divisions are thought to be important for maintenance of progenitor cell identity, whereas oblique asymmetric divisions result in neurogenic cell cycle exit. Both apico-basal polarity and regulation of cell cleavage plane depend on the MT network and centrosome function. Several centrosomal proteins regulating mitotic spindle orientation in neuronal progenitors have been identified (reviewed in Fietz and Huttner, 2010). Interestingly, some of these have been recently shown to interact with β-cat (Godin et al., 2010a, 2010b).

Here we have dissected the roles of the various β-catenin functions in neurogenesis in the embryonic midbrain using both temporally controlled inactivation of β-cat as well as replacement of β-cat with a non-phosphorylatable stabilized form. We find phosphorylated β-cat in centrosomes of neuroepithelial progenitors, and suggest involvement of p-β-cat in maintenance of MTs, orientation of the mitotic spindle and regulation of the neurogenic cell-cycle exit.

Methods

Generation and genotyping of mice and embryos

Generation and genotyping of an *Engrailed 1 (En1)* allele carrying Cre-recombinase knock-in (*En1*^{cre}; Kimmel et al., 2000), Rosa26 locus carrying tamoxifen inducible *R26cre-ert* allele (Vooijs et al., 2001), conditional *β-catenin* inactivating allele (*β-cat*^{flox}) (Brault et al., 2001), and the conditional stabilizing *β-catenin* allele (*β-catenin*$^{flox(ex3)}$) (Harada et al., 1999) were described elsewhere. Herein *R26*$^{cre\text{-}ert/+}$; *β-catenin*$^{flox/flox}$ embryos are called β-cat^{lof} (for loss of function), *En1*$^{cre/+}$; *β-catenin*$^{flox(ex3)/flox}$ as β-cat$^{non\text{-}phospho}$ and *En1*$^{cre/+}$; *β-catenin*$^{flox(ex3)/+}$ as β-cat^{stab} in accordance with (Chilov et al., 2010). For staging, the day of vaginal plug was counted as embryonic day 0.5 (E0.5). For BrdU incorporation analysis, pregnant females were given intraperitoneal injection of BrdU (3 mg/100 g body weight) 1 h before dissecting the embryos. To induce Cre-recombinase in *R26cre-ert* mice, pregnant females were given intraperitoneal injection of tamoxifen (Sigma) (8 mg/40 g body weight) 48 h prior to embryo harvesting. All animal work has been conducted according to relevant national and international guidelines. Approval has been obtained from the Finnish Committee of Experimental Animal Research.

Immunofluorescence

Immunofluorescent staining on paraffin sections was performed as described previously (Jukkola et al., 2006). Primary antibodies used were mouse monoclonals against BrdU (GE Healthcare); HuC/D (Invitrogen, Eugene, OR); pancadherins, γ-tubulin (Abcam, Cambridge, UK); aPKC, β-catenin, p27, EB1 (BD South San Francisco, CA); ZO1 (Zymed, South San Francisco, CA); and α-tubulin (Developmental Studies Hybridoma bank, Iowa City, IA). Rabbit polyclonal antibodies used were against SOX2, PAR3 (Millipore); β-catenin, γ-tubulin (Sigma, Saint Louis, USA); and phospho-β-catenin (Ser33/37/Thr41; Cell Signaling, #9561), α-tubulin (Cell Signaling), phosphohistone H3 (Upstate, Lake Placid, NY). All images were acquired using consecutive channel laser scanning.

Explant and cell culture

E9.5 embryos were dissected from NMRI females followed by isolation of rhombomere1/midbrain tissues. The neuronal tube was opened dorsally, placed on filters and positioned on organotypical culture grids. The explants and MDCK cells were cultured in DME medium with 10% fetal calf serum in presence or absence of 5 μM of BIO (Calbiochem) for 2 days. MDCK cells were obtained from ATCC (CCL-34).

Microscopy

Confocal images were acquired using the Leica TCS SP5 confocal system and LAS-AF software. Confocal stacks and images were processed and deconvoluted using Imaris 6.1 (Bitplane) and AutoQuantX (AutoQuant) software. All multi-color images were acquired using consecutive channel laser scanning. Co-localization of p-β-cat and γ-tubulin was analyzed using image-processing software Imaris. The program calculates a co-localization channel based on pixels that feature both green and red channel intensities above a certain threshold level. To increase stringency of calculation, pixels with intensities lower than 25% of average were cut off. EB1 particle numbers were calculated using ImageJ software.

Statistical analyses

The division plane angles were measured using ImageJ software and analyzed using two-sample *t*-test.

Results

To identify β-catenin functions in neurogenesis in the embryonic midbrain a strategy circumventing lethality associated with early deletion (Brault et al., 2001) was required. For this we generated mice with a widely expressed tamoxifen-inducible deletor allele (*R26cre-ert*) (Vooijs et al., 2001) together with a conditional *β-catenin* allele (Brault et al., 2001). Deletion of the floxed region of *β-catenin* in *R26*$^{cre\text{-}ert/+}$; *β-catenin*$^{flox/flox}$ embryos (abbreviated herein as β-cat^{lof} for loss of function) (Chilov et al., 2010) was induced by intraperitoneal injection of tamoxifen into pregnant dams at E9.5 (Brault et al., 2001; Vooijs et al., 2001) 2 days prior to harvesting of the embryos to analyze neurogenesis in the midbrain neuroepithelium.

Analysis of E11.5 midbrain sections of β-cat^{lof} embryos revealed occasional discontinuities in the neuroepithelial lining where cells were delaminated and bulged into the ventricle. β-cat staining revealed that cells in these bulges were β-cat protein null (Figs. 1e–h, arrows). In addition, there were areas of cells with altered β-cat staining within an apparently intact neuroepithelium (Figs. 1e–h). In these areas basolaterally localized β-cat staining was absent, whereas β-cat adjacent to apical cell edges was still present suggesting that these cells had undergone recombination recently and had partial loss of β-cat protein. These cells are referred to herein as "β-cat-low" to distinguish them from the genotypically identical β-cat-null cells.

β-catenin deficiency leads to loss of cell polarity

To investigate polarization of β-cat-low and β-cat-null cells, E11.5 midbrain sections were stained with the major apical polarity complex proteins PAR3 and atypical PKC (aPKC), which are critical for formation and the maintenance of mammalian neuroepithelial cell polarity, maintenance of cell asymmetry and thereby progenitor cell proliferation and differentiation (reviewed in Barnes and Polleux, 2009; Suzuki and Ohno, 2006). In β-cat-low cells with apically localized β-cat protein all polarity markers as well as cadherins are comparable to controls (Figs. 1e, f, g, and h). In contrast, in β-cat-null cells PAR3 is undetectable and aPKC is redistributed from the apical membrane (Figs. 1e and f, arrows). In addition the apical marker ZO1 is absent in β-cat-null cells (Fig. 1g, arrow). Thus, loss of β-cat leads to pronounced defects in polarity in midbrain neural progenitors. The delamination of the cells is indicative of defects also in cell adhesion,

