Current Biology 20, 2187–2192, December 21, 2010 ©2010 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2010.11.055

Report

Cnn Dynamics Drive Centrosome Size Asymmetry to Ensure Daughter Centriole Retention in *Drosophila* Neuroblasts

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Summary

Centrosomes comprise a pair of centrioles surrounded by an amorphous network of pericentriolar material (PCM). In certain stem cells, the two centrosomes differ in size, and this appears to be important for asymmetric cell division [1, 2]. In some cases, centrosome asymmetry is linked to centriole age because the older, mother centriole always organizes more PCM than the daughter centriole, thus ensuring that the mother centriole is always retained in the stem cell after cell division [3]. This has raised the possibility that an "immortal" mother centriole may help maintain stem cell fate [4, 5]. It is unclear, however, how centrosome size asymmetry is generated in stem cells. Here we provide compelling evidence that centrosome size asymmetry in *Drosophila* neuroblasts is generated by the differential regulation of Cnn incorporation into the PCM at mother and daughter centrioles. Shortly after centriole separation, mother and daughter centrioles organize similar amounts of PCM, but Cnn incorporation is then rapidly downregulated at the mother centriole, while it is maintained at the daughter centriole. This ensures that the daughter centriole maintains its PCM and so its position at the apical cortex. Thus, the daughter centriole, rather than an "immortal" mother centriole, is ultimately retained in these stem cells.

Results and Discussion

Drosophila neural stem cells divide asymmetrically generating a large self-renewing neuroblast and a small ganglion mother cell (GMC). The neuroblast inherits a single centrosome, containing a mother and a daughter centriole, which migrates to the apical cell cortex where the centrioles move apart, forming two distinct centrosomes. One centrosome continues organizing PCM and microtubules (MTs), thus maintaining a connection to the apical cell cortex; the other centrosome loses PCM and begins random movements throughout the cytoplasm [1, 2, 6]. The pre-positioning of one centrosome at the apical cell cortex ensures subsequent apical-basal spindle orientation, which is important for the correct segregation of cell fate determinants [7]. Similar centrosome behavior occurs in *Drosophila* male germline stem cells (GSCs), where the mother centriole always organizes the larger centrosome and so is ultimately retained in the stem cell [3]. Moreover, the mother centrosome is preferentially retained in mouse neural progenitor cells [8]. Perturbing correct centrosome inheritance in these cells, or in *Drosophila* male GSCs, dramatically decreases their proliferative capacity [8, 9], suggesting that an "immortal" mother centriole helps maintain stem cell fate [4, 5]. It is unclear, however, how centrosome size asymmetry is generated in these stem cells.

Elsewhere in this issue of *Current Biology*, we showed that the conserved PCM component centrosomin (Cnn) helps determine centrosome size (defined as the amount of PCM organized by the centrioles) [10]. Using increasing centriolar GFP-PACT fluorescence as a marker of increasing centriole age, we also showed that mother centrioles organize more Cnn than their daughters during centrosome separation, because daughter centrioles only start to incorporate Cnn into their PCM after they separate from their mothers. We postulated that this could help explain how centrosome size asymmetry is generated in stem cells.

We therefore examined larval neuroblasts expressing GFP-PACT and RFP-Cnn, reasoning that, as in embryos [10], GFP-PACT fluorescence might distinguish mother and daughter centrioles. In these neuroblasts, separating centriole pairs were almost always asymmetrically labeled by GFP-PACT (30 of 32 neuroblasts exhibited obvious GFP-PACT asymmetry). Moreover, as in embryos [10], the brighter GFP-PACT centriole initially organized more RFP-Cnn than the dimmer GFP-PACT centriole (Figure 1A, t = −20:00; Figure 1B, t = −13:00), strongly suggesting that the brighter GFP-PACT centriole was indeed the mother. Surprisingly, however, the brighter centriole gradually lost its RFP-Cnn, while the dimmer centriole accumulated RFP-Cnn, and in 30 of 30 neuroblasts, the dimmer centriole ultimately organized more Cnn and remained stably associated with the apical cell cortex (Figures 1A and 1B; see also Movie S1 and Movie S2 available online). This behavior was also observed when we examined MTs: the brighter centriole initially associated with more MTs than the dimmer centriole, but this gradually reversed (Figure 1C; Movie S3). We confirmed that the dimmer centriole was ultimately inherited by the neuroblast, whereas the brighter centriole was ultimately inherited by the GMC (Figure 2A; Movie S4). Thus, it seems that neuroblasts specifically retain their daughter centriole.

