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Review Article

SAPs as a new model to probe the pathway of centriole and centrosome assembly

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Centrioles are important cellular organelles involved in the formation of both cilia and centrosomes. It is therefore not surprising that their dysfunction may lead to a variety of human pathologies. Studies have identified a conserved pathway of proteins required for centriole formation, and investigations using the embryo of the fruit fly *Drosophila melanogaster* have been crucial in elucidating their dynamics. However, a full understanding of how these components interact has been hampered by the total absence of centrioles in null mutant backgrounds for any of these core centriole factors. Here, I review our recent work describing a new model for investigating these interactions in the absence of bona fide centrioles. Sas-6 Ana2 Particles (SAPs) form when two core centriole factors, Sas-6 and Ana2, are co-over-expressed in fruit fly eggs. Crucially, they form even in eggs lacking other core centriole proteins. I review our characterisation of SAPs, and provide one example of how they have been used to investigate the role of a core centriole protein in PCM formation. I then consider some of the strengths and weaknesses of the SAP model, and discuss them in the context of other models for centriole study in *Drosophila*. Similar aggregates have been seen in other systems upon expression of centriole factors, so SAPs may also be a useful approach to study centriole proteins in other organisms.

Introducing centrioles and centrosomes

Centrioles are cylindrical-shaped organelles, formed by a central cartwheel with a 9-fold radial symmetry that is surrounded by microtubule (MT) walls (as observed by EM, see Figure 1A) [1]. They are found in most eukaryotic cells and form two important structures — cilia and centrosomes. Cilia are formed when the centriole migrates to the plasma membrane and generates a MT-based protrusion that can be either motile or non-motile. When motile, cilia can either be involved in the propulsion of the cell itself or cause the movement of air/liquids across its surface. When non-motile, cilia form the basis of a specialised membrane domain where signalling molecules are concentrated. Centrioles can also form centrosomes, the main microtubule organising centres of the cell, and play an important part in organising the poles of the mitotic spindle [2,3].

In light of these important roles, it is not surprising that centriole dysfunction is of medical importance. Disruption of centriole formation or function can result in malfunctioning cilia, leading to a variety of human pathologies commonly known as 'ciliopathies' [3,4]. Meanwhile, defects in centrosome function can give rise to microcephaly, a condition characterised by an abnormally small brain [5,6]. In addition, centrosome defects can lead to problems in mitotic spindle formation and alignment. As a result, centrosome malfunction has been associated with several types of cancer [7,8].

Centrosomes are composed of a centriole surrounded by a cloud of proteinaceous material known as the pericentriolar material (PCM) [9]. The PCM, nucleated from around the older 'mother' centriole (see below), appears unstructured by EM, but super-resolution microscopy reveals higher order organisational features [10–13]. The exact nature of the PCM remains controversial, and there is evidence
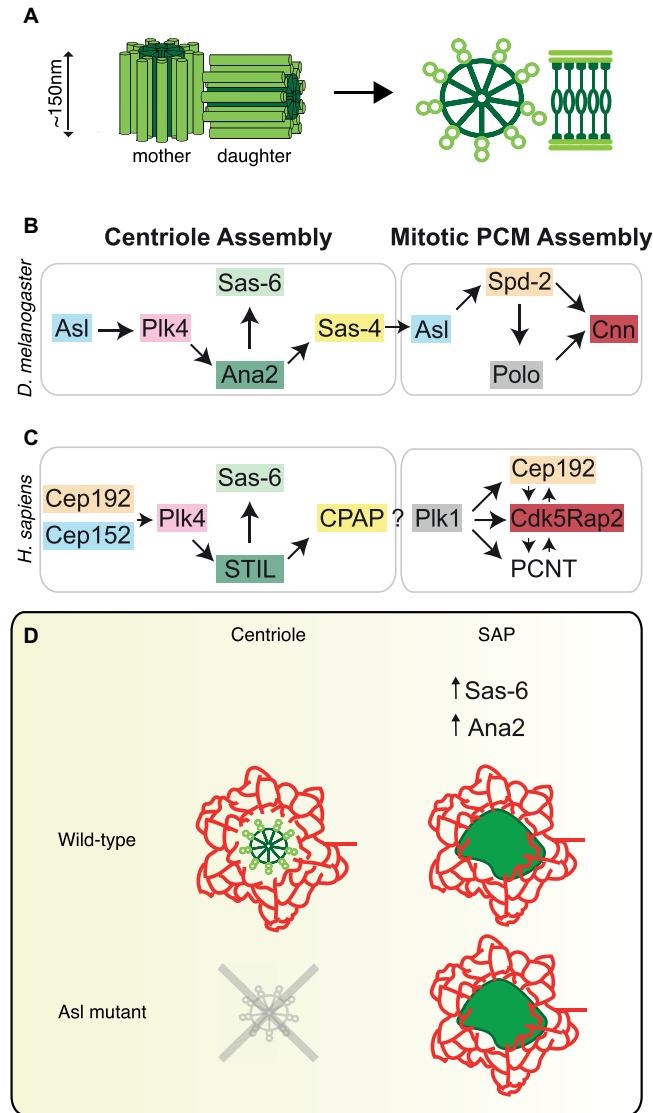
Figure 1. Centriole and SAP formation.