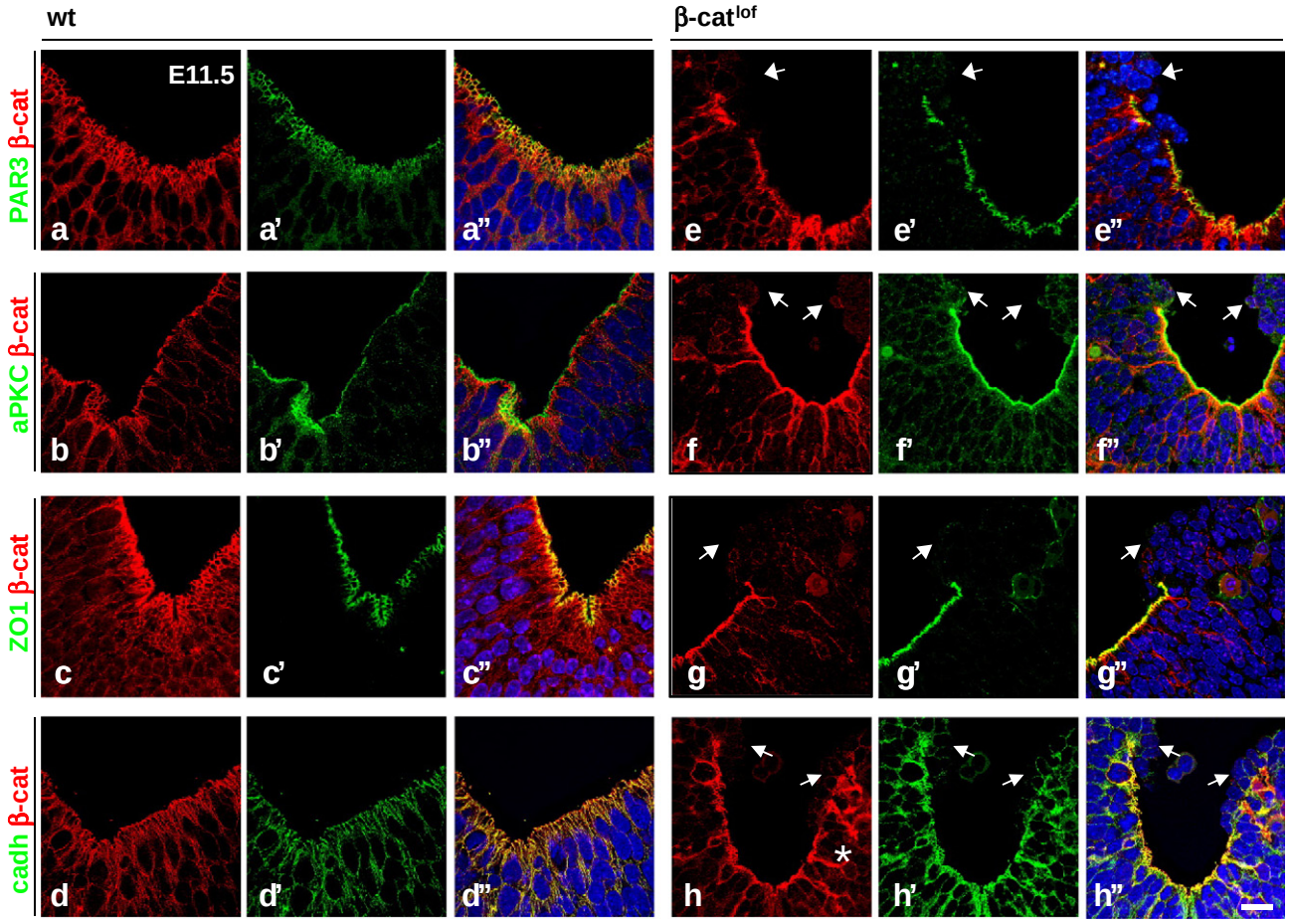

Fig. 1. β-catenin deficient cells lose cell polarity. Immunofluorescence analysis of coronal sections through ventral midbrains of wt (a–d") and β-cat^{lof} (e–h") embryos with anti β-cat antibody (red) and antibodies against PAR3 (a', e'), aPKC (b', f'), ZO1 (c', g'), or Cadherins (d', h') (green) also shown as overlay with nuclear DAPI stain (a"–h"). Images show areas with partial loss of β-catenin where basolateral β-cat staining is mostly undetectable, whereas β-cat on apical surfaces is still present (β-cat-low cells). In areas where β-cat is undetectable (β-cat-null cells) bulges form and cells lack polarity based on PAR3, ZO1, aPKC and Cadherin staining (arrows). Size bar = 10 μm.

although cadherin staining was still detectable also in β-cat-null bulges (Fig. 1h, arrow).

β-catenin is involved in the neurogenic switch in developing midbrain

To investigate neural differentiation in β-cat-low and β-cat-null cells distributions of proliferative ventricular zone progenitors (Sox2) and postmitotic neural precursors (HuC/D; Tuj1) were subsequently analyzed. In β-cat-low cells the normally even Sox2 staining in the ventricular zone had changed with low or undetectable staining in many cells leading to striking heterogeneity (Fig. S1b). β-cat-low cells in the ventricular zone were characterized by frequent ectopic expression of postmitotic markers HuC/D and Tuj1 (Figs. S1d and f, open arrows) as well as by p27 staining (Fig. S3b). The altered expression patterns noted in β-cat-low cells were also noted in bulges of β-cat-null cells (Figs. S2 and S3, bulge). The premature differentiation was prominently observed in the ventro-lateral midbrain, where it was not associated with significant changes in proliferation in either β-cat-low or β-cat-null cells as detected by either BrdU incorporation (Figs. S2a and b), Cyclin D1 (Figs. S2e–h) or phosphohistone H3 (Fig. S2i). Proliferation in the floor plate was significantly reduced consistent with previous results (Tang et al., 2009). Taken together the results indicate that decreased β-cat induces precocious differentiation of some ventricular zone progenitors prior to detectable alterations in polarity or adhesion. However, loss of β-cat may not directly lead to cell cycle exit, as increased differentiation was observed in a mosaic pattern and many β-cat protein negative progenitors were still found to proliferate despite the loss of their polarity.

β-catenin is required for centrosomes and microtubules in the neuroepithelium

In an attempt to identify mechanisms underlying the loss of cell polarity, we next analyzed centrosomes and MTs strongly implicated in cell polarity (reviewed in Gonczy, 2008; Higginbotham and Gleeson, 2007) and in neurogenesis (Bond et al., 2005; de Anda et al., 2005; Higginbotham et al., 2006; Shu et al., 2006; Yokota et al., 2009). Centrosomes in ventral zone neural progenitor cells as detected by γ-tubulin (γ-tub) were primarily localized close to the apical surface in wild-type cells as expected (Fig. 2a, open arrow). In β-cat low cells γ-tubulin staining was irregular, with decreased or undetectable staining in some cells along the intact apical surface (Fig. 2b, arrows). Centrosomal defects were even stronger in the β-cat null cells, which were devoid of γ-tubulin staining even when analyzed using consecutive sections (Fig. 3b'). In these cells also MTs as detected by α-tubulin were collapsed or barely detectable (Figs. 3d' and d", filled arrow), whereas in β-cat-low cells α-tubulin staining did not reveal MT deficiencies (see also below). These results for the first time suggest that β-cat is not only a component of centrosomes, but also required for centrosome maintenance in the neuroepithelial cells.

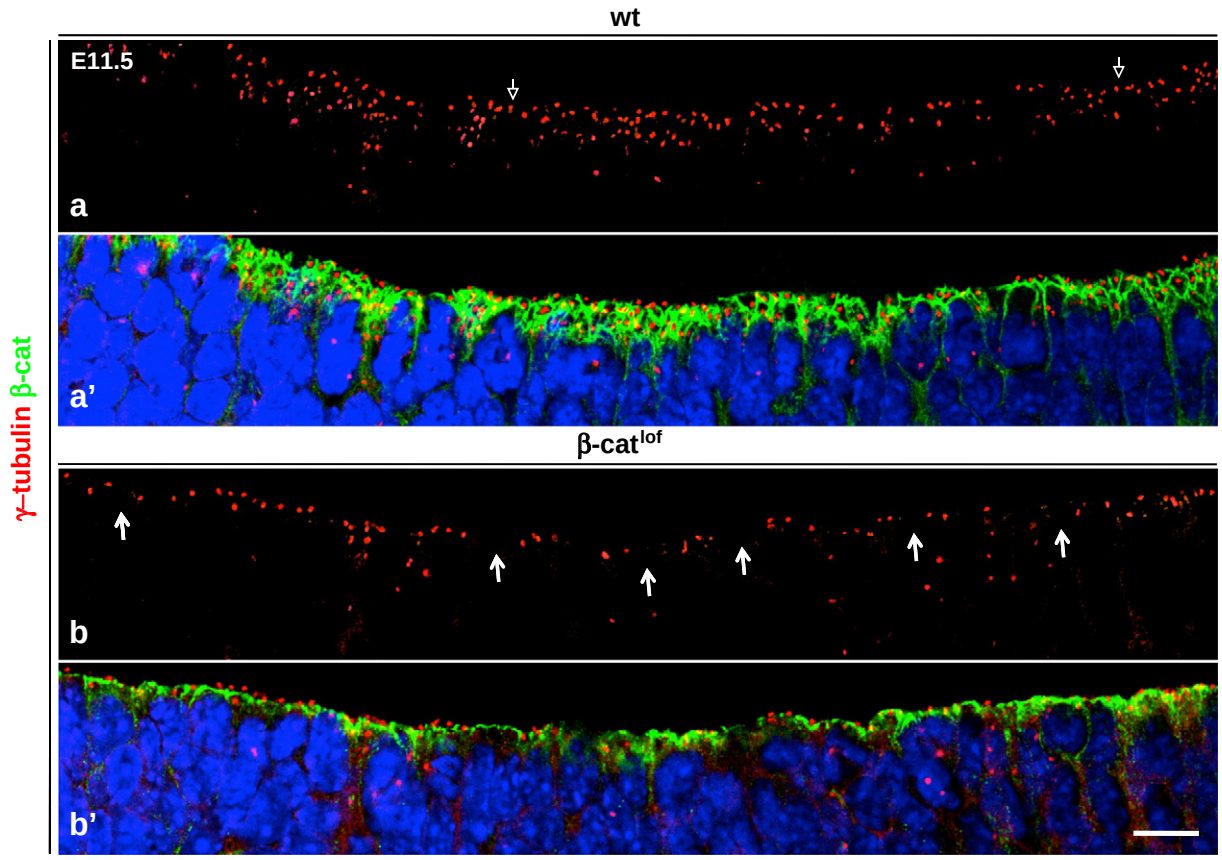

Fig. 2. Centrosomal abnormalities in β-cat-low cells. Coronal sections through midbrain of wt (a) and β-cat^{lof} (b) embryos were stained with antibodies against γ-tubulin also shown as overlay with β-cat and nuclear DAPI staining (a′,b′). Open arrows show normal apical localization of centrosomes in wt embryos. Arrows indicate intact apical areas (continuous β-cat staining) with irregular centrosomes (γ-tubulin) in β-cat-low cells. Size bar = 10 μm.

