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Centrioles: Some Self-Assembly Required

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Summary

Centrioles play an important role in organizing microtubules and are precisely duplicated once per cell cycle. New (daughter) centrioles typically arise in association with existing (mother) centrioles (canonical assembly), suggesting that mother centrioles direct the formation of daughter centrioles. However, under certain circumstances, centrioles can also self-assemble free of an existing centriole (de novo assembly). Recent work indicates that the canonical and de novo pathways utilize a common mechanism and that a mother centriole spatially constrains the self-assembly process to occur within its immediate vicinity. Other recently identified mechanisms further regulate canonical assembly so that during each cell cycle, one and only one daughter centriole is assembled per mother centriole.

Introduction

Since its discovery in the late nineteenth century, the centrosome has fascinated scientists interested in its central role in cell division. Researchers have been drawn to this organelle not only by its biological activities—the centrosome can organize microtubules into radial arrays during interphase and bipolar spindles during M phase—but by its exquisite structure and its mode of replication. As its name implies, the centrosome is often found near the cell's center and unlike other organelles, lacks a bounding membrane. At its center is a pair of centrioles, barrel-shaped organelles structurally related to the basal bodies that organize cilia and flagella. The two centrioles often lie in an orthogonal configuration and are surrounded by a cloud of pericentriolar material (PCM), which possesses microtubule nucleating and anchoring activities.

An intriguing aspect of the centrosome is how its behavior is so closely tied to that of chromosomes. At the beginning of each cell cycle two copies of each chromosome and one pair of centrioles are present. During S-phase, both chromosomes and centrioles duplicate and during mitosis both the duplicated sets of chromosomes and centrioles are segregated equally to daughter cells to re-establish the original copy numbers. Interestingly, while each chromosome consists of an old and a new DNA strand, each centrosome contains one old (mother) and one new (daughter) centriole. The resemblance between the chromosome and centrosome cycles is more than just superficial however as the two cycles share common regulators [1,2].

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In most cells, new centrioles typically arise only in association with pre-existing centrioles and a one to one correspondence between old and new centrioles ensures a precise doubling. However, this so-called canonical pathway is not the sole mode of centriole assembly. In multiciliated cells and in some embryonic systems, centrioles/basal bodies arise de novo [3-6]. That is, they self-assemble in the cytoplasm free of any pre-existing centriole. Also, de novo assembly can occur in dividing cells when existing centrioles are damaged or lost [7-9]. The de novo pathway however is normally suppressed by the presence of an intact centriole which restricts assembly to canonical pathway [9,10].

Many questions remain about the process of duplication including the nature of the molecules required for centriole assembly, how centriole structure is determined, and how the cell ensures a precise doubling of centrioles. While these issues at one time appeared intractable, the application of modern genetic, biochemical, and cell biological methods has yielded significant new insights. Here, we review recent results that address these issues.

Centrioles: assembled to the nines

The centriole is a complex structure whose outer wall is formed by a nine-fold rotationally symmetric array of microtubules singlets, doublets, or triplets. In addition, centrioles often possess other structural features including intraluminal spokes, filaments, and tubes. The nematode *C. elegans*, which has played a pivotal role in centriole biology, contains centrioles that are relatively small and simple in structure; its centrioles are composed of a central tube surrounded by nine singlet microtubules [11]. Based on the simplicity of their structure and assembly, it has been proposed that mature *C. elegans* centrioles may be analogous to procentrioles (i.e. immature centrioles) described in other organisms [12]. Thus, worms provide a less complex system for dissecting the steps of centriole assembly.

