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Regulation of centrosome movements by Numb and the Collapsin Response Mediator Protein during *Drosophila* sensory progenitor asymmetric division

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SUMMARY

Asymmetric cell division generates cell fate diversity during development and adult life. Recent findings have demonstrated that during stem cell divisions, the movement of centrosomes is asymmetric in prophase and that such asymmetry participates in mitotic spindle orientation and cell polarization. Here, we have investigated the dynamics of centrosomes during *Drosophila* sensory organ precursor asymmetric divisions and find that centrosome movements are asymmetric during cytokinesis. We demonstrate that centrosome movements are controlled by the cell fate determinant Numb, which does not act via its classical effectors, Sanpodo and α-Adaptin, but via the Collapsin Response Mediator Protein (CRMP). Furthermore, we find that CRMP is necessary for efficient Notch signalling and that it regulates the duration of the pericentriolar accumulation of Rab11-positive endosomes, through which the Notch ligand, Delta is recycled. Our work characterizes an additional mode of asymmetric centrosome movement during asymmetric divisions and suggests a model whereby the asymmetry in centrosome movements participates in differential Notch activation to regulate cell fate specification.

KEY WORDS: Notch signalling, Asymmetric cell division, Centrosome, *Drosophila*, Cytokinesis, Rab11

INTRODUCTION

Cell fate diversity during development and adult life is in part generated by asymmetric cell divisions (for a review, see Tajbakhsh et al., 2009). The centrosome plays a key function during asymmetric cell division by participating in mitotic spindle orientation and positioning (for a review, see Morin and Bellaïche, 2011). Accordingly, the dynamics of centrosomes are highly regulated during progenitor or stem cell asymmetric divisions (Yamashita and Fuller, 2008). In particular, recent studies have highlighted the existence of an asymmetric behaviour of centrosomes. This was initially discovered during the asymmetric division of *Drosophila* male germline stem cells (GSCs) and also shown to take place in *Drosophila* neural stem cell progenitors: the neuroblasts (Rebollo et al., 2007; Rusan and Peifer, 2007; Yamashita et al., 2003; Yamashita et al., 2007). In each case, the two centrosomes manifest distinct dynamics during prophase: one centrosome is rather static, likely due to its cortical anchoring; the other moves to the opposite pole prior to the formation of the mitotic spindle. Labelling of the mother and daughter centrosomes has elegantly demonstrated that asymmetric centrosome dynamics correlate with an intrinsic difference in centrosome age (Conduit and Raff, 2010; Januschke et al., 2011; Yamashita et al., 2007). Importantly, the distinct centrosome dynamics in prophase are proposed to participate in the mitotic spindle orientation and to ensure the propagation of cell polarization from one division to the next (Januschke and Gonzalez, 2010; Rebollo et al., 2009; Rebollo et al., 2007; Rusan and Peifer, 2007). Accordingly, the disruption of centrosome structure or the loss of centrosomes have profound consequences on mitotic spindle orientation, stem cell fitness and cell polarization (Basto et al., 2006; Giansanti et al., 2001; Lucas and Raff, 2007; Megraw et al., 2001; Rebollo et al., 2007; Rusan and Peifer, 2007; Yamashita et al., 2003; Yamashita et al., 2007). The characterization of the mechanisms controlling the asymmetric centrosome dynamics remains an important challenge to better understand the regulation of cell fate specification during asymmetric cell division.

Notch signalling controls key cell fate specification events during stem cell and progenitor divisions, including during the asymmetric cell division of the *Drosophila* sensory organ precursor cell (SOP or pI cell) (for a review, see Furman and Bukharina, 2011). In the dorsal thorax, pI cells divide along the *Drosophila* body axis to generate a posterior pIIa cell and an anterior pIIb cell, which subsequently divide to form the external and internal sensory organ cells, respectively (Fichelson and Gho, 2003; Gho et al., 1999). The anterior-posterior (AP) orientation of the pI division is regulated by the *Drosophila* NuMA homologue Mud (Ségalen et al., 2010). The specification of the pIIa and pIIb cell fates is controlled by the Notch pathway, which is activated in the pIIa daughter cell (de Celis et al., 1991; Hartenstein and Posakony, 1990). The differential activation of Notch signalling in the pIIa and pIIb daughter cells relies on complementary and redundant mechanisms: the segregation of the Numb and Neuralized (Neur) cell fate determinants (Le Borgne and Schweisguth, 2003b; Rhyu et al., 1994), and the trafficking of the Notch receptor and its ligand Delta via the Rab11 and Sara endosomal compartments (Coumailleau et al., 2009; Emery et al., 2005; Jafar-Nejad et al., 2005).

Numb and Neur are asymmetrically localized at the anterior cortex of the pI cell and segregate in the anterior daughter cell (Le Borgne

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and Schweisguth, 2003b; Rhyu et al., 1994). Their asymmetric localization is regulated in metaphase by the posterior Bazooka (Baz)/Atypical Protein K (DaPKC)/Par-6 (DmPar-6) complex, the anterior Discs large (Dlg)/Partner of Inscuteable (Pins)/Gαi complex and the Aurora A kinase (Bellaïche et al., 2001a; Bellaïche et al., 2001b; Berdnik and Knoblich, 2002; Hutterer et al., 2006; Lee et al., 2006; Petronczki and Knoblich, 2001; Roegiers et al., 2001; Rolls et al., 2003; Schaefer et al., 2001; Schaefer et al., 2000; Schober et al., 1999; Siegrist and Doe, 2005; Wirtz-Peitz et al., 2008). Yet, in telophase, a 'telophase rescue' mechanism promotes their asymmetric localization in the absence of Baz or Pins (Raps – FlyBase) activity (Bellaïche et al., 2001b). During cytokinesis and in the anterior daughter cell, Numb and α-Adaptin (Ada) promote the endocytosis of the Notch receptor, via Sanpodo (Spdo)-dependent and -independent mechanisms, thereby reducing the plasma membrane accumulation of Notch at the pI daughter cell interface (Babaoglan et al., 2009; Benhra et al., 2011; Berdnik et al., 2002; Couturier et al., 2012; Hutterer and Knoblich, 2005; O'Connor-Giles and Skeath, 2003; Roegiers et al., 2005; Santolini et al., 2000; Tong et al., 2010). In parallel, Delta is activated in the anterior pI daughter by a Neur-dependent mechanism. Neur promotes Delta endocytosis in the anterior pI daughter cell (Benhra et al., 2010; Le Borgne and Schweisguth, 2003b). The Delta recycling to the apical pIIa/pIIb interface is then accomplished through the Rab11 positive endosomes (Benhra et al., 2011; Benhra et al., 2010; Emery et al., 2005). Among other activities, Delta recycling requires the exocyst complex and the formation of apical actin-rich (ARS) structure at the level of the apical midbody, which forms upon pI cytokinesis (Jafar-Nejad et al., 2005; Langevin et al., 2005b; Rajan et al., 2009). Two additional mechanisms have been shown to participate in the differential activation of Notch in pIIa versus pIIb. First, the directional trafficking of Notch and Delta in Sara endosomes promotes the localization of active Notch in the posterior pI daughter cells during cytokinesis (Coumailleau et al., 2009). Second, the Rab11 recycling endosomes specifically accumulate around the anterior pI centrosome in late anaphase (Emery et al., 2005). This anterior accumulation is proposed to promote Delta recycling in the anterior pI daughter cell and to act redundantly with Numb and Neur to bias the activation of Notch signalling in the posterior pI daughter cell (Emery et al., 2005; Jafar-Nejad et al., 2005). The onset of Rab11 accumulation around the anterior centrosome is independent of Numb and Neur activity, and the mechanisms controlling Rab11 asymmetric accumulation around the anterior centrosome are poorly understood.

Here, we have found that during pI cell division the centrosomes behave asymmetrically during cytokinesis and that the asymmetry of their movements is controlled by Numb and the *Drosophila* Collapsin Response Mediator Protein (CRMP), the mammalian homologue of which, CRMP2 (DPYSL2), is a microtubule-binding protein that directly interacts with mammalian Numb to control the axonal growth (Arimura et al., 2005; Nishimura et al., 2003; Yoshimura et al., 2005). Furthermore, we show that CRMP controls the duration of Rab11 accumulation at the anterior centrosome and we propose that CRMP participates in Delta activation or trafficking to promote efficient Notch signalling. Our results put forward a functional link between asymmetric centrosome dynamics, endosome dynamics and Notch signalling during asymmetric cell division.

