Dab2–VEGFR-3 complex formation, and inhibition of aPKC in cultured cells led to accelerated VEGFR-2 and VEGFR-3 internalization. In line with these observations, aPKC inhibition enhanced the VEGF-A- and VEGF-C-induced ERK1/2 activation. Together, these findings suggest a role for aPKC-mediated Dab2 phosphorylation in negatively regulating VEGF receptor endocytosis and signalling (Fig. 1). In the neonatal retina, immunostaining for activated aPKC was weak at the sprouting front, but stronger in the quiescent proximal vasculature, implying that a graded aPKC activity may cause the differences in VEGFR-2 endocytosis between angiogenic and quiescent endothelial cells. In support of this interpretation, endothelial-specific ablation of aPKC-λ increased the accumulation of labelled VEGF and reduced VEGFR-2 and VEGFR-3 immunostaining at central locations, where the vessels also acquired characteristics of a sprouting phenotype. Strikingly, and consistent with the proposed mechanism, this phenotype was corrected by simultaneously deleting aPKC-λ together with Dab2 or PAR-3.

In summary, these results identify aPKC-λ as an inhibitor of Dab2/PAR-3-dependent VEGFR endocytosis and signalling in retinal angiogenesis. It remains to be elucidated how aPKC adopts its differential activity along the retinal vasculature, and how VEGFR signalling from the endosomal compartment is qualitatively and quantitatively different from its signalling at other subcellular locations. Although biochemical evidence suggests that VEGFR-2 endocytosis is required for full ERK1/2 and RAC1 activation, it would be valuable to see the many possible signalling pathways downstream of VEGFR-2 being investigated by endothelial-specific gene knockout in the retina, using the same rigorous standards as Nakayama *et al.* This would help us understand which pathways are physiologically relevant for angiogenesis *in vivo*, and whether these coincide with the pathways delineated in *in vitro* studies. RAC1 has been implicated in cell migration downstream of VEGFRs *in vitro*, and embryonic endothelial-specific deletion of RAC1 leads to early lethality associated with vascular defects[12]. However, the analysis done so far has not revealed whether defective cell migration, altered cell death or even other mechanisms are causing this phenotype. Surprisingly, RAC1 deletion in the neonatal retina failed to provoke an obvious angiogenic phenotype[13].

What should we expect from future research in angiogenic signalling? Although genetic toolbox and imaging techniques develop quickly, high-resolution live imaging of angiogenesis in mammals is still technically challenging and seldom reaches a satisfactory resolution. This is in marked contrast to zebrafish, where this approach has pushed the field significantly forward. Currently, we study the vast majority of developmental and pathological processes through 'snapshots'. However, as most biological processes during development and disease are dynamic, we would certainly benefit from live imaging at good resolution. Efforts to develop fluorescent markers and mouse lines to address this issue will definitely pay off.

COMPETING FINANCIAL INTERESTS
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The dominant force of Centrobin in centrosome asymmetry

Paul T. Conduit

Centrosomes play an important role in *Drosophila melanogaster* stem cells, where the different size and activity of the two centrosomes help these cells divide asymmetrically. The molecular basis of the centrosome asymmetry has remained unclear, but new work highlights the centrosomal protein Centrobin as a key player in this process.

Stem cells divide repeatedly to generate the cells that make up an organism, both during development and during adult tissue homeostasis. They can divide symmetrically, to increase or replenish the stem cell pool, but predominantly divide asymmetrically to reproduce the original stem cell and produce a cell whose progeny are destined to differentiate. To ensure tissue homeostasis and to avoid the overproduction of stem cells, the decision between symmetric and asymmetric stem cell division has to be tightly regulated. There is evidence that stem cell overproduction causes malignant tumour formation in *Drosophila*[1] and contributes to cancer progression in mice[2]. Whether a stem cell divides symmetrically or asymmetrically often depends on the orientation of the mitotic spindle, and this is dictated by centrosomes, which organise the spindle poles. During *Drosophila* development, neuroblasts divide asymmetrically to generate the central nervous system, and here the two centrosomes display differential activity early in the cell cycle that ultimately helps to establish spindle orientation later in mitosis[3,4]. It has remained unclear, however, how the centrosome asymmetry is

