

CORRECTION

Correction: The *Drosophila* centriole – conversion of doublets into triplets within the stem cell niche (doi: 10.1242/jcs.172627)

Marco Gottardo, Giuliano Callaini and Maria Giovanna Riparbelli

There was an error published in *J. Cell Sci.* (2015) **128**, 2437-2442 (doi: 10.1242/jcs.172627). In Fig. 1, the image in A was inadvertently duplicated in D. The corrected figure is shown below and the authors apologise to readers for any inconvenience caused.

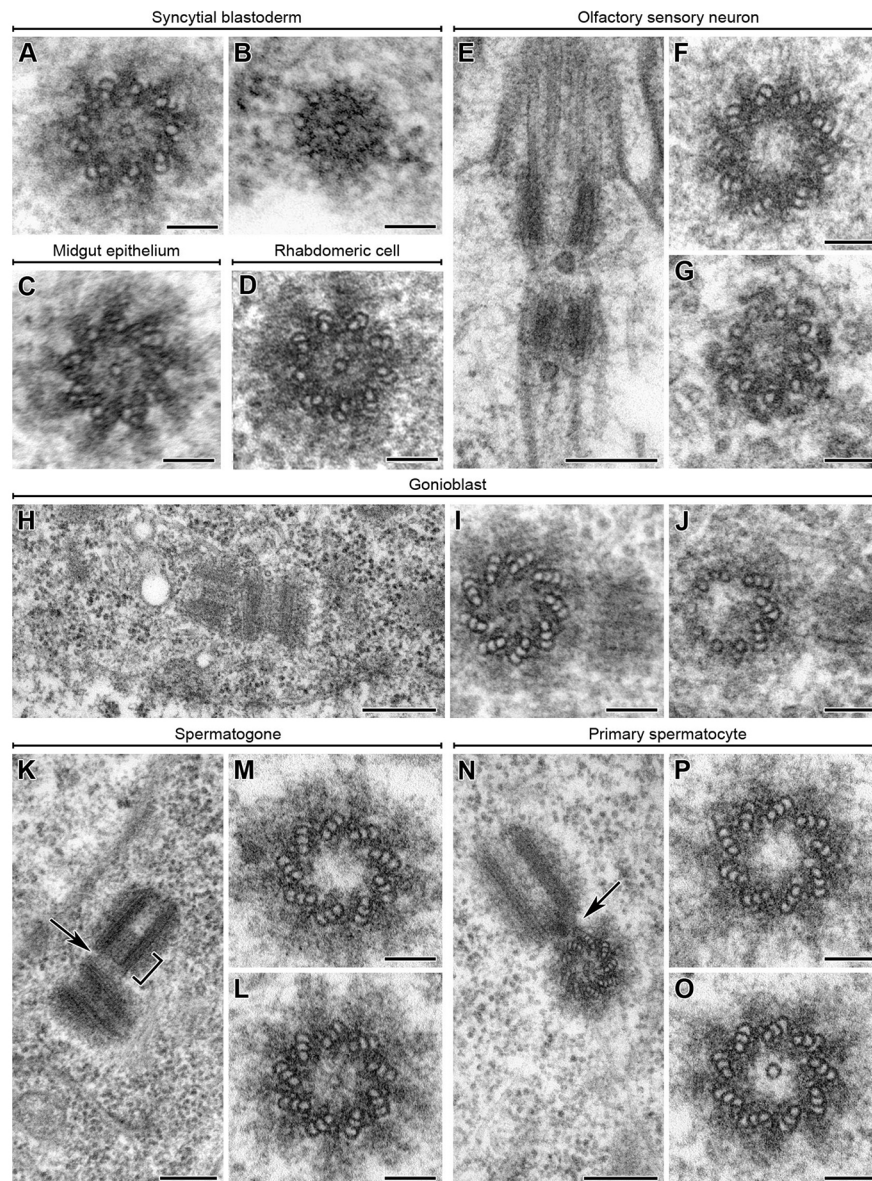


Fig. 1. Double centriole lineage in *Drosophila* cells. Transmission electron microscopy images of centrioles in (A,B) the syncytial blastoderm of the early embryo during the 11th nuclear division cycle, (C) midgut epithelial cells during the germ-band shortening, (D) rhabdomeric cells of the eye imaginal disc, (E–G) the olfactory neuron of the third antennomere, (H–J) gonioblasts, (K–M) spermatogones, and (N–P) early prophase primary spermatocytes. At the onset of duplication the daughter centriole is only composed of the A-tubules (B). The sensory neurons (E) display two distinct centrioles that are aligned in tandem: the mother centriole nucleates the ciliary axoneme and is composed by nine doublets (F), the daughter centriole is shorter and composed by singlets and some doublets (G). The basal and sub-basal regions of the germ cell centrioles display a distinct cartwheel (I,L; bracket in K) or an isolated central tube (O), respectively, whereas the lumen of the subapical region of the centrioles does not contain distinct structures (M,P). A dense fibrillar material is often seen between the basal end of the daughter centriole and the lateral wall of its mother (K,N, arrows). Scale bars: 250 nm (E,H,K,N); 100 nm (A–D,F,G,I,J,L,M,O,P).

