Centrosome organization and functions

Michel Bornens

Version of Record: https://www.sciencedirect.com/science/article/pii/S0959440X20301986
Manuscript_e14878dbc4ceab1350f409ec939052fa

Current Opinion in Structural Biology **ISSUE THEME:** VOLUME 6: Centrosomal organization and assemblies

Centrosome Organization and Functions

Abstract

The centrosome, discovered near 1875, was named by Boveri when proposing the chromosomal theory of heredity. After a long eclipse, a considerable amount of molecular data has been accumulated on the centrosome and its biogenesis in the last 30 years, summarized regularly in excellent reviews. Major questions are still at stake in 2021 however, as we lack a comprehensive view of the centrosome functions. Cell autonomous polarity and its transmission through cell division, appears as a unifying theme for the numerous centrosome functions [1], but specific mechanisms are often unknown. I will first try to see how progress towards a unified view of the role of centrosomes during evolution is possible, and then review recent data on only some of the many important questions raised by this organelle.

Towards a unified view of the centrosome in Evolution?

The membrane-less centrosome is formed about the conserved plasma membrane-associated and conserved centriole/basal body of protozoans, which is a derived character of eukaryotes containing hundreds of conserved proteins [2-6]. In vertebrates, the beating flagellum/cilium is conserved only in the male germ line, and in some of their specialized epithelial cell lineages having numerous beating cilia at their apical side [7]. Instead, depending on the cell type or in the same cell at different moments, the centrosome associates to the plasma membrane by its mother-centriole distal end, and grow a non-motile (9+0) primary cilium acting as a sensory design [8-11]. The presence of ciliated centrosomes in organisms may depend on the topological environment of individual cells [12].

The centrosome plays a key role in the asymmetry of division as it contains two centrioles with one or several generations between them: the two centrosomes of a stem cell distribute according to a specific pattern in all differentiating systems [13,14]. As recently demonstrated for the yeast Spindle Pole Body (SPB) [15], this differential distribution may be critical for animal cell ageing [16]. It is present almost exclusively in two multicellular groups forming the super-clade of Amorphea [17, 18; see Azimzadeh contribution]. First in the Opisthokonta, as a primary cilium/centrosome organ in the animal lineage, and as an acentriolar centrosome, the SPB in the fungus lineage. Second, in the Amoebozoa, as a Nucleus Associated Body (NAB), in unicellular organisms like *D. discoideum,* that are able to make transient multicellular organisms [19]. A comprehensive account of the functional evolution of the primary cilium/centrosome organ will have to clarify the significance of having two centrioles, mother and daughter, and why it is present in most cells of multicellular organisms capable of locomotion, yet being absent in some other cells, like myotubes [20]. It will have also to build a mechanistic description on how centrosome and cell periphery are permanently communicating, making a sort of unique compartment ensuring the interface between the cell inside and the outside [21]. Finally, the functional significance of non-canonical centrioles in Ecdysozoa will have to be clarified. They have lost a great number of centriole proteins that are present in both unicellular protozoans and vertebrates [22]. As for the simpler acentriolar centrosome, why is it present as a SPB only in

multicellular fungi (the unicellular yeasts are the result of secondary transitions) that are unable of locomotion, and as a NAB in cells that are able to make transient multicellular organisms able of locomotion? The NAB shares with the centrosome the property of being cytoplasmic and linked to the nucleus in a flexible manner [31], contrasting with the SPB which is tightly associated with the nuclear surface or even inserted into the nuclear envelope. The mode of association to the nucleus, as well as the three dimensional core structure, must have functional correlates through the cytoskeleton organized by the centrosome: these are likely to bear on cell and tissue morphogenesis, but how? The morphogenetic power of the centrosome-based microtubule aster is shown during *Drosophila* early embryogenesis, which leads to about 6 000 nuclei within a common cytoplasm, each with an associated cytoplasmic body without plasma membrane. And yet, the secretory membrane system is organized in functionally compartmentalized units around individual nuclei, with the normal polarity before cell formation [23]. Uncoupling centrosome and nuclear divisions in the syncytium before nuclei migration to the embryo periphery in a *gnu* locus mutation [24], demonstrated that centrosomes were able to double at the correct pace and reorganize microtubules, actin and spectrin networks and plasma membrane in the syncytium. Thus the centrosome has a major role in organizing and polarizing the perinuclear cytoplasm in the absence of plasma membrane. It coordinates all the early embryo stages until cell formation, although it is largely dispensable for further development [25,26]. This suggests that major functions concentrated on the centrosome in vertebrate distal and subdistal appendages on the mother centriole in *Drosophila* development are either not necessary or associated with other structures in Ecdysozoa, allowing the effects of centrosome loss to be buffered. The absence of the distal part of centrioles and thus of the would support this view [27].

A burning question is the evolutionary origin of the centrosome, and first that of the centriole/basal body and the 9+2 axoneme. They are only speculations so far [28,29]. Micro-organisms having the ancestral means for gliding motility, a very ancestral mode of locomotion [30], would also be candidates for showing organelles that predate centriole/basal body function or behavior. For example, mycoplasma such as *M. pneumonia,* so far known for their small size, minute genome, and total lack of a cell wall, are polarized, fusiformed bacteria which, instead of a polarized flagellum or a stalk like *Caulobacter crescentus* [31], have a cytoskeletal 'attachment organelle' involved in polarity, adherence, motility and cell division [32]. Moreover, its biogenesis seems to predate that of basal bodies: a new 'attachment organelle' forms in the vicinity of the old one through an apparent conservative mechanism and migrates to the other pole of the cell before its division together with the new nucleoid to which it is attached [32].