(**A**) Cartoon depicting mother and daughter centrioles from *Drosophila* embryos, showing a central, 9-fold, cartwheel (dark green) surrounded by doublet MTs (light green). (**B**). In *Drosophila*, centriole duplication requires five essential proteins (Plk4, Sas-6, Ana2, Sas-4 and Asl) and mitotic PCM formation requires three proteins (Polo, Spd-2 and Cnn). (**C**) These components and the pathway for centriole formation is well conserved in humans. The coloured boxes in **B** and **C** represent homologous proteins. Both sequence and function appear to be highly conserved throughout most higher eukaryotes. (**D**) SAPs, formed in eggs when Sas-6 and Ana2 are co-over-expressed to moderate levels, can be used to probe the PCM formation pathway in the absence of bona fide centrioles. In the example described here, from our recent paper [41], we used SAPs to show that the PCM can form, albeit inefficiently, in the absence of Asl.

supporting both a liquid condensate and/or a solid scaffold structure [14–16]. Regardless, the PCM provides a site for the nucleation of MTs, as well as a site for the concentration of many signalling molecules [17].

Centrosomes are dynamic structures that change throughout the cell cycle. At the beginning of a cycle, each centrosome contains one centriole, from which a 'daughter' centriole grows in an orthogonal orientation during S phase. At the end of the cell cycle the centriole pair separates, and the daughter centriole is able to mature and nucleate its own daughter and PCM [18]. The size of the PCM and its ability to recruit MTs also changes. Throughout much of the cell cycle centrosomes present very little PCM. In preparation for mitosis,

the PCM dramatically expands in size, in a process termed 'centrosome maturation' [19]. This prepares the organelle to nucleate the increased number of MTs required for spindle assembly.

The general morphology of centrioles, such as their 9-fold radial symmetry and the presence of MT walls, is well conserved throughout the evolution of eukaryotes, although some organisms have subsequently lost their ability to form centrioles, centrosomes or both [20]. However, the exact structure (e.g. length, composition, etc) can vary between species and even between cells within a single organism [21].

Over the years, both genetic and biochemical studies have identified the key components required for centriole assembly and PCM formation. Although several hundred proteins localise to the centrosome [22], only a handful are essential for centriole assembly (Plk4, Sas-6, Ana2, Sas-4 and Asl) and centrosome formation (Polo, Cnn and Spd-2) [3,23–27]. The fly pathway is summarised in Figure 1B with *Drosophila* names given hereon. Both the molecular players and the pathway that drives centriole formation appear to be remarkably well conserved (human pathway is shown in Figure 1C). Although great strides have been made, the exact function of these key components, and how they interact in both space and time to form the centrosome, is still unclear.

Fruit fly embryos as a model to study centrioles and centrosomes

The embryo of the fruit fly *Drosophila melanogaster* is an excellent system for studying centrosome and centriole dynamics. During the first three hours of development, the fruit fly embryo is a syncytium, where centrosomes grow and divide rapidly and in synchrony, as successive cell cycles take place within a common cytoplasm [28,29] . This enables many dynamic events, such as the growth of the cartwheel, duplication of centrioles and PCM growth, to be measured relatively quickly and in large numbers, leading to less experimental noise and hence the ability to detect more subtle effects [30–34]. This is also facilitated, from an imaging perspective, by the preferential localisation of centrioles and centrosomes to the cortex of the embryo, where they are in close proximity to the coverslip. There are also other advantages. In fruit flies, the very first centriole of the developing embryo is brought in by the sperm upon fertilisation. As such, unfertilised *Drosophila* eggs (which females naturally lay, facilitating their collection) provide a powerful control system, where no centrioles or centrosomes exist, but which contain all necessary components to form these structures [35–37].

These advantages place *Drosophila* embryos at the forefront of centriole and centrosome quantitative imaging, and hence the dynamic study of these organelles. *Drosophila* embryo centrioles also lack additional structural features (such as appendages) and other protein components found in higher organisms. They therefore represent a simpler form of this organelle that contains only its fundamental, conserved components and lacks the redundancies and complexities of other systems.

Despite these advantages, one particular issue has limited the study of the pathway of centriole and centrosome assembly in *Drosophila*. Knock out mutations of any of the five core centriole assembly factors (Plk4, Sas-6, Ana2, Sas-4, Asl) leads to the absence of centrioles [32,35,38–40]. This complete absence makes it hard to tease apart the interaction between these essential proteins during centriole formation. As centrosomal PCM formation requires the presence of a centriole around which to form, this also frustrates investigations to identify their precise role in guiding PCM assembly.

We recently published a paper presenting an experimental model that allows us to study the relationship between these core centriole duplication factors and their role in PCM recruitment in the absence of core factors in the fruit fly embryo [41]. Here, I discuss in some detail how Sas-6 Ana2 Particles (SAPs) resemble (and differ) from centrioles, and how they can be used to probe specific questions in centriole and centrosome biology. I also place them in the wider context of the various models available in the field.