Indeed, work in *C. elegans* embryos has yielded the first description of a morphogenetic pathway for the assembly of a centriole [11]. Centriole assembly begins with formation of a small central tube that then elongates (Figure 1). This is followed by the addition of the nine single microtubules to form the outer wall. This assembly process requires the activity of five core duplication factors, including the kinase ZYG-1 [13], and the coiled-coil proteins SPD-2 [14,15], SAS-4 [16,17], SAS-5 [18], and SAS-6 [19,20]. When any one of these gene products is maternally depleted, centriole duplication fails. SPD-2 and ZYG-1 localize to the site of centriole assembly first and are required to recruit SAS-5 and SAS-6, which are needed to form the central tube [11,21]. Finally SAS-4 is recruited by SAS-5 and SAS-6 and is needed for the addition of the microtubules to the outer wall. SAS-4 initially associates loosely with the nascent centriole but becomes stably incorporated upon γ-tubulin-mediated addition of singlet microtubules [12]. Interestingly, in the absence of SAS-4, a central tube lacking singlet microtubules can form but its stability is compromised [11].

The morphogenetic pathway described in *C. elegans* appears widely conserved as orthologs of SPD-2, ZYG-1, SAS-4, and SAS-6 have been identified in both flies and humans. While ZYG-1, SAS-4, and SAS-6 orthologs have been shown to play essential roles in centrosome duplication [20,22-25], the involvement of SPD-2 orthologs in this process is less clear. In worms, flies, and humans, SPD-2 is required for PCM assembly [14,15,26-29]. However, SPD-2 does not appear to be required for centrosome duplication in flies [26,27] and conflicting results have been reported for humans [28,29]. Thus, the role of SPD-2 in centriole assembly may not be absolutely conserved. Nonetheless, centriole assembly in humans and worms appears similar, suggesting that many aspects of the molecular pathway are conserved [11, 21,25].

Although species can differ with respect to the structural intermediates in centriole assembly, the end product of all such pathways is the same: a cylinder with nine-fold radial symmetry.

While in worms the central tube is thought to act as a scaffold [11], many other organism lack a central tube. Some species possess a cartwheel, which appears early during centriole assembly and consists of a central hub and nine radial spokes [30]. Interestingly, *Chlamydomonas* SAS-6 has been shown to be a component of the central portion of the cartwheel and is required to specify the nine-fold symmetry and position of the microtubule triplets [31]. Likewise Bld10p, a component of the spoke-tip, is also required for nine-fold symmetry [32]. As SAS-6 is required to form the central tube in worms (and perhaps flies), the cartwheel and central tube might be analogous structures that provide a framework to specify the nine-fold symmetry of the centriole.

Managing centriole number

Recent work has uncovered a number of pathways contributing to the maintenance of the correct centriole number. These discoveries have highlighted three different levels of control. Temporal control ensures coordination of the centrosome cycle and the cell cycle, limiting centriole duplication to once per cell cycle. Spatial control ensures that the new centriole forms in association with the mother centriole, whilst numerical control limits the number of daughter centrioles formed. Due to the complex nature of the centriole duplication process, there obviously needs to be interplay among these different regulatory circuits.

Temporal control: an important engagement

Cell fusions were used to demonstrate that a G2 (duplicated) centriole returned to S-phase will not reduplicate, suggesting the existence of a centriole-intrinsic block to centriole reduplication within a single cell cycle [33]. Until recently, the nature of this block, and the mode by which it is relieved with the appropriate timing had remained unclear. However a reexamination of ultrastructural data lead to the proposal of an elegant model by which centriole duplication is limited to once per cell cycle: Following centriole duplication the mother and daughter remain closely associated, or ‘engaged’. This configuration blocks further duplication and persists until the end of mitosis when the mother and daughter lose their close association, becoming ‘disengaged’ and permissive to duplication [34]. Centriole disengagement therefore constitutes the licensing event for centriole duplication, whilst assembly of a daughter centriole is restricted to S-phase under the control of cell cycle regulators such as cyclin E. In this way centriole duplication is limited to once per cell cycle. Evidence from both *Xenopus* extracts and human cells have implicated the protease separase (Box) in centriole disengagement [1,35]. Although this model provides an attractive link between the centrosome and cell cycles many details remain to be elucidated, including the mechanism that maintains the engaged state and the target(s) of separase whose cleavage alleviates this block.