A physical view of the centrosome organization

The centrosome is a compact organelle with a toroidal organization of the pericentriolar material (PCM) with respect to the mother centriole, revealed by super-resolution optical microscopy [2,33-34]. The PCM increases considerably its size and capacity to nucleate microtubules during mitosis [35,36]. In 2014, mitotic centrosomes from *C. elegans* embryos were described as autocatalytic droplets of pericentriolar material organized by centrioles [37,38]. This view drew the attention of the field to the importance of phase transition mechanisms [39,40]. A growing interaction between cell biologists and polymer physicists has developed on the genesis

and stability of membrane-less organelles in time and space and on the regulation of phase transitions. (See Woodruff contribution).

How can the mature centriole organize PCM in mitosis? The inner layer of molecules is recruited by the centriole, whereas molecules in the outer layer are recruited through self-assembly or self-oligomerization. The recruitment of PCM proteins apparently correlates with the developing structure of the daughter centriole [41,42]. Thus, beyond its direct structural aspect, the PCM-centriole interface is an important question as it bears on how the daughter centriole become a mother centriole, i.e a centrosome. How can centrioles provide a unique and highly specific platform in cells that is recognized at physiological concentration by centriolar MT-binding proteins? Several proteins have been shown to participate in the process of daughter centriole acquiring the ability to reproduce, such as SAS7 in the worm *C. elegans,* which has a limited homology with Chibby in *Drosophila,* implicated in centriole-to-basal body conversion [42-44]. Thus, the maturation of daughter centrioles and the participation of centrioles in cilia formation may involve similar mechanism. The luminal centriole protein CEP44, an essential protein in human cells that binds to the A-microtubules and interacts with the inner protein POC1B, has been shown to affect centriole structure and centriole conversion in RPE1 h-TERT cells [41]. The loading of CEP44 and POC1B to the lumen of centrioles downstream of Cep295 and the action of δ- and ε-tubulin would result in the formation of the centriole blades, showing that the developing centriolar structure is necessary for recruitment of PCM proteins. Interestingly, there is no CEP44 homologue in *D. melanogaster* suggesting once more important differences between vertebrates and insects in the maturation of the daughter centriole [41].

During interphase, the so-called satellites are another type of membrane-less structures concentrated in the vicinity of the centrosome. Their functions have long been puzzling. Recently, several reports have significantly progressed in understanding their functions [45-47]. This question is fully addressed in F. Gergely contribution.

The distal and the subdistal appendages

Correlative super-resolution microscopy and EM has been used to determine where distal appendage proteins localize in relation to the centriole microtubules and appendage electron densities [48]. Distal appendages undergo a dramatic ultra-structural reorganization before mitosis, during which they temporarily lose outer components, while inner components maintain a nine-fold organization. Mammalian distal appendages associate with two adjacent centriole microtubule triplets via an elaborate filamentous base and appear as almost radial finger-like protrusions. Thus, these findings modify seriously the traditional view of mammalian distal appendages as a pinwheel-like structures that are maintained throughout mitosis. Instead of a space between each pinwheel blade, distal appendages reveal an unprecedented architectural and functional organization at the base of mammalian primary cilia [49]. Classical transmission EM had clearly indicated the proximity of distal and subdistal appendages, although they have very different functions. The super-resolved molecular architecture of subdistal appendages has suggested that they are indeed structurally or functionally related to distal appendages, implicating a regulatory mechanism of microtubule anchoring around the centrosome [50]. It has been convincingly shown that distal appendages are displaced from the mother centriole prior to mitosis

under the control of Nek2 kinase [51]. Overexpression of active Nek2A, but not kinase-dead Nek2A, prematurely displaced distal appendages from the interphase centrosomes in (RPE1) cells.

The centrosome as an actin-nucleating center

The way in which the actin and the tubulin networks interact and regulate each other is a critical question for a better understanding of cell dynamics and cell polarity. A ground-breaking result in the field was the demonstration in 2016 that the centrosome can also nucleate actin microfilaments [52,53]. The mechanism involves the regulation of the nucleation of microtubules by actin microfilaments, thus explaining the nucleation by the centrosome of either microfilaments or microtubules [54]. The presence of actin at the centrosome provides an additional spatial region where the two filaments systems can interact to regulate one another's behavior. By using both *in vitro* and *in vivo* assays to study centrosomal actin nucleation as cells pass through mitosis, it was shown that mitotic exit is accompanied by a burst in cytoplasmic actin filament formation, leading to the accumulation of actin around centrosomes as cells enter anaphase and to a corresponding reduction in the density of centrosomal microtubules [55]. Mitotic regulation of centrosomal WASH and the Arp2/3 complex seems to control local actin nucleation, to tune the levels of centrosomal microtubules during passage through mitosis. Another work showed that Arp2/3 complex inhibition decreased spindle actin [56]. As a consequence, mitotic spindle formation was impaired, which resulted in disorganized chromosomes congression and ultimately mitotic defects in non-transformed cells. Thus centrosomal Arp2/3 complex activity plays a role in the maintenance of genomic integrity during mitosis. It was also shown independently that prophase specific perinuclear actin in connection with polymerizing microtubules regulates the positioning of centrosome separation before nuclear envelope breakdown [57]. This depends apparently on the interaction between the LINC complex and actin: breaking this link results in centrosome mis-positioning and an increase in sister chromatid segregation errors. It is likely that the actin nucleating activity of centrosomes plays a role also in this mis-positioning. Revisiting the function of the centrosome in neuronal differentiation, a recent review has argued that centrosomal F-actin could be important to understand how neurons achieve distinct functional domains, one axon and several dendrites, which allow them to connect properly [58].