MATERIALS AND METHODS

Drosophila stocks and genetics

The following stocks were used in this study: *neur-Gal4* (Bellaïche et al., 2001a), *pUAS-His2B::mRFP* (Langevin et al., 2005a), *UAS-GFP::cnn* (Megraw et al., 2002), *Ubi-YFP::Asl* (Varmark et al., 2007), *UAS-D82glued* (Allen et al., 1999), *UAS-Myc-numb* (Yaich et al., 1998), *pUAS-mRFP::Pon* (Emery et al., 2005), *UAS-spdo^{RNAi}* (VDRC #104092KK), *UAS-cdk5^{RNAi}* (VDRC #104491KK), *UAS-crmp^{RNAi}* (VDRC #101510KK or VDRC #15320GD), *UAS-rab11^{RNAi}* (VDRC #108382KK), *crmp^{supK1}* (Morris et al., 2012), *crmp^{suplA1}* (Morris et al., 2012), *cnn^{hk21}* (Bloomington #5039), *mud^{f01205}* (Ségalen et al., 2010), *sas-4^{S2214}* (Basto et al., 2006), *pins^{P62}* (Yu et al., 2000), *FRT40A numb^{15}* (Berdnik et al., 2002), *FRT40A ada^{ear4}* (Berdnik et al., 2002), *FRT82B spdo^{3R6}* (Hutterer and Knoblich, 2005) and *FRT9-2 baz^{xi106}* (Wodarz et al., 1999) mutant alleles have been previously described. *N^{ts}* and *Dl^{RF}* are a Notch and a Delta thermosensitive alleles, respectively (Gho et al., 1996; Parody and Muskavitch, 1993). Rab11 dynamics were analysed using a *Ubi-CherryFP::rab11* transgene. The *crmp^{supK1}*, *crmp^{suplA1}*, *crmp^{RNAi}* alleles disrupt both the CRMP and pyrimidine catabolism activities of the *Drosophila crmp* gene (Morris et al., 2012).

The expression of transgenes and dsRNA in the pI cell were achieved by the Gal4/UAS system (Brand and Perrimon, 1993). Somatic mutant clones were generated using the FLP/FRT technique (Golic and Lindquist, 1989; Xu and Rubin, 1993), using either the *hs-flp* stock (Golic and Lindquist, 1989) or the *Ubx-flp* stock (Hutterer and Knoblich, 2005).

Genetic interactions between *N^{ts}* and *crmp^{sup}*, as well as *Dl^{ts}* and *crmp^{sup}*, were performed as follows: the pupae were grown at 18°C until 15 hours after puparium formation (APF) and then shifted to 25°C until dissection at 26-28 hours APF.

Molecular biology and transgenesis

A full-length CRMP cDNA (isoform-A) was Gateway cloned into pUbi-GFP-W (*Drosophila* Genomics Resource Center) creating ubi-CRMP::GFP. The PTB domain of Numb (amino acids 1-227) was inserted by Gateway cloning in pActin-Flag-W (*Drosophila* Genomics Resource Center) resulting in pActin-Flag::Numb^{PTB}. The N-terminal domain of the Dynein Heavy Chain (amino acids 1-600, cDNA IP16365) was introduced by Gateway cloning into the pActin-Flag-W vector creating a pActin-Flag::DHC$^{1-600}$ vector.

To generate *pUAS-crmp::eGFP*, the *crmp* open reading frame was amplified by PCR from HL02693 cDNA (BDGP DGC clones), using the following primers: 5′-CACCATGTCGACCAGCCCGAAAC-3′ and 5′-CCAGAATCCAGAAGACTTTCCG-3′. The resulting PCR product was cloned by Gateway cloning in the destination vector *pUAS-PWG* (Gateway Drosophila Vector Collection, Terence Murphy). *Ubi-Cherry::Rab11* was created by cloning the a N-terminal fusion of CherryFP and Rab11 cDNA downstream of the Ubiquitin promoter. Transgenesis was carried out using BestGene.

Co-immunoprecipitation

Drosophila S2 cells were maintained at 25°C in Schneider's *Drosophila* Medium (Gibco), containing 10% fetal bovine serum inactivated at 65°C, penicillin (50 mg/ml) and streptomycin (50 mg/ml) (Gibco). Cells (1.8×10^7) were transfected with 2 μg expression vectors by the Effecten method (Qiagen). Seventy-two hours post-transfection, cells were lysed at 4°C in lysis buffer [either 50 mM Tris-HCl (pH 8), 150 mM NaCl, 1 mM EDTA, 5 mM glycophosphate, 0.1% Triton for the GFP::CRMP and Flag::DHC$^{1-600}$ immunoprecipitation or 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 50 μg/ml PMSF, 1% NP-40 for the GFP::CRMP and Flag::Numb^{PTB} immunoprecipitation]. Lysis buffers were supplemented with protease inhibitor cocktail (Sigma P-8340). Total protein levels were determined by the Bradford method (Bio-Rad). For immunoprecipitation, lysates were incubated with rabbit-anti-GFP antibodies coupled to protein A agarose beads. Cell lysates and immunoprecipitates were separated by SDS-PAGE, transferred to PVDF membranes (Millipore), probed with mouse anti-Flag primary antibodies (Sigma) and subsequently with HRP-conjugated anti-mouse secondary antibodies (Jackson ImmunoResearch). Western blots were visualized by an enhanced chemiluminescence (ECL) detection system according to the manufacturer's instructions (Amersham Biosciences).

Immunostaining

Nota were dissected from staged pupae, and fixed and stained as describe by Jauffred and Bellaiche (Jauffred and Bellaiche, 2012). For

immunostaining, the following antibodies were used: guinea-pig anti-Numb (1/500, a gift from Y. N. Jan, UCSF, CA, USA), rabbit anti-Bazooka (1/2000, a gift from A. Wodarz, Göttingen Graduate School for Neurosciences, Biophysics and Molecular Biosciences, The Netherlands), mouse anti-Senseless (1/500, Agrobio), rat anti-Su(H) (1/2000, a gift from F. Schweisguth, Institut Pasteur, Paris, France), Cy3 goat anti-HRP (1/400, Jackson Laboratory), mouse anti-Cut (1/20, DSHB), mouse anti-γ Tubulin (1/1000, Sigma-Aldrich clone GTU-88). The Cy3- and Cy5-coupled secondary antibodies were from Jackson Laboratory and Alexa-488-coupled secondary antibodies were from Molecular Probes. Images were acquired on Zeiss LSM 510, Zeiss LSM710 and on Leica SP2 confocal microscopes.

Live-imaging, quantification, image processing and statistics

Pupae staged at 16.5 hours APF were imaged under Zeiss LSM 510 and LSM710 confocal microscopes. Pupae are mounted as described by Jauffred and Bellaiche (Jauffred and Bellaiche, 2012). Live imaging was performed by acquiring *z*-stacks every 60 seconds. Kymographs were generated using ImageJ. Images were processed and assembled with ImageJ, Adobe Photoshop and Adobe Illustrator. The timing of the apical movement of each centrosome relative to anaphase onset was quantified as follows: the timing of anaphase onset was determined as the frame at which the chromosomes (labelled by His2B::mFRP) separate. The timing of apical centrosome (labelled by GFP::Cnn or YFP::Asl) movements was determined as the frame at which the centrosome moves towards the apical midbody. The duration of Rab11 accumulation at the anterior centrosome was determined by the time difference between the frames at which mCherryFP::Rab11 appears and disappears around the GFP::Cnn-labelled anterior centrosome.

Statistical significances was assessed using Student's *t*-test (centrosome movement asymmetry and duration of Rab11 accumulation) and using χ^2 test (genetic interaction).

RESULTS

Asymmetric centrosome dynamics during cytokinesis

As an asymmetry in centrosome movements during prophase participates in mitotic spindle orientation during GSC and in neuroblast divisions (Rebollo et al., 2007; Rusan and Peifer, 2007), we investigated whether the centrosome movements are asymmetric during pI cell division by imaging pI cells expressing the pericentriolar marker GFP::Centrosomin (GFP::Cnn) (Megraw et al., 2002) and Histone2B::mRFP (His2B::mRFP) (Fig. 1A-E; supplementary material Movie 1). In contrast to GSC and neuroblast asymmetric cell division, we observed that the two centrosomes of the dividing pI have a symmetric behaviour from late interphase to late anaphase (supplementary material Fig. S1A-B′). In prophase, both centrosomes move basally to the equatorial plane of the pI cell. Then during prometaphase, they rotate to align with the AP axis. At anaphase onset, both centrosomes undergo a small shift towards the anterior cortex of the pI cell and they then move apart during anaphase (Fig. 1A). Strikingly, the anterior and posterior centrosomes start to behave differently at the onset of pI cell cytokinesis that generates the posterior pIIa and the anterior pIIb cells (Fig. 1C,D, *t*=441 seconds, at *t*=819 seconds) (supplementary material Fig. S1C,C′). The posterior centrosome first starts to move towards the apically located midbody (Fig. 1C). Later on, and following a small movement under the nucleus (Fig. 1B), the anterior centrosome moves towards the apical midbody (Fig. 1D,E). The delay between the onset of apical movements of the posterior and anterior centrosome creates an asymmetry in their movements during cytokinesis (supplementary material Fig. S1D-E′).

To illustrate the asymmetry of the centrosome movements, we built a kymograph of the centrosome positions along a line passing by both centrosomes from metaphase onwards (Fig. 1F). This illustrates that the posterior centrosome moves towards the midbody prior to the anterior one. We quantified this asymmetry by measuring the time at which each centrosome starts to migrate towards the apical midbody relative to anaphase onset, which is precisely determined by chromatid separation. The posterior centrosome begins its movement on average 408±70 seconds (*n*=40) after anaphase onset, whereas the anterior one moves on average 819±132 seconds after anaphase onset (*n*=40) (Fig. 1G). An identical asymmetry in centrosome movements was observed using the centriolar marker YFP::Asterless (YFP::Asl; data not shown). Furthermore, on fixed tissues, the labelling of centrosomes by γ-tubulin (Fig. 1H) or Cnn (not shown) antibodies illustrates that during pI cell cytokinesis, the posterior centrosome can be found in an apical position above the condensing nucleus of the posterior pI daughter cell (Fig. 1H, red arrow), whereas the anterior one is located in a basal position nearby the condensing nucleus of the anterior pI daughter cell (Fig. 1H, blue arrow).