SHORT REPORT

The *Drosophila* centriole – conversion of doublets into triplets within the stem cell niche

Marco Gottardo, Giuliano Callaini* and Maria Giovanna Riparbelli

ABSTRACT

We report, here, that two distinct centriole lineages exist in *Drosophila*: somatic centrioles usually composed by microtubule doublets and germ line centrioles characterized by triplets. Remarkably, the transition from doublets to triplets in the testis occurs within the stem cell niche with the formation of the C-tubule. We demonstrated that the old mother centriole, which stays in the apical cytoplasm of the male germline stem cells (GSCs), is invariably composed of triplets, whereas its daughter is always built of mixed doublets and triplets. This difference represents the first documentation of a structural asymmetry between mother and daughter centrioles in *Drosophila* GSCs and might reflect a correlation between the architecture of parent centrioles and their ability to recruit centrosomal proteins. We also found that the old mother centriole is linked to the cell membrane by distinct projections that might play an important role in keeping its apical position during centrosome separation.

KEY WORDS: Centriole, Structural asymmetry, Germcell niche, *Drosophila*

INTRODUCTION

Centrioles are small cylindrical organelles that play the dual role of centrosome organizers and axoneme constructors in cilia and flagella of vertebrate and invertebrate cells (Bettencourt-Dias and Glover, 2007). Dysfunctions in these organelles are associated with a wide range of inherited diseases and cancer (Nigg and Raff, 2009; Bettencourt-Dias et al., 2011). Vertebrate cells usually begin interphase with a centrosome that contains one pair of centrioles that have distinctive morphologies and different abilities to nucleate microtubules (Bornens and Gönczy, 2014). The mother centriole recruits more centrosomal materials and displays characteristic distal appendages involved in microtubule binding and membrane docking. Only the mother centriole can assemble the primary cilium in non-dividing cells (Nigg and Stearns, 2011). The daughter centriole will acquire the distal appendages at the end of interphase and the ability to organize a primary cilium a cell cycle later. Parent centrioles in *Drosophila* lack a distinct structural dimorphism like that observed in vertebrate centrioles (Callaini et al., 1997). They can only be recognized by specific markers such as centrobins and asterless or by the accumulation of the mother centriole of some centrosomal proteins (Reina and Gonzalez, 2014).

Usually the centriole structure remains constant within different tissues of the same organism. Although certain *Drosophila* tissues

present centrioles with doublets, whereas others show triplets, it is actually unclear if these structural differences are correlated with different centriolar lineages. Here, we show that somatic centrioles are usually composed by microtubule doublets whereas triplets characterize the centrioles of the germ cell line. We also found that the old mother centriole, which stays in the apical cytoplasm of the male GSC (Yamashita et al., 2007), is invariably composed of triplets, whereas its daughter is always built from mixed doublets and triplets. This difference represents the first documentation of a structural asymmetry between mother and daughter centrioles in *Drosophila* GSCs and might reflect a correlation between the architecture of the parent centrioles and the unequal distribution of centrosomal proteins. Moreover, the mother centriole is bound to the cell membrane by discrete links that might help to maintain its apical positioning during centrosome separation.

RESULTS AND DISCUSSION

Somatic and germline centrioles – different lineages with common origin

Although the structural features of the animal centriole have been carefully investigated (Winey and O'Toole, 2014), the available evidence regarding the proper architecture of the centrioles in the early *Drosophila* syncytial embryo is rather contradictory (Gonzalez et al., 1998). Hence, we performed a detailed analysis and defined that mature embryonic centrioles were invariably built by nine doublet microtubules and a distinct cartwheel (Fig. 1A). We also found centrioles with nine singlet microtubules (Fig. 1B) often showing lateral hooks that might correspond to forming B-tubules. Therefore, these structures might represent intermediate stages in centriole assembly that will acquire the final architecture later in interphase. The overall morphology of the embryonic centrioles was conserved during further developmental stages (Fig. 1C,D). Centrioles of cultured cells also lack the C-tubule (Cunha-Ferreira et al., 2009). We never found structural differences between mother and daughter centrioles in the somatic cells examined. Parent centrioles could be only recognized by their reciprocal disposition: the daughter centriole is orthogonal to the proximal end of the mother centriole. By contrast, parent centrioles were unusually aligned in tandem and easily distinguishable in auditory and olfactory sensilla that contained sensory neurons (Fig. 1E). The distal centriole that nucleated the ciliary axoneme was built by nine doublet microtubules (Fig. 1F), whereas the proximal one was composed by singlets and one or two doublets (Fig. 1G). This suggests that the daughter centrioles remained incomplete during development of the sensory organ.