Characterising SAPs

SAPs were first observed when our group mildly co-over-expressed both Sas-6 and Ana2, two of the five core centriole duplication proteins, in the *Drosophila* egg [42]. As mentioned above, these eggs are unfertilised, and hence do not contain centrioles. Yet, we could observe the formation of 'particles' in the cytoplasm, which were not present when either Sas-6 or Ana2 were over-expressed individually. This suggested that Sas-6 and Ana2 are able to co-assemble into higher order structures in unfertilised eggs.

Further investigation allowed us to characterise these structures in more detail [41]. We found that SAPs are similar to bona fide centrioles in several ways. First, when SAPs were taken from unfertilised eggs and injected into developing embryos (which are fertilised, and hence have hundreds of centrosomes duplicating every cell

cycle), they were able to organise robust MT asters whose dynamics cycled in synchrony with the MTs nucleated from endogenous centrosomes. This intriguing result suggested that SAPs can recruit MT nucleation factors in a manner indistinguishable from the recruitment by centrosomes, and that this recruitment is subject to the same cell-cycle regulation cues.

We therefore investigated whether SAPs were able to recruit other centriole and PCM components, and whether this organisation mimicked that seen in bona fide centrosomes. We showed by super-resolution microscopy that this is indeed the case.

In cycling *Drosophila* embryos, recruitment and scaffold formation of the PCM components Spd-2 and Cnn is dependent on their phosphorylation, with the cytoplasmic pool of these proteins remaining unphosphorylated [43–45]. We showed that both Spd-2 and Cnn recruited by SAPs contained similar phosphorylation modifications to those observed in centrosomes, suggesting that the recruitment of a PCM scaffold by SAPs mimics that of bona fide centrioles.

SAPs: not just non-specific aggregates

Our initial characterisation suggested that SAPs are able to act as a platform for centriole and PCM component recruitment. But are they just non-specific aggregates or do these structures rely on specific, and biologically relevant, interactions between Sas-6 and Ana2 proteins?

Sas-6 is one of the five essential centriole duplication proteins, and the major component of the characteristic 9-fold symmetric cartwheel structure at the centre of centrioles (Figure 1A). Crystal structures of Sas-6 from various species (including fruit flies) have been solved, providing an insight to the role of this protein [46–49]. Nine Sas-6 dimers multimerise through specific interactions in their N-terminal headgroups to form the central hub. Meanwhile, the extended coiled coil and C-terminus of each dimer gives rise to the spokes of the cartwheel. Point mutations in conserved headgroup residues (e.g. *Drosophila* F143 → D) disrupts essential interactions and prevents cartwheel and centriole formation [46,48,49]. We found that such mutant forms of Sas-6 were unable to form SAPs when over-expressed in combination with wild type Ana2.

Similarly, Ana2 contains several conserved domains: a C-terminal STAN domain; a central coiled-coil region; and a short N-terminus region [42,48,50]. Crystal structures have shown that Ana2 can homo-oligomerise via the central coiled-coil region, and that this oligomerisation is essential for centriole formation [48,51]. SAP formation was also prevented when a form of Ana2 lacking this coiled-coil domain was co-over-expressed with Sas-6.

The interaction between Sas-6 and Ana2 is dependent on the STAN domain located in the C-terminus of Ana2 [52–54], although the exact position of binding within Sas-6 is still unclear [55]. This interaction is driven by the phosphorylation of the STAN domain [52–54]. SAPs failed to form when we co-over-expressed Sas-6 and a mutant form of Ana2 lacking the STAN domain. Expression of phospho-null versions that inhibit Ana2 binding to Sas-6 reduced, although did not completely prevent, SAP formation [52–54]. In endogenous centriole formation, it is thought that the kinase Plk4 is responsible for the phosphorylation of the STAN domain [52–54]. Surprisingly, SAPs still form in the absence of Plk4, suggesting that, at least in the context of SAP formation, a second redundant kinase maybe be responsible for this phosphorylation.

Together, this set of experiments led us to conclude that SAP formation relies on many of the molecular interactions between Sas-6 and Ana2 and their regulators that are known to be necessary for bona fide centriole formation. We are therefore confident that they are not simply non-specific aggregates of Sas-6 and Ana2 molecules.

SAPs: a 'useful artefact'

Although our observations show that SAPs share many properties with centrioles, it is also clear that they are not centrioles. SAPs are much larger, have higher levels of Sas-6 and Ana2, and appear to lack any observable cartwheel structure by EM. The lack of a cartwheel structure, however, may be the reason why these SAPs can form even in eggs that lack certain essential centriole assembly factors. We discuss one example here of how this has allowed us to investigate the pathway of SAP assembly.

SAPs can form in eggs that lack the core centriole duplication factor Asterless (Asl) [41]. Whilst the role of Asl in centriole duplication has been well established [56], there is both evidence for [57–59] and against [40,60] a role for Asl in PCM formation. This observation provided a unique opportunity to examine whether PCM could form in the absence of Asl.