We then investigated whether the asymmetric centrosome movements are specific to the pI cell division by following centrosomes movements in the surrounding epithelial cells. In dividing epithelial cells expressing YFP::Asl and His2B::mRFP, centrosome movements are symmetric from late interphase to cytokinesis (Fig. 1I-L). In contrast to the pI cell division, both centrosomes simultaneously move towards the apical domains of the epithelial cells on average 613±95 seconds (*n*=18) after the anaphase onset (Fig. 1J).

We conclude that, during pI cell division, the centrosome movements are asymmetric, as revealed by the difference in the onset of apical movements of the posterior and anterior centrosomes during cytokinesis.

Centrosome movement asymmetry is regulated by Numb

In both GSCs and neuroblasts, the asymmetry in centrosome dynamics correlates with the intrinsic age difference of centrosomes (Conduit and Raff, 2010; Januschke et al., 2011; Yamashita et al., 2007). We therefore analysed whether an intrinsic centrosome difference might determine the asymmetry in the movement of centrosomes during pI cytokinesis. If the asymmetry in centrosome movements depended only on an intrinsic age difference, the centrosome dynamics should be independent of the unequal segregation of cell fate determinants. We therefore randomized the mitotic spindle orientation to induce the equal segregation of cell fate determinants in telophase and analysed whether centrosome movements were affected in cytokinesis. We imaged GFP::Cnn and Partner of Numb tagged with mRFP (mRFP::Pon) in *mud* pI cells, and we quantified the centrosome movements upon equal segregation of mRFP::Pon into both daughter cells due to spindle misalignment. Strikingly, the difference in the timing of apical movements between the two centrosomes was reduced in such 'symmetric' division (*n*=5). Although we cannot exclude that Mud directly affects centrosome dynamics, this experiment suggests that cortical polarity, and not only a centrosome-intrinsic age difference, controls the asymmetry of centrosome movements during cytokinesis.

To further test this hypothesis, we explored whether the polarity complexes and the cell fate determinants control the asymmetry of centrosome movements. In *baz* or *pins* mutant pI cells, the centrosome asymmetry was identical to the one observed in the wild-type pI cells (Fig. 2). Likewise, reducing *neur* expression by

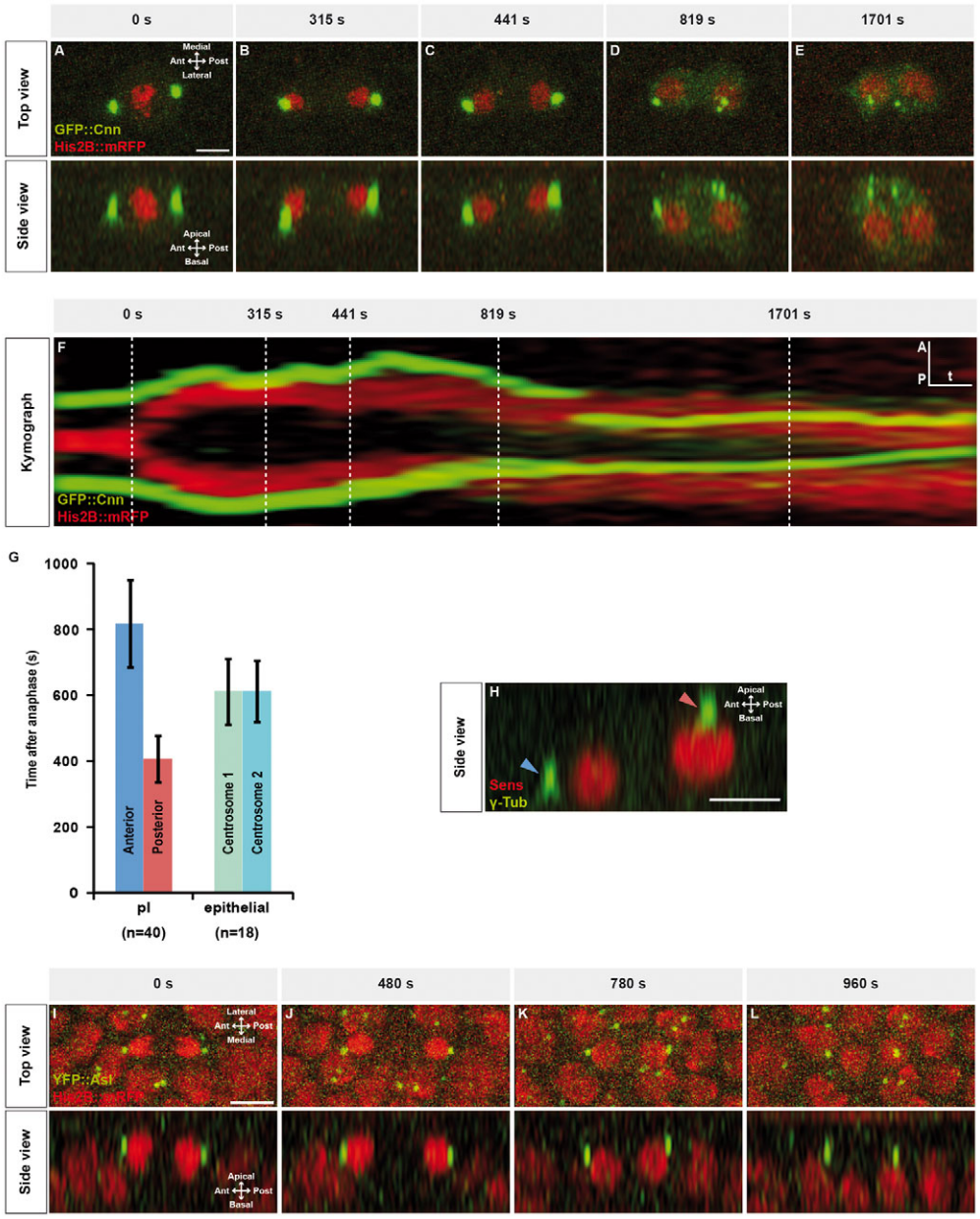

Fig. 1. Centrosome dynamics during the asymmetric division of the pI cells. (A-E) Time-lapse of His2B::mRFP (red) and GFP::Cnn (green) during pI cell division at different time points following anaphase. Top panels are top views corresponding to a maximal projection along the apical-basal axis of the dividing pI cell (anterior, leftwards; medial, upwards). Bottom panels are side views corresponding to lateral maximal projections (anterior, leftwards; apical, upwards). (**A**) The chromatids separate at *t*=0 seconds. (**B**) The anterior centrosome undergoes a movement underneath the nucleus (*t*=315 seconds). (**C**) The posterior centrosome moves towards the apex of the pI cell (*t*=441 seconds). (**D**) The anterior centrosome moves towards the apex of the anterior daughter of the pI cell (*t*=819 seconds). (**E**) Both centrosomes are located at the cell apex (*t*=1701 seconds). The mitotic spindle is slightly tilted along the apical basal axis due to the activity of Fz signalling pathway (Ségalen et al., 2010). (**F**) Kymograph of the pI division shown in A. His2B::mRFP (red) and GFP::Cnn (green). (**G**) The timing of the anterior (blue) and posterior (red) apical centrosome movements in the pI cells and of the timing of apical centrosome (green and turquoise) movement in epithelial cells. (**H**) Lateral section of a pI cell stained for the pI cell-specific marker Senseless (Sens, red) and the centrosomal marker γ-Tubulin (γ-Tub, green). Red and blue arrowheads indicate posterior and anterior centrosomes, respectively. (I-L) Time-lapse of His2B::mRFP (red) and YFP::Asl (green) during an epithelial cell division. (**I**) The chromatids separate at *t*=0 seconds. (**J**) Both centrosomes are located on the lateral side of the epithelial cells (*t*=480 seconds). (**K**) Both centrosomes move towards the apex of the cell (*t*=780 seconds). (**L**) Both centrosomes are located at the cell apex (*t*=960 seconds). Scale bars: 5 μm.