The centriole of the germline stem cell (GSC) daughter, the gonio blasts, had nine triplet microtubules (Fig. 1H–J). Sections of its distal end often displayed isolated A-tubules together with doublets and triplets (Fig. 1J), suggesting that tubule elongation is asynchronous. The B- and C-tubules are added sequentially and grow from the basal region of the centriole, unlike in cultured

Department of Life Sciences, University of Siena, 53100 Siena, Italy.

*Author for correspondence (callaini@unisi.it)

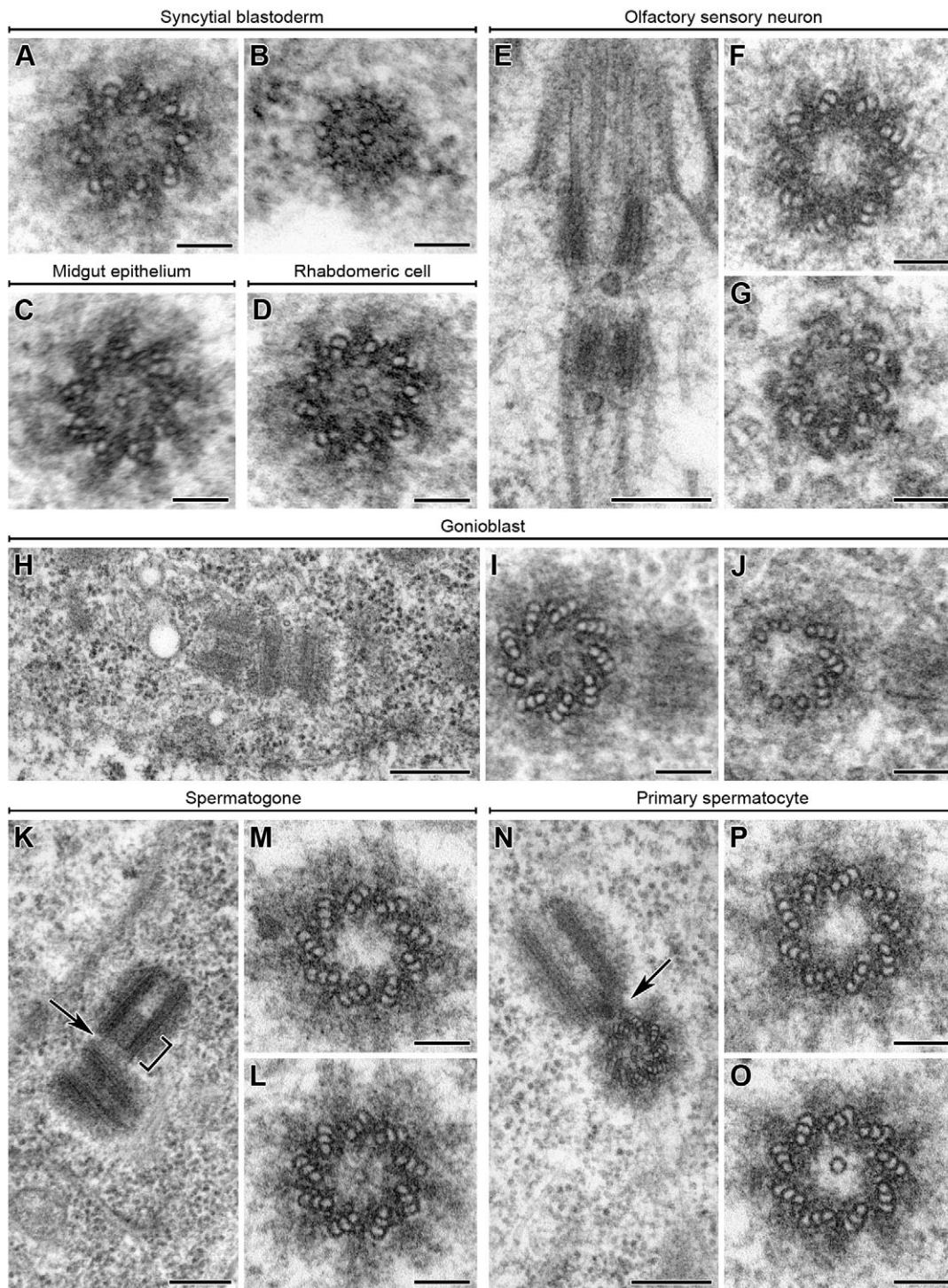


Fig. 1. Double centriole lineage in *Drosophila* cells. Transmission electron microscopy images of centrioles in (A,B) the syncytial blastoderm of the early embryo during the 11th nuclear division cycle, (C) midgut epithelial cells during the germ-band shortening, (D) rhabdomic cells of the eye imaginal disc, (E–G) the olfactory neuron of the third antennomere, (H–J) gonioblasts, (K–M) spermatogones, and (N–P) early prophase primary spermatocytes. At the onset of duplication the daughter centriole is only composed of the A-tubules (B). The sensory neurons (E) display two distinct centrioles that are aligned in tandem: the mother centriole nucleates the ciliary axoneme and is composed by nine doublets (F), the daughter centriole is shorter and composed by singlets and some doublets (G). The basal and sub-basal regions of the germ cell centrioles display a distinct cartwheel (I,L; bracket in K) or an isolated central tube (O), respectively, whereas the lumen of the subapical region of the centrioles does not contain distinct structures (M,P). A dense fibrillar material is often seen between the basal end of the daughter centriole and the lateral wall of its mother (K,N, arrows). Scale bars: 250 nm (E,H,K,N); 100 nm (A–D,F,G,I,J,L,M,O,P).