If mother centrioles are segregated into the GMCs, then the oldest (and therefore brightest) centrioles should be found in the GMCs. We therefore analyzed GFP-PACT fluorescence in neuroblasts and their associated GMC progeny. Consistent with our interpretation, both the neuroblasts and GMCs contained a dim centriole (the presumptive daughters) and a bright centriole (the presumptive mothers), but the brightest centrioles were present in the GMCs (Figure 2B). By far the simplest interpretation of these results is that centriolar GFP-PACT fluorescence increases as centrioles age in neuroblasts and GMCs, and that neuroblasts retain the daughter centriole (see below for further discussion).

We previously showed that in syncytial embryos, centrioles can influence centrosome size by regulating how much Cnn is incorporated into the PCM per unit time (CnnIN) [10]. To examine whether mother centrioles in neuroblasts lose their PCM because they downregulate CnnIN, we performed fluorescence recovery after photobleaching (FRAP) analyses of separating centrosomes in neuroblasts expressing GFP-PACT and RFP-Cnn. When we bleached RFP-Cnn from centrosomes immediately after mitosis, both centrioles initially

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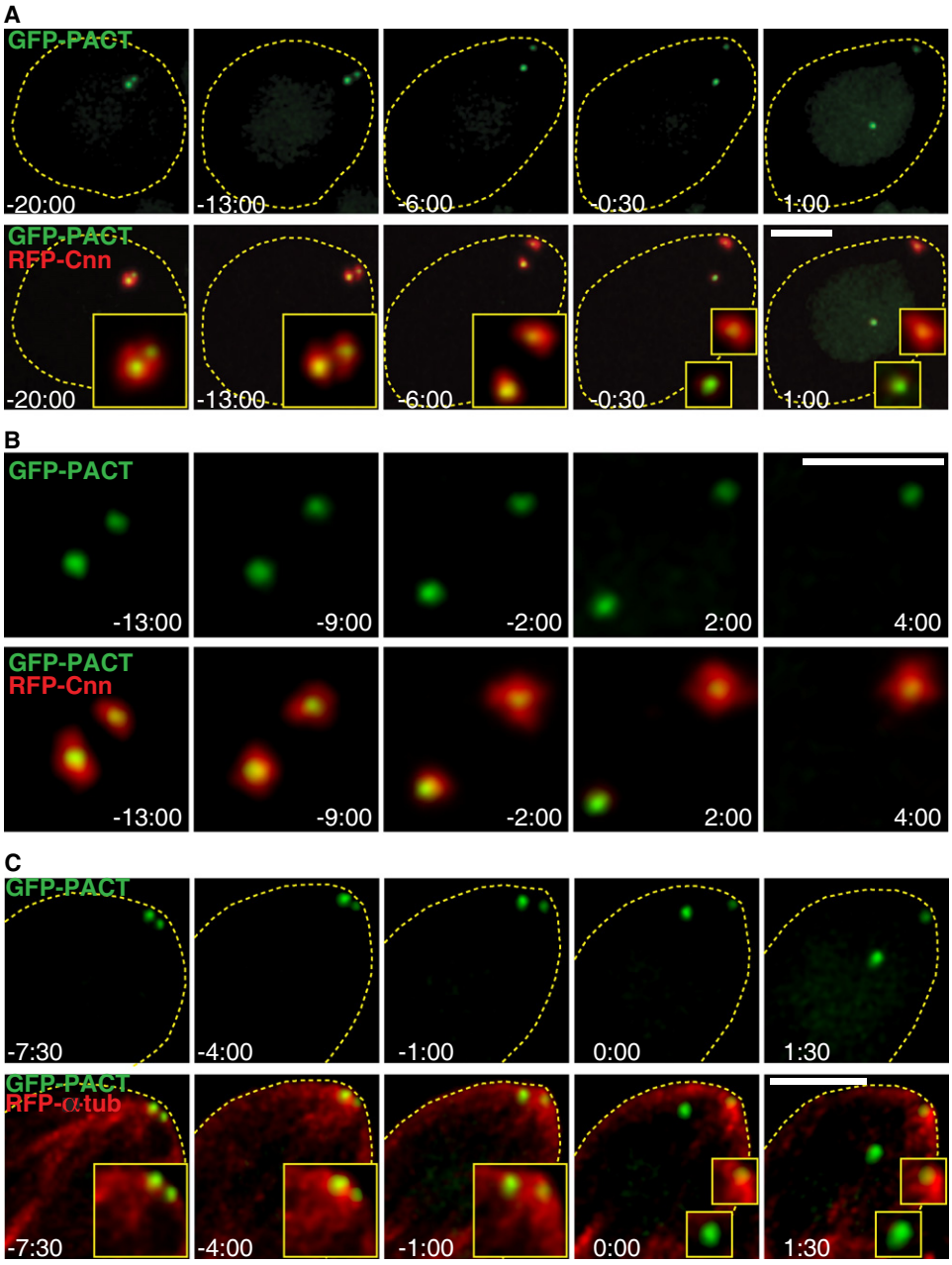