Drosophila genome-wide RNAi screens for genes involved in mitotic PCM formation found three significant hits: Polo, Spd-2 and Cnn (RNAi to Asl in these screens resulted in an absence of centrioles, and hence its role

in PCM formation could not be established) [61–63]. In *Drosophila* embryos, Spd-2 is recruited to the centriole wall and fluxes outwards, forming a scaffold [34]. Polo kinase can then bind to this scaffold and phosphorylate Cnn [12,44]. This phosphorylation then causes Cnn to form a scaffold [43,45]. The Spd-2/Cnn scaffold provides the platform that subsequently recruits other PCM components, including those required for MT nucleation. The exact nature of Spd-2 recruitment to the centriole wall is unclear, although previous evidence strongly points to a role for Asl [59].

In eggs expressing a truncated form of Asl (which supports centriole duplication, but not PCM formation, in other *Drosophila* tissues [40,58]), PCM recruitment to SAPs is blocked. Surprisingly, in the complete absence of Asl, PCM could still form around SAPs (although less efficiently than around SAPs in WT eggs). This suggests that, in normal conditions, Asl plays a role in PCM formation but, in the total absence of this protein, an alternative pathway is present that can recruit PCM, albeit less efficiently. This alternative PCM formation pathway is dependent on Spd-2, as SAPs lacking both Asl <u>and</u> Spd-2 form no PCM.

Using SAPs, we have confirmed a role for Asl in PCM recruitment, as well as demonstrated the existence of an alternative PCM recruitment pathway that primarily relies on Spd-2. This is only one example of how SAPs could be used to probe outstanding questions regarding the role of essential centriole duplication proteins, and we direct you to our paper for further examples [41].

SAPs in *Drosophila* eggs: just one of many models

Despite their many similarities, SAPs are not centrioles. This can be advantageous, enabling experiments and assays not possible with bona fide centrioles [41]. Although the organisation of proteins around SAPs mimics those around the centriole wall, the exact behaviour, organisation and function of these proteins may not be identical in the two structures. In addition, many of our observations in SAPs have taken place in unfertilised eggs, where standard cell cycle cues are not present. Therefore, and although they are a useful model, any conclusions with regards to how events are regulated must be taken with caution.

Other *in vivo Drosophila* approaches have been used over the years to probe the role of core centriole duplication factors in PCM formation. The use of hypomorphic alleles has helped to identify potential roles for these proteins (*asl[1]* hypomorph) [40,58]. The injection of antibodies that inhibit the function of core centriole duplication factor proteins into cycling WT embryos has provided further insight into the relationship between core centriole proteins and the PCM [33,59,64]. Similarly, the injection of mRNA into embryos or transgenic lines encoding mutated forms of centriole duplication factors have also been used to identify important residues and domains [56,65].

The role of core centriole factors has also been investigated in other tissues, such as the male germline of *Drosophila*. A useful genetic trick can be used by looking at spermatocyte cells that are homozygous for a centriole duplication mutation but which derive from heterozygous mothers. When the male germ cells are forming in this embryo, there is still enough wild type protein in the cytoplasm (through maternal contribution) to allow the formation of a few centrioles. Further in development the wild type protein is completely depleted, allowing us to examine 'wild type' centrioles in a 'mutant' cytoplasmic context [40,60,66,67]. In such a system it is only possible to study the role of proteins that exchange actively with the cytoplasm rather than those that were incorporated into centrioles during their formation.

Although all these approaches can provide insights into the role of core centriole proteins, none of them categorically assesses the effect of complete protein absence. Rather, they reduce protein levels/function, and therefore residual protein may obscure certain phenotypes; or they use centrioles that were formed in the presence of these proteins. In such cases, the advantages of a complementary analysis using SAPs in a complete mutant background is apparent, as illustrated in the case of Asl.

SAPs in *Drosophila* eggs may not be the only 'SAP-like' model available in the toolbox of the centriole/centrosome biologist. Previous observations from our lab shows that the co-over-expression of Sas-6 and Ana2 in *Drosophila* spermatocytes also generates aggregates in this tissue. Interestingly, these 'testes SAPs' do not nucleate PCM or MTs but instead display an ultrastructure strikingly similar to the centriole cartwheel [68]. This suggests that these SAPs may be useful to probe the structural role of centriole duplication proteins in a way that 'egg SAPs' cannot. Intriguingly, similar structures are seen in *Drosophila* spermatocytes when Sas-6 and another centriole duplication factor, Cep135/Bld10, are co-over-expressed, suggesting that other proteins may be involved in forming these aggregates [69].

Fascinatingly, the formation of SAP-like structures does not seem to be unique to *Drosophila*. For example, the expression of Plk4 can give rise to aggregates that are able to organise centriole and PCM factors and

nucleate MTs, not unlike our observations, in *Xenopus* cells [70]. Similarly, ectopic expression of Cep63 and Cep152 (the human homologue of Asl) as well as Plk4 in human cells gives rise to similar aggregates [71,72]. This suggests that this useful, self-organising capacity of centriole proteins might be a wider phenomenon that can be exploited for investigations in other model systems, no doubt with unique features that will allow other aspects of centriole and centrosome biology to be explored.

