Centrosomes: **Central no more?**

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It has recently been found that the zygotic development of a morphologically normal fly can occur without properly functioning mitotic centrosomes. Does this mean that centrosomes are not required for cell division in animals at all?

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The centrosome is the main microtubule-organising centre in animal cells. For most of the last century, cell biologists bestowed upon the centrosome an almost mystical importance, especially for mitosis [1,2]. In a similar manner to a cell's chromosomal DNA, centrosomes replicate once, and only once, per cell cycle, generating two centrosomes which then form the two poles of the mitotic spindle. In anaphase, the sister chromatids separate and move to opposite poles. The two spindle poles then somehow position the cleavage furrow half way between them, ensuring that the replicated chromosomes are partitioned into two daughter cells.

There was strong evidence that animal cells could not divide without centrosomes. This was perhaps most dramatically demonstrated in experiments in which frog eggs, which do not contain a centrosome, were artificially activated by pricking with a needle. This mimicked fertilisation and triggered cell-cycle oscillations in the egg, but, unless a centrosome — normally provided by the fertilizing sperm — was co-injected into the egg, the egg would not divide [3,4]. Conversely, if a cell had too many centrosomes, it would try to divide halfway between all of the centrosomes [5].

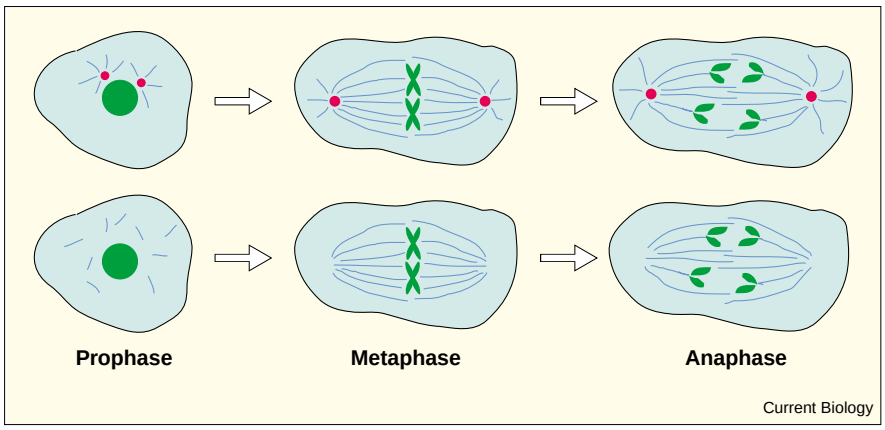
Given these observations, few people doubted the importance of the centrosome for mitotic cell division in animal cells — even though higher plant cells and some animal meiotic systems appeared to lack centrosomes. Recently, however, several experiments have questioned the central role of the centrosome in animal cell division [6,7]. Remarkably, as reported in a recent issue of *Current Biology*, Megraw *et al.* [8] have now shown that the zygotic development of an adult fruit fly can occur almost perfectly normally without properly functioning mitotic centrosomes. How can this be?

The first hint that centrosomes may not be required for animal cell mitosis came with the striking demonstration by Heald *et al.* [6] that bipolar spindles can form around chromatin beads in *Xenopus* egg extracts in the absence of centrosomes. This spindle formation was dependent on the ability of chromatin to locally stabilise microtubules, and on several microtubule motor proteins [9]. This result was perhaps not that surprising, as it was already known that bipolar meiotic spindles could form around chromosomes in the absence of centrosomes in several meiotic systems [10,11]. Nevertheless, this finding dispelled much of the mystique of the centrosome: you do not need two centrosomes to generate a bipolar spindle — chromatin, microtubules and motor proteins can do it alone.

Many in the centrosome field argued that the ability to form bipolar spindles in the absence of centrosomes might be restricted to cells that can normally form spindles without centrosomes. This refuge was, literally, blown away when Khodjakov *et al.* [7] showed that normal bipolar spindles can form and proceed through anaphase in cultured vertebrate cells in which the centrosomes had been destroyed by laser ablation (Figure 1). Still, many in the centrosome field took comfort from the fact that these experiments were performed in culture, where it is difficult to determine the long-term consequences of dividing without centrosomes. Surely, acentrosomal cell divisions would not be accurate or fast enough to build an animal, where many thousands of cell divisions have to be completed accurately within a strict developmental context. Moreover, in animal development some cell divisions are asymmetric, producing two daughters of unequal size and with different developmental fates [12]. Astral microtubules emanating from the centrosome are thought to be crucial for accurately positioning spindles during asymmetric divisions [13] (Figure 2), and acentrosomal spindles are invariably anastral.