Figure 1. The Daughter Centriole Is Retained at the Apical Cell Cortex after Neuroblast Cell Divisions

(A and B) Images from movies of neuroblasts expressing GFP-PACT (green) and RFP-Cnn (red). Top panels show the GFP signal alone, bottom panels show the merged signal, and insets at bottom in (A) show a magnified view of the centrosomes. Time before and after one of the centrosomes begins rapid movements (t = 0:0 min:s) is indicated.

(A) In a typical nascent neuroblast, the two centrioles are closely associated at the apical cortex (t = −20:00): GFP-PACT fluorescence is asymmetric, and the brighter centriole (presumably the mother centriole) associates with more Cnn than the dimmer centriole (presumably the daughter centriole). Thereafter, the mother centriole progressively associates with less Cnn, while the daughter centriole progressively associates with more Cnn (t = −13:00 through t = −0:30). Eventually, the mother centriole starts to move about randomly within the cytoplasm (t = 1:00).

(B) This sequence of events is easier to visualize in the neuroblast shown here, because the centrioles were particularly well separated. This behavior was observed in 30 of 30 neuroblasts.

(C) Images from a movie of a neuroblast expressing GFP-PACT (green) and RFP-α-tubulin (red). The microtubules initially preferentially associate with the mother centriole and then "switch" to the daughter centriole. This behavior was observed in 2 of 2 neuroblasts.

Scale bars represent 5 μm. See also Movie S1, Movie S2, and Movie S3.

recovered similar levels of RFP-Cnn fluorescence (Figure 3A, t = 0:00 and t = 2:52). When we bleached centrosomes slightly later, however, we only detected significant fluorescence recovery around the daughter centriole (Figure 3B), demonstrating that mother centrioles rapidly downregulate CnnIN shortly after centrosome separation. Consistent with this, the