Perspectives

- Centrioles are cellular organelles of medical importance, and their dysfunction may result in ciliopathies, microcephaly or cancer. Yet, a full understanding of how their molecular components interact is hampered by the total absence of these structures when core components are absent.

- Sas-6 Ana2 particles (SAPs) are an experimental model that overcomes these limitations and is allowing us to probe at important molecular interactions in these organelles.

- The self-organising capacity of centriole proteins demonstrated by SAPs may be a wider phenomenon that can be exploited for investigations of centriole biology in other model systems.

Abbreviations
MT, microtubule; PCM, pericentriolar material; SAPs, Sas-6 Ana2 Particles.

References

1 LeGuennec, M., Klena, N., Aeschlimann, G., Hamel, V. and Guichard, P. (2020) Overview of the centriole architecture. *Curr. Opin. Struct. Biol.* **66**, 58–65 https://doi.org/10.1016/j.sbi.2020.09.015

2 Nigg, E.A. and Raff, J.W. (2009) Centrioles, centrosomes, and cilia in health and disease. *Cell* **139**, 663–678 https://doi.org/10.1016/j.cell.2009.10.036

3 Nigg, E.A. and Holland, A.J. (2018) Once and only once: mechanisms of centriole duplication and their deregulation in disease. *Nat. Rev. Mol. Cell Biol.* **19**, 297–312 https://doi.org/10.1038/nrm.2017.127

4 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 https://doi.org/10.1016/j.tig.2011.05.004

5 Jayaraman, D., Bae, B.-I. and Walsh, C.A. (2018) The genetics of primary microcephaly. *Annu. Rev. Genom. Hum. Genet.* **19**, 177–200 https://doi.org/10.1146/annurev-genom-083117-021441

6 Nano, M. and Basto, R. (2017) Consequences of centrosome dysfunction during brain development. *Adv. Exp. Med. Biol.* **1002**, 19–45 https://doi.org/10.1007/978-3-319-57127-0_2

7 Goundiam, O. and Basto, R. (2020) Centrosomes in disease: how the same music can sound so different? *Curr. Opin. Struct. Biol.* **66**, 74–82 https://doi.org/10.1016/j.sbi.2020.09.011

8 Chan, J.Y. (2011) A clinical overview of centrosome amplification in human cancers. *Int. J. Biol. Sci.* **7**, 1122–1144 https://doi.org/10.7150/ijbs.7.1122

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9   Pimenta-Marques, A. and Bettencourt-Dias, M. (2020) Pericentriolar material. *Curr. Biol.* **30**, R687–R689 https://doi.org/10.1016/j.cub.2020.04.064

10   Mennella, V., Keszthelyi, B., McDonald, K.L., Chhun, B., Kan, F., Rogers, G.C. et al. (2012) Subdiffraction-resolution fluorescence microscopy reveals a domain of the centrosome critical for pericentriolar material organization. *Nat. Cell Biol.* **14**, 1159–1168 https://doi.org/10.1038/ncb2597

11   Sonnen, K.F., Schermelleh, L., Leonhardt, H. and Nigg, E.A. (2012) 3D-structured illumination microscopy provides novel insight into architecture of human centrosomes. *Biol Open.* **1**, 965–976 https://doi.org/10.1242/bio.20122337

12   Fu, J. and Glover, D.M. (2012) Structured illumination of the interface between centriole and peri-centriolar material. *Open Biol.* **2**, 120104 https://doi.org/10.1098/rsob.120104

13   Lawo, S., Hasegan, M., Gupta, G.D. and Pelletier, L. (2012) Subdiffraction imaging of centrosomes reveals higher-order organizational features of pericentriolar material. *Nat. Cell Biol.* **14**, 1148–1158 https://doi.org/10.1038/ncb2591

14   Woodruff, J.B., Wueseke, O. and Hyman, A.A. (2014) Pericentriolar material structure and dynamics. *Philos. Trans. R. Soc. Lond., B Biol. Sci.* **369**, 20130459 https://doi.org/10.1098/rstb.2013.0459

15   Woodruff, J.B. (2020) The material state of centrosomes: lattice, liquid, or gel? *Curr. Opin. Struct. Biol.* **66**, 139–147 https://doi.org/10.1016/j.sbi.2020.10.001

16   Raff, J.W. (2019) Phase separation and the centrosome: a fait accompli? *Trends Cell Biol.* **29**, 612–622 https://doi.org/10.1016/j.tcb.2019.04.001

17   Wu, J. and Akhmanova, A. (2017) Microtubule-Organizing centers. *Annu. Rev. Cell Dev. Biol.* **33**, 51–75 https://doi.org/10.1146/annurev-cellbio-100616-060615

18   Fu, J., Lipinszki, Z., Rangone, H., Min, M., Mykura, C., Chao-Chu, J. et al. (2016) Conserved molecular interactions in centriole-to-centrosome conversion. *Nat. Cell Biol.* **18**, 87–99 https://doi.org/10.1038/ncb3274

19   Palazzo, R.E., Vogel, J.M., Schnackenberg, B.J., Hull, D.R. and Wu, X. (2000) Centrosome maturation. *Curr. Top. Dev. Biol.* **49**, 449–470 https://doi.org/10.1016/S0070-2153(99)49021-0