RNA interference (RNAi) did not affect the dynamics of the centrosomes. In sharp contrast, in *numb* mutant pI cells, the difference in the timing of centrosome apical movements was strongly reduced as the posterior centrosome movement towards the cell apex was delayed (Fig. 2; supplementary material Movie 2). This shows that Numb is necessary to promote an early posterior apical centrosome movement. Furthermore, the overexpression of Numb induces a premature movement of both the anterior and the posterior centrosomes, and an absence of difference in their timing of apical movements (Fig. 2). Together, our results demonstrate that

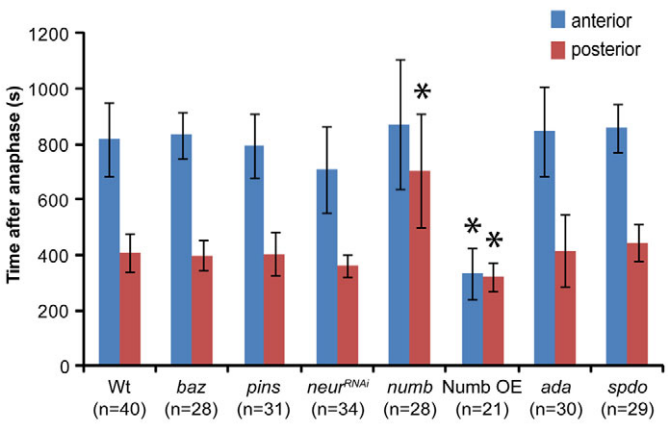

Fig. 2. Polarity genes and the control of centrosome movements. The average timing of anterior (blue) and posterior (red) apical movement in wild-type (anterior, 819±132, *n*=40; posterior, 408±70, *n*=40), *baz* (anterior, 833±83, *n*=28; posterior, 399±55, *n*=28), *pins* (anterior, 794±118, *n*=31; posterior, 405±76, *n*=31), *neur*^{RNAi} (anterior, 708±155, *n*=34; posterior, 362±41, *n*=34), *numb* (anterior, 871±232, *n*=28; posterior, 703±205, *n*=28), *ada* (anterior, 845±162, *n*=30; posterior, 416±58, *n*=30) and *spdo* (anterior, 859±89, *n*=29; posterior, 445±66, *n*=29) mutant pI cells as well as in Numb-overexpressing pI cells (Numb OE; anterior, 333±105, *n*=21; posterior, 321±51, *n*=21). The significant differences ($P<10^{-3}$) between mutant and wild-type pI cells are indicated by asterisks.

Numb controls the onset of apical centrosome movement during cytokinesis. As both Pins and Baz regulate Numb asymmetric localization at metaphase but are dispensable for Numb polarization in telophase and cytokinesis (Bellaïche et al., 2004; Bellaïche et al., 2001a; Bellaïche et al., 2001b), our results further suggest that the asymmetric localization of Numb in telophase and cytokinesis regulates the difference in the timing of apical centrosome movements during cytokinesis.

Numb controls centrosome movements independently of Sanpodo and α-Adaptin

Our genetic findings demonstrate that Numb delays the posterior centrosome movement, while Numb is localized at the anterior cortex. This strongly suggests that Numb indirectly regulates centrosome dynamics and proposes a possible model whereby the anterior localization of Numb restricts the activity of a negative regulator of centrosome movements in the anterior part of the pI cell during cytokinesis. To further investigate this model, we characterized whether known interactors of Numb regulate centrosome dynamics during pI cell division.

During the pI division, Numb acts via two downstream effectors, α-Adaptin (Ada) and Sanpodo (Spdo), to regulate Notch endocytosis and signalling (Berdnik et al., 2002; Hutterer and Knoblich, 2005; O'Connor-Giles and Skeath, 2003). Yet in *ada* and *spdo* mutant pI cells, the centrosome movements proceed as observed in wild-type pI cells (Fig. 2). We therefore concluded that Numb regulates the centrosome movements independently of its previously well-characterized downstream effectors during asymmetric cell division. As Ada and Spdo participate in Notch endocytosis and signalling, as well as in the pIIa and pIIb cell fate specification (Berdnik et al., 2002; Hutterer and Knoblich, 2005), this further indicates that centrosome movements are not a consequence of Notch endocytosis and of the specification of the pIIa and pIIb cell fates by Notch signalling.

CRMP and Cdk5 regulate the movement of the anterior centrosome

Previous studies in mammals have demonstrated that Numb interacts with collapsin response mediator protein 2 (CRMP2), a microtubule-binding protein that regulates axonal growth (Nishimura et al., 2003) and neuronal polarity (Arimura et al., 2005; Yoshimura et al., 2006; Yoshimura et al., 2005). The *Drosophila crmp* gene shares 64% homology with the mouse *Crmp2* (*Dpysl2*) gene and it has been shown to be required for pyrimidine metabolism, learning and memory (Morris et al., 2012; Rawls, 2006). In agreement with findings made with mammalian proteins, we found that the PTB domain of Numb tagged with a Flag epitope (Flag::Numb^{PTB}) is able to immunoprecipitate full-length CRMP tagged with GFP (GFP::CRMP) in *Drosophila* S2 cell extracts (Fig. 3A). CRMP function is controlled being phosphorylated by cyclin-dependent kinase 5 and its interactor p35 (Cdk5/p35) (Arimura et al., 2000; Brown et al., 2004). No study has so far addressed the putative role of CRMP and the Cdk5/p35 complex during asymmetric cell division in *Drosophila* and mammals.

We analysed centrosome movements in mutant pupae for two *crmp*-null alleles. The inactivation of CRMP function leads to an almost complete loss of asymmetry in centrosome movements. Indeed, the anterior centrosome moves earlier than in wild-type pI cells, leading to both centrosomes moving almost simultaneously towards the apical midbody (Fig. 3B; supplementary material Movie 3). The function of CRMP is autonomous to the pI cell because a similar loss of centrosome movement asymmetry is observed upon the expression of a *crmp* hairpin (*crmp*^{RNAi}) specifically in the pI cells (Fig. 3B,J). We then analysed whether Cdk5 also controls centrosome movement during pI cell division. The downregulation of Cdk5 function by RNAi leads to a phenotype similar to the one observed in *crmp* mutant pI cells (Fig. 3B). Neither CRMP nor Cdk5 acts by disrupting pI cell polarization or mitotic spindle orientation because, in *crmp* mutant or *cdk5*^{RNAi} pI cells, Numb is correctly localized at the anterior cortex (Fig. 3C-D′) and the mitotic spindle is oriented along the AP axis (Fig. 3E). We therefore concluded that CRMP and the Cdk5 kinase delay the anterior centrosome movement and thereby promote asymmetric centrosome movement during pI cell division. Furthermore, a CRMP::GFP tagged version was homogeneously distributed in the pI cell (Fig. 3F,G), suggesting that CRMP regulates centrosome movements by being differentially activated at the anterior and the posterior poles of the pI cell in telophase. In keeping with the known interaction between the mammalian Numb and CRMP counterparts, and the opposing activity of Numb and CRMP on centrosome movements, we propose that Numb loss of function leads to an ectopic activation of CRMP at the posterior cortex, and that this in turn induces a delay in posterior centrosome movement. Accordingly, loss of CRMP function in *numb* mutant pI cells leads to a phenotype similar to the one observed in *crmp* mutant pI cells, showing that CRMP function is needed in absence of Numb to regulate centrosome movements (Fig. 3B).

Recent findings have shown that CRMP directly interacts with the Dynein motor and suggested that CRMP interferes with Dynein (Dhc64C) activity in COS7 cells (Arimura et al., 2009). We found that CRMP::GFP can be co-immunoprecipitated with the N-terminal domain of the Dynein Heavy Chain tagged with a Flag epitope (Flag::DHC$^{1\text{-}600}$) in *Drosophila* S2 cell extracts (Fig. 3H) and we therefore analysed whether centrosome movements are regulated by Dynein activity during pI cell division. To inhibit Dynein function, we expressed either Dynamitin (Dmn) or D82-Glued, both of which inhibit Dynein function by preventing the