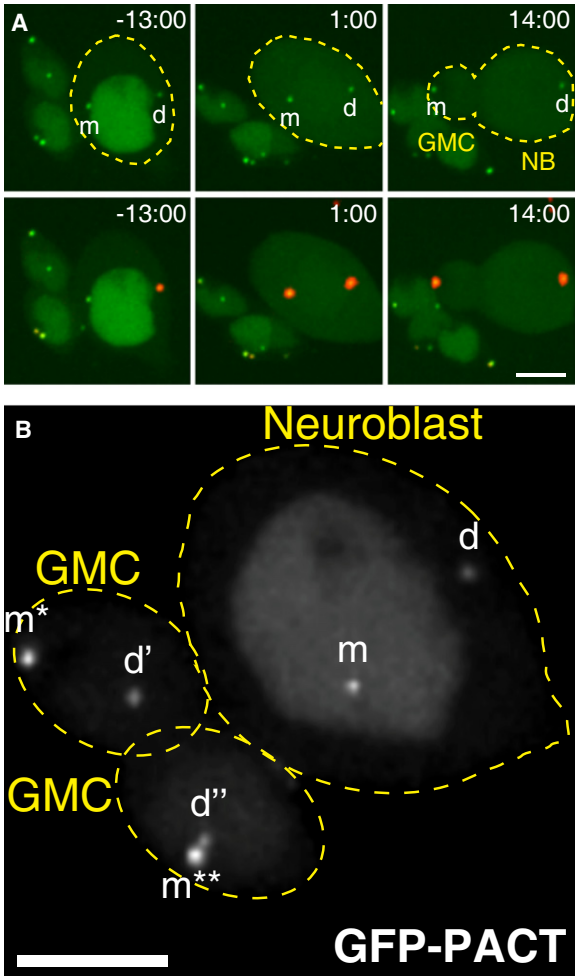

Figure 2. The Daughter Centriole Is Retained in the Stem Cell during Larval Neuroblast Divisions

(A) Images from a movie of a neuroblast expressing GFP-PACT (green) and RFP-Cnn (red). Top panels show the GFP signal alone; bottom panels show a merge of the GFP and RFP signals. Time before and after nuclear envelope breakdown (t = 0:0 min:s) is indicated. In this movie, the brighter, mother (m) centriole has already lost its pericentriolar material (PCM) and is moving randomly in the cytoplasm; the dimmer, daughter (d) centriole organizes some Cnn and remains stably positioned at the apical cortex (t = −13:00). On entry into mitosis, both centrioles accumulate Cnn (t = 1:00) and the mother centriole is ultimately segregated into the ganglion mother cell (GMC) (t = 14:00).

(B) Image of a neuroblast and its associated GMC progeny that are expressing GFP-PACT. The distribution of GFP-PACT fluorescence is consistent with the interpretation that the mother centriole is inherited by the GMC during these stem cell divisions. Each cell contains a dim, daughter centriole (d, d′, d″) and a brighter, mother centriole (m, m*, m**), but the brightest (and so presumably oldest) mothers are found in the GMCs. This pattern was observed in 4 of 4 instances where we observed a neuroblast that had separated from the main brain mass while still remaining associated with its GMC progeny.

Scale bars represent 5 μm. See also Figure S2 and Movie S4.

mother centrioles that were bleached early and so had initially recovered RFP-Cnn fluorescence eventually lost their RFP-Cnn signal (Figure 3A, t = 7:22).

If the downregulation of CnnIN at mother centrioles generates centrosome size asymmetry in neuroblasts, it should occur prior to the loss of any PCM from around the mother centrioles. To test whether this was the case, we first examined separating centrosomes in neuroblasts expressing both GFP-Cnn and RFP-Cnn. This allowed us to measure CnnIN (by bleaching the GFP-Cnn signal) while assessing the total amount of Cnn associated with the centrioles (by monitoring the unbleached RFP-Cnn signal). We bleached GFP-Cnn at centrosomes shortly after centrosome separation and confirmed that only one centrosome recovered significant amounts of GFP-Cnn fluorescence (Figure 4A). Importantly, however, at the time of photobleaching and shortly thereafter, both centrioles organized similar amounts of RFP-Cnn (Figure 4A; t = 0:00 and t = 1:20). Thus, CnnIN is downregulated at mother centrioles before they begin to lose their associated Cnn.

