Centrosome segregation and its relevance in asymmetric cell division

Mariana Pliego Caballero

Master in Biomedical Sciences

Examiner: Prof. Dr. Jan Jacob Schuringa

Department of Experimental Hematology

October 2017

Abstract

Stem cells can divide asymmetrically, giving rise to a self-renewing daughter stem cell and another cell that undergoes differentiation. Cell intrinsic and extrinsic factors are involved in the fate determination of the progeny. Since the last decade, centrosomes have also been suggested to contribute to the cell fate through their stereotypical segregation. While some types of stem cells seem to retain the older of the parental centrosomes and the differentiating sister inherits the younger centrosome, for other types of stem cells the inheritance is inversed. In this assay, the asymmetric inheritance of centrosomes is reviewed, with the aim to clarify whether this influences the cell fate in asymmetric stem cell divisions.

Introduction

Asymmetric cell division results in the generation of two daughter cells with different characteristics. In stem cells, the asymmetric cell division yields one cell that conserves the self-renewal potential and one that undergoes differentiation. This process is especially important for adult stem cells in order to maintain tissue homeostasis, since deviation from the balance between self-renewal and differentiation could lead to either tissue degeneration or tumorigenesis[1]. Asymmetric cell division can be regulated by extrinsic cues, for example through the exposure of daughter cells to different delimited environments or gradients of extracellular factors and also by intrinsic cues, for example by the polarization of fate determinants [2]. Recently, centrosomes have gained interest as a potential fate determinant in asymmetric cell divisions.

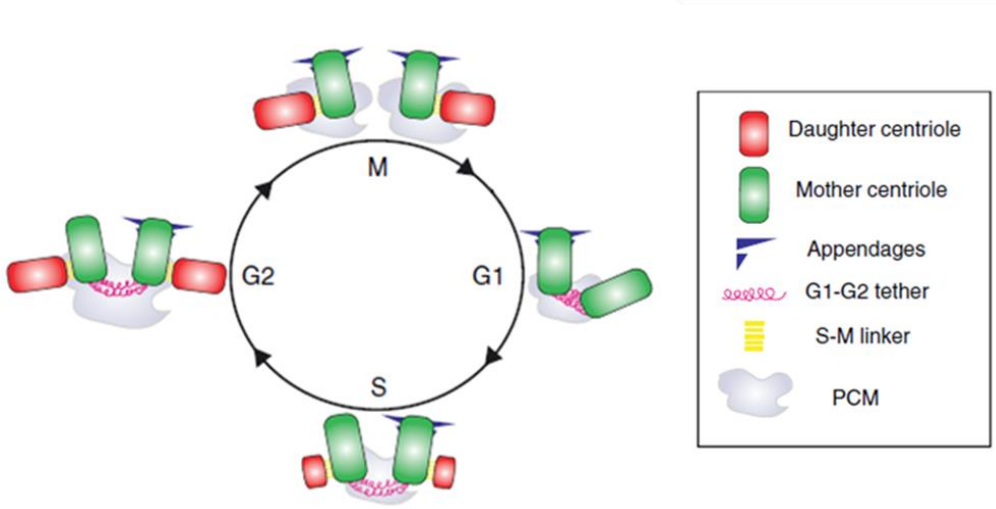

Figure 1. The centrosome cycle. During the M/G1 phase the S-M linker is lost and replaced by a loose connection through the G1-G2 tether. Procentriole formation starts along the S phase and are elongated during the G2 phase. Before mitosis, the G1-G2 tether is dissolved and centrosomes take opposite positions inside the cell. PCM: pericentriolar material. Modified from Yamashita 2012.

Centrosomes are the microtubule-organizing centers (MTOC) of animal cells and participate in many processes such as spindle formation, chromosome segregation, cell motility and intracellular transport; they are also important for cell shape and polarity [3–5]. Centrosomes are composed of a pair of centrioles, cylindrical structures formed by a 9-fold symmetric array of microtubules and surrounded by the pericentriolar material (PCM) that contains microtubule (MT) nucleating factors [3].

At the beginning of the cell cycle, animal cells contain a single centrosome, however, in preparation for cell division, centrosomes must duplicate once following a semiconservative synthesis. The centrosome cycle starts at the M/G1 phase of the cell cycle *(Fig. 1)*, when the perpendicularly oriented parental centrioles lose this position due to a process known as disengagement, where the S-M1 tether that holds them together is degraded. Simultaneously, a longer linker called the G1-G2 tether is created resulting in a more loose connection. During the G1/S transition, a procentriole will be formed perpendicularly to each parental centriole and these will elongate along the S/G2 phases. In late G2 phase, the amount of PCM surrounding the centrioles increases and this will be followed by the dissolution of the G1-G2 tether, that allows centrosomes to separate and take opposite positions in the cell for the formation of the spindle apparatus [3,6]. Although this centrosome cycle is well stablished, some exceptions exist: in *Drosophila* for example, centrosomes divide before centriole duplication[7].