20   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 https://doi.org/10.1083/jcb.201011152

21   Gupta, A. and Kitagawa, D. (2018) Ultrastructural diversity between centrioles of eukaryotes. *J. Biochem.* **164**, 1–8 https://doi.org/10.1093/jb/mvy031

22   Alves-Cruzeiro, J.M.D.C., Alves-Cruzeiro, J.M.D.C., Nogales-Cadenas, R., Nogales-Cadenas, R., Pascual-Montano, A.D. and Pascual-Montano, A.D. (2014) CentrosomeDB: a new generation of the centrosomal proteins database for human and *Drosophila melanogaster*. *Nucleic Acids Res.* **42**, D430–D436 https://doi.org/10.1093/nar/gkt1126

23   Banterle, N. and Gönczy, P. (2017) Centriole biogenesis: from identifying the characters to understanding the plot. *Annu. Rev. Cell Dev. Biol.* **33**, 23–49 https://doi.org/10.1146/annurev-cellbio-100616-060454

24   Gönczy, P. and Hatzopoulos, G.N. (2019) Centriole assembly at a glance. *J. Cell Sci.* **132**, jcs228833 https://doi.org/10.1242/jcs.228833

25   Lattao, R., Kovacs, L. and Glover, D.M. (2017) The centrioles, centrosomes, basal bodies, and cilia of drosophila melanogaster. *Genetics* **206**, 33–53 https://doi.org/10.1534/genetics.116.198168

26   Loncarek, J. and Bettencourt-Dias, M. (2018) Building the right centriole for each cell type. *J. Cell Biol.* **217**, 823–835 https://doi.org/10.1083/jcb.201704093

27   Conduit, P.T., Wainman, A. and Raff, J.W. (2015) Centrosome function and assembly in animal cells. *Nat. Rev. Mol. Cell Biol.* **16**, 611–624 https://doi.org/10.1038/nrm4062

28   Foe, V.E. and Alberts, B.M. (1983) Studies of nuclear and cytoplasmic behaviour during the five mitotic cycles that precede gastrulation in drosophila embryogenesis. *J. Cell Sci.* **61**, 31–70 PMID:6411748

29   Callaini, G. and Riparbelli, M.G. (1990) Centriole and centrosome cycle in the early Drosophila embryo. *J. Cell Sci.* **97**, 539–543 PMID:2127418

30   Megraw, T.L., Kilaru, S., Turner, F.R. and Kaufman, T.C. (2002) The centrosome is a dynamic structure that ejects PCM flares. *J. Cell Sci.* **115**, 4707–4718 https://doi.org/10.1242/jcs.00134

31   Lerit, D.A., Jordan, H.A., Poulton, J.S., Fagerstrom, C.J., Galletta, B.J., Peifer, M. et al. (2015) Interphase centrosome organization by the PLP-Cnn scaffold is required for centrosome function. *J. Cell Biol.* **210**, 79–97 https://doi.org/10.1083/jcb.201503117

32   Aydogan, M.G., Wainman, A., Saurya, S., Steinacker, T.L., Caballe, A., Novak, Z.A. et al. (2018) A homeostatic clock sets daughter centriole size in flies. *J. Cell Biol.* **217**, 1233–1248 https://doi.org/10.1083/jcb.201801014

33   Novak, Z.A., Conduit, P.T., Wainman, A. and Raff, J.W. (2014) Asterless licenses daughter centrioles to duplicate for the first time in Drosophila embryos. *Curr. Biol.* **24**, 1276–1282 https://doi.org/10.1016/j.cub.2014.04.023

34   Conduit, P.T., Richens, J.H., Wainman, A., Holder, J., Vicente, C.C., Pratt, M.B. et al. (2014) A molecular mechanism of mitotic centrosome assembly in drosophila. *eLife* **3**, e03399 https://doi.org/10.7554/eLife.03399

35   Peel, N., Stevens, N.R., Basto, R. and Raff, J.W. (2007) Overexpressing centriole-replication proteins in vivo induces centriole overduplication and de novo formation. *Curr. Biol.* **17**, 834–843 https://doi.org/10.1016/j.cub.2007.04.036

36   Pimenta-Marques, A., Bento, I., Lopes, C.A.M., Duarte, P., Jana, S.C. and Bettencourt-Dias, M. (2016) A mechanism for the elimination of the female gamete centrosome in *drosophila melanogaster*. *Science* **353**, aaf4866 https://doi.org/10.1126/science.aaf4866

37   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 https://doi.org/10.1126/science.1142950

38   Basto, R., Lau, J., Vinogradova, T., Gardiol, A., Woods, C.G., Khodjakov, A. et al. (2006) Flies without centrioles. *Cell* **125**, 1375–1386 https://doi.org/10.1016/j.cell.2006.05.025

39   Baumbach, J., Novak, Z.A., Raff, J.W. and Wainman, A. (2015) Dissecting the function and assembly of acentriolar microtubule organizing centers in Drosophila cells in vivo. *PLoS Genet.* **11**, e1005261 https://doi.org/10.1371/journal.pgen.1005261