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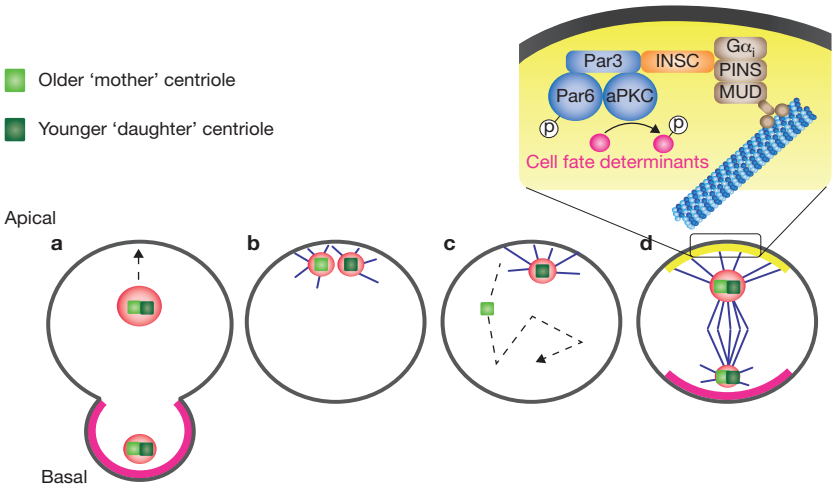

Figure 1 The asymmetric divisions of *Drosophila* neuroblasts. (**a**) At the end of mitosis, the pair of centrioles inherited by the neuroblast (top cell) migrate to the apical cell cortex. (**b**) The centrioles become anchored to the apical cortex through a microtubule–cortex interaction and begin to separate. The cortical factors that bind the microtubules at this stage are so far unknown. (**c**) The 'mother' centriole then loses its PCM and begins to move through the cytoplasm, while the 'daughter' centriole retains its PCM and remains positioned at the apical cell cortex. (**d**) Towards the end of interphase, apical (yellow) and basal (pink) cortical crescents are established: aPKC becomes localized and then activated as part of a Par3–Par6–aPKC complex at the apical cell cortex, where it then phosphorylates cell fate determinants (pink circles). These are then thought to be excluded from the apical cortex and subsequently localize to the basal cell cortex. At this stage in the cell cycle, the microtubules from the apical centrosome are thought to interact with MUD (which is located at the apical cortex as part of a $G\alpha_i$–PINS–MUD complex, linked to the Par3–Par6–aPKC complex through INSC) through the dynein–dynactin complex (light brown). The positioning of the daughter centrosome at the apical cell cortex forces the mitotic spindle to form along the apical–basal cell axis, ensuring the correct asymmetric segregation of the cell fate determinants.

established. An exciting report by Januschke *et al.*[5] now describes the centrosomal protein Centrobin (CNB) as a key regulator of this asymmetric centrosome behaviour.

Centrosomes are the main microtubule organizing centres in animal cells. A newly born cell inherits a single centrosome composed of a pair of centrioles that organize a complex matrix of proteins known as the pericentriolar material (PCM). The PCM contains a huge variety of proteins and enables the centrosome to nucleate and anchor large numbers of microtubules. In most *Drosophila* cells, the PCM is disassembled shortly after mitosis and the centrioles organize little or no PCM during interphase. The two centrioles move apart and duplicate to produce two centrosomes, each containing an older 'mother' centriole and a younger 'daughter' centriole. When the cell re-enters mitosis, the PCM is re-assembled at both centrosomes in a process known as centrosome maturation, and centrosomal microtubules contribute to mitotic spindle formation and spindle positioning. After cell division, each centrosome is inherited by one of the two daughter cells and the cycle can repeat.