To test whether the rapid downregulation of Cnn incorporation at mother centrioles is a feature common to all PCM components, we performed FRAP analyses in neuroblasts expressing RFP-Cnn and γ-tubulin-GFP. In this instance, we bleached both proteins, allowing us to monitor the rate of their recovery at the same time. When we bleached newly separated centrosomes containing similar amounts of Cnn and γ-tubulin, only one centrosome recovered RFP-Cnn fluorescence, whereas both centrosomes initially recovered similar amounts of γ-tubulin-GFP fluorescence (Figure 4B, t = 0:00 and t = 2:00). The levels of γ-tubulin at the centrosome that was not incorporating RFP-Cnn eventually decreased (Figure 2B, t = 5:20), presumably as a consequence of the downregulation of CnnIN, and therefore loss of Cnn from this centrosome. Taken together, these data show that mother centrioles downregulate the rate of Cnn incorporation (but not γ-tubulin incorporation) into their PCM shortly after centriole separation. Shortly thereafter, centrosomes become asymmetric in size as Cnn and γ-tubulin levels at the mother centriole decrease. Thus, the downregulation of CnnIN at mother centrioles appears to be a very early event in the generation of centrosome asymmetry in neuroblasts.

To test whether Cnn is important for the specific retention of the daughter centriole in neuroblasts, we examined the behavior of centrioles in *cnn* null mutant neuroblasts expressing GFP-PACT. As in wild-type neuroblasts, the centrioles in these cells were usually positioned at the apical cortex at the end of mitosis, but in the absence of Cnn, both centrioles lost their connection to the apical cortex and moved randomly in the cytoplasm (Figure 4C; Movie S5). This defect should lead to the random segregation of centrioles in these cells, and consistent with this, the majority of *cnn* null mutant neuroblasts contained too many centrioles, and some contained no centrioles at all (Movie S6; data not shown). In neuroblasts with too many centrioles, the centrioles tended to be clustered in the apical region of the cell cortex shortly after mitosis, but subsequently they all appeared to move randomly in the cytoplasm (Movie S6). We conclude that Cnn is required to allow the daughter centriole to continue organizing PCM and so to maintain its MT-dependent connection to the apical cortex. Thus, Cnn appears to play an important part not only in generating centrosome size asymmetry but also in ensuring the preferential retention of the daughter centriole within the stem cell (see Figure S1 for schematic).

It is unclear how Cnn incorporation is differentially regulated at mother and daughter centrioles in neuroblasts. Proximity to the apical cortex does not seem to be an important factor, because we observed instances where either the mother or the daughter centriole was positioned closest to the apical cortex, but the mother centriole always lost its associated Cnn. We suspect that Asl and/or DSpd-2, two centriolar