Basal bodies/centrioles from epithelial ciliated cells are known for long to be associated with acto-myosin [59]. However, only recently has the functional significance of this association been clarified in the brain ependymal cells [60]: a coordination between ciliary beating and actin organization exists that contributes to the protection of cilia and basal bodies/centrioles against the shear stress generated by ciliary beating and the associated fluid flow. Amazingly, disruption of the apical actin network causes basal bodies/centrioles to detach from the apical surface of ependymal cell. Thus, it is the cilia beating that controls the apical actin network around basal bodies/centrioles to protect them.

The centrosome positioning and cell polarity

Amongst all the organelles, the centrosome, as a single copy-organelle able to organize the cytoskeleton, and thus the distribution of the other organelles, at the cell scale, is bound to have a

dominant role in defining cell polarity [12, 61]. Its positioning has been looked at from different approaches [62,63, for a review 64], demonstrating, as expected, a pleiotropic regulation for this organelle positioning. The centrosome is a 'central body' mainly in eggs, and in free-living animal cells such as white blood cells. In cells within tissues, the centrosome is not at the cell center, but close to the cell periphery on the contrary. Indeed, it was demonstrated with kidney cells cultured on adhesive micro-patterns, that cell confinement could control centrosome positioning and cell polarity [65]. Actin play a specific role, not only for centrosome segregation during mitosis, but also in centrosome positioning during interphase [66]. It is the interaction of the MTs with the actin network rather than the cell contour itself which matters.

When the centrosome is ciliated, i-e in the majority of post-mitotic cells in the body, the mother centriole is physically associated with the plasma membrane, where it templates a primary cilium and controls the intra-ciliary traffic. Regulation of microtubule dynamics nucleated by the mother centriole is critical for its repositioning [67]. Centrosome repositioning was shown to be an early event of epithlial-to-mesenchymal transition in cultured conditions, before cells scattering proper, in elegant experiments using adhesive micro patterns. This demonstrated the critical role of this organelle in controlling cell polarity [68].

However, half of the MT nucleation in human RPE1 cells occurs at the Golgi Apparatus (GA) and relies on AKAP450 interaction with GM130 [69]. Is there a crosstalk between the GA and the centrosome? At the G2/M transition, when the centrosome MT nucleation activity increases, the activity at the GA is switched off and fully recovers after cell division. To examine the situation in interphase, gene ablation of four γ-TuRC canonical receptors and the Plk4 inhibitor centrinone to induce centrosome loss were used [70]. It showed that MT nucleation is a hierarchically regulated process: ablation of the centrosome strongly enhanced MT nucleation at the GA, while inhibition of GA-associated MT nucleation did not affect centrosome activity. Only silencing both MT nucleation activities laid to the formation of aggregates able to nucleate MTs in the cytoplasm.

The centrosome in neurogenesis

The critical involvement of the primary cilium/centrosome organ in microcephaly [20], suggested an important role of this organ in brain development. Two papers have significantly progressed in the mechanisms by which centrosomes influence brain development [71,72]. In neural progenitor cells that generate neurons and glia in the cerebral cortex, the centrosome is positioned away from the nucleus, anchored to the apical membrane of the ventricular zone of the cerebral cortex. This positioning apparently controls the mechanical properties of cortical cells in mice [81]. Disruption of the anchorage of the centrosome to the apical membrane results in the disorganization of microtubules and stretching and stiffening of the apical membrane as measured by AFM. As a consequence, their mitotic behavior and the size and formation of the cortex are modified. Disruption of the anchorage of the centrosome, done by the elimination of CEP83, also activates the YAP protein and promotes the over-proliferation of progenitor cells, followed by overproduction of intermediate progenitor cells, leading to the formation of an enlarged cortex with abnormal folding. Suppression of this effect by the simultaneous elimination of YAP suggests a new role of the centrosome in regulating the mechanical features of neural progenitor

cells and the size and configuration of the mammalian cerebral cortex. An important outcome of this work is to show a centrosome-dependent relationship between the mechanical properties of precursor cells and the size and formation of the cortex.

Basal progenitors then delaminate and settle in the sub-ventricular zone in enlarged brain regions. The length of time that cells stay in the sub-ventricular zone is essential for controlling further amplification and fate determination. In a search for regulators of sub-ventricular zone generation and expansion, AKNA [73], first annotated as an AT-hook transcription factor, was interesting as the level of its mRNA correlated with the time at which the sub-ventricular zone was generated [72]. This work shows that it is in fact a novel integral component of sub-distal appendages and that it is binding to microtubules, which reassembles at the centrosomes during late telophase and early G1 phase. AKNA loss of function impairs delamination, and leads to the retention of more basal progenitor cells in the ventricular zone, whereas AKNA overexpression induced a fast delamination. Moreover, AKNA-induced delamination occurs in interphase, during which AKNA localizes at centrosomes, and overexpression of the cytoplasmic form AKNA which lacks the centrosome-binding domain does not induce delamination. Importantly, AKNA efficiently recruited components of the γ-tubulin ring complex but no other centrosome- and microtubule-associated proteins. AKNA also co-immuno-precipitates with proteins having a role in microtubule organization (EB1, p150-glued, ODF2), thus appearing as capable of regulating microtubule nucleation at the centrosome, specifically in the brain. Apparently, AKNA regulates the delamination process by increasing centrosomal MTOC activity, weakening complexes at the cell junctions and promoting contraction of the apical side. The delamination from the ventricular zone resembles the epithelial–mesenchymal transition and indeed AKNA had some effect on EMT in other tissues. Live imaging in slices after AKNA overexpression or knockdown *in vivo* demonstrated that AKNA is key not only in bringing cells into the sub-ventricular zone but also in retaining them there. The switch in morphology and the radial migration of neurons apparently require a switch from a centrosomal to more non-centrosomal MTOC via endogenous downregulation of AKNA. Thus, microtubule organization differs profoundly for cell migration into the sub-ventricular zone, which requires AKNA and a centrosomal MTOC, versus migration out of the sub-ventricular zone, which requires the loss of AKNA.