Decrease of EB1 in β-cat-low cells

To further explore defects in MTs EB1 distribution and levels were analyzed in β-cat-low cells. EB1 is associated with MT plus ends and with centrosomes and is important for MT dynamics, linking MTs to the cell cortex and centrosomes and in positioning of the mitotic spindle (Askham et al., 2002; Lansbergen and Akhmanova, 2006; Tirnauer and Bierer, 2000). Staining with EB1 antibodies demonstrated the expected particulate staining (Vaughan, 2005) where the number as well intensity of total EB1 spots was significantly decreased in β-cat-low cells in ventral midbrain (Figs. 3e and f). Also a specific reduction in apical EB1 staining was noted in ventral midbrain consistent with the irregular centrosomal staining (Figs. 2b and 5b,d). In the floor plate region, where β-cat levels are not significantly decreased, EB1 staining was not altered (Figs. 3g and h), whereas in the β-cat-null bulges EB1 was further decreased as expected based on the lack of centrosomes and MTs. The decrease of EB1 thus correlates with decline in β-cat levels and is consistent with alterations in centrosome/MT dysfunction prior to the loss of cell polarity.

β-catenin regulates the plane of cleavage of progenitor cells

Neurogenesis is determined by mitosis of proliferative progenitors, where asymmetric cleavage leads to differentiation of one of the daughter cells. The centrosome is a critical regulator of the mitotic spindle orientation (Haydar et al., 2003; Kosodo et al., 2004; Wilcock et al., 2007). To investigate the possibility that centrosomal defects in β-cat low cells affect mitotic spindle positioning, we investigated orientation of division planes of mitotic cells. The majority of division planes in control embryos were close to perpendicular (spindle angle 75°–90°; example in Fig. 4a). The spindle angle below 54° could be expected in 5% of wt but in more than 15% of β-cat-low cells ($p = 0.0004$). The latter frequently exhibited horizontal cleavage (Fig. 4b), which was rare in the midbrain of wt embryos. The results suggest that there are increased asymmetric cleavages, which may contribute to precocious differentiation in β-cat-low cells.

Phosphorylated β-catenin localizes to centrosomes in neural progenitors in vivo

The requirement of β-cat for centrosomes/MTs prompted us to investigate the localization and role of S33/S34/T41-phosphorylated β-cat (p-β-cat) in the noted phenotypes in vivo, as p-β-cat has been identified in centrosomes in cultured mammalian cells (Bahmanyar et al., 2008; Hadjihannas et al., 2010; Huang et al., 2007). p-β-cat staining demonstrated a readily detectable punctuate staining, which was not detected following phosphatase treatment or blocking with a β-cat phosphopeptide (Fig. S5). Further indication of specificity was provided by significant co-localization of signal with γ-tubulin in MDCK cells (Fig. S6). P-β-cat staining in the midbrain neuroepithelium was concentrated on apical surfaces of neural progenitors especially in the ventral midbrain (Fig. S7c), and was markedly reduced in this area in β-cat-low cells (Fig. S7d). A clear colocalization was noted in p-β-cat staining with γ-tubulin (Figs. 5a" and 6a) and pericentrin (Fig. 5c") consistent with centrosomal staining. In the β-cat-low cells colocalization was not clear due to weaker staining and irregular centrosomes (Figs. 5b and d) characteristic of the β-cat low cells (see Fig. 2). In the β-cat-null cells no specific staining was noted (Figs. 5d and S7d, e). Thus, we demonstrate that GSK3β-phosphorylated β-cat localizes to centrosomes in developing neuronal progenitors.

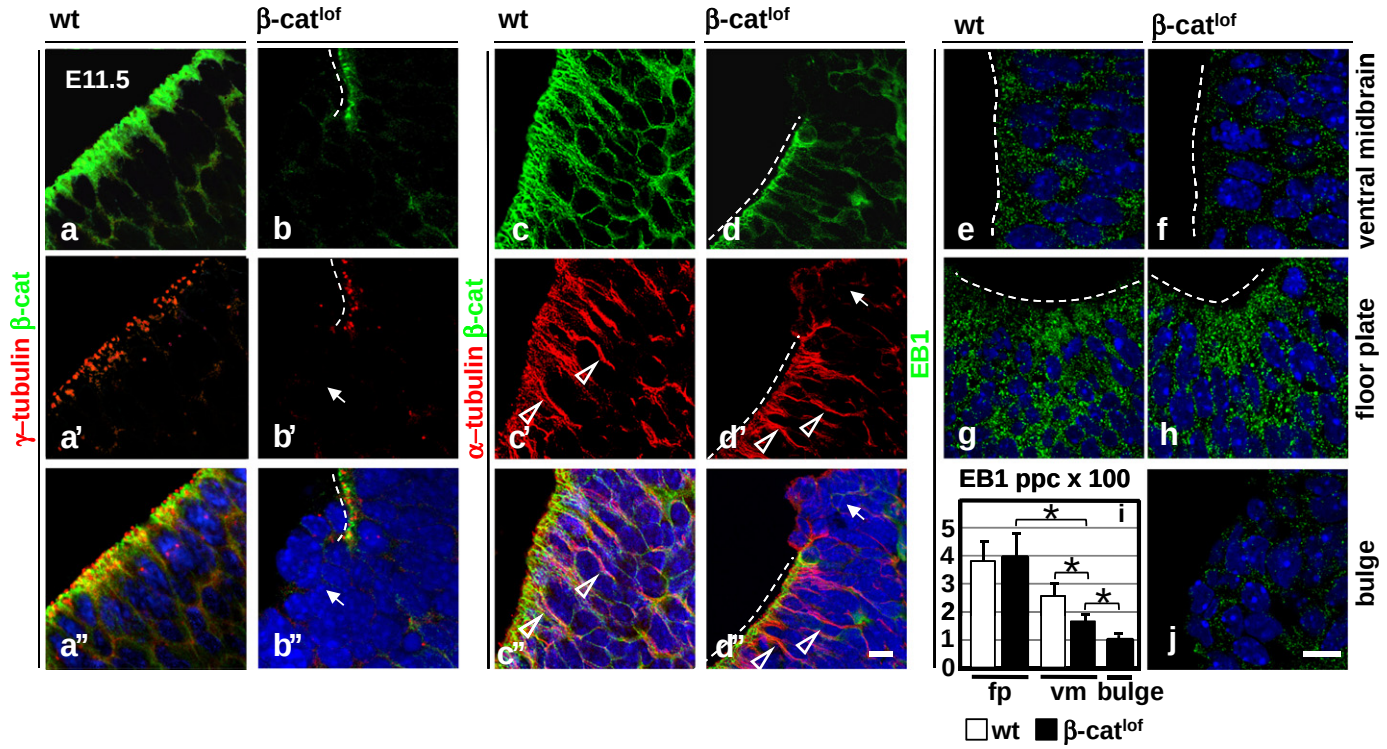

Fig. 3. β-catenin is involved in centrosome stability and microtubule organization. Coronal sections through midbrain of wt (a, c) and β-cat^{lof} (b, d) embryos were stained with antibodies against β-cat and γ-tubulin (a–b) or β-cat and α-tubulin (c–d) also shown as overlay with nuclear DAPI stain (a"–d"). β-cat-null bulges are devoid of γ-tubulin and feature collapsed microtubules (filled arrows). Areas of β-cat-low cells with intact apical surface (dash lines) contain clearly detectable slightly irregular centrosomes and no detectable defects in microtubules (c–d; open arrowheads). Coronal sections through ventral midbrain (e, f, j) and floor plate (g, h) of wt (e, g) and β-cat^{lof} (f, h, j) embryos were stained with anti EB1 antibody. Intact apical surface marked by dashed lines. Chart (i) depicts the number of EB1 particles per cell (ppc, see Methods) from indicated genotypes and areas (fp — floor plate, vm — ventral midbrain, wt and β-cat^{lof} — white and black columns, respectively). At least 150 cells from minimally three different embryos were used for calculations. Lines indicate standard error and significant differences in EB1 between samples are shown by brackets and asterisks. Size bar = 10 μm.