Given the semiconservative synthesis of centrosomes, an inherent asymmetry can be noticed. In every centrosome, one of the centrioles was formed just in the previous cycle whereas the other was synthesized earlier: they are referred to as the mother and daughter centrioles. Once the pair of centrioles splits and duplicates, two centrosomes will arise. The mother centrosome, carrying the oldest of the centrioles plus one just synthesized and the daughter centrosome carrying the younger of the parental centrioles plus another newly formed. Finally, upon cell division this asymmetry will be segregated to the progeny, since once cell inherits the mother centrosome and the other the daughter centrosome[4] *(Fig. 2).*

It has been reported that in progenitor or stem cells, centrosomes are not segregated randomly but in a stereotypical fashion and it is suggested that centrosomes might act as fate determinants in asymmetric cell division. In this essay the evidence of asymmetric centrosome segregation will be reviewed with the aim to analyze if this process indeed plays a role in cell fate.

Differences between daughter and mother centrosomes

Apart for the clear difference in age, the mother and daughter centrioles present some differences at the molecular level as well. In vertebrates, the mother centriole harbors two structures that the daughter centriole lacks: the subdistal and distal appendages *(Fig. 2)*, these structures are acquired by the daughter centriole 1.5 cycles after its synthesis. While subdistal appendages are involved in microtubule anchoring, the distal appendages are necessary for the formation of a primary cilium [3,8,9]. This raises a functional difference, since only the old centriole can nucleate a primary cilium [9,10]. In *Drosophila* the centrioles do not present appendages, however, it is reported that the size of the younger centriole is smaller during most of the cycle [11]. In addition, older centrioles accumulate more pericentriolar material than the younger ones [4]. Finally, some proteins like the outer dense fibre protein 2 (ODF2), Ninein and the centrosomal protein 164 (CEP164) are only present

in the mother centriole, whereas Centrobin (Cnb) is exclusive expressed in the daughter centriole [12].

Regarding the duplicated centrosomes, some differences can also be noticed between the mother and daughter centrosomes. The most remarkable difference is given by the differential MTOC activity (discussed below). Additionally the differential expression of proteins between centrioles are also reflected in differences between centrosomes, for example, in *Drosophila* neuroblasts, Cnb (Centrobin)[7] and Polo localizes in the daughter centrosome, whereas PLP expression is higher in the mother centrosome [13]. The protein Ninein (Nin) shows a higher expression in the mother centrosome of mice radial glial progenitors[14]. The differential expression of some of these proteins contributed to the findings of asymmetric centrosome segregation in stem cells and progenitors.

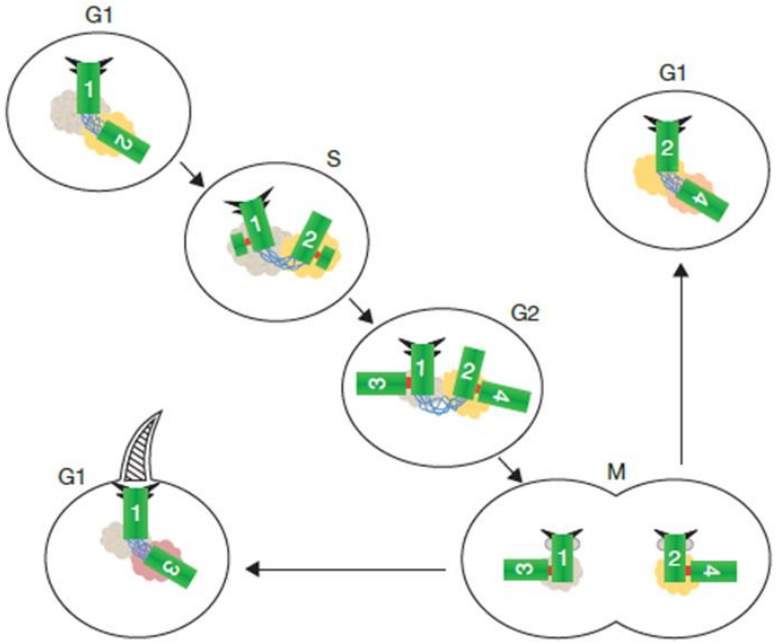

Figure 2. Centrosome asymmetries and segregation. The older of the parental centrioles, mother centriole and the younger, daughter centriole, are represented with number 1 and 2, respectively. In G1 the centriolar disengagement occurs, followed by procentriole formation in S phase and elongation of the new centrioles (3 and 4) in G2 phase. After mitosis, one of the cells inherits the mother centrosome (left side) and the other cell retains the daughter centrosome (right side). Distal and subdistal appendages are represented in black triangles. As it can be observed, the younger parental centriole (2) acquires the appendages at the G1 phase of the cell cycle that proceeded its synthesis (1.5 cycles later). In addition, the primary cilium (dashed peak), nucleates earlier in the cell inheriting the mother centrosome (lower left side of the figure). From Stearns 2014.