Thus, AKNA orchestrates the delamination process by increasing centrosomal microtubule nucleation and recruiting nucleation factors and minus-end stabilizers, by destabilizing microtubules at the adherens junctions and by mediating constriction of the apical side. Regulating AKNA levels orchestrates delamination and retention in the sub-ventricular zone. Interestingly, this process is particularly relevant in species with highly proliferative cells in an enlarged sub-ventricular zone. Thus, the function of AKNA in ontogeny may have profound relevance in phylogeny.

Brain development may become the best experimental model to unravel centrosome functions.

The centrosome biogenesis during cell cycle progression

Long seen as the division organ only, most effort was first devoted to the coupling between centrosome biogenesis and cell cycle progression and its deregulation in disease [74]. The inhibition of centriole duplication either by an inhibitor of PLK4 [75] or by the auxin inducible degradation system [76] were very important to reveal the p53-dependent specific pathway for

monitoring centriole assembly [77]. The specificity of the drug was then questioned [78] and recently, PLK4 signaling outside centriolar networks was investigated by mass spectrometry of cellular protein phosphorylation in stable PLK4-expressing U2OS human cells exposed to centrinone. Surprisingly, sequence interrogation of ~300 significantly down-regulated phosphoproteins reveals an extensive network of centrinone sensitive [Ser/Thr]Pro phosphorylation sequence motifs, which might be either direct or indirect targets of PLK4 [79].

The initiation of centriole assembly by the transition from a uniform ring of PLK4 surrounding the mother centriole to a single PLK4 spot, corresponds to what physicists call a symmetry breaking. How this is achieved is the matter of different models, which involve several ways to regulate the activity of PLK4 [80,81; see Kitagawa contribution]. Important new ideas on how centrosome-specific oscillations in the association of a regulatory molecule like PLK4 could time its biogenesis have recently pointed to the ground-breaking possibility that perhaps many different biological processes have the ability to oscillate [82] : first, it was shown in fly early embryo that PLk4 functions as a clock, establishing both the rate and period of daughter centriole growth and thus the relationship between them to ensure that daughter centrioles grow to the correct size : using Sas-6-GFP or Ana2-GFP as a proxy to measure daughter centriole length in *D. melanogaster* embryos, since fly centrioles are short and since the cartwheel extends throughout the length of the daughter centriole, this work provided a quantitative description of the kinetics of daughter centriole growth and showed that the cartwheel grows from the proximal end [90]. Thus, Plk4 helps coordinate the inverse relationship between centriole growth and growth period. Then it was shown that PLK4 oscillation, which is normally entrained to the cell-cycle oscillator, can run autonomously [83]. This is indeed a very important result as it could illuminate the question of the physical and molecular principles that cells use to regulate the biogenesis of their organelles. In any case, it could potentially explain why centrioles can assemble independently of cell-cycle progression. The authors importantly discuss the possibility that the Plk4 centriole oscillation could be an example of a general mechanism describing the regulation of organelle biogenesis: oscillations in the levels/activity of key regulatory factors essential for organelle biogenesis could precisely time the initiation and duration of the growth process, ensuring that organelles grow at the right time and to the appropriate size. In such a model, cell cycle engine and circadian clocks could entrain the phase of a network of autonomous oscillators to ensure that biological processes occur in a coordinated manner.

Conclusion

Cellular functions such as microtubule-mediated nucleocytoplasmic transports, signaling pathways controlling the positioning of the centrosome, or centrosome in diseases, including non-cell-autonomous effects, could not be covered in this brief review. However, its contribution to so many cellular processes already, both as a signaling hub and a mutually exclusive organizing center for both tubulin- and actin-based polymers, together with the fact that centrosome biogenesis could be an example of a more general mechanism of organelle biogenesis, makes of the primary cilium/centrosome a critical organ for cell economy and polarity in Metazoa, including for the mechanical properties of the polarized cells during brain development. The current exploding research on the centrosome and primary cilium should lead to a better understanding of how time and space are controlled in animal cells.