Phosphorylated β-catenin is required for maintenance of centrosomes and polarity

In contrast to the cytoplasmic p-β-cat, which is rapidly degraded, centrosomal p-β-cat is readily detectable in cultured cells ((Hadjihannas et al., 2010), Fig. S6) and in neural progenitors (Fig. 5) suggesting that p-β-cat may have a specific role in centrosomes in the developing brain. To investigate this, we analyzed mice carrying a β-cat mutant lacking the GSK3β phosphorylation sites (Harada et al., 1999) and accordingly acting as a stabilized constitutively active allele for the β-cat coactivator function in developing midbrain (Chilov et al., 2010). This allele would by contrast be expected to act as a loss-of-function allele for β-cat functions requiring phosphorylated β-cat. Using a modification of a strategy recently described (Chilov et al., 2010) we generated mice only carrying the non-phosphorylatable allele in the developing midbrain (*En1*$^{cre/+}$; *β-catenin*$^{flox(ex3)/flox}$; embryos called β-cat$^{non\text{-}phospho}$). These β-cat$^{non\text{-}phospho}$ embryos featured neuronal closure defects (data not shown) and developed large delaminated areas devoid of p-β-cat and γ-tub (Fig. 6b). These areas also lack PAR3 and ZO1 (Fig. 6g) and feature collapsed MTs (Fig. 6l). Importantly, β-cat$^{non\text{-}phospho}$ embryos still have abundant membrane-associated β-cat and to some extent nuclear β-cat (Fig. 6l). This implies that adherence junctions remain despite the loss of cell polarity in β-cat$^{non\text{-}phospho}$ cells. In addition, we used a specific inhibitor of GSK3β, 6-bromoindirubin-3′oxime (BIO) to block phosphorylation of β-cat in ex vivo cultured midbrain explants (Meijer et al., 2003). Treatment of the explants with BIO for 2 days caused formation of foci with lost epithelial polarity and disintegrated microtubules similar to β-cat$^{non\text{-}phospho}$ embryos (Figs. 6e, j, and o). In contrast, accumulation of stabilized β-cat in the presence of phosphorylatable β-cat in β-cat^{stab} embryos does not reduce adhesion (Fig. 6h and Chilov et al., 2010; Gounari et al., 2002; Heiser et al., 2006) and does not deteriorate centrosome/MT network (Figs. 6c and m). Also, both β-cat^{lof} and β-cat$^{non\text{-}phospho}$ embryos feature similar collapsed polarity phenotype despite opposing effect on Wnt target gene expression (Chilov et al., 2010). Importantly, whereas loss of β-cat causes enhanced differentiation (Fig. S1), accumulation of stabilized β-cat suppresses differentiation both in β-cat$^{non\text{-}phospho}$ as well as in β-cat^{stab} embryos (Fig. S4). Thus, loss of cell polarity does not appear to simply correlate with mitotic cell cycle exit. Taken together, these data strongly suggest that β-cat maintains MT/centrosome stability and hence cell polarity via non-transcriptional mechanisms. The results are consistent with a requirement for p-β-cat in maintenance of centrosomes, microtubules network and neural progenitor polarity.

Discussion

The study reveals new critical roles for β-cat in mammalian neurogenesis through the use of a temporally regulated deletion of β-cat in ventral midbrain and through the generation of neuronal progenitors only expressing a β-cat lacking the N-terminal phosphorylation sites. β-cat was identified as a regulator of spindle orientation and to be required for maintenance of symmetric cleavages of proliferating progenitors. β-cat was also found to be required for maintenance of MTs, polarity and centrosomes during neuronal development. The inability of the non-phosphorylatable stabilized β-cat to rescue MT, polarity, centrosome maintenance and adhesion defects suggests a specific function for p-β-cat phosphorylated on S33/S37/T41. Alterations in spindle orientation, MTs and centrosomes

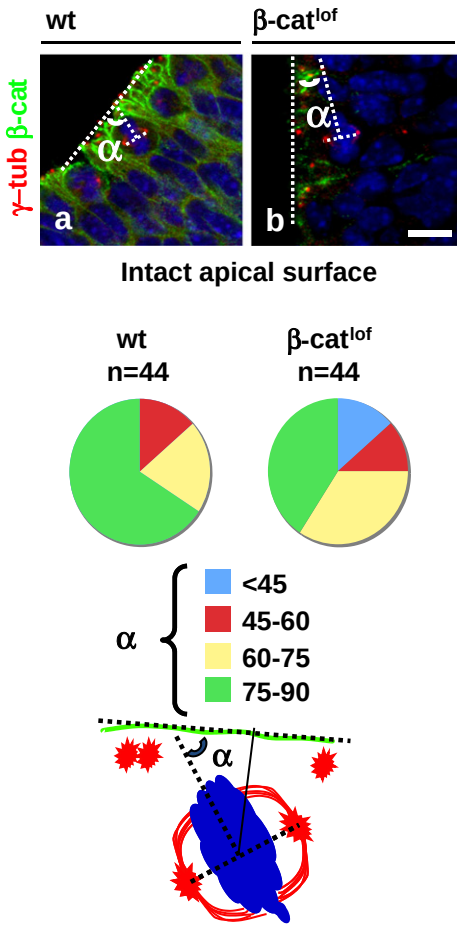

Fig. 4. β-catenin regulates the plane of cleavage of progenitor cells. Coronal sections through midbrain of wt (a) and β-cat^{lof} (b) embryos from β-cat-low areas with intact apical surfaces were stained with antibodies against β-cat and γ-tubulin also shown as overlay with nuclear DAPI stain. In a–b and diagram below mitotic spindle angle (α) was measured as the angle between the division plane and apical surface plane as shown in example images (a–b; dashed lines mark centrosome plane, division plane, and apical surface) and schematic at bottom. The pie chart shows fractions of cells with spindle angles in indicated angle ranges from the indicated genotypes. Size bar = 10 μm.

preceded loss of polarity and adhesion, defects which have previously been associated with conditional depletion of β-cat in the forebrain and midbrain floor plate (Machon et al., 2003; Tang et al., 2009).

Identification of β-catenin as a regulator of spindle orientation in mammalian neurogenesis

In the developing midbrain at E11 95% of proliferative progenitors divide with the mitotic spindle planes perpendicular to the surface of the neuroepithelium consistent with symmetric divisions. One of the early changes noted in the β-cat cells was a very significant increase in mitoses where the spindle had rotated and the spindle plane was close to parallel with the surface (Fig. 4). Identified regulators of spindle orientation in mammalian neurogenesis include several MT and spindle pole interacting proteins, such as mInsc (Zigman et al., 2005), the dynein/dynactin complex component Lis-1 (Yingling et al., 2008), doublecortin (Dcx) (Pramparo et al., 2010), Aspm (Fish et al., 2006), Cdk5rap2 (Buchman et al., 2010; Lizarraga et al. 2010), and huntingtin (htt) (Godin et al., 2010a). Htt in this regard is of special interest as it interacts with and regulates β-cat (Godin et al., 2010b). Htt is thought to regulate spindle orientation through interactions with dynein/dynactin and NuMA (Godin et al., 2010a) and is localized to centrosomes and spindle poles. Also dynein interacts with β-cat, and the interaction has been implicated to be important for centrosome and MT organization (Ligon et al., 2001). Dynein/dynactin and NuMA are involved in regulating organization of MTs at the spindle poles (Merdes et al., 2000) as well as in mediating the interaction of astral MTs with the cell cortex in complex with LGN (Du and Macara, 2004). An attractive hypothesis based on these observations is that the mechanism by which β-cat regulates spindle orientation involves interactions with huntingtin and dynein affecting centrosomes and the MT network (Fig. 7).

ß-catenin promotes symmetric cleavages and proliferative progenitors

Precise regulation of spindle orientation especially in the proliferative progenitors is critical for normal neural development (Yingling et al., 2008), and abundant evidence suggests that spindle orientation determines the fates of the daughter cells (Bond et al., 2005; Fietz and Huttner, 2010; Haydar et al., 2003; Shu et al., 2006; Tabler et al., 2010; Yingling et al., 2008; Zigman et al., 2005). The compromised self-renewal of neural progenitors and a bias of cell fate decisions towards cell cycle exit in β-cat-low cells are therefore likely to be mediated at least in part by spindle orientation changes leading to increased asymmetric cleavages. Recent studies have suggested that strict regulation of the mitotic spindle orientation in a dividing neural progenitor cell is needed to ensure inheritance of both apical and basal components by the daughter cells and to maintain their proliferative progenitor character. Apically located proteins required for neural progenitor character include Par3, which regulates Notch activity (Bultje et al., 2009). Although Par3 was eventually lost in β-cat-null cells, this may not be the primary cause of increased neurogenesis, since defects in the spindle orientation and increased neurogenesis was observed in cells still expressing Par3. Recently, also the basal process has been implicated in the maintenance of proliferative progenitor character (Konno et al., 2008). The molecular details of the basal process function are unknown, but may include signals from the basement membrane (Lahti et al., 2010). Also distribution of other cellular organelles can be asymmetric. Of special interest is the asymmetric division of the centrosome itself, as during neurogenesis inheritance of the older mother centrosome was observed to correlate with the maintenance of proliferative apical progenitor identity in the developing cortex (Wang et al., 2009).