Megraw *et al.* [8], however, have demonstrated that it is possible to build a fly without fully functional mitotic centrosomes. They found that *Drosophila* cells lacking the centrosomal protein centrosomin have normal looking centrosomes in interphase, but the centrosomes lose several characteristic markers as they enter mitosis. These centrosomes fail to nucleate astral microtubules properly, and the mitotic spindles formed resemble the anastral spindles seen in acentrosomal meiotic spindles. Remarkably, centrosomin-deficient flies develop at normal rates and form almost perfectly normal adult flies which are born at normal Mendelian ratios. Their only reproducible visible defect is that they have a few small clumps of abnormally organised tissue in the wing. The reason for this defect is unclear. All of the mutant flies, however, are sterile (see below).

Figure 1



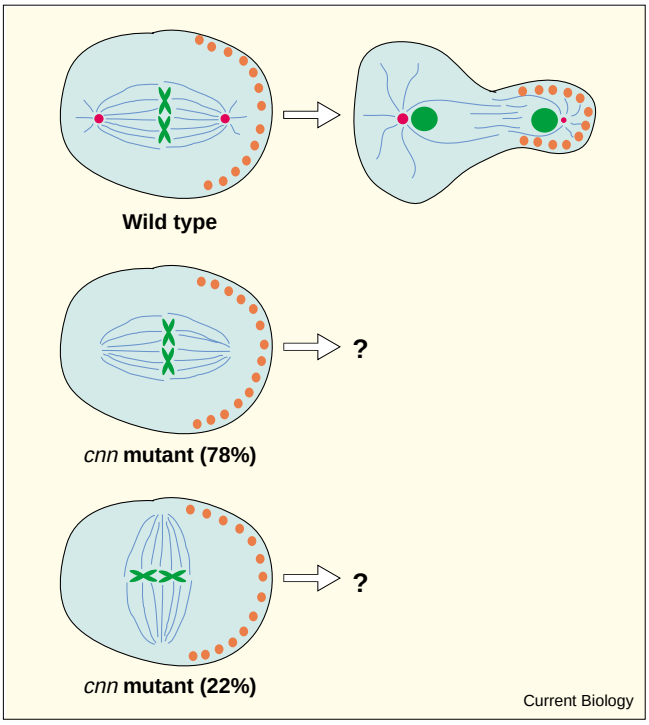
Schematic drawings of cell division in a typical animal cell in culture (top panel) and a similar cell in which both centrosomes have been destroyed at prometaphase by laser ablation (bottom panel). Centrosomes are shown in red, microtubules in blue and chromosomes in green. Shortly after the centrosomes have been destroyed in a prometaphase cell, the microtubules in the cell become disorganised. Remarkably, the cells eventually organise a relatively normal-looking bipolar spindle around the mitotic chromosomes, and appear to enter anaphase as normal.

Do these flies finally establish that centrosomes are not required for normal cell division in animals? Perhaps not. First, the *centrosomin* (*cnn*) mutation does not inactivate centrosomes; rather, it inactivates a centrosomal component that compromises centrosome function during mitosis. Although the centrosomin-deficient mitotic centrosomes nucleate many fewer microtubules, it is not clear that these centrosomes are completely non-functional. Second, mitotic centrosomes do appear to be essential during early embryonic development in *Drosophila*. The *cnn* mutant flies are sterile, so that all homozygous *cnn* flies must have developed from heterozygous mothers. These mothers supply enough centrosomin protein to allow the embryos they lay to proceed through the first 13 rounds of rapid nuclear division that occur within the giant single cell (syncytium) of the early embryo. Embryos laid by homozygous *cnn* mothers have no centrosomin protein supplied to them, and they die due to an accumulation of mitotic defects during these early nuclear divisions. The astral microtubules that emanate from normal centrosomes are apparently essential to prevent the nuclei from colliding with each other in the common cytoplasm of the syncytial embryo [14]. Finally, as discussed above, there is compelling evidence that centrosomes are required for cleavage in at least some cells [3,4]. Moreover, recent evidence suggests that, although cultured vertebrate cells in which the centrosomes have been removed by microsurgery or laser ablation can form bipolar spindles and enter anaphase, cytokinesis often then fails (T. Hinchcliffe and G. Sluder, and A. Khodjakov and C. Rieder, personal communications).