References

1. Bornens M, Gönczy P: **Centrosomes back in the limelight.** *Phil. Trans. R. Soc.B 2004* **369**: 20130452. http://dx.doi.org/ 10.1098/rstb.2013.0452
2. Conduit P T, Wainman A, and. Raff J W **Centroso*m*e function and assembly in animal cells.** *Nat Rev Mol Cell Biol 2015,* **16:** 611-624.
3. Sanchez AD, Feldman JL: **Microtubule-organizing centers: from the centrosome to non-centrosomal sites.** *Curr. Opin. Cell Biol. 2017* **44**: 93–101. doi:10.1016/j.ceb.2016.09.003
4. Vertii A, Hehnly H, Doxsey S: **Centrosome, a Multitalented Renaissance Organelle.** *Cold Spring Harb Perspect Biol 2016*; **8**:a025049
5. Ishikawa T, Ueno H, Omori T, Kikuchi K. **Cilia and centrosomes: Ultrastructural and mechanical perspectives.** *Seminars in Cell and Developmental Biology 2020*, https://doi.org/10.1016/j.semcdb.2020.03.007
6. Gönczy, P: **Towards a molecular architecture of centriole assembly**. *Nat. Rev.Mol. Cell Biol 2012,* **13**: 425–435.
7. Mercey O, Al Jord A, Rostaing P, *et al.* **Dynamics of centriole amplification in centrosome-depleted brain multiciliated progenitors**. *Sci Rep 2019,* **9:** 13060 (2019https://doi.org/10.1038/s41598-019-49416-2
8. Goetz SC, Anderson KV. **The primary cilium: a signalling centre during vertebrate development**. *Nat. Rev. Genet. 2010,* **11**: 331–344. (doi:10.1038/nrg2774)
9. Nachury MV. **How do cilia organize signaling cascades?** *Phil. Trans. R. Soc. B 2014,* **369**: 20130465. (doi:10.1098/rstb.2013.0465)
10. Gerdes JM, Davis EE, Katsanis N. **The Vertebrate Primary Cilium in Development, Homeostasis, and Disease,** *Cell 2009,* **137**: 32-45, DOI 10.1016/j.cell.2009.03.023
11. Chavali PL, Pütz M, Gergely F. **Small organelle, big responsability: the role of centrosomes in development and disease.** *Phil Trans. R. Soc. B 2014,* **369**: 20130468. http://dx.doi.org/10.1098/rstb.2013.0468
12. Bornens M. **Cell polarity: having and making sense of direction—on the evolutionary significance of the primary cilium/centrosome organ in Metazoa**. *Open Biology 2018*, **8**: 180052. (doi:10.1098/rsob.180052
13. Yamashita YM, Jones DL, Fuller MT, **Orientation of asymmetric stem cell division by the APC tumor suppressor and centrosome.** *Science 2003*, **301**: 1547–1550
14. Reina I, and Gonzalez C. **When fate follows age: unequal centrosomes in asymmetric cell division.** *Phil. Trans. R. Soc. B 2014*, **369**: 20130466. http://dx.doi.org/ 10.1098/rstb.2013.0466
15. Manzano-López J, Matellán L, Álvarez-Llamas A, Carlos Blanco-Mira J and Monje-Casas F. **Asymmetric inheritance of spindle microtubule organizing centres preserves replicative lifespan.** *Nature Cell Biol 2019*, **21**: 952–965 (doi:10.1038/s41556-019-0364-8)
    - **A demonstration, using a *S. cerevisiae* strain that displays a constitutively inverted SPB, that the asymmetric segregation of these structures between the mother and daughter cells severely accelerates cellular ageing.**

16. Lengefeld J, Barral Y. **Asymmetric Segregation of Aged Spindle Pole Bodies During Cell Division: Mechanisms and Relevance Beyond Budding Yeast**? *BioEssays, 2018*, **40**: 1800038 DOI: 10.1002/bies.201800038
    - **A thorough analysis of the molecular mechanisms enabling budding yeast to recognize SPBs based on their age, and guide their non-random mitotic segregation. Revisiting other molecules and structures that partition asymmetrically in yeast and other systems, the authors conclude that non-random segregation may facilitate asymmetric cell fate determination and thereby indirectly ageing and rejuvenation.**
17. Azimzadeh J, Bornens M, **The centrosome in evolution.** In *The centrosome in development and disease 2004,* (ed. E Nigg), pp. 93–121. New York, NY: Wiley.
18. Loncarek J Bettencourt-Dias M: **Building the right centriole for each cell type.** *J Cell Biol 2018,* **217**: 823-835. (doi:10.1083/jcb.201704093)
19. Gräf R, Batsios P, Meyer I. **Evolution of centrosomes and the nuclear lamina: amoebozoan assets.** *Eur. J. Cell Biol 2015* **94**: 249–256. (doi:10.1016j.ejcb.2015.04.004)
20. Tassin AM, Maro B, Bornens M. **Fate of microtubule-organizing centers during myogenesis in vitro.** *J Cell Biol 1985* **100**: 35–46. (doi:10.1083/jcb.0.1.35)
21. Arquint C, Gabryjonczyk AM, Nigg EA. **Centrosomes as signaling centres**. *Phil. Trans. R. Soc. B 2014* **369**: 20130464. http://dx.doi.org/ 10.1098/rstb.2013.0464
22. Bornens M, Azimzadeh J. **Origin and evolution of the centrosome.** *Adv. Exp. Med. Biol. 2007* **607**:119–129. (doi:10.1007/978-0-387-74021-8_10)
23. Mavrakis M, Rikhy R, Lippincott-Schwartz J. **Plasma membrane polarity and compartmentalization are established before cellularization in the fly embryo.** *Dev. Cell 2009* **16**: 93–104. (doi:10.1016/j.devcel.2008.11.003)
24. Freeman M, Nüsslein-Volhard C, Glover DM. **The dissociation of nuclear and centrosomal division in *gnu*, a mutation causing giant nuclei in Drosophil**a. Cell 1986, **46**: 457–468. (doi:10.1016/0092-8674(86)90666-5)
25. Blake-Hedges C and Megraw TL. **Coordination of Embryogenesis by the Centrosome in Drosophila melanogaster** in The Golgi Apparatus and Centriole, *Results and Problems in Cell Differentiation 2020*, **67** https://doi.org/10.1007/978-3-030-23173-6_12
26. Basto R, Lau J, Vinogradova T, Gardiol A, Woods CG, Khodjakov A, Raff JW. **Flies without centrioles.** *Cell 2006* **125**: 1375–1386. doi:10.1016/j.cell.2006.05.025
27. Gottardo M, Callaini G, Riparbelli MG. **The Drosophila centriole: conversion of doublets into triplets within the stem cell niche**. *J Cell Sci 2015* **128** : 2437–2442. doi:10.1242/jcs.172627
28. Mitchell DR, **Evolution of Cilia**, *Cold Spring Harb Perspect Biol 2017*;9:a028290
29. Salisbury JL, **A mechanistic view on the evolutionary origin for centrin-based control of centriole duplication.** *J. Cell. Physiol. 2007* 213: 420-428 (DOI:10.1002/jcp.21226)
30. Saito A et al. **Gliding movement in *Peranema trichophorum* is powered by flagellar surface motility**. *Cell Motil. Cytosk. 2003* **55** : 244–253. (doi:10.1002/cm.10127),
31. Bowman GR, Lyuksyutova AI, Shapiro L. **Bacterial polarity**. *Curr. Opin Cell Biol. 2011* **23**: 71–77. (doi:10.1016/j.ceb.2010.10.013)