Evidence of asymmetric centrosome segregation

The first report of asymmetric centrosome segregation in stem cells came from observations in Drosophila's male germline stem cells (mGSC) made by Yamashita et al.[15] In the fly's testis, mGSC achieve an asymmetric cell division partially through their asymmetric interaction with the niche, formed by the hub cells at the apical side of the

testis. Once the cell division is completed, the self-renewing daughter cell remains attached to the hub, whereas the differentiated daughter is displaced away. Through the use of a fusion of the green fluorescent protein (GFP) with the C-terminus domain of the pericentrin-like protein (PACT), a protein that localizes at centrioles, it was possible to differentially label the mother and daughter centrosomes. This strategy led to the observation that, during mGSC division, the mother centrosome remained close to the hub cells and was inherited by the mGSC, whereas the daughter centrosome migrated to the opposite side and was inherited by the cell committed to differentiation, this pattern was repeated through multiple rounds of cell divisions (*Fig. 3a)*.

Similar results were observed in radial glial progenitors at the ventricular zone of the developing neocortex in mice[14]. The radial glia cells are key progenitors in the developing nervous system of vertebrates, due to asymmetric cell divisions they can either self-renew or give rise to neurons. Using a fusion of centrin 1 (CENT1), a component of centrioles, with the photoconvertible fluorescent protein Kaede, it was possible to label and track the mother centrosome. This led to the observation that the mother centrosome is inherited most of the times by the self-renewing cell, whereas the daughter centrosome was inherited by the differentiating neuron (*Fig. 3d)*. In addition, when the expression of Nin, a protein necessary for centriole maturation, was blocked by the use of short hairpin (shRNA) or small interfering RNAs (siRNA), the asymmetric segregation of centrosomes was lost. This resulted in a premature depletion of the radial glia cells and an increase in differentiated neurons, suggesting that the asymmetric inheritance of centrosomes is required for the maintenance of the radial progenitors.

After these observations it was suggested that stem cells or progenitors may always inherit the mother centrosome, studies made in *Drosophila* neuroblasts initially supported this idea. Neuroblasts are neural progenitor cells that divide asymmetrically, giving rise to a self-renewing neuroblasts and a ganglion mother cell (GMC), it was observed that upon division, one of the centrosomes always remained at the apical cell cortex and was inherited by the self-renewing cell [16,17], due to the results obtained in mGSC it was assumed that the mother centrosome was the one inherited by the neuroblasts.

However, further studies made by Conduit & Raff demonstrated the opposite[18]. Through the measurement of GFP-PACT intensity as a marker of centrosome age, in combination with the incorporation of centrosomin (Cnn), that reflects the organization of the PCM at the centrosome, it was possible to distinguish mother and daughter centrosome. Contrary to what was expected, it was observed that the daughter centrosome was inherited by the neuroblasts[18] (*Fig. 3b)*. Interestingly, after centrosome duplication, the mother centrosome initially incorporated more Cnn and interacted with more microtubules (MT). However, as the centrosomes divided, the incorporation of Cnn and MT interaction gradually decreased in the mother centrosome while it increased in the daughter centrosome. These results suggested that in neuroblasts, the daughter centrosome retained the microtubule organizing activity and this allowed it to remain attached to the neuroblast. Similarly Januschke et al. also observed the inheritance of the daughter centrosome by neuroblasts with the use of a photoconvertible PACT[7].

Related to the observations in *Drosophila* neuroblasts, neuroblastoma human cell lines also showed asymmetric centrosome segregation[19]. Neuroblastoma is a type of cancer derived from neuroblasts. In these cells, the localization of the nuclear mitotic apparatus protein (NuMa) was used as a marker of symmetric/asymmetric cell division. Since some cell lines showed a polarized expression of NuMa at the cell cortex, it was suggested that these cells undergo asymmetric cell divisions. Along with these observations, when an

antibody against ODF2/cenexin, a component of the centriolar appendages, was used to differentiate the mother centrosome, it was noticed that the daughter centrosome was preferentially segregated with the cell that expressed NuMa at the cell cortex (*Fig. 3e)*. Taking as a reference previous studies where self-renewing neuroblasts inherit the NuMA crescent [20–22], it was proposed that the cells carrying the daughter centrosome probably contained the self-renewal potential. However this was not further characterized.

The most recent study that demonstrated the stereotypical segregation of centrosomes was performed in *Drosophila* female GSC (fGSC) and a segregation pattern comparable to what is reported for neuroblasts was observed. Similar to the fly's testis, the cap cells present in the ovary form the niche to which fGSC attach and provide a signaling necessary to maintain their stemness. Once asymmetric cell division is completed, the cell in contact with the cap cells will self-renew whereas the displaced cell will undergo differentiation. With the use of centrobin-yellow-fluorescent-protein (Cnb-YFP) as a marker of the daughter centrosome, it was observed that this was preferentially inherited by the fGSC [23], while the mother centrosome was retained by the differentiated cystoblast (*Fig. 3c)*.

Table 1 summarizes the observations of the above mentioned studies.