40   Blachon, S., Gopalakrishnan, J., Omori, Y., Polyanovsky, A., Church, A., Nicastro, D. et al. (2008) Drosophila asterless and vertebrate Cep152 Are orthologs essential for centriole duplication. *Genetics* **180**, 2081–2094 https://doi.org/10.1534/genetics.108.095141

41   Gartenmann, L., Vicente, C.C., Wainman, A., Novak, Z.A., Sieber, B., Richens, J.H. et al. (2020) Drosophila Sas-6, Ana2 and Sas-4 self-organise into macromolecular structures that can be used to probe centriole and centrosome assembly. *J. Cell Sci.* **133**, jcs244574 https://doi.org/10.1242/jcs.244574

42 Stevens, N.R., Dobbelaere, J., Brunk, K., Franz, A. and Raff, J.W. (2010) Drosophila Ana2 is a conserved centriole duplication factor. J. Cell Biol. **188**, 313–323 https://doi.org/10.1083/jcb.200910016

43 Conduit, P.T., Feng, Z., Richens, J.H., Baumbach, J., Wainman, A., Bakshi, S.D. et al. (2014) The centrosome-specific phosphorylation of Cnn by Polo/Plk1 drives Cnn scaffold assembly and centrosome maturation. Dev. Cell **28**, 659–669 https://doi.org/10.1016/j.devcel.2014.02.013

44 Alvarez Rodrigo, I., Steinacker, T.L., Saurya, S., Conduit, P.T., Baumbach, J., Novak, Z.A. et al. (2019) Evidence that a positive feedback loop drives centrosome maturation in fly embryos. eLife **8**, D430 https://doi.org/10.7554/eLife.50130

45 Feng, Z., Caballe, A., Wainman, A., Johnson, S., Haensele, A.F.M., Haensele, A.F.M. et al. (2017) Structural basis for mitotic centrosome assembly in flies. Cell **169**, 1078–1089.e13 https://doi.org/10.1016/j.cell.2017.05.030

46 van Breugel, M., Hirono, M., Andreeva, A., Yanagisawa, H.-A., Yamaguchi, S., Nakazawa, Y. et al. (2011) Structures of SAS-6 suggest its organization in centrioles. Science **331**, 1196–1199 https://doi.org/10.1126/science.1199325

47 van Breugel, M., Wilcken, R., McLaughlin, S.H., Rutherford, T.J. and Johnson, C.M. (2014) Structure of the SAS-6 cartwheel hub from L

48 Cottee, M.A., Muschalik, N., Johnson, S., Leveson, J., Raff, J.W. and Lea, S.M. (2015) The homo-oligomerisation of both Sas-6 and Ana2 is required for efficient centriole assembly in flies. eLife **4**, e07236 https://doi.org/10.7554/eLife.07236

49 Kitagawa, D., Vakonakis, I., Olieric, N., Hilbert, M., Keller, D., Olieric, V. et al. (2011) Structural basis of the 9-fold symmetry of centrioles. Cell **144**, 364–375 https://doi.org/10.1016/j.cell.2011.01.008

50 Cottee, M.A., Muschalik, N., Wong, Y.L., Johnson, C.M., Johnson, S., Andreeva, A. et al. (2013) Crystal structures of the CPAP/STIL complex reveal its role in centriole assembly and human microcephaly. eLife **2**, 30 https://doi.org/10.7554/eLife.01071

51 David, A., Amartely, H., Rabinowicz, N., Shamir, M., Friedler, A. and Izraeli, S. (2016) Molecular basis of the STIL coiled coil oligomerization explains its requirement for de-novo formation of centrosomes in mammalian cells. Sci. Rep. **6**, 24296 https://doi.org/10.1038/srep24296

52 Dzhindzhev, N.S., Tzolovsky, G., Lipinszki, Z., Schneider, S., Lattao, R., Fu, J. et al. (2014) Plk4 phosphorylates Ana2 to trigger Sas6 recruitment and procentriole formation. Curr. Biol. **24**, 2526–2532 https://doi.org/10.1016/j.cub.2014.08.061

53 Ohta, M., Ashikawa, T., Nozaki, Y., Kozuka-Hata, H., Goto, H., Inagaki, M. et al. (2014) Direct interaction of Plk4 with STIL ensures formation of a single procentriole per parental centriole. Nat. Commun. **5**, 5267 https://doi.org/10.1038/ncomms6267

54 Kratz, A.-S., Kratz, A.S., Barenz, F., Bärenz, F., Richter, K.T., Richter, K.T. et al. (2015) Plk4-dependent phosphorylation of STIL is required for centriole duplication. Biol. Open **4**, 370–377 https://doi.org/10.1242/bio.201411023

55 Fatalska, A., Dzhindzhev, N.S., Dadlez, M. and Glover, D.M. (2020) Interaction interface in the C-terminal parts of centriole proteins Sas6 and Ana2. Open Biol. **10**, 200221 https://doi.org/10.1098/rsob.200221