32. Balish MF. ***Mycoplasma pneumoniae*, an underutilized model for bacterial cell biology**. *J Bacteriology 2014*, **196**: 3675-3682 (doi:10.1128/JB.01865-14)
33. Fu J, Glover DM, **Structured illumination of the interface between centriole and peri-centriolar material.** *Open Biol 2012* **2**, 120104
34. Lawo S, Hasegan M, Gupta GD, Pelletier L. **Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material**. *Nat. Cell Biol. 2012* **14**: 1148–1158
35. Pelletier L, et al. **The *Caenorhabditis elegans* centrosomal protein SPD-2 is required for both pericentriolar material recruitment and centriole duplication**. *Curr Biol 2004* **14**: 863–873.
36. Hamill DR, Severson AF, Carter JC, Bowerman B. **Centrosome maturation and mitotic spindle assembly in *C. elegans* require SPD-5, a protein with multiple coiled coil domains.** *Dev Cell 2002* **3**: 673–684.
37. Zwicker D, Decker M, Jaensch, Hyman AA, Jülicher F. **Centrosomes are autocatalytic droplets of pericentriolar material.** *Proc Natl Acad Sci U S A, 2014* **111**: E2636-45. doi: 10.1073/pnas.1404855111
38. Woodruff JB, Wueseke O, Hyman AA. **Pericentriolar material structure and dynamics.** *Phil. Trans. R. Soc. B 2014* **369**: 20130459. http://dx.doi.org/ 10.1098/rstb.2013.0459.
39. Itakura AK, Futia RA, Jarosz DF. **It pays to be in phase**. *Biochemistry 2018* **57**: 2520−2529 doi: 10.1021/acs.biochem.8b00205.
40. Gomes E, Shorter J, **The molecular language of membraneless organelles.** *J Biol Chem 2019* **294**: 7115–7127, doi:10.1074/jbc.TM118.001192
41. Atorino ES, Hata S, Funaya C, Neuner A, Schiebel E. **CEP44 ensures the formation of bona fide centriole wall, a requirement for the centriole-to-centrosome conversion.** *Nat. Commun. 2020* **11**: 903, doi:10.1038/s41467-020-14767-2
    - **Analyzing the proteins that promote centriole biogenesis, this work shows that the developing centriole structure helps drive centriole conversion. It demonstrates that ablation of Cep44 affects both centriole structure and centriole conversion.**
42. Sugioka K, Hamill DR, Lowry JB, McNeely ME, Enrick M, Richter AC, Kiebler LE, Priess JR, Bowerman B. **Centriolar SAS-7 acts upstream of SPD-2 to regulate centriole assembly and pericentriolar material formation.** *eLife 2017* **6**: e20353. doi: 10.7554/eLife.20353
43. Enjolras C, Thomas J, Chhin B, Cortier E, Duteyrat JL, Soulavie F, Kernan MJ, Laurenc¸on A, Durand B. ***Drosophila* chibby is required for basal body formation and ciliogenesis but not for wg signaling**. *J Cell Biol 2012* **197**: 313–325. doi: 10.1083/jcb. 201109148, PMID: 22508513
44. Lee YL, Sante´ J, Comerci CJ, Cyge B, Menezes LF, Li FQ, Germino GG, Moerner WE, Takemaru K, Stearns T. **Cby1 promotes Ahi1 recruitment to a ring-shaped domain at the centriole-cilium interface and facilitates proper cilium formation and function**. *Mol Biol Cell 2014* **25**:2919–2933. doi: 10.1091/mbc.E14-02-0735, PMID: 25103236
45. Prosser SL, Pelletier L. **Centriolar satellite biogenesis and function in vertebrate cells**. *J Cell Sci. 2020* **133**: doi:10.1242/jcs.239566.

46. Odabasi E, Batman U, Firat-Karalar EN. **Unraveling the mysteries of centriolar satellites: Time to rewrite the textbooks about the centrosome/cilium complex.** *Mol Biol Cell 2020* **31**: 866–872, doi:10.1091/mbc.E19-07-0402.
47. Quarantotti V, Chen JX, Tischer J, et al. **Centriolar satellites are acentriolar assemblies of centrosomal proteins.** *EMBO J 2019* **38**: e101082. doi:10.15252/embj.2018101082
48. Bowler M, Kong D, Sun S, Nanjundappa R, Evans L, Farmer V, Holland A, Mahjoub MR, Sui H, Loncarek J. **High-resolution characterization of centriole distal appendage morphology and dynamics by correlative STORM and electron microscopy.** *Nat Commun 2019* **10**: 993.DOI: https://doi.org/10.1038/s41467-018-0216-4,PMID: 30824690
49. Yang TT, Chong WM, Wang W-J, Mazo G, Tanos B, Chen Z, Tran TMN, Chen Y- D, Weng RR, Huang C-E, Jane W-N, Bryan Tsou M-F, Liao J-C. **Super-resolution architecture of mammalian centriole distal appendages reveals distinct blade, and matrix functional components.** *Nat Commun 2018* **9**: 2023 DOI: 10.1038/s41467-018-04469-1

    •• **A three-dimensional nanoscopic resolution of the numerous proteins participating in distal appendages from intact cells, reaching a level of detail not previously achieved by current structure biology techniques.**
50. Chong WM, Wang WJ, Lo CH, et al. **Super-resolution microscopy reveals coupling between mammalian centriole subdistal appendages and distal appendages**. *eLife 2020* **9**: e53580 doi:10.7554/eLife.53580