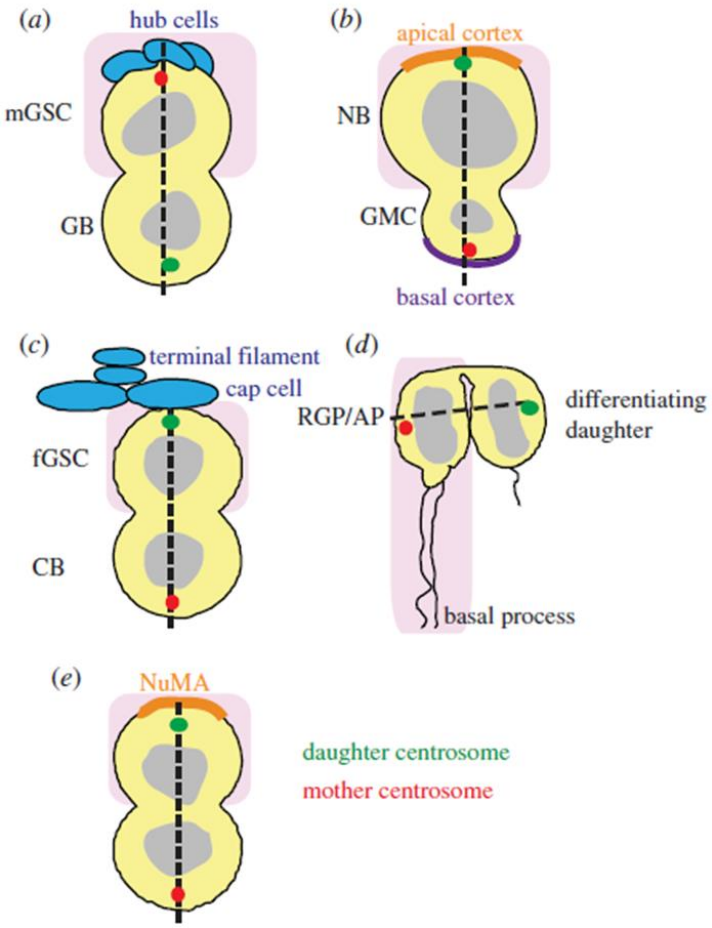

Figure 3. Stereotypical centrosome segregation in asymmetric cell divisions.
A) *Drosophila* male germline stem cells, mGSC; b) *Drosophila* neuroblasts; c) *Drosophila* female germline stem cells (fGSC); d) Mouse radial glial progenitors or apical progenitors (RGP/AP); d) human neuroblastoma cell lines with asymmetric polarization of NuMA, representing the possible self-renewing cell. In green, the daughter centrosome is represented; in color red, the mother centrosome. Dashed lines represent the plane of division. In color blue the niche is pictured. GB: gonialblast; NB: neuroblast; CB: cystoblast. From Reina 2014

Table 1. Summary of the observations of stereotypical centrosome segregation.

Model	Progenitor/ stem cell inherited:	Method to distinguish centrosomes	Molecules involved	Reference
Drosophila male germline stem cells	Mother centrosome (90%)	GFP–pericentrin/AKAP450 C-terminus (GFP-PACT)	Centrosomin (Cnn) In mutants for this protein, segregation of centrosomes is randomized	Yamashita Y (2007) *Science*
Mouse radial glial progenitors	Mother centrosome (78%)	EGFP–ninein (GFP-Nin) and Photoconvertible Kaede- Centrin (Kaede-CETN1)	Ninein (Nin) shRNA against Nin disrupted the asymmetric segregation of centrosomes	Wang X (2009) *Nature*
Drosophila neuroblasts	Daughter centrosome (NR)	GFP-PACT and Red Fluorescent Protein-Cnn (RFP-Cnn)	Centrosomin (Cnn) On its absence centrosomes lose connection to apical cortex	Conduit P (2010) *Current Biology*
Drosophila neuroblasts	Daughter centrosome (NR)	Photo convertible PACT–d2Eos and YFP-Centrobin (YFP-Cnb)		Januschke J (2011) *Nat Comm*
Human neuroblastoma cell lines	Daughter centrosome (65-85%)	Antibody for the mother centrosome marker ODF2/cenexin		Izumi H (2012) *PNAS*
Drosophila female germline stem cells	Daughter centrosome (70%)	YFP-Cnb		Salzmann V (2013) *MBoC*

Numbers in the second column represent the percentage of stem cells inheriting the mentioned centrosome. NR: not reported.

Relationship with primary cilium

The primary cilium is an organelle that nucleates from the basal body derived from centrioles. It has an antenna-like structure that projects from the surface of almost all vertebrate cells and is composed of a tubular structure, axoneme, made of microtubules with a 9-fold symmetry, and covered by a ciliary membrane. The primary cilium plays an important role as a sensory organ[9].

In mouse fibroblasts cell line (3T3), through the use of a tagged tubulin to distinguish the mother and daughter centrosomes, it was observed that the primary cilium grew earlier in the sister cell inheriting the mother centrosome. Additionally, these cells also showed a differential expression of PDGFR at the cilia in time, as well as a faster response to the presence of the signaling molecule Sonic hedgehog (Shh) [24].

Further studies made in the apical progenitors of the developing neocortex of mice and cell lines, demonstrated the reason why the primary cilium is first assembled at the daughter cell inheriting the mother centrosome[25]. This is because opposite to what was previously thought, the primary cilium is not fully disassembled before mitosis. Instead, the membrane covering it, ciliary membrane (CM), is internalized to the cell that inherits the mother centrosome and remains attached to the appendages of the mother centriole during mitosis. Interestingly, most of the times, the cell inheriting the mother centrosome

and the CM kept the stem cell character. The asynchronic ability to respond to signaling molecules given by the asymmetric grow of the primary cilium, has been suggested as the mechanism linking the differences in stem cell potential with the asymmetric segregation of centrosomes. The earlier growth of the primary cilium could affect the response of the daughter cells to external signals, influencing cell fate.