Towards this, depletion of a small splice variant of Shugoshin, termed sSgo1, was recently shown to lead to aberrant centriole separation [2]. This suggests that sSgo1 protects a ‘cohesive factor’ at the centrosome from separase, thus preventing untimely centriole disengagement. If this is indeed the case it would mean that Sgo1 plays a similar role at centrosomes and chromatids (Box). However this model remains speculative as the identity/existence of the ‘cohesive factor’ that sSgo1 protects from degradation remains unknown. Moreover how sSgo1 is regulated to permit centriole disengagement at the appropriate time is unclear as it localizes at the centrosome throughout the cell cycle. Nevertheless this work suggests that the roles of proteins long associated with chromatid cohesion and timely separation, may also be conserved in the regulation of centriole duplication. In the future it will be interesting to see whether additional proteins associated with chromatid cohesion are also moonlighting in centriole duplication control.

Spatial control: the PCM comes into focus

Although centrioles can arise *de novo* in many cell types, in the presence of a resident centriole this pathway is suppressed and new centrioles form only in close association with the pre-existing centriole. How is this strict spatial control imposed? It was recently shown that daughter centrioles form in the PCM rather than in direct contact with the mother centriole and that the PCM itself may direct the formation of the daughter centrioles [36]. This implies that the primary role of the mother centriole is to maintain the PCM as a compact focus enforcing the formation of a daughter centriole in close association with the mother. Presumably in the context of centriole duplication the PCM serves to concentrate centriole assembly factors allowing duplication to efficiently proceed. This hypothesis is very attractive as it not only explains spatial control of canonical centriole duplication, but can also explain why in the absence of a centriole *de novo* formation is possible: when a central focus is lacking, random PCM aggregation can reach a critical mass that allows centriole formation. Moreover, it explains why the loss of some PCM proteins leads to a reduced efficiency of centriole duplication [19].

Numerical control: planned-parenthood for centrioles

Just as it is important to limit centriole duplication to once each cell cycle it is also important to ensure that only a single daughter centriole is formed next to each mother. It is clear that the mother centriole is capable of simultaneously forming multiple daughters [25,37,38], thus the actual number must be actively specified. Increasing the quantity of PCM at the centrosome results in the formation of extra centrioles, presumably by increasing the concentration of centriole assembly factors in the vicinity of the mother centriole [36]. Over-expression of either Plk4/Sak or SAS-6 leads to centriole over-duplication [24,38-40] which in the case of the human proteins has been shown to occur through the formation of multiple daughter centrioles in association with a single mother [25,38]. Plk4/Sak is required for recruitment of SAS-6 to the centriole and Plk4 induced centriole over-duplication is SAS-6 dependent [25] suggesting that SAS-6 is a key determinant of daughter centriole number. Thus the regulation of SAS-6 levels is crucial to ensure the formation of a single daughter centriole per mother. In human cells SAS-6 levels are cell cycle regulated, increasing from S-phase until the end of mitosis, when they rapidly decrease due to proteosomal degradation directed by APC^{Cdh1}[38]. This control is essential for limiting the levels of SAS-6 and preventing the formation of extra daughter centrioles. The exact period during the cell cycle when SAS-6 levels dictate the number of daughter centrioles formed remains to be determined. However, given the role for SAS-6 in central hub formation [31] it is tempting to speculate that limiting the availability of SAS-6 in early S-phase is crucial to the control of centriole number.

Although for simplicity we have conceptually separated the three levels of control, it is very likely that at the molecular level these controls are intimately related. For example, spatial and numerical control involve regulation of the localization and levels of the same factors. Moreover the cell cycle controls that provide temporal regulation of centriole duplication also regulate the levels of key centriole duplication proteins such as SAS-6. As we learn more about the regulation of centriole duplication we will surely come to understand the links among these various processes.