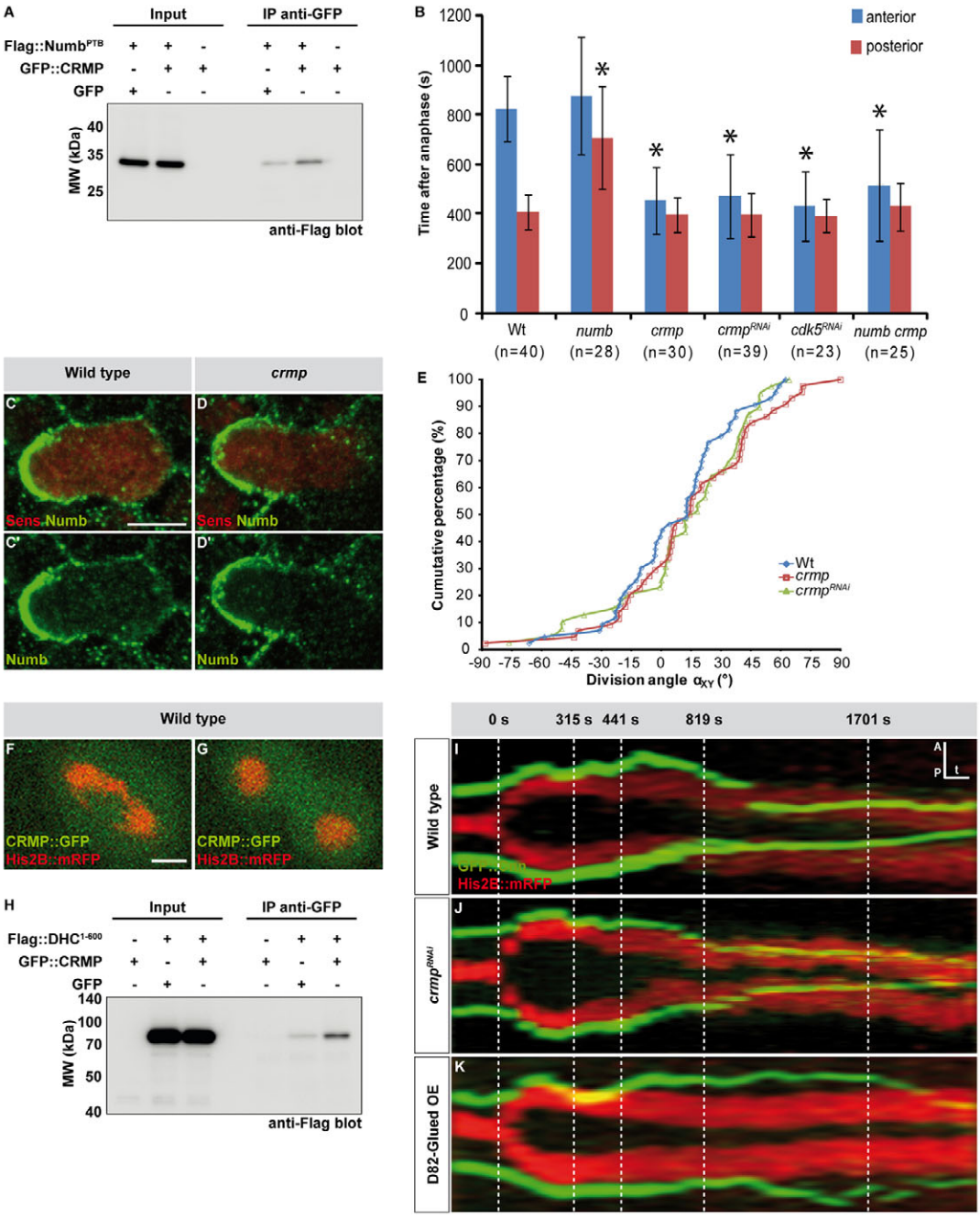

Fig. 3. CRMP and Cdk5 regulate centrosome movement asymmetry. (**A**) Anti-Flag blot of GFP immunoprecipitates from cells expressing Flag::Numb^{PTB}, GFP; Flag::Numb^{PTB} and GFP::CRMP; GFP::CRMP. Molecular weight marker (MW) are in kDa. Although a non-specific GFP binding was observed, a larger amount of Flag::Numb^{PTB} was reproducibly co-precipitated with GFP::CRMP (n=3). (**B**) The average timing of anterior (blue) and posterior (red) apical movement in wild-type (anterior, 819±132, n=40; posterior, 408±70, n=40), *numb* (anterior, 871±232, n=28; posterior, 703±205, n=28), *crmp* (anterior, 455±133, n=30; posterior, 393±68, n=30), *crmp*^{RNAi} (anterior, 464±180, n=39; posterior, 378±92, n=39) and *cdk5*^{RNAi} (anterior, 431±139, n=23; posterior, 390±65, n=23) mutant and *crmp, numb* double-mutant (anterior, 512±224, n=25; posterior, 426±98, n=25) pI cells. The significant differences ($P<10^{-3}$) between mutant and wild-type pI cells are indicated by asterisks. The anterior and posterior centrosome movements in double *crmp numb* mutant pI cells are significantly different from the anterior and posterior centrosome movement in *numb* mutant pI cells ($P<10^{-3}$) and not significantly different from the one of the *crmp* pI cells ($P\geq10^{-1}$). The wild-type and *numb* data are identical to those shown in Fig. 2 and are shown for comparison with the *crmp*, *cdk5* and double *crmp*, *numb* mutant phenotypes. (**C-D′**) Localization of Numb (green) in wild-type (C,C′) and *crmp* (D,D′) pI cells stained by Senseless (Sens, red, C, D) at anaphase. (**E**) Cumulative mitotic spindle orientation in wild-type (blue), *crmp* (red) and *crmp*^{RNAi} (green) pI cells. (**F**,**G**) Localization of CRMP::GFP (green) in anaphase (F) and telophase (G) in dividing pI cells identified by His2B::mRFP (red) expression. (**H**) Anti-Flag blot of GFP immunoprecipitates from cells expressing Flag::DHC$^{1-600}$, GFP; Flag::DHC$^{1-600}$ and GFP::CRMP; GFP::CRMP. Molecular weight marker (MW) in kDa. Although a non-specific GFP binding was observed, a larger amount of Flag::DHC$^{1-600}$ was reproducibly co-precipitated with GFP::CRMP (n=2). (**I-K**) Kymograph of a wild-type (I), *crmp*^{RNAi} (J) and D82-Glued-overexpressing (D82-Glued OE; K) dividing pI cells labelled by His2B::mRFP (red) and GFP::Cnn (green). Out of the 40 D82-Glued overexpressing pI analysed, 32 pI cells showed no centrosome movement and eight pI cells showed weak and erratic movements without any clear movement towards the midbody. Scale bars: 5 μm.

interaction between the Dynactin and the Dynein motor (Allen et al., 1999; Januschke et al., 2002). Similar results were obtained for D82-Glued (Fig. 3K; supplementary material Movie 4) or Dmn overexpression (not shown). In our overexpression conditions, we found that divisions proceed normally from prophase to late anaphase (Fig. 3I,K). In telophase, both centrosomes labelled by GFP::Cnn failed to move towards the apex of the pI daughter cells (Fig. 3K). This shows that dynein-dynactin interaction is necessary for the apical centrosome movements at the end of the pI cell division. Furthermore this result accounts for a model whereby a higher anterior activity of CRMP delays the anterior centrosome movement by inhibiting Dynein function.

CRMP promotes Notch signalling to specify pI daughter cell fate

The identification of specific regulators of centrosome movement allows us to address for the first time the respective contributions of centrosomes and of asymmetric centrosome movements in cell fate specification during asymmetric division. This was achieved by comparing the defects in pIIa/pIIb cell fate specification in the absence of the function of Cnn (contribution of centrosomes) and of CRMP (contribution of centrosome movements). Whereas Cnn loss of function results in 0.5% of pIIa-to-pIIb cell fate transformation (n=383 organs, not shown), the CRMP loss of function did not affect pIIa/pIIb cell fate specification (n=110 organs, Fig. 4A-A‴). This demonstrates that asymmetric centrosome movement is not strictly required for cell fate specification. It is established that several redundant processes concur to promote the differential activation of Notch signalling between the pIIa and pIIb daughter cells. Hence, the contribution of asymmetric centrosome movements in cell fate specification might be revealed by investigating whether CRMP loss of function exacerbates or rescues a partial loss of Notch signalling. We therefore analysed whether loss of CRMP function modulates the phenotype of thermosensitive Notch and Delta (N^{ts} and Dl^{ts}) mutant pupae placed at the semi-permissive temperature at the time of pI division. Under this condition, the partial loss of Notch or Delta activity results in 7.3% (n=320 organs) or in 3.6% (n=663 organs) of pIIa-to-pIIb cell fate transformation, respectively (Fig. 4B-B‴,D-D‴). Strikingly, under identical experimental conditions but in the context of CRMP loss of function, we observed increases in the number of pIIa-to-pIIb cell fate transformations in both N^{ts} mutant background (17.7%, n=351 organs, $P<10^{-3}$, Fig. 4C-C‴) and Dl^{ts} background (13.2%, n=927 organs, $P<10^{-3}$, Fig. 4E-E‴). These results establish that CRMP activity promotes Notch signalling and suggests that asymmetric centrosomes movement participates in the acquisition of distinct daughter cell fates during asymmetric division.

CRMP controls the duration of Rab11 accumulation around the anterior centrosome

As previously shown (Emery et al., 2005), we observed that the Rab11 endosomal recycling compartment transiently accumulates around the anterior centrosome during pI telophase and cytokinesis (Fig. 5A-C′). Although the Rab11 function during pI cell division has not been directly tested owing to its role in cytokinesis, several findings indicate that the Delta ligand transits the Rab11 endosome to reach the pI daughter cell interface to promote Notch signalling and the specification of the pIIa cell fate (Benhra et al., 2011; Benhra et al., 2010; Emery et al., 2005; Jafar-Nejad et al., 2005). Accordingly, we observed that fluorescently labelled internalized