Mechanisms influencing the asymmetric segregation of centrosomes

In *Drosophila* neuroblasts, as it has been mentioned, centriole duplication takes place after division[7], this means that individual centrioles divide and take opposite positions within the mother cell before they form a typical centrosome with a pair of centrioles. This difference has helped to better understand the mechanisms involved in the asymmetry of centrosomes.

As previously mentioned, in *Drosophila,* the neural stem cells or neuroblasts inherit the daughter centrosome, whereas the GMC retains the mother centrosome. Before this was known, it was noticed by Rebollo et al [16]. that only one microtubule aster was visible at the apical side of the neuroblast during most of the cell cycle, whereas the second one would appear at the basal side just before mitosis. In addition, it was observed that the PCM of the basal centrosome was downregulated after centrosome division and it was upregulated just before spindle assembly. When centrosome movement was tracked, they found that the apical centrosome was almost static, while the other centrosome presented long and fast movements along the whole cell, until it finally migrated to the basal side and became stable upon spindle positioning. It was later proposed that the apical centrosome through the retention of PCM formed a dominant MTOC, whereas the other did not present MTOC activity due to the PCM shedding just after centrosome duplication[17]. In 2010 it was demonstrated that in Drosophila neuroblasts, this “dominant” centrosome is the daughter, whereas the basal one is the mother centrosome [18].

After these observations revealed an asymmetric behavior in centrosomes, many research groups focused on studying the mechanisms involved. It is now known that the kinase Polo, through its asymmetric location at the daughter centrosome, phosphorylates Cnb, a protein necessary for PCM retention at the centrosome [26,27]. This results in the retention of γ-tubulin, Cnn and other PCM components, on the contrary, the pericentrin-like protein (PLP) along with Bld10 negatively regulate this process in the mother centrosome by shedding Polo and along with this, the PCM [28,29]. Since the PCM is necessary for MT nucleation, a difference in MTOC activity is present between both centrosomes and affects their mobility. While the daughter centrosome remains stably attached to the apical side of the neuroblasts, the mother centrosome is free to move around until it reaches the basal side of the cell. This asymmetry continues until early prophase, when centrosome maturation results in the recruitment of PCM proteins to the mother centrosome [12,16,17,30].

Recently, the *Drosophila* homologue of WD40 repeat protein 62 (Wrd62) was found to also regulate centrosome asymmetry through the stabilization of MT that are necessary to recruit and maintain Polo at the PCM, further promoting MTOC activity in the daughter centrosome. In addition, it was suggested that Cnb exert a negative regulation upon PLP, avoiding its localization at the daughter centrosome, which explains the mother-centrosome-exclusive PCM shedding[30].

These mechanisms, however, do not explain what gives the mother/daughter centrosome its identity and how is it defined that one of them will be an active MTOC and the other will migrate; it is also not known how this positioning is regulated.

Consequences of the loss of asymmetric centrosome segregation

Little is known about the effect that the loss of asymmetric centrosome segregation has on the cell or the whole organism. In the above mentioned studies focused in dissecting the mechanisms involved in the asymmetry of centrosomes, some of the proteins involved in PCM recruitment and MTOC activity were mutated and the effect in centrosome segregation was tested. In most of these studies the stereotypical segregation of centrosomes was altered, showing either an inverse segregation of the mother/daughter centrosome [11,26,29,30] or the presence of cells carrying too many centrosomes or none at all [18,28]. However, after testing for other abnormalities, no differences were observed in comparison to the respective controls. The average cell cycle length, orientation of the plane of division, distribution of polar proteins and the asymmetry of cell division remained unchanged [5,7,29,30]. In addition, in *Drosophila* larval brains, the number of neuroblasts and brain size were analyzed and no difference was found in flies carrying Bld10 or Plp deletions[28,29]. In contrast, *wrd62* mutant *Drosophila*, showed a decrease in brain volume, however this effect seems to be unrelated to the altered centrosome segregation since this effect was not observed in *cnn* mutants [30].

One common feature observed in most of this studies, was a disruption in the orientation of the spindle apparatus; since the location of the centrosomes was affected, initially the spindle presented a distorted alignment, however this was frequently corrected during the progression of mitosis through rotation of the spindle [17,26,28,29] *(Fig. 4 and Table 2).* Only one study reported a low frequency of cells where the initial positioning of the spindle was too aberrant that could not be corrected, although it was suggested that it might lead to symmetric division, this was not confirmed [17]. Hence, it is proposed that centrosome asymmetry might act as an initial mechanism for spindle positioning, however, it is probably not essential since it seems that a backup mechanism exists to ensure its proper orientation and with this, conserve the asymmetry of cell division[17,26,28–30].

Finally, it was reported by Wang et al. that removal of the centriolar protein Nin by shRNA, disrupted the asymmetric segregation of centrosomes in the developing mouse neocortex. This resulted in a premature depletion of radial glial progenitors and an increase in the number of differentiated neurons [14]. However, Nin is a component of the centriole appendages and its depletion prevents centrosome maturation; since the appendages are involved in the primary cilium formation and this is involved in sensing and signaling, it cannot be discarded that this effect might be related to alterations of the primary cilium instead of a direct consequence of centrosome mis-segregation.