vertebrate cells in which the growth of the B- and C-tubules is bidirectional (Guichard et al., 2010). Triplet microtubules elongated further during the spermatogonial mitoses (Fig. 1K–M) and

throughout prophase of the first meiosis (Fig. 1N,O) to reach their full length at the onset of the first prometaphase. We were unable to find ultrastructural details to distinguish parent centrioles

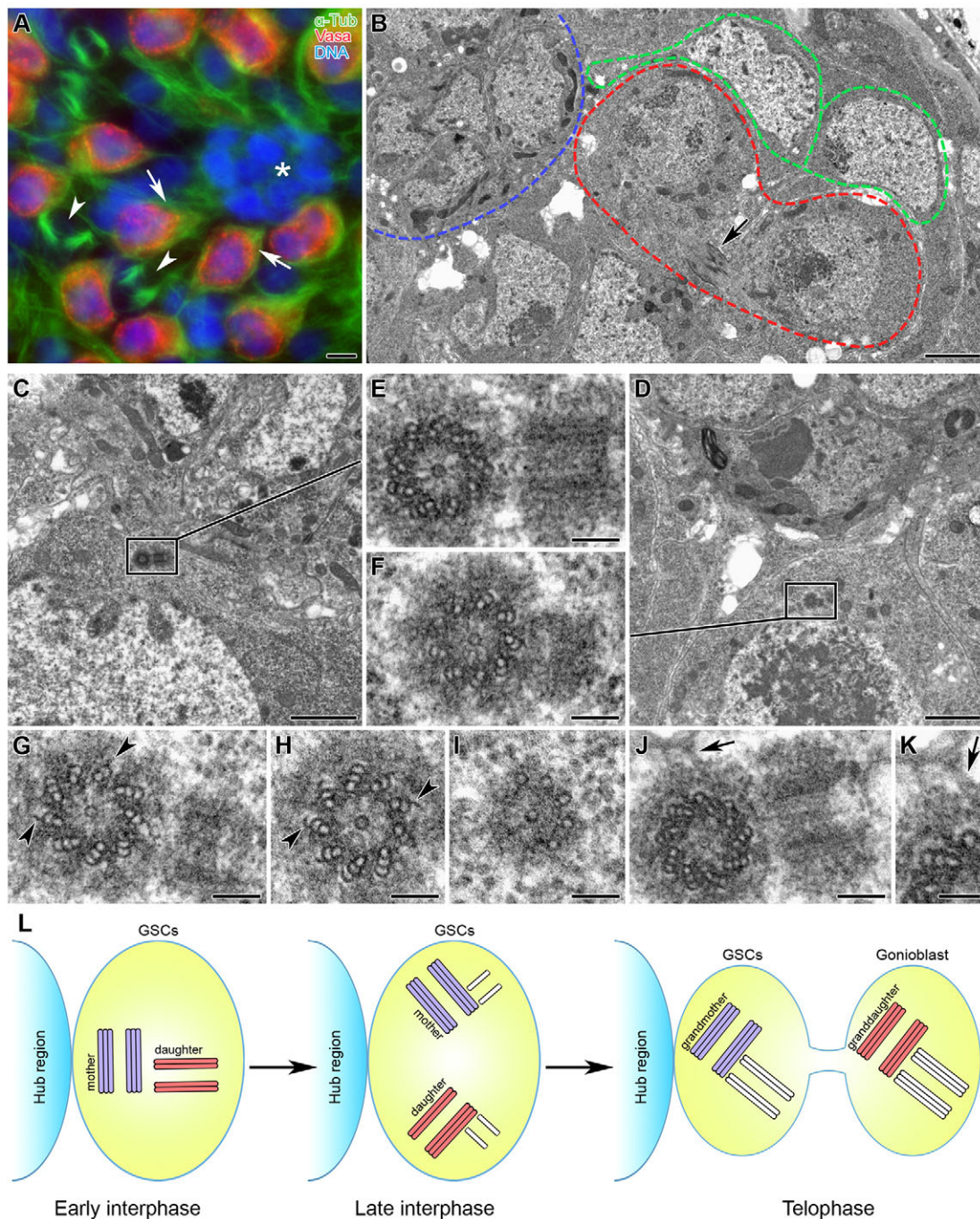


Fig. 2. *Drosophila* stem cell niche – asymmetric organization of the parent centrioles. (A) The stem cell niche is composed of an apical cluster of postmitotic hub cells (asterisk) that is surrounded by GSCs (arrows) and CySCs (arrowheads). Germ cells are visualized in red by anti-Vasa antibody staining, microtubules are green, DNA is blue. The picture shows a slight asynchrony among the divisions of the GSCs and CySCs: the GSCs are in interphase, whereas the two flanking CySCs are in metaphase. This asynchrony is also confirmed by the transmission electron microscopy picture (B) in which CySCs (green dotted line) are in interphase, whereas the GSCs (red dotted line) have completed telophase, as judged by the midbody remnant (arrow). Both GSCs and CySCs contact the hub region (blue dotted line). Low magnification (C,D) and details of centriole pairs (E–G) at the apical side of the GSCs. The mother centrioles are composed by triplets (E), doublets (F), or mixed doublets and triplets (G). The mixed organization also characterizes the daughter centrioles (H) found in the apical region of the GSCs. The incomplete triplets of both parent centrioles show lateral hooks of different extensions (arrowheads). Procentrioles (I) at the onset of centriole duplication look like those found in somatic cells and are formed by nine single peripheral microtubules placed on a thin ring of dense material and by a central tube from which some radial spokes elongate. The mother centrioles (J) at the apical end of the interphase GSC are often linked to the cell membrane by distinct fibers (J,K, arrows). (L) Schematic representation of the centriole dynamics during the asymmetric division of the male GSCs: the old mother and daughter centrioles are blue and red, respectively, the new daughters are white. Scale bars: 2.5 μ m (A); 1 μ m (B–D); 100 nm (E–J); 50 nm (K).

among them. Rather, the ability of both the centrioles within the same pair to nucleate a ciliary axoneme points to the loss of daughter characteristics upon the acquisition of mother

properties. Often parent centrioles were linked together by a dense fibrillar material (Fig. 1K,N) that might play a role in centriole engagement.