The centrosome cycle is modified in *Drosophila* neuroblasts, where one of the two centrosomes continues to organize PCM and microtubules during interphase[3,4] (Fig. 1). This 'dominant' centrosome was recently shown to be the younger 'daughter' centrosome[6,7]. After mitosis in these cells, the centrosome inherited by the neuroblast migrates to the apical cell cortex and becomes anchored there by the microtubules it organizes. A short time later, the mother centriole in this centrosome loses its PCM and moves away from the apical cortex, whereas the daughter centriole retains its PCM and remains anchored to the apical cortex throughout interphase. When the cell re-enters mitosis and both centrosomes fully mature, the positioning of the daughter centrosome at the apical cell cortex forces the spindle to align along the apical–basal cell axis. The importance of spindle positioning becomes apparent when considering the segregation of cell fate determinants, which are positioned at the apical and basal cortices during early mitosis. The apical–basal orientation of the mitotic spindle ensures that these apical and basal crescents are segregated asymmetrically between the two daughter cells[8,9].

Exactly why the daughter centrosome retains PCM during interphase, whereas the mother centrosome does not, has remained mysterious. In a previous report, the Gonzalez lab had identified the *Drosophila* homologue of CNB and showed that it, like its human counterpart, localized specifically to the daughter centriole[7]. This prompted them to ask whether the asymmetric localization of CNB could drive the asymmetric centrosome behaviour observed in *Drosophila* neuroblasts. In the current report[5], Januschke *et al.* began by examining neuroblasts deficient of CNB. They found that, although the initial interphase microtubule aster was established and the centrioles migrated to the apical cortex, both centrioles subsequently lost their PCM and were released from the apical cell cortex. PCM and microtubule loss were interphase-specific, because when the cells entered mitosis both centrosomes could recruit PCM and organize microtubules. The mitotic spindle was assembled normally but often formed in a different orientation to the previous cell cycle. Such a phenotype has been observed in other mutant conditions, and a process known as 'telophase rescue' normally ensures that the cortical crescents are redistributed over the spindle poles and the cells still divide asymmetrically[10]. Indeed, the authors found that the CNB-deficient neuroblasts still divided into two cells of different size, and presumably still segregated cell fate determinants correctly, although this was not directly shown. Their data do show, however, that CNB is necessary for the daughter centriole to retain PCM and organize microtubules during interphase in neuroblasts.

Next, the authors wanted to establish whether CNB was sufficient for interphase PCM recruitment. Thus, they targeted CNB to both mother and daughter centrioles by fusing the centriole-targeting domain PACT to CNB. Strikingly, this induced both centrioles to recruit PCM and organize microtubules during interphase. Furthermore, both centrosomes remained close to the apical cortex for the majority of interphase, presumably tethered by their microtubule asters. Remarkably, the two centrosomes, despite still organizing microtubules, moved away from the apical cortex at the end of interphase and separated to form a bipolar spindle. These spindles were often misoriented, but they rotated back to match the original apical–basal axis and the cells divided asymmetrically. Taken together, these findings

clearly show that CNB is a key driver of PCM recruitment during interphase in neuroblasts.

Interestingly, the authors found that CNB did not drive interphase PCM recruitment in other cell types, even though it localized to one of the two centrosomes. Moreover, they found that CNB was not required for mitotic PCM recruitment in *Drosophila*. Thus, CNB seems to function in a neuroblast- and interphase-specific PCM recruitment pathway. Surprisingly, the authors showed that the cortical polarity protein PINS also functions in this pathway. PINS is known to recruit the microtubule-interacting protein MUD to the apical cell cortex during prophase, and *pins* and *mud* mutants have problems aligning their metaphase spindles[11,12]. Januschke *et al.* found that *pins* mutants also have defects in interphase PCM recruitment, whereas *mud* mutants do not. Thus, PINS seems to have a role in interphase PCM recruitment that is independent from its role in apical MUD localization.