56 Dzhindzhev, N.S., Yu, Q.D., Weiskopf, K., Tzolovsky, G., Cunha-Ferreira, I., Riparbelli, M. et al. (2010) Asterless is a scaffold for the onset of centriole assembly. Nature **467**, 714–718 https://doi.org/10.1038/nature09445

57 Bonaccorsi, S., Giansanti, M.G. and Gatti, M. (1998) Spindle self-organization and cytokinesis during male meiosis in asterless mutants of Drosophila melanogaster. J. Cell Biol. **142**, 751–761 https://doi.org/10.1083/jcb.142.3.751

58 Varmark, H., Llamazares, S., Rebollo, E., Lange, B., Reina, J., Schwarz, H. et al. (2007) Asterless is a centriolar protein required for centrosome function and embryo development in Drosophila. Curr. Biol. **17**, 1735–1745 https://doi.org/10.1016/j.cub.2007.09.031

59 Conduit, P.T., Brunk, K., Dobbelaere, J., Dix, C.I., Lucas, E.P. and Raff, J.W. (2010) Centrioles regulate centrosome size by controlling the rate of Cnn incorporation into the PCM. Curr. Biol. **20**, 2178–2186 https://doi.org/10.1016/j.cub.2010.11.011

60 Galletta, B.J., Jacobs, K.C., Fagerstrom, C.J. and Rusan, N.M. (2016) Asterless is required for centriole length control and sperm development. J. Cell Biol. **213**, 435–450 https://doi.org/10.1083/jcb.201501120

61 Bettencourt-Dias, M., Giet, R., Sinka, R., Mazumdar, A., Lock, W.G., Balloux, F. et al. (2004) Genome-wide survey of protein kinases required for cell cycle progression. Nature **432**, 980–987 https://doi.org/10.1038/nature03160

62 Dobbelaere, J., Josué, F., Suijkerbuijk, S., Baum, B., Tapon, N. and Raff, J. (2008) A genome-wide RNAi screen to dissect centriole duplication and centrosome maturation in drosophila. PLoS Biol. **6**, e224 https://doi.org/10.1371/journal.pbio.0060224

63 Goshima, G., Wollman, R., Goodwin, S.S., Zhang, N., Scholey, J.M., Vale, R.D. et al. (2007) Genes required for mitotic spindle assembly in Drosophila S2 cells. Science **316**, 417–421 https://doi.org/10.1126/science.1141314

64 Ramani, A., Mariappan, A., Gottardo, M., Mandad, S., Urlaub, H., Avidor-Reiss, T. et al. (2018) Plk1/Polo phosphorylates Sas-4 at the onset of mitosis for an efficient recruitment of pericentriolar material to centrosomes. Cell Rep. **25**, 3618–3630.e6 https://doi.org/10.1016/j.celrep.2018.11.102

65 Novak, Z.A., Wainman, A., Gartenmann, L. and Raff, J.W. (2016) Cdk1 phosphorylates drosophila Sas-4 to recruit polo to daughter centrioles and convert them to centrosomes. Dev. Cell **37**, 545–557 https://doi.org/10.1016/j.devcel.2016.05.022

66 Riparbelli, M.G. and Callaini, G. (2011) Male gametogenesis without centrioles. Dev. Biol. **349**, 427–439 https://doi.org/10.1016/j.ydbio.2010.10.021

67 Blachon, S., Cai, X., Roberts, K.A., Yang, K., Polyanovsky, A., Church, A. et al. (2009) A proximal centriole-like structure is present in Drosophila spermatids and can serve as a model to study centriole duplication. Genetics **182**, 133–144 https://doi.org/10.1534/genetics.109.101709

68 Stevens, N.R., Roque, H. and Raff, J.W. (2010) DSas-6 and Ana2 coassemble into tubules to promote centriole duplication and engagement. Dev. Cell **19**, 913–919 https://doi.org/10.1016/j.devcel.2010.11.010

69 Jo, K.H., Jaiswal, A., Khanal, S., Fishman, E.L., Curry, A.N. and Avidor-Reiss, T. (2019) Poc1b and Sas-6 function together during the atypical centriole formation in Drosophila melanogaster. Cells **8**, 841 https://doi.org/10.3390/cells8080841

70 Montenegro Gouveia, S., Zitouni, S., Kong, D., Duarte, P., Ferreira Gomes, B., Sousa, A.L. et al. (2018) PLK4 is a microtubule-associated protein that self-assembles promoting de novoMTOC formation. J. Cell Sci. **132**, jcs219501 https://doi.org/10.1242/jcs.219501

71 Ahn, J.I., Park, J.-E., Meng, L., Zhang, L., Kim, T.-S., Kruhlak, M.J. et al. (2020) Phase separation of the Cep63•Cep152 complex underlies the formation of dynamic supramolecular self-assemblies at human centrosomes. Cell Cycle **19**, 3437–3457 https://doi.org/10.1080/15384101.2020.1843777

72 Park, J.-E., Zhang, L., Bang, J.K., Andresson, T., DiMaio, F. and Lee, K.S. (2019) Phase separation of Polo-like kinase 4 by autoactivation and clustering drives centriole biogenesis. Nat. Commun. **10**, 23–19 https://doi.org/10.1038/s41467-018-07919-y