Why would the centriole architecture be different in somatic and germ line cells? It has been suggested that the evolutionary pressure applied to the ciliary and flagellar axoneme might affect the architecture of the basal bodies (Carvalho-Santos et al., 2011). Thus, the structural differences we observed might be explained by the special role of the germline centrioles to nucleate the cilium-like projections of the primary spermatocytes, which at the onset of spermatid differentiation elongate in the sperm axoneme (Gottardo et al., 2013; Basiri et al., 2014). However, the female germ cells also have centrioles with triplets (Mahowald and Strassheim, 1970) but lack ciliary projections. Moreover, the presence of the C-tubule seems to be dispensable in *Drosophila* for the proper assembly of an axoneme since the sensory cilia of auditory and olfactory sensilla are assembled from basal bodies composed of only doublets. However, when the C-tubule dynamics is defective in *unc* *Drosophila* mutants the growth of centrioles and axonemes is severely affected (Gottardo et al., 2013). Thus, at least during *Drosophila* male gametogenesis, the C-tubule seems to be essential for the proper dynamics of centrioles and to the correct assembly of the ciliary projections.

Transition from doublets to triplets – the stem cell niche

Given that the precursors of the germ line, the pole cells, have centrioles with doublet microtubules (data not shown), whereas the centrioles of the gonoblasts have triplets (Fig. 1I), the modification of the centriole architecture might occur within the stem cell niche (Fig. 2A,B). Thus, we analyzed the ultrastructure of the centrioles in interphase GSCs, when the centriole pairs are found near the hub

boundary (Fig. 2C,D). We easily distinguished parent centrioles among them by their reciprocal position. In images showing the mother centriole in cross section, the daughter centriole was always visible in a longitudinal orientation (Fig. 2E–G), whereas images showing cross sections of the daughter never contained the mother centriole (Fig. 2H,I). This is because the daughter centriole is always orthogonal to the basal end of the mother. We found three classes of mother centrioles: centrioles with nine triplets (Fig. 2E; 15 out of 39 centrioles), centrioles with nine doublets (Fig. 2F; 5 out of 39 centrioles) and centrioles with mixed doublets and triplets (Fig. 2G; 19 out of 39 centrioles).

Centrosomes of different ages have been suggested to have different functions during the asymmetric divisions of neuroblasts (Conduit and Raff, 2010; Januschke et al., 2011), and male (Yamashita et al., 2007) and female (Salzmann et al., 2014) germ stem cells in *Drosophila*. Specifically in male gametogenesis, the mother centrosome remains in the GSC close to the hub boundary, whereas the daughter one is displaced into the differentiating gonoblast. These centrosomes are distinct from each other owing to their age and to their different ability to organize a functional microtubule-organizing center (MTOC), which might enable the mother centriole to maintain its apical position (Yamashita et al., 2007). Therefore, to define whether the different structural patterns of mother centrioles were correlated with their age, we took serial sections throughout the apical cytoplasm of the GSCs, the region where centriole duplication occurred. We expected one centriole pair in early interphase and two distinct centriole pairs in late interphase. We found that mothers with triplets were usually