Although the neuroblast–interphase PCM recruitment pathway is distinct from the normal mitotic PCM recruitment pathway, it still seems to share some common features. Januschke *et al.* found that CNB interacted with several centrosome components strongly implicated in mitotic PCM recruitment, including Centrosomin, which has previously been implicated in the establishment of centrosome asymmetry in neuroblasts[6]. CNB was also found to interact with POLO kinase, a key driver of mitotic PCM assembly. Low-dose inhibitor assays showed that POLO was important for CNB function, and the authors identified three putative POLO phosphorylation sites in CNB that were essential for CNB's role in interphase PCM recruitment. Moreover, they found that a phospho-mimetic version of CNB could no longer promote interphase PCM assembly after the cells were treated with the POLO inhibitor, suggesting that POLO also regulates other molecules in the interphase PCM assembly pathway.

In summary, the report by Januschke *et al.* provides a simple model for asymmetric centrosome behaviour in neuroblasts (Fig. 2) and raises interesting questions for both the centrosome and stem cell fields. It has revealed an interphase- and neuroblast-specific pathway of PCM recruitment that needs to be considered when discussing centrosome assembly. Although human CNB has previously been implicated in mitotic PCM assembly[13], PINS has not. How CNB and PINS function in interphase PCM recruitment, and how interphase PCM recruitment is limited to neuroblasts, will need to be investigated further. Moreover, it will be important to elucidate the dynamics of CNB localization, particularly regarding how CNB is eventually removed from the daughter centriole.

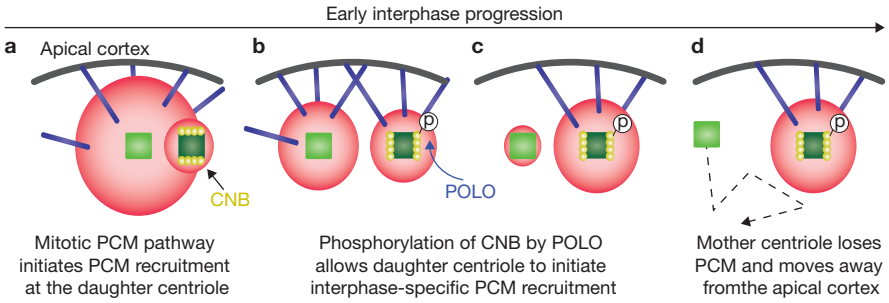

Figure 2 A model for asymmetric centrosome behaviour in *Drosophila* neuroblasts. (**a**) During mitosis, only the mother centriole is thought to organize PCM, but as the centrioles disengage towards the end of mitosis, the daughter centriole begins to recruit its own PCM pool. (**b**) Both centrioles initially become anchored to the apical cell cortex through microtubule–cortex interactions. The mitotic PCM recruitment pathway is downregulated but the phosphorylation of CNB at the daughter centriole by POLO initiates an interphase-specific PCM recruitment pathway that allows the daughter centriole to recruit PCM and organize microtubules during interphase. (**c**) The mother centriole does not contain CNB and thus cannot initiate the interphase PCM recruitment pathway. (**d**) This causes the mother centriole to be lost from the apical cortex.

For stem cell biology, the findings draw attention to previous data showing that centrosome positioning during interphase dictates the cortical polarity axis[14]. Indeed, if the daughter centrosome is lost from the apical cortex during interphase, either when CNB is absent[5] or when microtubules are depolymerized[14], a new polarity axis is established. In contrast, when both centrosomes are forced to associate with the apical cortex throughout interphase[5], the polarity axis is maintained, even though the spindle often forms in the wrong orientation and has to rotate into position. It is still unclear how the apical aster signals to the cortex to dictate the position of cortical polarity, especially given that it doesn't seem to rely on the ability of microtubules to induce PINS cortical localization[14]. Moreover, the cortical factors that provide the attachment sites for the apical centrosome during interphase remain completely unknown.

It will be important to further unravel the molecular details of stem cell divisions, which may not always be the same in different cell types. For example, *Drosophila* male germline stem cells retain their mother centrosome[15], essentially excluding a role for CNB in directing asymmetric centrosome behaviour in these cells. Regardless of what mechanisms are in place, however, it is clear that centrosomes and stem cells are intimately linked, and their relationship will be the focus of future research.

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