Instead of progenitor division plane affecting neural differentiation, neuronal differentiation might also have an impact on the orientation of progenitor cell division. Thus, increased neuronal differentiation might contribute to the alterations in the cell polarity and cell division planes, which we observed in the β-cat^{lof} mutants. However, loss of cell polarity and defects in centrosomes were also observed in the β-cat$^{non\text{-}phospho}$ mutants despite the continuous cell cycle and greatly reduced differentiation. Also, many progenitors were still able to proliferate efficiently despite the loss of cell polarity in the β-cat^{lof} mutants. These results suggest that loss of cell polarity is not simply downstream of mitotic cell cycle exit in the β-cat mutants and that the phosphorylated β-cat has a cell-differentiation independent function in regulation of cell polarity.

Phosphorylated centrosomal β-catenin is implicated in maintenance of microtubules and polarity

Previous β-cat overexpression studies suggest that β-cat is important together with dynein for both tethering of MTs to adherens junctions (Bellett et al., 2009; Ligon et al., 2001) and for organization of MTs at centrosomes (Askham et al., 2002; Ligon et al., 2001). Also β-cat knockdown studies in tissue culture have implicated β-cat in MT regulation (Huang et al., 2007; Kaplan et al., 2004). In these studies MTs were severely affected both after depletion of β-cat and when only the non-phosphorylatable β-cat was expressed suggesting a role

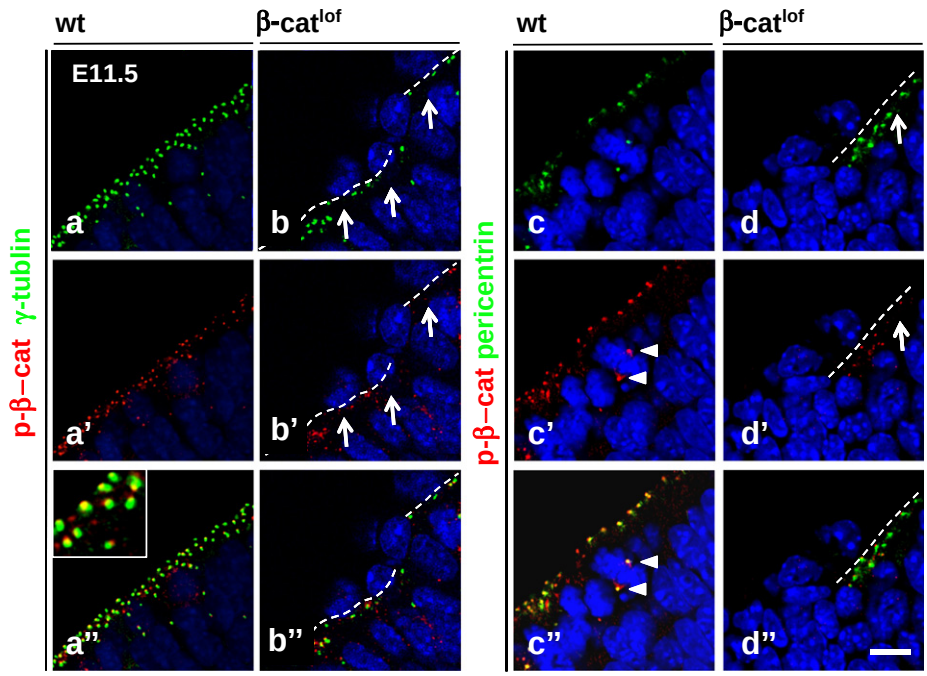

Fig. 5. Phosphorylated β-catenin localizes to centrosomes. Coronal sections through midbrain of wt (a, c) and β-cat^{lof} (b, d) embryos from β-cat-low areas with intact apical surfaces were stained with antibodies against phosphorylated β-cat (p-β-cat) and either γ-tubulin (a–b) or pericentrin (c–d). In the overlay images (a"–d") colocalization is indicated by yellow (for calculation see Methods). Arrowheads point to p-β-cat in mitotic centrosomes. Arrows indicate reduced p-β-cat and irregular γ-tubulin or pericentrin staining in β-cat-low cells. Dashed lines mark intact apical surface. Inset shows high magnification γ-tubulin/p-β-cat staining. Size bar = 10 μm.

for p-β-cat in regulating MTs. This is in agreement with the observation that a phospho-mimick β-cat mutant (S33/37/T41E-b-cat) displayed MT aberrancies (Huang et al., 2007). Localization studies in both neuronal tissues (this study) as well as in various other cell types (Bahmanyar et al., 2008, 2010; Corbit et al., 2008; Hadjihannas et al., 2010; Huang et al., 2007) detect p-β-cat specifically at centrosome thus implicating centrosomal p-β-cat in maintaining MTs. Interestingly, the β-cat binding protein EB1 is required for the formation and maintenance of the radial MT array anchored at the centrosome (Askham et al., 2002; Louie et al., 2004). Furthermore, EB1 colocalizes with p-β-cat at centrosomes, and this localization requires β-cat (Huang et al., 2007) suggesting that p-β-

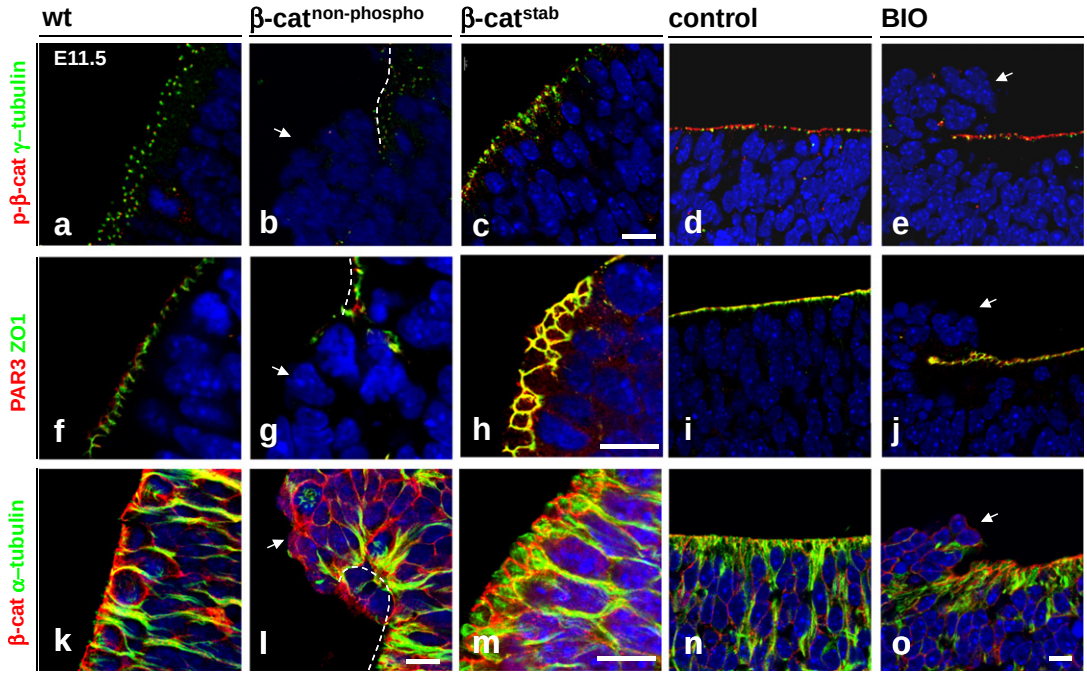

Fig. 6. GSK3β mediated phosphorylation of β-catenin is required to maintain centrosome stability and cell polarity. Coronal sections through midbrain of wt (a, f, k), β-cat$^{non\text{-}phospho}$ (b, g, l) and β-cat^{stab} (c, h, m) embryos. Sections were co-stained with antibodies against p-β-cat and γ-tubulin (a, b, c, d, e), PAR3 and ZO1 (f, g, h, i, j) β-cat and α-tubulin (k, l, m, n, j). Note the delaminated bulges (arrows) lacking signal for p-β-cat, γ-tubulin, PAR3, ZO1 and α-tubulin despite abundant expression of unphosphorylated β-cat. Dash lines mark intact apical surfaces. Sections through explants isolated from midbrain of wt embryos and cultured for 2 days in the presence of DMSO (control: d, i, n) or 5 μM BIO (e, j, o).