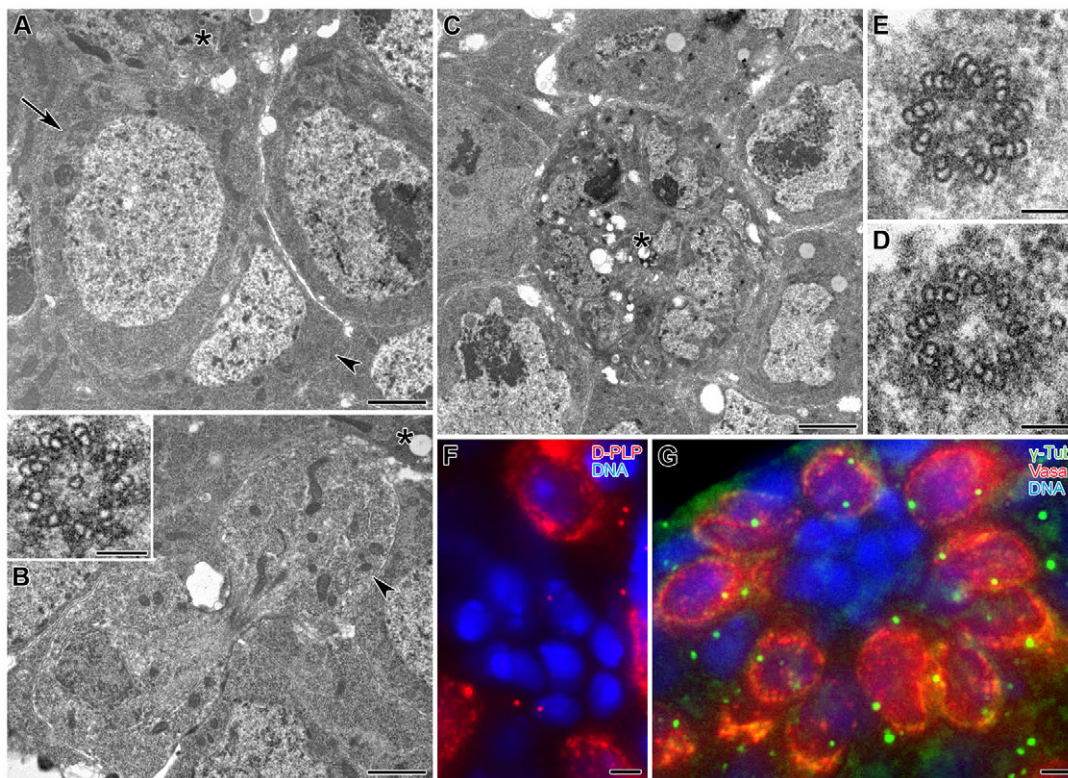


Fig. 3. Hub and CySCs – the somatic counterparts of GSCs. (A) Low magnification of a stem cell niche showing a GSC (arrow) with its accompanying CySC (arrowheads), both in contact with the hub region (asterisk). (B) Detail of a CySC during late telophase: the daughter cell (arrowhead) in contact with the hub (asterisk) maintains its stem cell properties; the inset represents a CySC centriole. (C) Low magnification of the hub region (asterisk) surrounded by the stem cells. (E,D) Two consecutive sections of a centriole found in the hub region showing the lack of a distinct cartwheel and the incomplete microtubule pattern. Localization of D-PLP (F, red) and γ -tubulin (G, green) in the stem cell niche; note that both the proteins are found in GSCs and CySCs, but they are barely detectable in the hub region. DNA is blue, germ cells are stained for Vasa (red). Scale bars: 1 μ m (A–C); 100 nm (B inset, E,D); 2.5 μ m (F,G).

associated with fully elongated daughters that had a smaller diameter (Fig. 2E) likely due to the incomplete C-tubule. Serial sections failed to reveal additional centrioles in this region. Therefore, stem cells were at the beginning of interphase and the centriole pairs examined were inherited from the previous cell cycle. Mothers with doublets or mixed tubules were always associated with short procentrioles (Fig. 2F,G) and were found within the apical cytoplasm of the GSCs together with an additional centriole pair. Daughter centrioles never displayed a complete set of nine triplets when they resided in the apical side of the GSC, but had triplets mixed with doublets that held lateral hooks of various lengths (Fig. 2H). Procentrioles consisting of nine single A-tubules and a central tube were often found (Fig. 2I). Centrioles with triplets were found in early interphase and might represent the older mothers, whereas centrioles with mixed tubules are likely to be the daughters that are turned into new mothers at the beginning of centriole duplication. Our observations suggest that the daughter centrioles acquire the complete set of microtubule triplets during the migration to the opposite pole of the nucleus. By contrast, the newborn daughter associated with the stationary mother fully elongates, but seems to complete its microtubule wall during the next cell cycle (Fig. 2L).