Discussion

Along this essay, evidence for the stereotypical segregation of centrosomes in asymmetric cell division has been presented. Whereas in *Drosophila* mGSC and the mice radial glial progenitor, the self-renewing stem cell inherits the mother centrosome and the differentiated daughter cells inherit the daughter centrosome[14,15]; the opposite occurs in *Drosophila* fGSC and neuroblasts[7,18,23]. It has been suggested that centrosomes may be relevant for the cell fate in asymmetric cell division; however, more studies need to be performed before we can clearly confirm or reject this hypothesis.

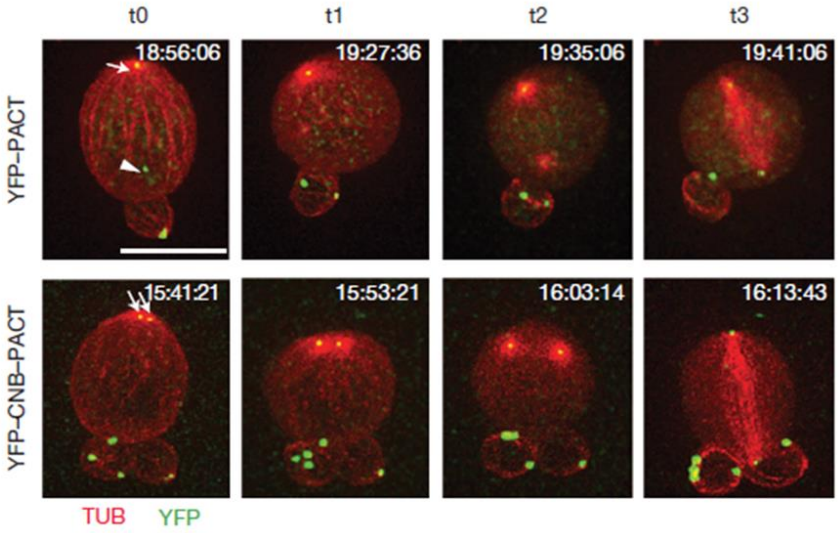

Figure 4. Altered centrosome positioning does not affect spindle orientation. In this figure, neuroblast centrosomes are labeled with YFP-PACT (green) and tubulin with mCherry (red). Upper part: centrosomes take opposite positions in the cell (t0), at t1 only the apical centrosome retains the the MTOC activity. Later on t2 the basal centrosome regains the MTOC activity. At t3 the mitotic spindle can be observed. Lower part: In neuroblasts expressing ectopic CNB, both centrosomes retain the MTOC activity and are positioned at the apical side of the cell (t0-t2). However the mitotic spindle is correctly oriented (t3). Modified from Januschke 2013

Table 2. Summary of the studies reporting spindle misorientation in response to the mutation of proteins involved in the asymmetric MTOC activity of centrosomes.

Mutated centrosomal protein	Protein function	Spindle misorientation resolved during:	Reference
Asterless (Asl) deletion	Centriole component Involved in centriole duplication and elongation*	In Metaphase	Rusan N (2007) *JCB*
Centrobin (Cnb) ectopycally expressed	Daughter centriole protein. Involved in PCM retention*	In Prometaphase	Januschke J (2013) *Nature Cell Biology*
Pericentrin-like protein (Plp) deletion	Mother-centriole-enriched protein. Involved in PCM organization. Negative regulator*	In Metaphase	Lerit D (2013) *JCB*
Bld10 / Cep 135 truncated protein	MT binding protein Necessary for centrosome asymmetry*	In Metaphase	Singh P (2014) *Current Biology*
Wdr62 truncated protein	MT binding protein Suggested to stabilize MT and necessary to recruit Polo #	Not mentioned	Ramdas Nair A (2016) *Cell Reports*

All studies were performed in *Drosophila* neuroblasts.
*: Information obtained at the fly base. #: Information obtained from Ramdas Nair (2016).

One point against this hypothesis is the absence of deleterious effects upon the alteration of centrosome segregation. If centrosome inheritance was a determinant of cell fate, it would be expected to observe a shift in the number of stem cells and the differentiated cells, however this has only been reported in one study after depletion of Nin protein levels [14]. Since this protein has been suggested to participate in centriole maturation[14], it is possible that the observed premature depletion of the radial glial progenitors is given by a lack or alteration of the primary cilium, since this structure is derived from the mother centriole and is dependent on the centriole maturation. As a matter of fact, Paridaen et al.[25], reported that the ciliary membrane is carried by the mother centrosome and inherited preferentially by the progenitor, this results in an earlier accumulation of the transducer protein Smoothened (Smo). Taking these observations into account, it seems plausible that centrosome mis-segregation in the radial glial progenitors results in an altered cilia formation and this affect the sensing of signaling involved in fate determination. It would be interesting to test if the mother centriole is segregated to the daughter cell that needs to respond first to an environmental signal.