    •• **The follow-up of the preceding work showing that sub-distal and distal appendages are structurally or functionally related to each other, implicating a regulatory mechanism of microtubule anchoring around the centrosome.**
51. Viol L, Hata S, Pastor-Peidro A, et al. **Nek2 kinase displaces distal appendages from the mother centriole prior to mitosis**. *J Cell Biol 2020* **219**: e201907136. doi:10.1083/jcb.201907136

    • **This work demonstrates convincingly that Nek2 is an important kinase that regulates distal appendages before mitosis.**
52. Farina F, Gaillard J, Guérin C, Couté Y, Sillibourne J, Blanchoin L, & Théry M. **The centrosome is an Actin-organizing Centre**. *Nat Cell Biol 2016* **18:** 65–75. https://doi.org/10.1038/ncb3285
53. Obino D, Farina F, Malbec O, Sáez PJ, Maurin M, Gaillard J, …Lennon-Duménil AM. **Actin nucleation at the centrosome controls lymphocyte polarity.** *Nat Commun 2016* **7**: 10969. https://doi.org/10.1038/ncomms10969]
54. Inoue D, Obino D, Pineau J, Farina F, Gaillard J, Guerin C, Blanchoin L, Lennon-Duménil AM, Théry M. **Actin filaments regulate microtubule growth at the centrosome.** *EMBO J 2019* **38**: e99630].

    ••**This work demonstrates, both *in vivo* and *in vitro*, using adhesive micro-patterns, that actin filaments constitute a physical barrier blocking elongation of nascent microtubules. It also shows that cell adhesion and cell spreading lead to lower densities of centrosomal actin, thus resulting in higher microtubule growth. The authors propose a novel mechanism, by which the number of centrosomal microtubules is regulated by cell adhesion and actin-network architecture.**

55. Farina F, Ramkumar N, Brown L, et al. **Local actin nucleation tunes centrosomal microtubule nucleation during passage through mitosis.** *EMBO J 2019* **38**: e99843. doi:10.15252/embj.201899843
    - **••Using in vitro and in vivo assays, this work shows that a WASH and Arp2/3 complex-dependent burst in cytoplasmic actin filament formation at mitotic exit that leads to accumulation of actin around centrosomes as cells enter anaphase and to a corresponding reduction in the density of centrosomal microtubules.**
56. Plessner M, Knerr J, Grosse R. **Centrosomal Actin Assembly Is Required for Proper Mitotic Spindle Formation and Chromosome Congression.** *iScience 2019* **15**: 274-281. doi:10.1016/j.isci.2019.04.022
57. Stiff T, Echegaray-Iturra FR, Pink HJ, Herbert A, Reyes-Aldasoro CC, Hochegger H. **Prophase-Specific Perinuclear Actin Coordinates Centrosome Separation and Positioning to Ensure Accurate Chromosome Segregation**. *Cell Rep 2020* **31**:107681. doi:10.1016/j.celrep.2020.107681
58. Meka DP, Scharrenberg R, Calderon de Anda F. **Emerging roles of the centrosome in neuronal development.** *Cytoskeleton 2020* **77***:* 84-96. https://doi.org/10.1002/cm.21593
59. Klotz C, Bordes N, Laine MC, Sandoz D, Bornens M. **Myosin at the apical pole of ciliated epithelial cells as revealed by a monoclonal antibody**. J. Cell Biol 1986 **103**: 613–619. (doi:10.1083/jcb.103.2.613)
60. Mahuzier A, Shihavuddin A, Fournier C, et al. **Ependymal cilia beating induces an actin network to protect centrioles against shear stress**. *Nat Commun 2018* **9** :1-15. https://doi-org.insb.bib.cnrs.fr/10.1038/s41467-018-04676-w
    - **A demonstration that it is the ciliary beating that controls the apical actin network around basal bodies/centrioles to protect them against the shear stress generated by ciliary beating and the associated fluid flow.**
61. Bornens, M - **Organelle positioning and cell polarity**. *Nat Rev Mol Cell Biol 2008* **9**: 874-86 DOI: 10.1038/nrm2524
62. Zyss D, Ebrahimi H, Gergely F. **Casein kinase I delta controls centrosome positioning during T cell activation.** *J Cell Biol 2011*, **195**: 781–797;
63. Liu X, Kapoor TM, Chen JK, Huse M. **Diacylglycerol promotes centrosome polarization in T cells via reciprocal localization of dynein and myosin II**. *Proc. Natl. Acad. Sci. USA 2013* **110**: 11976–11981
64. Burakov AV, Nadezhdina E. **Centering and shifting of centrosomes in cells** . *Cells 2020* **9:** 1351; doi: 10.3390/cells9061351
65. Rodríguez-Fraticelli AE, Auzan M, Alonso MA, Bornens M, Martín-Belmonte F. **Cell confinement controls centrosome positioning and lumen initiation during epithelial morphogenesis**. *J Cell Biol. 2012* **198**: 1011–1023
66. Jimenez AJ, De Pascalis C, Letort G, Vianay B, Goldman RD, Bornens M, Piel M, Blanchoin L, Théry M. **Acto-myosin network geometry defines centrosome position**. *BioRxiv 2020* doi:10.1101/2020.01.07.896969.
    - **Using cytoplasts adhering on different triangular micropatterns, this work shows that the shift of the centrosome positioning to the centroid was proportional to the symmetry of the actin network.**