The failure to complete the microtubule set does not prevent centriole duplication in *Drosophila*, unlike daughter centrioles in vertebrate cells, which cannot support the assembly of a new procentriole until completion of their maturation process. However, only centrioles with a full set of nine triplets organize functional centrosomes in *Drosophila* GSCs. Therefore, the acquisition of the C-tubule and the completion of the triplet set might represent an aspect of the maturation process of the male germline centrioles. This could explain why the mother centrosome supports the nucleation of a robust aster of microtubules that might enable its anchoring at the apical side of the GSC (Yamashita et al., 2007). In this respect, the distinct projections that link the old mother centriole to the cell membrane (Fig. 2J,K) might play an important role in keeping the centriole to the apical position.

The somatic counterpart in the stem cell niche – hub cells and CySCs

Male GSCs are flanked in the niche by a pair of cyst stem cells (CySCs) (Fig. 3A). The asymmetric divisions of these cells were slightly uncoupled, so that each GSC daughter had his accompanying somatic cell before its division was completed (Fig. 2B; Fig. 3B). The centrioles in CySCs looked like those of the somatic centrioles, and were built from nine doublet microtubules with a distinct cartwheel (Fig. 3B, inset).

The centrioles of the hub cells (Fig. 3C) resembled those of GSCs because they were built by mixed doublets and triplets (Fig. 3D,E), although they lacked lateral hooks and a distinct cartwheel. Despite examining serial sections of different hub cells ($n=21$), we only found single centrioles and we never observed procentrioles. Therefore, we assessed whether the centriole number was reduced by staining larval, pupal and adult testes with antibodies against *Drosophila* Pericentrin-like protein (D-PLP) (Martinez-Campos et al., 2004) and γ -tubulin. Unlike GSCs and CySCs, which showed one or two spots, a few hub cells displayed a distinct staining (Fig. 3F,G) with these antibodies. The missing cartwheel, the incomplete triplets, the lack of daughters and the unsuccessful recruitment of γ -tubulin suggests that the hub centrioles are not functional. This agrees with the general view that the hub is composed of post-mitotic cells that are terminally differentiated. We, indeed, never found mitotic figures within the hub regions

examined ($n=87$). However, the conversion of quiescent hub cells into functional CySCs has been recently reported (Hétié et al., 2014), suggesting that the hub cells might be able to rescue their mitotic activity. Although this possibility contrasts with the findings of abnormal centrioles, we cannot exclude the possibility that these unusual centrioles convert into functional ones or that new centrioles can assemble *ex novo*.

MATERIALS AND METHODS

Indirect immunofluorescence staining

Oregon R flies were raised on standard *Drosophila* medium at 24°C. Testes were dissected from larvae, pupae and adults in PBS and fixed as previously reported (Riparbelli et al., 2009). We used the following antibodies: mouse anti- α -tubulin (1:100; Sigma-Aldrich), mouse anti- γ -tubulin GTU88 (1:100; Sigma-Aldrich); anti-D-PLP (1:1500; Rodrigues-Martins et al., 2007) and rabbit anti-Vasa (1:400; Lasko and Ashburner, 1988). After washing in PBS with 0.1% bovine serum albumin (BSA) the samples were incubated for 1 h at room temperature with the appropriate Alexa-Fluor-488- and -555-conjugated secondary antibodies. DNA was visualized by incubation for 3–4 min in Hoechst 33258 (Sigma-Aldrich). Samples were observed by using an Axio Imager Z1 (Carl Zeiss) microscope equipped with an AxioCam HR camera (Carl Zeiss). Grayscale digital images were collected separately and then pseudocolored and merged using Adobe Photoshop V.7.0 software (Adobe Systems).

Transmission electron microscopy

Testes dissected from pupae were fixed overnight in 2.5% glutaraldehyde in PBS at 4°C and processed as described previously (Gottardo et al., 2013). Ultrathin sections were stained with uranyl acetate and lead citrate, and then observed with a Tecnai G2 Spirit EM (FEI) equipped with a Morada CCD camera (Olympus).

Acknowledgements

The authors would like to thank Paul Lasko (Department of Biology, McGill University, Canada) and Ana Rodrigues-Martins (Instituto Gulbenkian de Ciência, Portugal) for generously providing the antibodies.

Competing interests

The authors declare no competing or financial interests.

Author contributions

M.G., M.G.R. and G.C. conceived the project. M.G. and M.G.R. performed experiments. G.C. wrote the manuscript.

Funding

This work was supported by a grant from PRIN2012 to G.C.