In support of a role of the asymmetric centrosome segregation on cell fate, it is known that polarization of cell components is highly relevant to achieve asymmetric cell division. In *Drosophila* neuroblasts, for example, it is reported that the protein complex formed by the atypical protein kinase C (aPKC), partition defective-6 (Par-6) and Par-3, localize at the apical side of the dividing neuroblasts. This results in the phosphorylation and release of Numb from the apical side which allows the activation of Notch, a transcription factor that promotes stemness. On the contrary, Miranda accumulates at the basal side of the cell and recruits Brat and Prospero, this results in the transcriptional activation of the differentiation process and repression of self-renewing[31]. Polarization is also present in asymmetric cell divisions regulated extrinsically, for example, *Drosophila* GSC are exposed to an asymmetric environment where cap/ hub cells have contact with only one side of the GSC and promote self-renewal, whereas lack of contact on the other side promotes differentiation[1]. These asymmetries are not only observed in *Drosophila,* but are conserved among other species [31]. Thus, considering the importance of asymmetry and polarity of internal and external cell factors, it seems plausible that the mother vs daughter centrosome segregations could also play a role in cell fate in asymmetric cell divisions.

It is reported that in response to the mutation of proteins important for the asymmetric MTOC activity of centrosomes, misoriented spindles are observed. However, in most of the cases, the orientation is corrected before metaphase, suggesting that the stereotypical segregation of centrosomes serves as a rough mechanism to set spindle position, followed by a second refined mechanism that helps to properly align it when the first one fails[17,26,28–30]. Hence, in the absence of the refined mechanism, centrosome positioning may be more relevant for fate determination through the orientation of the mitotic spindle. An example of this situation could be during ageing: it is reported that in aged flies there is an increase in the number of GSC with centrosomes misoriented with respect to the niche. Although no misoriented spindles are observed, it seems that cells with misoriented centrosomes arrest until they are properly oriented, probably resulting in the reduction of spermatogenesis and with this an involution of testes [32]. Perhaps during ageing, the stereotypical segregation of centrosomes plays an essential role for the correct positioning of the mitotic spindle, contributing to achieve the asymmetry of cell division.

As future perspectives, since four of the six studies where stereotypical segregation of centrosomes has been observed were performed in *Drosophila,* it would be interesting to analyze what happens in other model organisms like rat, zebra fish, *C. elegans* or chicken in order to compare if this mechanism is conserved among species. In addition, since the type of cells that have been analyzed are related to either neuronal progenitors or the germline; analyzing the centrosome segregation pattern in other cell types like skin of intestinal progenitors might be helpful. Perhaps the inheritance of the mother or daughter centrosomes is influenced by the characteristics of the stem cell or the niche.

Finally, the studies evidencing the asymmetric centrosome segregation also mention that the stereotypical segregation of centrosomes is not always followed, there is a percentage of the cells (15-30%) showing an inverse centrosome inheritance[14,15,19,23]. It might be helpful to track this population and analyze if they present certain abnormalities that could shed light into the consequences of inheriting the "wrong" centrosome under conditions were the observed effect cannot be given by unrelated functions of mutant centrosome proteins.

One standing question is what determines the centrosome that has to be retained by the stem cell. This stereotypical segregation may suggest that something inherent to the centrosomes determines its identity as mother or daughter, so that the PCM regulators localize asymmetrically and this grants the centrosomes different MTOC activity. In a simplistic view, the inherent differences between mother and daughter centrosomes and the fact that they are segregated in a stereotypical way, suggest that this has a biological relevance. It would be easier to retain a random centrosome in the GSC and segregate the other to the differentiated sister, instead of having mechanisms to specifically retain one of the centrosomes.

In conclusion, while it is clear that asymmetric centrosomes are not segregated randomly during asymmetric cell divisions, further research is needed in order to understand, if so, the role that centrosomes have in cell fate, as well as the consequences of not inheriting centrosomes in the stereotypical fashion.

References:

1. Inaba, M. & Yamashita, Y. M. Asymmetric stem cell division: Precision for robustness. *Cell Stem Cell* **11,** 461–469 (2012).

2. Chen, C., Fingerhut, J. M. & Yamashita, Y. M. The ins(ide) and outs(ide) of asymmetric stem cell division. *Curr. Opin. Cell Biol.* **43,** 1–6 (2016).

3. Nigg, E. A. & Stearns, T. The centrosome cycle: Centriole biogenesis, duplication and inherent asymmetries. *Nat. Cell Biol.* **13,** 1154–1160 (2011).

4. Pelletier, L. & Yamashita, Y. M. Centrosome asymmetry and inheritance during animal development. *Curr. Opin. Cell Biol.* **24,** 541–546 (2012).

5. Lerit, D. A., Smyth, J. T. & Rusan, N. M. Organelle asymmetry for proper fitness, function, and fate. *Chromosom. Res.* **21,** 271–286 (2013).

6. Fujita, H., Yoshino, Y. & Chiba, N. Regulation of the centrosome cycle. *Mol. Cell. Oncol.* **3,** e1075643 (2016).

7. Januschke, J., Llamazares, S., Reina, J. & Gonzalez, C. Drosophila neuroblasts retain the daughter centrosome. *Nat. Commun.* **2,** 243 (2011).