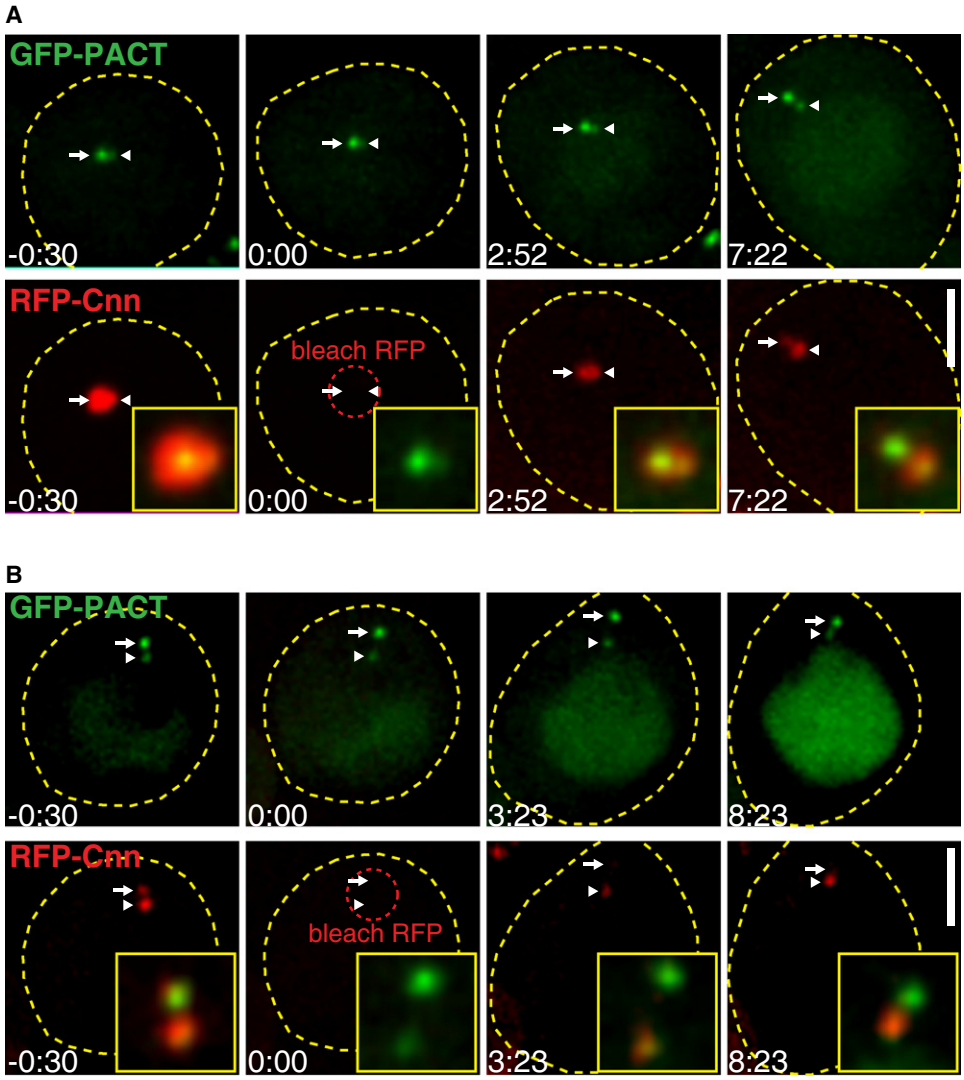

Figure 3. Incorporation of Cnn into the PCM Is Differentially Regulated at Mother and Daughter Centrioles in Neuroblasts

FRAP analyses in neuroblasts expressing GFP-PACT (green, top panels) and RFP-Cnn (red, bottom panels). Insets show a merged and magnified view of the centrosomes; arrows and arrowheads indicate mother and daughter centrioles, respectively. Time before and after photobleaching of the RFP signal (t = 0:0 min:s) is indicated.

(A) These centrosomes were bleached shortly after mitosis. Initially, both the mother and the daughter centriole incorporate similar amounts of RFP-Cnn (t = 2:52), but the mother centriole eventually begins to lose its previously recovered RFP-Cnn fluorescence (t = 7:22); 3 of 3 neuroblasts bleached at this time exhibited this behavior.

(B) These centrosomes were bleached slightly later, as they were starting to separate. Although the mother centriole was still associated with some Cnn (t = −0:30), we could only detect significant fluorescence recovery at the daughter centriole (t = 3:23 and t = 8:23), indicating that the mother centriole had downregulated Cnn incorporation by this stage; 8 of 8 neuroblasts bleached at this time exhibited this behavior.

Scale bars represent 5 μm.

proteins that are required for Cnn incorporation into the PCM [10], are differentially regulated at mother and daughter centrioles, and this may be controlled by the asymmetric localization or activation of protein kinases such as Polo/Plk1 [2].

Our conclusion that the daughter centriole is specifically retained in neuroblasts is unexpected and depends on our ability to distinguish mother and daughter centrioles. *Drosophila* mother and daughter centrioles are morphologically indistinguishable at the electron microscopy level [11], and so we have used GFP-PACT fluorescence intensity to infer centriole age. In *Drosophila* embryos, centriolar GFP-PACT fluorescence intensity increases as centrioles age [10]. This also appears to occur in neuroblasts, because the separating centrioles display different levels of GFP-PACT fluorescence and, as in embryos, the bright centriole initially organizes more Cnn than the dim centriole. GFP-PACT is known to irreversibly incorporate into centrioles [12, 3], and so for the daughter centriole to be brighter than the mother centriole in neuroblasts, either the GFP-PACT molecules would have to become less fluorescent as they age or GFP-PACT would have to be partially removed from the mother centriole before it separates from its daughter. Moreover, given our observations of neuroblasts and their GMC progeny, if