Perhaps the most surprising implication of the findings reported by Megraw *et al.* [8] is that mitotic centrosomes appear to be dispensable for the asymmetric cell divisions that occur during fly development. This was also suggested by Bonaccorsi *et al.* [15] who recently showed that *Drosophila* larval neuroblasts mutant for another gene, *asterless*, can apparently divide asymmetrically even though, like *cnn* mutants, they have drastically reduced numbers of astral microtubules. Are astral microtubules really not required for asymmetric divisions? Megraw *et al.* [8] looked at asymmetric divisions in the larval neuroblasts of *cnn* mutants. Normally, the cell-fate determinant Prospero is asymmetrically localised to one side of the cortex in these cells, and the mitotic spindle reorients to ensure that Prospero is inherited by only the smaller of the two daughter cells when the cell divides (Figure 2). In *cnn* mutant neuroblasts, the spindle is misaligned relative to Prospero in approximately 22% of neuroblast divisions (Figure 2). If only completely misaligned spindles — as depicted in Figure 2 — were included in this 22%, it is possible that spindle orientation relative to Prospero may have been completely randomised in the mutant neuroblasts. In any case, it is clear that spindle orientation is abnormal in many *cnn* mutant neuroblasts. Thus, the development of morphologically and behaviourally normal *cnn* mutant flies may be more a testament to the ability of the nervous system to compensate for mistakes than to the ability of cells to divide asymmetrically without centrosomes.

Megraw *et al.* [8] did not examine whether the *cnn* mutant neuroblasts with misaligned spindles divide asymmetrically to produce daughters of different sizes. Such asymmetric divisions were observed in *asterless* mutant larval neuroblasts [15], although it is not clear how often they occur, or whether they segregate cell-fate determinants normally. It would be interesting to study the asymmetric divisions of *cnn* or *asterless* mutant neuroblasts in embryonic, rather than larval tissues, as in the former case the plane of division is defined by the dorsal–ventral axis of the embryo and therefore can be predicted [16], which is not the case in larval neuroblasts. This would allow one to determine whether cell-fate determinants, the spindle or both are misaligned, and how often cells of different sizes are produced.

Figure 2



Schematic drawings of asymmetric cell divisions in *Drosophila* neuroblasts. Prospero protein is shown in orange. In dividing neuroblasts, Prospero is concentrated at the cortex at one side of the cell. During metaphase, the spindle reorients so that the Prospero is partitioned exclusively into the smaller daughter cell when the neuroblast divides. Astral microtubules appear to be essential for properly positioning the spindle during asymmetric divisions in many systems. In *Drosophila* neuroblasts, the centrosome (red) and its associated astral microtubules (blue) increase in size in the larger of the two daughter cells during anaphase [16]. In *cnn* mutant neuroblasts, astral microtubules are absent, or greatly reduced, and the spindle fails to align properly in relation to Prospero in 22% of these divisions. It is not clear whether these cells go on to divide asymmetrically, although this appears to be the case in at least some asterless mutant neuroblasts [15].

In summary, it is now clear that mitotic spindles have a remarkable capacity to self-assemble and direct animal cell division without centrosomes. With hindsight, this should not have been so surprising. Centrosomes have at their core centrioles, which are themselves complicated microtubular structures. Thus, animal cells must have been using microtubules to divide accurately for many hundreds of thousands of years before they evolved centrosomes, and higher plant cells have continued to do so. Centrosomes, however, are required to produce astral microtubules, which appear to be essential for syncytial embryonic development, and are perhaps required for properly coordinated asymmetric cell division and for efficient cytokinesis in animal cells.

Is the centrosome, then, only required to generate astral microtubules? Recent results suggest not. Cultured vertebrate cells in which the centrosomes have been removed by either microsurgery or laser ablation (T. Hinchcliffe and G. Sluder, and A. Khodjakov and C. Rieder, personal communications) can proceed through mitosis, but they then arrest prior to the entry into the next S phase, even though they reform a relatively normal-looking microtubule cytoskeleton. These findings suggest that, either centrosomes contain a factor essential for S-phase initiation, or that animal cells have a checkpoint mechanism that detects the lack of a centrosome and arrests the cell cycle prior to the initiation of S phase. Thus, while centrosomes may not be as important in animal cell division as previously thought, they may surprise us yet.

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