8. Paintrand, M., Moudjou, M., Delacroix, H. & Bornens, M. Centrosome organization and centriole architecture: Their sensitivity to divalent cations. *J. Struct. Biol.* **108,** 107–128 (1992).

9. Hoyer-Fender, S. Centriole maturation and transformation to basal body. *Semin. Cell Dev. Biol.* **21,** 142–147 (2010).

10. Azimzadeh, J. & Marshall, W. F. Building the centriole. *Curr. Biol.* **20,** R816–R825 (2010).

11. Yamashita, Y. M. & Fuller, M. T. Asymmetric centrosome behavior and the mechanisms of stem cell division. *J. Cell Biol.* **180,** 261–266 (2008).

12. Reina, J. & Gonzalez, C. When fate follows age: unequal centrosomes in asymmetric cell division. *Philos. Trans. R. Soc. B Biol. Sci.* **369,** 20130466–20130466 (2014).

13. Roubinet, C. & Cabernard, C. Control of asymmetric cell division. *Curr. Opin. Cell Biol.* **31,** 84–91 (2014).

14. Wang, X. *et al.* Asymmetric centrosome inheritance maintains neural progenitors in the neocortex. *Nature* **461,** 947–955 (2009).

15. Yamashita, Y. M., Mahowald, A. P., Perlin, J. R. & Fuller, M. T. Asymmetric Inheritance of Mother Versus Daughter Centrosome in Stem Cell Division. *Science (80-.).* **315,** 518–521 (2007).

16. Rebollo, E. *et al.* Functionally Unequal Centrosomes Drive Spindle Orientation in Asymmetrically Dividing Drosophila Neural Stem Cells. *Dev. Cell* **12,** 467–474 (2007).

17. Rusan, N. M. & Peifer, M. A role for a novel centrosome cycle in asymmetric cell division. *J. Cell Biol.* **177,** 13–20 (2007).

18. Conduit, P. T. & Raff, J. W. Cnn dynamics drive centrosome size asymmetry to ensure daughter centriole retention in Drosophila neuroblasts. *Curr. Biol.* **20,** 2187–2192 (2010).

19. Izumi, H. & Kaneko, Y. Evidence of asymmetric cell division and centrosome inheritance in human neuroblastoma cells. *Proc. Natl. Acad. Sci.* **109,** 18048–18053 (2012).

20. Lechler, T. & Fuchs, E. Asymmetric cell divisions promote stratification and differentiation of mammalian skin. *Nature* **437,** 275–280 (2005).

21. Izumi, Y., Ohta, N., Hisata, K., Raabe, T. & Matsuzaki, F. Drosophila Pins-binding protein Mud regulates spindle-polarity coupling and centrosome organization. *Nat. Cell Biol.* **8,** 586–593 (2006).

22. Siller, K. H., Cabernard, C. & Doe, C. Q. The NuMA-related Mud protein binds Pins and regulates spindle orientation in Drosophila neuroblasts. *Nat. Cell Biol.* **8,** 594–600 (2006).

23. Salzmann, V. *et al.* Centrosome-dependent asymmetric inheritance of the midbody ring in Drosophila germline stem cell division. *Mol. Biol. Cell* **25,** 267–75 (2014).

24. Anderson, C. T. & Stearns, T. Centriole Age Underlies Asynchronous Primary Cilium Growth in Mammalian Cells. *Curr. Biol.* **19,** 1498–1502 (2009).

25. Paridaen, J. T. M. L., Wilsch-Bräuninger, M. & Huttner, W. B. Asymmetric inheritance of centrosome-associated primary cilium membrane directs ciliogenesis after cell division. *Cell* **155,** 333–344 (2013).

26. Januschke, J. *et al.* Centrobin controls mother–daughter centriole asymmetry in Drosophila neuroblasts. *Nat. Cell Biol.* **15,** 241–248 (2013).

27. Gramates LS, Marygold SJ, dos Santos G, Urbano J-M, Antonazzo G, Matthews BB, Rey AJ, Tabone CJ, Crosby MA, Emmert DB, Falls K, Goodman JL, Hu Y, Ponting L, Schroeder AJ, Strelets VB, Thurmond J, Z. P. and the F. C. Fly Base. (2017).

28. Lerit, D. A. & Rusan, N. M. PLP inhibits the activity of interphase centrosomes to ensure their proper segregation in stem cells. *J. Cell Biol.* **202,** 1013–1022 (2013).

29. Singh, P., Ramdas Nair, A. & Cabernard, C. The centriolar protein Bld10/Cep135 is required to establish centrosome asymmetry in drosophila neuroblasts. *Curr. Biol.* **24,** 1548–1555 (2014).

30. Ramdas Nair, A. *et al.* The Microcephaly-Associated Protein Wdr62/CG7337 Is Required to Maintain Centrosome Asymmetry in Drosophila Neuroblasts. *Cell Rep.* **14,** 1100–1113 (2016).

31. Mukherjee, S. & Brat, D. J. in *Results and problems in cell differentiation* **61,** 401–421 (2017).

32. Cheng, J. *et al.* Centrosome misorientation reduces stem cell division during ageing. *Nature* **456,** 599–604 (2008).