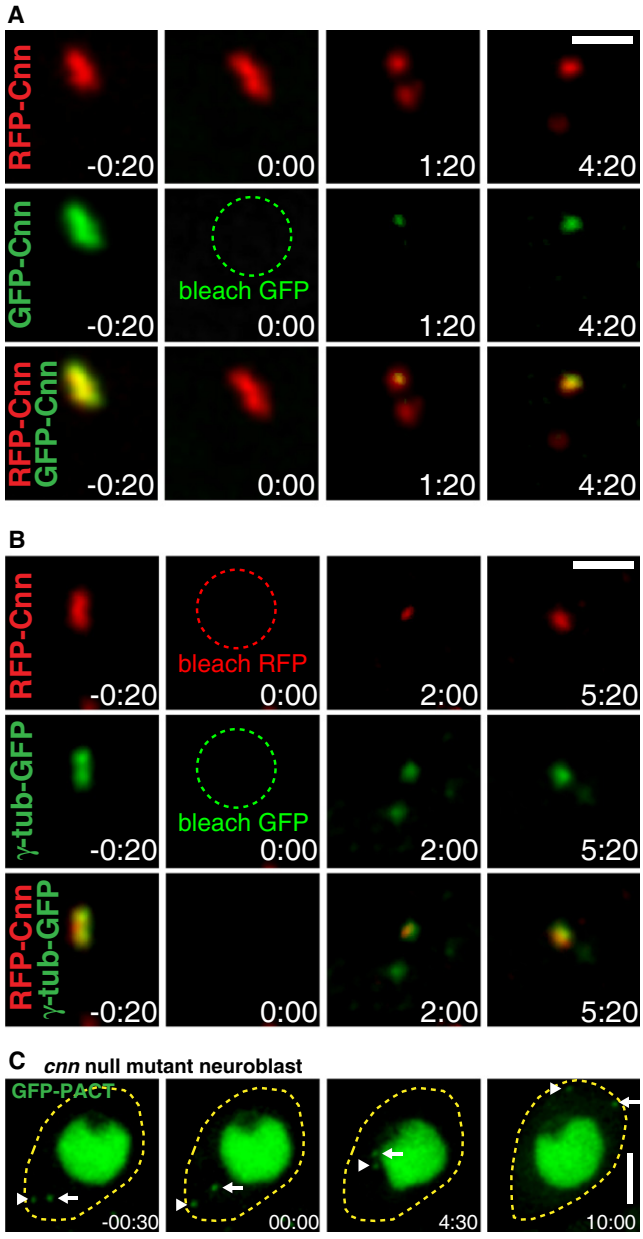

Figure 4. Mother Centrioles Lose Their Associated PCM because they Downregulate the Incorporation of Cnn into Their PCM

FRAP analysis in neuroblasts expressing RFP-Cnn (red, top panels) and either GFP-Cnn (green, middle panels in A) or γ-tubulin-GFP (green, middle panels in B); bottom panels show a merge of the RFP and GFP signals. Time before and after photobleaching of the GFP signal (A) or of the GFP and RFP signals (B) (t = 0:0 min:s) is indicated.

(A) These centrosomes were bleached as they were starting to separate. Although both centrosomes contained similar amounts of Cnn during and shortly after photobleaching (t = 0:00 and t = 1:20), only one centrosome recovered a significant amount of GFP-Cnn fluorescence (t = 1:20 and t = 4:20). The centrosome that did not recover GFP-Cnn fluorescence, and had therefore downregulated Cnn incorporation, gradually lost its associated RFP-Cnn and moved away from the cortex. Three of three neuroblasts examined exhibited this behavior.