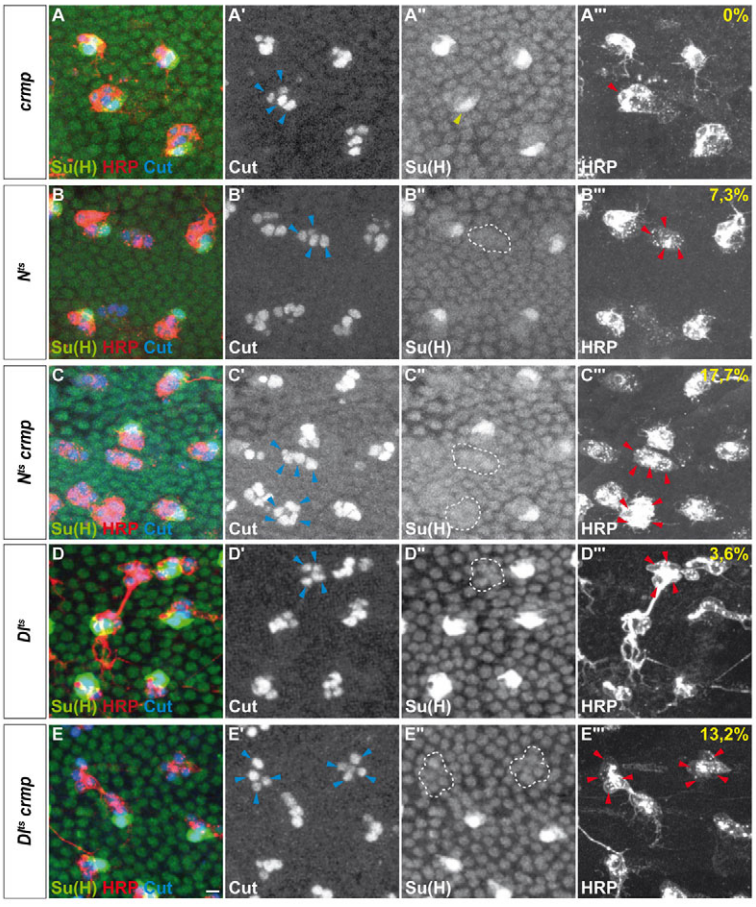

Fig. 4. CRMP enhances Notch and Delta loss of function. (**A-E‴**) *crmp* (A-A‴), N^{ts} (B-B‴), N^{ts}; *crmp* (C-C‴), Dl^{ts} (D-D‴), Dl^{ts}; *crmp* (E-E‴) sensory organs labelled with: Cut (blue), which marked the four sensory organ cells; Suppressor of Hairless [Su(H), green], which is expressed in the socket cell, a pIIa daughter (blue); and anti-HRP (red), which labelled the neuron, a pIIb grand-daughter. No pIIa-to-pIIb cell fate transformations are observed in *crmp* organs (A-A‴, n=110) as all organs are composed of four Cut-positive cells with one socket and one neuron. 7.3% of the N^{ts} sensory organs (n=320, outlined in white, B″), 17.7% of the N^{ts}; *crmp* sensory organs (n=351, outlined in white, C″), 3.6% of the Dl^{ts} sensory organs (n=663, outlined in white, D″) and 13.2% of the Dl^{ts}; *crmp* sensory organs (n=927, outlined in white, E″) are composed of four HRP-positive, Cut-positive and Su(H)-negative cells, indicating a pIIa-to-pIIb cell fate transformation. Blue arrowheads (A′,B′,C′,D′,E′) indicate Cut-positive cells; yellow arrowhead (A″) indicate Su(H)-positive cells; red arrowheads (A‴,B‴,C‴,D‴,E‴) indicate HRP-positive cells. Anterior is leftwards; medial is upwards. Scale bar: 10 μm.

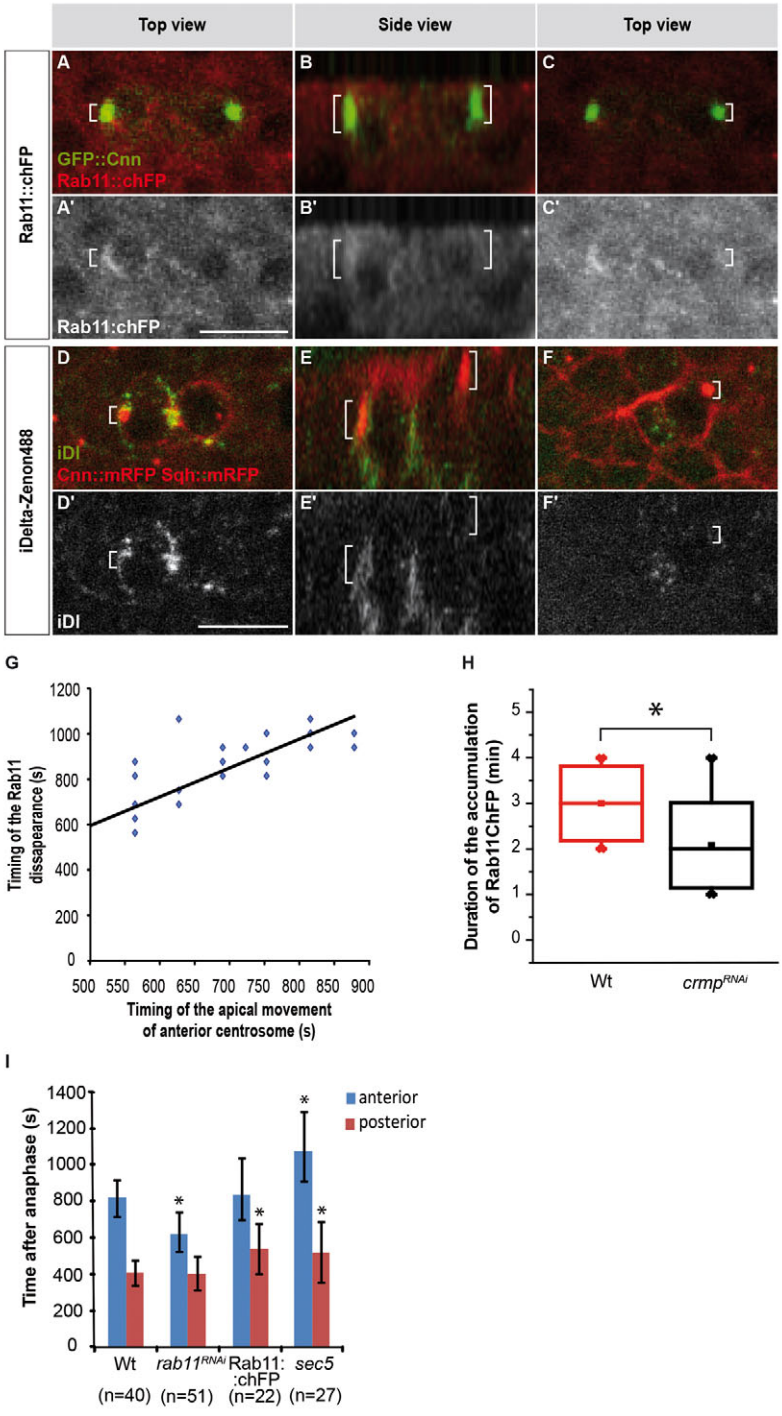

Fig. 5. CRMP controls the duration of Rab11 accumulation at the anterior centrosome. (**A-C′**) Localization of Rab11::chFP (red, A,B,C; white, A′,B′,C′) and Cnn::GFP (green, A,B,C) during pI cell cytokinesis (390 seconds after anaphase onset). (A,A′) *Z*-projection at the level of the anterior centrosome, (B,B′) lateral view and (C,C′) *z*-projection at the level of the posterior centrosome. Brackets indicate centrosome positions. Rab11 asymmetry was observed in 15 out of 22 dividing pI cells. (**D-F′**) Localization of internalized fluorescently labelled anti-Delta antibody (iDl, green, D,E,F; white, D′,E′,F′) in a pI cell expressing Cnn::mRFP and Sqh::mRFP (red, D,E,F) at cytokinesis (390 seconds after the anaphase onset). (D,D′) *Z*-projection at the level of the anterior centrosome, (E,E′) lateral projection and (F,F′) *z*-projection at the level of the posterior centrosome. Brackets indicate centrosome positions. Internalized Delta antibodies also strongly accumulated at the cytokinesis ring. Endocytosed Delta accumulated around the anterior centrosome in 12 out of 12 dividing pI cells. (**G**) The correlation between the timing of disappearance of Rab11 accumulation around the anterior centrosome and the timing of the anterior centrosome movement towards the apex of the pIIb cell. (**H**) The duration of Rab11 accumulation in wild-type (3.0±0.66 seconds, n=19) and $crmp^{RNAi}$ (2.07±0.87 seconds, n=26) pI cells ($P<10^{-3}$). (**I**) The average timing of anterior (blue) and posterior (blue) apical movement in wild-type (anterior, 819±132, n=40; posterior, 408±70, n=40), $rab11^{RNAi}$ (anterior, 616±122, n=51; posterior, 406±94, n=51), Rab11::chFP (anterior, 835±199, n=22; posterior, 539±139, n=22) and *sec5* (anterior, 1073±219, n=27; posterior, 520±165, n=27) loss-of-function pI cells. The significant differences ($P<10^{-3}$) between mutant and wild-type pI cells are indicated by asterisks.

Delta antibodies can be found localized around the anterior pI centrosome during cytokinesis (Fig. 5D-F′).

As CRMP inhibits the apical movement of the anterior centrosome where Rab11 accumulates, we explored whether CRMP might affect Notch signalling by regulating Rab11 dynamics. We observed that the apical movement of the anterior centrosome correlates with the disappearance of the Rab11 anterior centrosome accumulation (Fig. 5G). In *crmp* mutant pI cells, Rab11 is still found to accumulate preferentially at the anterior centrosome in telophase (not shown). Yet the duration of Rab11 accumulation was significantly reduced in comparison with the wild-type pI cells (Fig. 5H). We conclude that CRMP controls the duration of Rab11 accumulation around the anterior centrosome, through which Delta is proposed to be recycled.

The correlation between centrosome apical movement and Rab11 disappearance prompted us to determine whether Rab11 accumulation might also have a role in apical centrosome movement. We therefore analysed whether a reduction of Rab11 function decreases the asymmetry in centrosome movement. In $rab11^{RNAi}$ pI cells, the asymmetry in centrosome movement is reduced with the anterior centrosome moving earlier (Fig. 5I). Furthermore, the loss of Sec5 function that increases the Rab11 accumulation around the anterior centrosomes (Langevin et al., 2005b) promotes a delay in the apical movement of the anterior

centrosome (Fig. 5I). We therefore conclude that Rab11 is a negative regulator of apical centrosome movement.