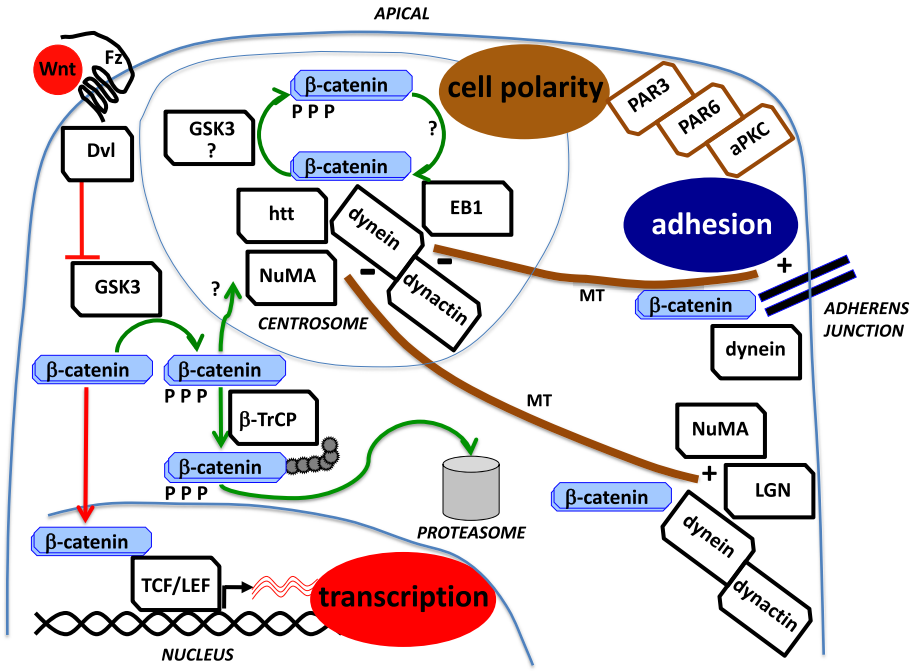

Fig. 7. Control of transcription, cell polarity and adhesion in neuronal progenitors by β-catenin. Binding of Wnts to cell surface receptors (Frizzled) inhibits phosphorylation of β-cat by GSK3 and results in accumulation and nuclear translocation of unphosphorylated β-cat, which acts as a co-activator for TCF/LEF to regulate transcription and promote proliferation during development. Phosphorylated β-cat is recognized by ubiquitin ligase β-TrCP and degraded in proteasomes. In contrast, phospho-β-cat remains in centrosomes, perhaps due to block in ubiquitin ligation, increased GSK3 or decreased phosphatase activity. In centrosomes and MT plus ends β-cat was shown to interact with huntingtin, dynein, EB1. Identified regulators of spindle orientation, MT organization and MT interaction with cell cortex include dynein/dynactin complex, huntingtin (htt), NuMA, LGN.

cat regulates MTs at least in part through EB1 (Fig. 7). This is also consistent with the observation that a decrease in EB1 puncta was noted prior to detectable alterations in MTs in this study.

Similarly to the collapse of MTs also loss of polarity as detected by absence of apical PAR3 and aPKC was a phenotype noted both after the loss of β-cat and in cells expressing the non-phosphorylatable β-cat. Loss of polarity was only noted in cells null for β-cat protein and which had lost epithelial integrity forming delaminated bulges and lacking adherent junctions. This late phenotype was similar to the one noted when β-cat was deleted from the floor plate (Tang et al., 2009). Based on the critical role the radial MT array plays in determining polarity (Li and Gundersen, 2008) it appears conceivable that loss of polarity is secondary to MT defects in these cells. This is also consistent with the observation that the non-phosphorylatable β-cat was cortically localized (Fig. 6) suggesting it was anchored through association with the cadherin complex.

A non-phosphorylated stabilized β-catenin does not rescue requirement of β-catenin in centrosome maintenance

Our results unexpectedly demonstrated loss of centrosomes both following depletion of β-cat as well as in cells only expressing a non-phosphorylatable stabilized β-cat suggesting an important function for the phosphorylated β-cat in maintaining centrosomes in neural progenitors. Earlier siRNA studies indicate that loss of β-cat leads to inhibition of centrosome maturation (Huang et al., 2007), monoastral spindles (Kaplan et al., 2004), and unseparated centrosomes at mitosis (Bahmanyar et al., 2008). By contrast, expression of non-phosphorylated β-cat has been observed to induce centrosome splitting (Hadjihannas et al., 2010). At least two alternative hypotheses could explain the loss of centrosomes in both the β-cat^{lof} and β-cat$^{non\text{-}phospho}$ embryos in our studies. If centrosomal p-β-cat is inactive protein the loss of centrosomes in β-cat^{lof} progenitors maybe due to the lack of centrosome maturation (Huang et al., 2007) or because of centrosome splitting (Bahmanyar et al., 2008). However, the loss of centrosomes in the β-cat$^{non\text{-}phospho}$ embryos is not consistent with the observed increase in aberrant centrioles and extra centrosomes in other models expressing similar non-phosphorylatable β-cat (Bahmanyar et al., 2010) arguing against this model.

An alternative model, where a common mechanism would underlie the loss of centrosomes, suggests that p-β-cat has a previously unidentified independent role in centrosome maintenance, which is lacking in both the β-cat^{lof} and β-cat$^{non\text{-}phospho}$ embryos. To notice the p-β-cat function requires cells expressing only the non-phosphorylatable mutant protein as noted by the lack of such phenotypes in *En1*$^{cre/+}$; *β-catenin*$^{flox(ex3)/+}$ embryos (Fig. 6 and Chilov et al., 2010) also expressing wild type β-cat. It would explain why this role has gone unnoted in studies considering the centrosomal p-β-cat only as a pool of inactive protein waiting to be degraded (Corbit et al., 2008) or to be activated for a splitting function prior to mitosis (Bahmanyar et al., 2008), where a non-phosphorylated β-cat has been expressed concomitantly with a wild type β-cat. A specific role for p-β-cat in centrosomes in neuronal cells is consistent with the observation that p-β-cat is abundant in centrosomes, that levels of phosphorlated β-cat at centrosomes can be regulated independently of non-phosphoryalted β-cat (Hadjihannas et al., 2010) and that overexpression of phospho-β-cat leads to neuronal cell death (Godin et al., 2010b). We show that lack of p-β-cat in β-cat-null cells leads to the loss of centrosomes thus overriding the centrosome cohesion-promoting effect expected in the absence of β-cat (Hadjihannas et al., 2010). It would be interesting to find out whether the loss of centrosomes is due to increased splitting in the absence of p-β-cat. Nek2 – a key kinase regulating centrosome splitting – phosphorylates centrosomal cohesion proteins C-Nap1 and Rootletin to induce splitting. Also β-cat is a Nek2 substrate (Bahe et al., 2005; Bahmanyar et al., 2008). Of note, over-expression of mutated centrosomal protein kinase Nek1, which lacks acidic domain leads to the loss of centrosomes (White and Quarmby, 2008). This raises a question whether p-β-cat and Nek1/2 are involved in the same pathway to centrosome stabilization. Most likely, p-β-cat is important for centrosomal maintenance in neural progenitors in complex with rootletin (Bahmanyar et al., 2008; Hadjihannas et al., 2010).

Stabilized β-catenin without wild-type: disruption instead of proliferation of neural progenitors

Comparison of the β-cat$^{non\text{-}phospho}$ (*En1*$^{cre/+}$; *β-catenin*$^{flox(ex3)/flox}$) embryos to the β-cat^{stab} (*En1*$^{cre/+}$; *β-catenin*$^{flox(ex3)/+}$) embryos revealed that a previously considered dominant active allele of β-cat when expressed without wild type β-cat behaves as a loss-of-function allele in maintenance of MTs and polarity. This underscores the importance of understanding the full spectrum of β-cat functions and in interpreting phenotypes in the plethora of mouse models utilizing similar β-cat alleles. In this regard it will also be interesting to study p-β-cat and its localization to centrosomes in circumstances where β-cat is stabilized through external regulation such as huntingtin mutations in Huntington's disease, presenilin-1 mutations in Alzheimers disease (Chevallier et al., 2005) and APC mutations in colorectal cancer. Stabilization of p-β-cat in Huntington's disease leads to neurotoxicity (Godin et al., 2010a), whereas stabilization by mutant presenilin-1 leads to altered neuronal differentiation (Chevallier et al., 2005).

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References

Andersson, E.R., Prakash, N., Cajanek, L., Minina, E., Bryja, V., Bryjova, L., Yamaguchi, T.P., Hall, A.C., Wurst, W., Arenas, E., 2008. Wnt5a regulates ventral midbrain morphogenesis and the development of A9-A10 dopaminergic cells in vivo. PLoS One 3, e3517.