(B) Both the RFP-Cnn and γ-tubulin-GFP signals were bleached from these centrosomes as they were starting to separate. Both centrosomes initially recovered similar amounts of γ-tubulin-GFP fluorescence (t = 2:00), but only one centrosome recovered significant amounts of RFP-Cnn fluorescence (t = 5:20). The centrosome that did not recover any RFP-Cnn fluorescence, and had therefore downregulated Cnn incorporation, gradually lost its previously recovered γ-tubulin-GFP fluorescence (t = 5:20). Three of three neuroblasts examined exhibited this behavior.

(C) Images from a movie of a *cnn* null mutant neuroblast expressing GFP-PACT (green). Arrows and arrowheads indicate the mother and daughter centriole, respectively. Time before and after the mother centriole begins random movements (t = 00:00 min:s) is indicated. As in wild-type cells, both centrioles initially remained close to the cell cortex (t = −00:30), but soon after, both centrioles began to move around rapidly in the cytoplasm (t = 4:30 and t = 10:00). The centrioles in 14 of 14 *cnn* mutant neuroblasts exhibited this behavior; however, the majority of *cnn* mutant neuroblasts contained more than two centrioles (see Movie S6).

Scale bars represent 3 μm in (A) and (B) and 5 μm in (C).

daughter centrioles were brighter than their mothers, not only would mother centrioles have to exhibit the same level of GFP-PACT fluorescence irrespective of their age, daughter centrioles of the same age would have to exhibit widely varying levels of GFP-PACT fluorescence (see Figure S2). Thus, it seems very likely that GFP-PACT preferentially labels the mother centrioles in neuroblasts and that the daughter centriole is specifically retained in these stem cells.

We can only speculate as to why it might be an advantage for neuroblasts to specifically retain their daughter centriole. It seems to be of benefit to neuroblasts to retain one centrosome at the apical cortex throughout interphase, because this helps to establish correct spindle orientation for the subsequent asymmetric division [1, 2, 6]. It is also easy to envisage the benefits of retaining only one centrosome at the cortex, because retaining both might hinder efficient centrosome separation prior to mitosis. Because several centriolar proteins are irreversibly incorporated into centrioles during their assembly [12–14], continuously selecting the mother centriole might lead to the retention in the neuroblast of a centriole that accumulates damage. Male GSCs, which appear to specifically retain their mother centriole, exist in a niche environment, and so defective stem cells can be replaced by cells that dedifferentiate [8, 9]; this option is probably not available to neuroblasts.

It is difficult to test whether perturbing the preferential retention of the daughter centriole would be detrimental to neuroblasts. The functional significance of randomizing centrosome inheritance (for example, by removing Cnn) is hard to assess, because *cnn* mutant brains appear largely morphologically normal [15, 16], but this is also true in flies that completely lack centrioles and centrosomes—even though ~30% of asymmetric neuroblast divisions fail in these cells [17]. Thus, centrosome defects in flies can lead to a high frequency of defective asymmetric neuroblast divisions without producing any obvious defects in brain morphology. Mutations in several human centrosomal proteins (including CDK5RAP2, the human homolog of Cnn) are linked to microcephaly, a condition where patients have perturbed brain development [18]. Thus, human brains may not be able to compensate for defects in asymmetric neural stem cell divisions in the same way that flies apparently can. It seems to be an emerging principle that neural progenitor/stem cell divisions are particularly sensitive to defects in centrosome function, and so potentially in asymmetric centrosome inheritance.

Supplemental Information

Supplemental Information includes two figures, Supplemental Experimental Procedures, and six movies and can be found with this article online at doi:10.1016/j.cub.2010.11.055.

Acknowledgments

We thank Caroline Fabre, Graham Stokes, and members of the Raff laboratory for critical reading of the manuscript and stimulating discussions. This work was supported by a research studentship from Cancer Research UK (P.T.C.) and by a program grant from Cancer Research UK (J.W.R.).

Received: August 23, 2010
Revised: October 26, 2010
Accepted: November 19, 2010
Published online: December 9, 2010

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