67. Pitaval A, Senger F, Letort G, Gidrol X, Guyon L, Sillibourne J, Théry M. **Microtubule stabilization drives 3D centrosome migration to initiate primary ciliogenesis.** *J Cell Biol 2017* **216**: 3713–3728.
68. Burute M, Prioux M, Blin G, Truchet S, Letort G, Tseng Q, Bessy T, Lowell S, Young J, Filhol O, et al. **Polarity Reversal by Centrosome Repositioning Primes Cell Scattering during Epithelial-to-Mesenchymal Transition**. *Dev. Cell 2017* **40:** 168–184.
69. Rivero S, Cardenas J, Bornens M, Rios RM. **Microtubule nucleation at the cis-side of the Golgi apparatus requires AKAP450 and GM130.** *EMBO J. 2009* **28** :1016-28.
70. Gavilan MP, Gandolfo P, Balestra F, Arias F, Bornens M, Rios RM. **The dual role of the centrosome in organizing the microtubule network in interphase.** *EMBO reports 2018* **19**: e45942 DOI 10.15252/embr.201845942

    - **This work contributes to the understanding of mechanisms conferring MT formation plasticity in interphase, introducing a new view of the centrosome as the leading MTOC exerting tight control on the other MTOC sites.**

71. Shao W, Yang J, He M, Yu X-Y, Lee CH, Yang Z, Alexandra L. Joyner AL, Anderson KV, Zhang J, Bryan Tsou M-F, Shi H, & Shi S-H. **Centrosome anchoring regulates progenitor properties and cortical formation**. *Nature 2020* https://doi.org/10.1038/s41586-020-2139-6

    ••Disruption of the anchorage of the centrosome to the apical membrane of the neurone precursor cells results in the disorganization of microtubules and stretching and stiffening of the apical membrane.

72. Camargo Ortega G, Falk S, Johansson PA, et al. **The centrosome protein AKNA regulates neurogenesis via microtubule organization.** *Nature 2019* **567**:113-117. doi:10.1038/s41586-019-0962-4

    ••The function of AKNA in the ontogeny of the brain is revealed: it modifies considerably the microtubule network organization during brain development. These results may have profound relevance in phylogeny.

73. AKNA derives its name from the word "mother" in Inuit and Mayan language. This connection is made from the observations of what occurs to mice lacking AKNA: their body very rapidly enters an increasing weakened state, very similarly to as if they did not have a mother to support them, and are not likely to live longer than 10 days. From Wilkipedia.
74. Nigg EA, Holland AJ. **Once and only once: mechanisms of centriole duplication and their deregulation in disease**. *Nat Rev Mol Cell Biol. 2018* **19**: 297-312. doi:10.1038/nrm.2017.127
75. Wong YL, Anzola JV, Davis RL, Yoon M, Motamedi A, Kroll A, Seo CP, HsiaJE, Kim SK, Mitchell JW et al. **Reversible centriole depletion with an inhibitor of Polo-like kinase 4.** *Science 2015* **348**: 1155–1160
76. Lambrus BG, Uetake Y, Clutario KM, Daggubati V, Snyder M, Sluder G, Holland AJ **p53 protects against genome instability following centriole duplication failure**. *J Cell Biol. 2015* **210**: 63-77

77. Lambrus BG, Daggubati V, Uetake Y, Scott PM, Clutario KM, Sluder G, Holland AJ. **A USP-28-53BP1-p53-p21 signaling axis arrests growth after centrosome loss or prolonged mitosis.** *J Cell Biol. 2016* **214**:143-153
78. Oegema K, Davis RL, Lara-Gonzalez P, Desai A, Shiau AK. **CFI-400945 is not a selective cellular PLK4 inhibitor.** *Proc. Natl Acad. Sci. U.S.A. 2018* **115**: E10808–E10809https://doi.org/doi:10.1073/pnas.1813310115
79. Byrne DP, Clarke CJ, Brownridge PJ, et al. **Use of the Polo-like kinase 4 (PLK4) inhibitor centrinone to investigate intracellular signalling networks using SILAC-based phosphoproteomics.** *Biochem J. 2020* **477**:2451-2475. doi:10.1042/BCJ20200309
    - **This work reveals the existence of an extensive network of centrinone sensitive [Ser/Thr]Pro phosphorylation sequence motifs, which might be either direct or indirect targets of PLK4.**
80. Yamamoto S, Kitagawa D. **Self-organization of Plk4 regulates symmetry breaking in centriole duplication**. *Nat Commun. 2019* **10**:1810. doi:10.1038/s41467-019-09847-x
81. Leda M, Holland AJ, Goryachev AB. **Autoamplification and Competition Drive Symmetry Breaking: Initiation of Centriole Duplication by the PLK4-STIL Network.** *iScience 2018* **8**:222-235. doi:10.1016/j.isci.2018.10.003
82. Aydogan MG, Wainman A, Saurya S, et al. **A homeostatic clock sets daughter centriole size in flies**. *J Cell Biol. 2018* **217**:1233-1248. doi:10.1083/jcb.201801014
    ••This work monitors the assembly of hundreds of centrioles in the syncytial *Drosophila* embryo as they duplicate in near-synchrony in a common cytoplasm, and demonstrates that centriole growth is homeostatic: when centrioles grow slowly, they grow for a longer period; when centrioles grow quickly, they grow for a shorter period. This is how centrioles grow to a consistent size.
83. Aydogan MG, Steinacker TL, Mofatteh M, et al. An Autonomous Oscillation Times and Executes Centriole Biogenesis. *Cell 2020* **181**:1566-1581.e27. doi:10.1016/j.cell.2020.05.018

    ••This remarkable work, which is a follow-up of the previous one, demonstrates that the Plk4 oscillation that initiates and times centriole biogenesis which is normally entrained to the cell-cycle oscillator can run autonomously.