Askham, J.M., Vaughan, K.T., Goodson, H.V., Morrison, E.E., 2002. Evidence that an interaction between EB1 and p150(Glued) is required for the formation and maintenance of a radial microtubule array anchored at the centrosome. Mol. Biol. Cell 13, 3627–3645.

Bahe, S., Stierhof, Y.D., Wilkinson, C.J., Leiss, F., Nigg, E.A., 2005. Rootletin forms centriole-associated filaments and functions in centrosome cohesion. J. Cell Biol. 171, 27–33.

Bahmanyar, S., Guiney, E.L., Hatch, E.M., Nelson, W.J., Barth, A.I., 2010. Formation of extra centrosomal structures is dependent on {beta}-catenin. J. Cell Sci. 123, 3125–3135.

Bahmanyar, S., Kaplan, D.D., Deluca, J.G., Giddings Jr., T.H., O'Toole, E.T., Winey, M., Salmon, E.D., Casey, P.J., Nelson, W.J., Barth, A.I., 2008. beta-Catenin is a Nek2 substrate involved in centrosome separation. Genes Dev. 22, 91–105.

Barnes, A.P., Polleux, F., 2009. Establishment of axon-dendrite polarity in developing neurons. Annu. Rev. Neurosci. 32, 347–381.

Bellett, G., Carter, J.M., Keynton, J., Goldspink, D., James, C., Moss, D.K., Mogensen, M.M., 2009. Microtubule plus-end and minus-end capture at adherens junctions is involved in the assembly of apico-basal arrays in polarised epithelial cells. Cell Motil. Cytoskeleton 66, 893–908.

Bond, J., Roberts, E., Springell, K., Lizarraga, S.B., Scott, S., Higgins, J., Hampshire, D.J., Morrison, E.E., Leal, G.F., Silva, E.O., Costa, S.M., Baralle, D., Raponi, M., Karbani, G., Rashid, Y., Jafri, H., Bennett, C., Corry, P., Walsh, C.A., Woods, C.G., 2005. A centrosomal mechanism involving CDK5RAP2 and CENPJ controls brain size. Nat. Genet. 37, 353–355.

Brault, V., Moore, R., Kutsch, S., Ishibashi, M., Rowitch, D.H., McMahon, A.P., Sommer, L., Boussadia, O., Kemler, R., 2001. Inactivation of the beta-catenin gene by Wnt1-Cre-mediated deletion results in dramatic brain malformation and failure of craniofacial development. Development 128, 1253–1264.

Buchman, J.J., Tseng, H.C., Zhou, Y., Frank, C.L., Xie, Z., Tsai, L.H., 2010. Cdk5rap2 interacts with pericentrin to maintain the neural progenitor pool in the developing neocortex. Neuron 66, 386–402.

Bultje, R.S., Castaneda-Castellanos, D.R., Jan, L.Y., Jan, Y.N., Kriegstein, A.R., Shi, S.H., 2009. Mammalian Par3 regulates progenitor cell asymmetric division via notch signaling in the developing neocortex. Neuron 63, 189–202.

Castelo-Branco, G., Rawal, N., Arenas, E., 2004. GSK-3beta inhibition/beta-catenin stabilization in ventral midbrain precursors increases differentiation into dopamine neurons. J. Cell Sci. 117, 5731–5737.

Castelo-Branco, G., Sousa, K.M., Bryja, V., Pinto, L., Wagner, J., Arenas, E., 2006. Ventral midbrain glia express region-specific transcription factors and regulate dopaminergic neurogenesis through Wnt-5a secretion. Mol. Cell. Neurosci. 31, 251–262.

Chenn, A., Walsh, C.A., 2002. Regulation of cerebral cortical size by control of cell cycle exit in neural precursors. Science 297, 365–369.

Chevallier, N.L., Soriano, S., Kang, D.E., Masliah, E., Hu, G., Koo, E.H., 2005. Perturbed neurogenesis in the adult hippocampus associated with presenilin-1 A246E mutation. Am. J. Pathol. 167, 151–159.

Chilov, D., Sinjushina, N., Saarimaki-Vire, J., Taketo, M.M., Partanen, J., 2010. beta-Catenin regulates intercellular signalling networks and cell-type specific transcription in the developing mouse midbrain-rhombomere 1 region. PLoS One 5, e10881.

Corbit, K.C., Shyer, A.E., Dowdle, W.E., Gaulden, J., Singla, V., Chen, M.H., Chuang, P.T., Reiter, J.F., 2008. Kif3a constrains beta-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms. Nat. Cell Biol. 10, 70–76.

de Anda, F.C., Pollarolo, G., Da Silva, J.S., Camoletto, P.G., Feiguin, F., Dotti, C.G., 2005. Centrosome localization determines neuronal polarity. Nature 436, 704–708.

Du, Q., Macara, I.G., 2004. Mammalian Pins is a conformational switch that links NuMA to heterotrimeric G proteins. Cell 119, 503–516.

Fietz, S.A., Huttner, W.B., 2011. Cortical progenitor expansion, self-renewal and neurogenesis-a polarized perspective. Curr. Opin. Neurobiol. 21, 23–25.

Fish, J.L., Kosodo, Y., Enard, W., Paabo, S., Huttner, W.B., 2006. Aspm specifically maintains symmetric proliferative divisions of neuroepithelial cells. Proc. Natl Acad. Sci. U. S. A. 103, 10438–10443.

Godin, J.D., Colombo, K., Molina-Calavita, M., Keryer, G., Zala, D., Charrin, B.C., Dietrich, P., Volvert, M.L., Guillemot, F., Dragatsis, I., Bellaiche, Y., Saudou, F., Nguyen, L., Humbert, S., 2010a. Huntingtin is required for mitotic spindle orientation and mammalian neurogenesis. Neuron 67, 392–406.

Godin, J.D., Poizat, G., Hickey, M.A., Maschat, F., Humbert, S., 2010b. Mutant huntingtin-impaired degradation of beta-catenin causes neurotoxicity in Huntington's disease. EMBO J. 29, 2433–2445.

Gonczy, P., 2008. Mechanisms of asymmetric cell division: flies and worms pave the way. Nat. Rev. Mol. Cell Biol. 9, 355–366.

Gounari, F., Signoretti, S., Bronson, R., Klein, L., Sellers, W.R., Kum, J., Siermann, A., Taketo, M.M., von Boehmer, H., Khazaie, K., 2002. Stabilization of beta-catenin induces lesions reminiscent of prostatic intraepithelial neoplasia, but terminal squamous transdifferentiation of other secretory epithelia. Oncogene 21, 4099–4107.

Grigoryan, T., Wend, P., Klaus, A., Birchmeier, W., 2008. Deciphering the function of canonical Wnt signals in development and disease: conditional loss- and gain-of-function mutations of beta-catenin in mice. Genes Dev. 22, 2308–2341.

Hadjihannas, M.V., Bruckner, M., Behrens, J., 2010. Conductin/axin2 and Wnt signalling regulates centrosome cohesion. EMBO Rep. 11, 317–324.

Harada, N., Tamai, Y., Ishikawa, T., Sauer, B., Takaku, K., Oshima, M., Taketo, M.M., 1999. Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. EMBO J. 18, 5931–5942.

Haydar, T.F., Ang Jr., E., Rakic, P., 2003. Mitotic spindle rotation and mode of cell division in the developing telencephalon. Proc. Natl Acad. Sci. U. S. A. 100, 2890–2895.

Heiser, P.W., Lau, J., Taketo, M.M., Herrera, P.L., Hebrok, M., 2006. Stabilization of beta-catenin impacts pancreas growth. Development 133, 2023–2032.

Higginbotham, H., Tanaka, T., Brinkman, B.C., Gleeson, J.G., 2006. GSK3beta and PKCzeta function in centrosome localization and process stabilization during Slit-mediated neuronal repolarization. Mol. Cell. Neurosci. 32, 118–132.

Higginbotham, H.R., Gleeson, J.G., 2007. The centrosome in neuronal development. Trends Neurosci. 30, 276–283.

Huang, H., He, X., 2008. Wnt/beta-catenin signaling: new (and old) players and new insights. Curr. Opin. Cell Biol. 20, 119–125.

Huang, P., Senga, T., Hamaguchi, M., 2007. A novel role of phospho-beta-catenin in microtubule regrowth at centrosome. Oncogene 26, 4357–4371.

Jukkola, T., Lahti, L., Naserke, T., Wurst, W., Partanen, J., 2006. FGF regulated gene-expression and neuronal differentiation in the developing midbrain-hindbrain region. Dev. Biol. 297, 141–157.

Junghans, D., Hack, I., Frotscher, M., Taylor, V., Kemler, R., 2005. Beta-catenin-mediated cell-adhesion is vital for embryonic forebrain development. Dev. Dyn. 233, 528–539.