Altogether, we conclude that Rab11 delays the apical centrosome movement and, in agreement with our findings that CRMP promotes Notch signalling, we find that CRMP controls the duration of the Rab11 accumulation around the anterior pI centrosome.

DISCUSSION

Centrosome dynamics play key roles in the regulation of mitotic and non-mitotic cell function (Vaughan and Dawe, 2010). We have uncovered a novel type of asymmetric centrosome dynamics during cytokinesis of asymmetrically dividing progenitor cells. By characterizing the function of CRMP during this process, our work suggests that asymmetrical centrosome dynamics play a role in the Notch signalling pathway by regulating the duration of Rab11 accumulation around the centrosome.

Asymmetrical centrosome dynamics during stem cell and progenitor cell division

Asymmetric centrosome dynamics are documented in both *Drosophila* GSCs and neuroblasts (Rebollo et al., 2007; Rusan and Peifer, 2007; Yamashita et al., 2007). In both types of stem cells, one of the centrosomes is anchored to the cortex, whereas the other becomes positioned at the opposite side of the cell in prophase and prior to mitotic spindle formation. In both cases, the asymmetric centrosome behaviour is proposed to play an important function in mitotic spindle orientation, and consequently in cell fate specification. In GSCs, the mitotic spindle orientation is shown to be essential for the correct positioning of daughter cells relative to the GSCs niche (Yamashita et al., 2003), whereas in neuroblasts the mitotic spindle orientation is essential for the correct segregation of cell fate determinants (Morin and Bellaïche, 2011). Strikingly, the anchored centrosome in GSCs is the mother centrosome (Yamashita et al., 2007), which is therefore inherited by the future gonioblast stem cell, whereas in the neuroblasts, the daughter centrosome is anchored and is inherited by the neuroblasts stem cells (Conduit and Raff, 2010; Januschke et al., 2011). Therefore, the asymmetric centrosome dynamics in prophase correlate with centrosome age, yet there is no general rule as to which centrosome is inherited by stem cells. So far, the role of asymmetric centrosome behaviour in prophase has been studied by affecting pericentriolar centrosome organization or by altering cell polarization (Yamashita et al., 2003; Yamashita et al., 2007; Rebollo et al., 2007).

Our work identifies and characterizes a different mode of asymmetric centrosome dynamics during asymmetric cell division. First, the asymmetric dynamics of centrosomes are observed during cytokinesis. Second, this asymmetry is likely to be independent of centrosome age and is regulated by the cell fate determinant Numb. Finally, our results suggest that this asymmetry regulates the accumulation of Rab11 recycling endosomes and thereby modulates Notch signalling.

Regulation of centrosome dynamics during pI cell division

We have characterized a novel regulator of dynein-dependent centrosome movement during asymmetric cell division. The

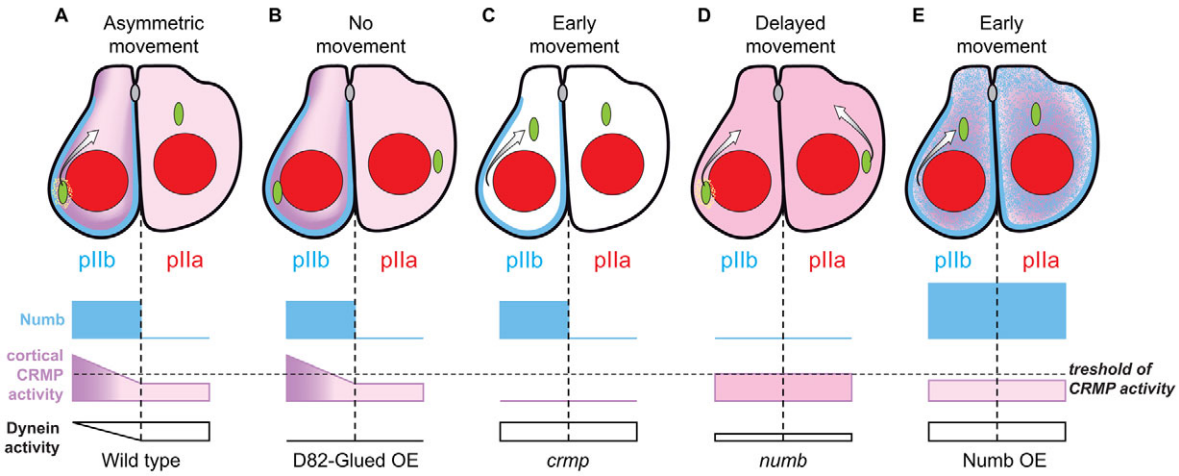

Fig. 6. Proposed model of asymmetric centrosome movements during pI cell cytokinesis. (**A**) During cytokinesis, Numb (blue) is enriched at the cortex of the anterior pI daughter cells (pIIb). The anterior centrosome (green) accumulates Rab11 (yellow)-positive endosomes and it remains associated with the anterior cortex, whereas the posterior one, which shows a weak or no accumulation of Rab11 moves apically towards the apical midbody (grey). Based on our analysis of Numb, CRMP, Rab11 and Dynein loss of function, we propose that the asymmetric distribution of Numb increases the activity of CRMP at the anterior cortex. In turn, CRMP (pink) delays centrosome movement by inhibiting Dynein activity or promoting the accumulation of Rab11, which also prevents the anterior centrosome apical movement. (**B**) In the absence of Dynein function, both centrosomes fail to move apically. This shows that apical centrosome movement is regulated by Dynein motor activity. (**C**) In absence of CRMP activity or upon Rab11 knock-down, the movement of centrosomes is almost symmetric, owing to an earlier apical movement of the anterior centrosome. This demonstrates that CRMP and Rab11 activities are necessary to maintain the anterior centrosome in a basal position. We proposed that in the absence of CRMP activity, Dynein activity is no longer inhibited at the anterior cortex and therefore both centrosomes move towards the apical domain simultaneously. In agreement with the asymmetric localization of Rab11 around the anterior centrosome, we proposed that Rab11 maintains the anterior centrosome in a basal position. The mechanisms by which Rab11 maintains the anterior centrosome basally remain to be characterized. (**D**) In the absence of Numb activity, centrosome movement becomes more symmetric owing to a delay in posterior centrosome movement. As we have demonstrated that CRMP negatively regulates anterior centrosome movement, we propose that Numb is necessary to restrict the activity of CRMP to the anterior cortex and that loss of Numb activity lead to inhibition of Dynein activity both at the anterior and posterior cortex, owing to misregulation of CRMP activity. (**E**) In the case of the overexpression of Numb, we propose that CRMP activity is titrated by the high amount of Numb. Therefore, Dynein activity is not inhibit at either the anterior or posterior cortex, leading to the early symmetric movement of both centrosomes.

anterior centrosome remains basally localized where it accumulates Rab11, whereas the posterior centrosome has already moved towards the pI daughter cell interface. Our genetic analysis of Pins, Baz and Numb loss of function suggest that centrosome movement is regulated by anteriorly asymmetrically localized Numb in telophase. Surprisingly, the loss of Numb function affects the movement of the posterior centrosome, showing that Numb is likely to indirectly control centrosome movements. Furthermore we speculate that asymmetric Numb distribution leads to the anterior enrichment of an activity that limits centrosome movement. Although CRMP is not asymmetrically distributed during cell division, our biochemical data and genetic epistasis data suggest that CRMP is the downstream effector of Numb in the regulation of apical centrosome movements. Further analysis of the role of Cdk5 and of the mechanisms of CRMP regulation will dissect where and when CRMP is active during pI cell division and how it inhibits Dynein activity to prevent centrosome movement (see Fig. 6 for model). Our results can be explained by two non-exclusive models regarding the mechanisms of CRMP activity on centrosome movement. First, CRMP might directly delay centrosome movement by inhibiting Dynein activity. Second, CRMP might promote pericentrosomal Rab11 accumulation, which prevents centrosome movement via Dynein. The latter model is consistent with the fact that Rab11 interacts with Dynein via Nuclear-fallout (Riggs et al., 2007; Riggs et al., 2003). Although Numb is proposed to regulate CRMP activity positively during axonal growth via α-Adaptin-dependent endocytosis of L1 receptor (Nishimura et al., 2003), our genetic analyses of centrosome movements in *numb*, *crmp*, double *numb*, *crmp* mutant pI cells, as well as in response to *numb* overexpression, show that Numb and CRMP act antagonistically to regulate centrosome movement. This demonstrates the existence of a different mode of regulation of CRMP by Numb, which is furthermore independent of α-Adaptin. Taken together, our work identifies one of the first regulators of asymmetric centrosome movement during cell division. It will be important to analyse the function of CRMP in neuroblasts and GSCs in order to investigate further the role of asymmetric centrosome behaviour during stem cell division.