References

- Basiri, M. L., Ha, A., Chadha, A., Clark, N. M., Polyakov, A., Cook, B. and Avidor-Reiss, T. (2014). A migrating ciliary gate compartmentalizes the site of axoneme assembly in *Drosophila* spermatids. *Curr. Biol.* **24**, 2622–2631.
- Bettencourt-Dias, M. and Glover, D. M. (2007). Centrosome biogenesis and function: centrosomes brings new understanding. *Nat. Rev. Mol. Cell Biol.* **8**, 451–463.
- Bettencourt-Dias, M., Hildebrandt, F., Pellman, D., Woods, G. and Godinho, S. A. (2011). Centrosomes and cilia in human disease. *Trends Genet.* **27**, 307–315.
- Bornens, M. and Gönczy, P. (2014). Centrosomes back in the limelight. *Phil. Trans. R. Soc. B Biol. Sci.* **369**, 20130452.
- Callaini, G., Whitfield, W. G. F. and Riparbelli, M. G. (1997). Centriole and centrosome dynamics during the embryonic cell cycles that follow the formation of the cellular blastoderm in *Drosophila*. *Exp. Cell Res.* **234**, 183–190.
- Carvalho-Santos, Z., Azimzadeh, J., Pereira-Leal, J. B. and Bettencourt-Dias, M. (2011). Evolution: Tracing the origins of centrioles, cilia, and flagella. *J. Cell Biol.* **194**, 165–175.
- Conduit, P. T. and Raff, J. W. (2010). Cnn dynamics drive centrosome size asymmetry to ensure daughter centriole retention in *Drosophila* neuroblasts. *Curr. Biol.* **20**, 2187–2192.
- Cunha-Ferreira, I., Bento, I. and Bettencourt-Dias, M. (2009). From zero to many: control of centriole number in development and disease. *Traffic* **10**, 482–498.
- Gonzalez, C., Tavosanis, G. and Mollinari, C. (1998). Centrosomes and microtubule organization during *Drosophila* development. *J. Cell Sci.* **111**, 2697–2706.

- Gottardo, M., Callaini, G. and Riparbelli, M. G.** (2013). The cilium-like region of the *Drosophila* spermatocyte: an emerging flagellum? *J. Cell Sci.* **126**, 5441-5452.
- Guichard, P., Chrétien, D., Marco, S. and Tassin, A.-M.** (2010). Procentriole assembly revealed by cryo-electron tomography. *EMBO J.* **29**, 1565-1572.
- Hétié, P., de Cuevas, M. and Matunis, E.** (2014). Conversion of quiescent niche cells to somatic stem cells causes ectopic niche formation in the *Drosophila* testis. *Cell Rep.* **7**, 715-721.
- Januschke, J., Llamazares, S., Reina, J. and Gonzalez, C.** (2011). *Drosophila* neuroblasts retain the daughter centrosome. *Nat. Commun.* **2**, 243.
- Lasko, P. F. and Ashburner, M.** (1988). The product of the *Drosophila* gene *vasa* is very similar to eukaryotic initiation factor-4A. *Nature* **335**, 611-617.
- Mahowald, A. P. and Strassheim, J. M.** (1970). Intercellular migration of centrioles in the germarium of *Drosophila melanogaster*: an electron microscopic study. *J. Cell Biol.* **45**, 306-320.
- Martinez-Campos, M., Basto, R., Baker, J., Kernan, M. and Raff, J. W.** (2004). The *Drosophila* pericentrin-like protein is essential for cilia/flagella function, but appears to be dispensable for mitosis. *J. Cell Biol.* **165**, 673-683.
- Nigg, E. A. and Raff, J. W.** (2009). Centrioles, centrosomes, and cilia in health and disease. *Cell* **139**, 663-678.
- Nigg, E. A. and Stearns, T.** (2011). The centrosome cycle: centriole biogenesis, duplication and inherent asymmetries. *Nat. Cell Biol.* **13**, 1154-1160.
- Reina, J. and Gonzalez, C.** (2014). When fate follows age: unequal centrosomes in asymmetric cell division. *Phil. Trans. R. Soc. B Biol. Sci.* **369**, 20130466.
- Riparbelli, M. G., Colozza, G. and Callaini, G.** (2009). Procentriole elongation and recruitment of pericentriolar material are downregulated in cyst cells as they enter quiescence. *J. Cell Sci.* **122**, 3613-3618.
- Rodrigues-Martins, A., Riparbelli, M., Callaini, G., Glover, D. M. and Bettencourt-Dias, M.** (2007). Revisiting the role of the mother centriole in centriole biogenesis. *Science* **316**, 1046-1050.
- Salzmann, V., Chen, C., Chiang, C.-Y. A., Tiyaboonchai, A., Mayer, M. and Yamashita, Y. M.** (2014). Centrosome-dependent asymmetric inheritance of the midbody ring in *Drosophila* germline stem cell division. *Mol. Biol. Cell* **25**, 267-275.
- Winey, M. and O'Toole, E.** (2014). Centriole structure. *Phil. Trans. R. Soc. B* **369**, 20130457.
- Yamashita, Y. M., Mahowald, A. P., Perlin, J. R. and Fuller, M. T.** (2007). Asymmetric inheritance of mother versus daughter centrosome in stem cell division. *Science* **315**, 518-521.