Kaplan, D.D., Meigs, T.E., Kelly, P., Casey, P.J., 2004. Identification of a role for beta-catenin in the establishment of a bipolar mitotic spindle. J. Biol. Chem. 279, 10829–10832.

Kimmel, R.A., Turnbull, D.H., Blanquet, V., Wurst, W., Loomis, C.A., Joyner, A.L., 2000. Two lineage boundaries coordinate vertebrate apical ectodermal ridge formation. Genes Dev. 14, 1377–1389.

Konno, D., Shioi, G., Shitamukai, A., Mori, A., Kiyonari, H., Miyata, T., Matsuzaki, F., 2008. Neuroepithelial progenitors undergo LGN-dependent planar divisions to maintain self-renewability during mammalian neurogenesis. Nat. Cell Biol. 10, 93–101.

Kosodo, Y., Roper, K., Haubensak, W., Marzesco, A.M., Corbeil, D., Huttner, W.B., 2004. Asymmetric distribution of the apical plasma membrane during neurogenic divisions of mammalian neuroepithelial cells. EMBO J. 23, 2314–2324.

Lahti, L., Saarimaki-Vire, J., Rita, H., Partanen, J., 2011. FGF-signaling gradient maintains symmetrical proliferative divisions of midbrain neuronal progenitors. Dev. Biol. 349, 270–282.

Lansbergen, G., Akhmanova, A., 2006. Microtubule plus end: a hub of cellular activities. Traffic 7, 499–507.

Li, R., Gundersen, G.G., 2008. Beyond polymer polarity: how the cytoskeleton builds a polarized cell. Nat. Rev. Mol. Cell Biol. 9, 860–873.

Ligon, L.A., Karki, S., Tokito, M., Holzbaur, E.L., 2001. Dynein binds to beta-catenin and may tether microtubules at adherens junctions. Nat. Cell Biol. 3, 913–917.

Lizarraga, S.B., Margossian, S.P., Harris, M.H., Campagna, D.R., Han, A.P., Blevins, S., Mudbhary, R., Barker, J.E., Walsh, C.A., Fleming, M.D., 2010. Cdk5rap2 regulates centrosome function and chromosome segregation in neuronal progenitors. Development 137, 1907–1917.

Louie, R.K., Bahmanyar, S., Siemers, K.A., Votin, V., Chang, P., Stearns, T., Nelson, W.J., Barth, A.I., 2004. Adenomatous polyposis coli and EB1 localize in close proximity of

the mother centriole and EB1 is a functional component of centrosomes. J. Cell Sci. 117, 1117–1128.
Machon, O., Backman, M., Machonova, O., Kozmik, Z., Vacik, T., Andersen, L., Krauss, S., 2007. A dynamic gradient of Wnt signaling controls initiation of neurogenesis in the mammalian cortex and cellular specification in the hippocampus. Dev. Biol. 311, 223–237.
Machon, O., van den Bout, C.J., Backman, M., Kemler, R., Krauss, S., 2003. Role of beta-catenin in the developing cortical and hippocampal neuroepithelium. Neuroscience 122, 129–143.
Megason, S.G., McMahon, A.P., 2002. A mitogen gradient of dorsal midline Wnts organizes growth in the CNS. Development 129, 2087–2098.
Meijer, L., Skaltsounis, A.L., Magiatis, P., Polychronopoulos, P., Knockaert, M., Leost, M., Ryan, X.P., Vonica, C.A., Brivanlou, A., Dajani, R., Crovace, C., Tarricone, C., Musacchio, A., Roe, S.M., Pearl, L., Greengard, P., 2003. GSK-3-selective inhibitors derived from Tyrian purple indirubins. Chem. Biol. 10, 1255–1266.
Merdes, A., Heald, R., Samejima, K., Earnshaw, W.C., Cleveland, D.W., 2000. Formation of spindle poles by dynein/dynactin-dependent transport of NuMA. J. Cell Biol. 149, 851–862.
Panhuysen, M., Vogt Weisenhorn, D.M., Blanquet, V., Brodski, C., Heinzmann, U., Beisker, W., Wurst, W., 2004. Effects of Wnt1 signaling on proliferation in the developing mid-/hindbrain region. Mol. Cell. Neurosci. 26, 101–111.
Prakash, N., Brodski, C., Naserke, T., Puelles, E., Gogoi, R., Hall, A., Panhuysen, M., Echevarria, D., Sussel, L., Weisenhorn, D.M., Martinez, S., Arenas, E., Simeone, A., Wurst, W., 2006. A Wnt1-regulated genetic network controls the identity and fate of midbrain-dopaminergic progenitors in vivo. Development 133, 89–98.
Pramparo, T., Youn, Y.H., Yingling, J., Hirotsune, S., Wynshaw-Boris, A., 2010. Novel embryonic neuronal migration and proliferation defects in Dcx mutant mice are exacerbated by Lis1 reduction. J. Neurosci. 30, 3002–3012.
Shu, T., Tseng, H.C., Sapir, T., Stern, P., Zhou, Y., Sanada, K., Fischer, A., Coquelle, F.M., Reiner, O., Tsai, L.H., 2006. Doublecortin-like kinase controls neurogenesis by regulating mitotic spindles and M phase progression. Neuron 49, 25–39.
Suzuki, A., Ohno, S., 2006. The PAR-aPKC system: lessons in polarity. J. Cell Sci. 119, 979–987.
Tabler, J.M., Yamanaka, H., Green, J.B., 2010. PAR-1 promotes primary neurogenesis and asymmetric cell divisions via control of spindle orientation. Development 137, 2501–2505.
Tang, M., Miyamoto, Y., Huang, E.J., 2009. Multiple roles of beta-catenin in controlling the neurogenic niche for midbrain dopamine neurons. Development 136, 2027–2038.
Tirnauer, J.S., Bierer, B.E., 2000. EB1 proteins regulate microtubule dynamics, cell polarity, and chromosome stability. J. Cell Biol. 149, 761–766.
Vaughan, K.T., 2005. TIP maker and TIP marker; EB1 as a master controller of microtubule plus ends. J. Cell Biol. 171, 197–200.
Vooijs, M., Jonkers, J., Berns, A., 2001. A highly efficient ligand-regulated Cre recombinase mouse line shows that LoxP recombination is position dependent. EMBO Rep. 2, 292–297.
Wang, X., Tsai, J.W., Imai, J.H., Lian, W.N., Vallee, R.B., Shi, S.H., 2009. Asymmetric centrosome inheritance maintains neural progenitors in the neocortex. Nature 461, 947–955.
White, M.C., Quarmby, L.M., 2008. The NIMA-family kinase, Nek1 affects the stability of centrosomes and ciliogenesis. BMC Cell Biol. 9, 29.
Wilcock, A.C., Swedlow, J.R., Storey, K.G., 2007. Mitotic spindle orientation distinguishes stem cell and terminal modes of neuron production in the early spinal cord. Development 134, 1943–1954.
Woodhead, G.J., Mutch, C.A., Olson, E.C., Chenn, A., 2006. Cell-autonomous beta-catenin signaling regulates cortical precursor proliferation. J. Neurosci. 26, 12620–12630.
Yingling, J., Youn, Y.H., Darling, D., Toyo-Oka, K., Pramparo, T., Hirotsune, S., Wynshaw-Boris, A., 2008. Neuroepithelial stem cell proliferation requires LIS1 for precise spindle orientation and symmetric division. Cell 132, 474–486.
Yokota, Y., Kim, W.Y., Chen, Y., Wang, X., Stanco, A., Komuro, Y., Snider, W., Anton, E.S., 2009. The adenomatous polyposis coli protein is an essential regulator of radial glial polarity and construction of the cerebral cortex. Neuron 61, 42–56.
Zechner, D., Fujita, Y., Hulsken, J., Muller, T., Walther, I., Taketo, M.M., Crenshaw III, E.B., Birchmeier, W., Birchmeier, C., 2003. beta-Catenin signals regulate cell growth and the balance between progenitor cell expansion and differentiation in the nervous system. Dev. Biol. 258, 406–418.
Zechner, D., Muller, T., Wende, H., Walther, I., Taketo, M.M., Crenshaw III, E.B., Treier, M., Birchmeier, W., Birchmeier, C., 2007. Bmp and Wnt/beta-catenin signals control expression of the transcription factor Olig3 and the specification of spinal cord neurons. Dev. Biol. 303, 181–190.
Zigman, M., Cayouette, M., Charalambous, C., Schleiffer, A., Hoeller, O., Dunican, D., McCudden, C.R., Firnberg, N., Barres, B.A., Siderovski, D.P., Knoblich, J.A., 2005. Mammalian inscuteable regulates spindle orientation and cell fate in the developing retina. Neuron 48, 539–545.