On the regulation of Notch signalling by centrosome movement

Notch signalling is central to numerous developmental processes (Artavanis-Tsakonas et al., 1999; Bray, 2006; Lai, 2004; Wang et al., 2009). Activation of the Notch ligand, Delta, has been show to depend on its endocytosis and/or recycling (Le Borgne and Schweisguth, 2003a; Yamamoto et al., 2010). During pI cell division, numerous studies have established the functional importance of Delta recycling in the regulation of cell fate acquisition (Le Borgne, 2006; Le Borgne et al., 2005). In particular, Delta recycling via the basal recycling endosomes and its trafficking towards the apical pI daughter cell interface is essential to the activation of the Notch receptor (Benhra et al., 2011; Benhra et al., 2010; Giagtzoglou et al., 2012; Rajan et al., 2009). Accordingly, the asymmetric Rab11 accumulation around the basally located anterior centrosome has been proposed to play a redundant role to control the activation of Notch signalling (Emery et al., 2005). The discovery that CRMP regulates the duration of the Rab11 accumulation links centrosome movement and Rab11 accumulation to the regulation Notch signalling. Our genetic interaction establishes that CRMP promotes Notch signalling during pI cell division. Furthermore, we could establish that CRMP is necessary for the prolonged accumulation of the Rab11 endosomal compartment at the basal side where Delta is likely to be recycled. However, we could not directly determine whether the reduction in duration of Rab11 accumulation around the anterior centrosomes in *crmp* mutant pI cells leads to a reduced Delta endocytosis as our Delta endocytosis assay does not allow precise quantification of the amount of endocytosed Delta around the anterior centrosome. Although CRMP might also regulate Notch signalling via other process, we propose that CRMP regulates apical centrosome movement and Rab11 accumulation to promote efficient Delta recycling, and consequently Notch signalling at the pI daughter cell interface. Taken together, our results suggest a functional link between asymmetric dynamics of centrosomes, endosome dynamics and Notch signalling during asymmetric cell division.

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Competing interests statement

The authors declare no competing financial interests.

Author contributions

B.J. and F.L. performed the experiments and their quantifications (except for Fig. 3A,H). B.S. discovered the asymmetry of centrosome movement and performed the initial characterization of the underlying mechanisms. Z.W. built the tagged constructs for co-immunoprecipitation and performed the co-immunoprecipitation (Fig. 3A,H). C.M. built the Rab11::ChFP construct. B.J., F.L and Y.B. wrote the manuscript with contributions from all other authors. Y.B. directed the study.

Supplementary material

Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.087338/-/DC1

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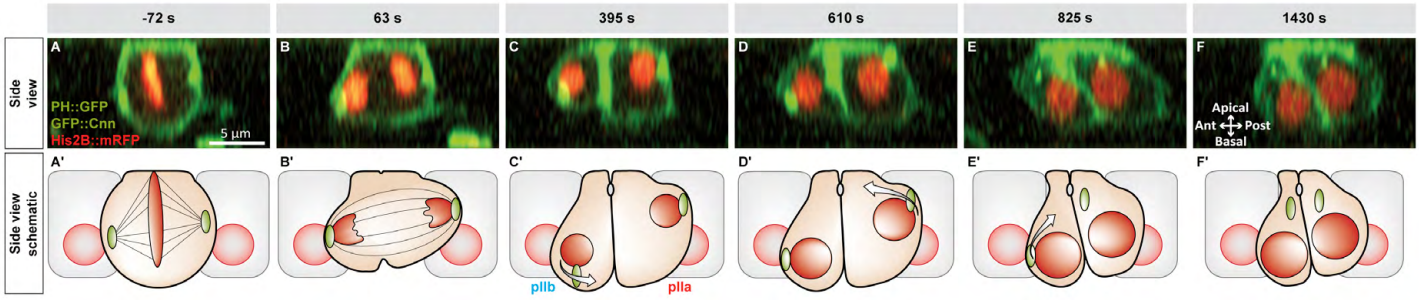
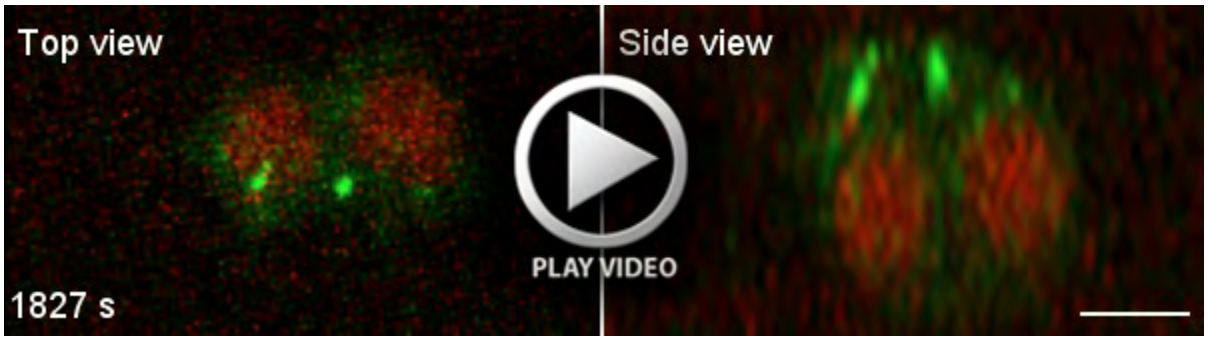


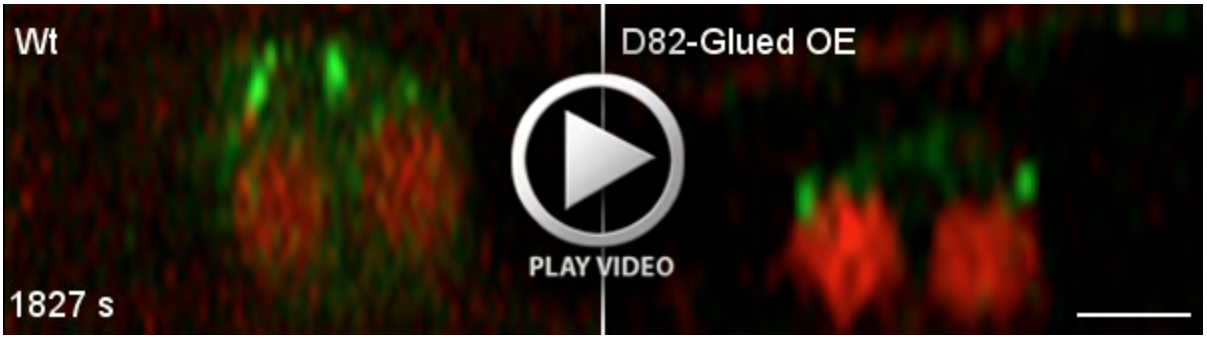
Fig. S1. Centrosome movement during pI cell division. (**A**) The centrosome, the membrane and the chromatin are labelled by GFP::Cnn (green), PH::GFP (green) and His2::mRFP (red). (**B**) Schematic of centrosome dynamics during pI cell division.



Movie S1. Asymmetric movement of the centrosomes during pI cell division. Time-lapse of His2B::mRFP (red) and GFP::Cnn (green) during pI cell division from metaphase onwards shown in Fig. 1. Left movie is a top view corresponding to a maximum projection along the apical-basal axis of the dividing pI cell (anterior, leftwards; medial, upwards). Right movie is a side view corresponding to a lateral maximal projections (anterior, leftwards; apical, upwards). The chromatids separate at *t*=0 seconds. The anterior centrosome undergoes a movement underneath the nucleus at *t*=315 seconds. The posterior centrosome moves towards the apex of the pI cell at *t*=441 seconds. The anterior centrosome moves towards the apex of the anterior daughter of the pI cell at *t*=819 seconds. Both centrosomes are located at the cell apex (*t*=1701 seconds). Scale bars: 5 µm.

Movie S2. Centrosome dynamics in wild-type and *numb* pI cells. Time-lapse of His2B::mRFP (red, expressed only in the pI cell) and YFP::Asl (green, ubiquitously expressed) during pI cell division from metaphase onwards. YFP::Asl is ubiquitously expressed, the centrioles of surrounding epithelial cells are also labelled. Left movie and right movies are side views corresponding to lateral maximal projections in a wild-type pI cell (left movie) and in a *numb* pI cell, marked by the absence of the nls::GFP expression (green, right movie). In *numb* pI cell, the posterior centrosome movement towards the cell apex is delayed, leading to both centrosomes moving almost simultaneously towards the apical midbody. Anterior is leftwards; apical is upwards. Scale bars: 5 µm.

Movie S3. Centrosome dynamics in wild-type and *crmp* pI cells. Time-lapse of His2B::mRFP (red, expressed only in the pI cell) and YFP::Asl (green, ubiquitously expressed) during pI cell division from metaphase onwards. YFP::Asl is ubiquitously expressed, the centriole of surrounding epithelial cells are also labelled. Left movie and right movies are side views corresponding to lateral maximal projections in a wild-type pI cell (left movie) and in a *crmp* pI cell (right movie). In *crmp* context, the anterior centrosome movement towards the cell apex is early, leading to both centrosomes moving almost simultaneously towards the apical midbody. Anterior is leftwards; apical is upwards. Scale bars: 5 µm.



Movie S4. Centrosome dynamics in the wild-type and overexpressing D82-Glued pI cells. Time-lapse of His2B::mRFP (red) and GFP::Cnn (green) during pI cell division from metaphase onwards. Left movie and right movies are side views corresponding to lateral maximal projections in a wild-type pI cell (left movie) and in a pI cell overexpressing D82-Glued (right movie). Upon D82-Glued overexpression, both centrosomes fail to reach the apical midbody. Anterior is leftwards; apical is upwards. Scale bars: 5 µm.