Concluding Remarks

Exciting discoveries obtained in the past few years have radically changed our view about the mechanism of centrosome duplication. In Figure 2 we present a model that attempts to unify a number of old and new observations. As shown, the first steps of centriole assembly likely take place within the PCM and involve the assembly of unstable rudimentary structures. During this initial assembly phase, these structures may compete for constituents. Eventually, one of

these structures is able to engage the mother centriole. Engagement could then initiate two events. First, it could stabilize the nascent structure. In *C. elegans* the immature central tube might represent such an unstable early intermediate [11]. Upon engagement, γ-tubulin associated with the mother centriole would promote the formation of the microtubule singlets thereby stabilizing the structure [12]. Second, engagement could generate a signal to end the initial phase of assembly by blocking any additional intermediates from docking with the mother. This signal would persist until the following anaphase when separase-mediated disengagement of mother and daughter licenses both centrioles for the next round of duplication [1].

As outlined in this review, centriole assembly is subject to multiple layers of regulation to ensure that proper centriole number is maintained from one generation to the next. Spatial control is provided by a system that monitors the status of centrioles, such that in the presence of an intact centriole, assembly is restricted to the high-fidelity canonical pathway, and in the absence of centrioles (due to loss or damage) the lower-fidelity de novo pathway is activated to regenerate these organelles (Figure 2). For canonical assembly, additional controls make sure that only one daughter is assembled per mother per cell cycle. Much work still needs to be done in order to fully understanding the complex control mechanisms responsible for precise duplication. In particular, it will be necessary to identify the relevant targets of separase and to flesh out the molecular pathway that limits the number of daughter centrioles formed. Thus, for the foreseeable future, the centrosome will continue to draw the attention of researchers.

BOX 1

The better known roles of separase and Shugoshin in chromosome segregation

Separase was first identified as a regulator of chromatid cohesion in mitosis. Once replicated, the sister chromatids must remain closely associated to allow their metaphase alignment and correct segregation in mitosis. This association depends upon the cohesin complex and is established during DNA replication. At the metaphase-to-anaphase transition the protease separase is activated leading to the cleavage of chromatid-associated cohesin and subsequently chromatid separation. A similar mechanism functions during meiosis although the segregation of homologs in meiosis I and of sister chromatids in meiosis II requires a strict control of cohesin cleavage. This is achieved by protection of centromeric cohesion in anaphase I by Shugoshin, which allows the separation of homologs at anaphase I but maintains sister chromatid cohesion until meiosis II.

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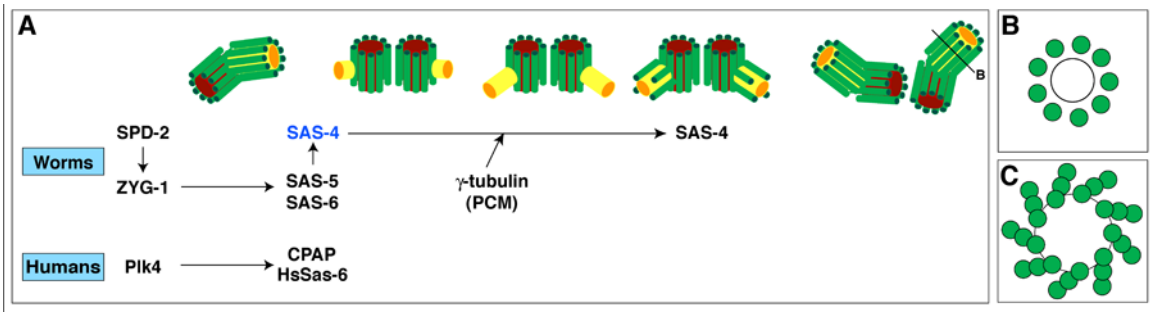

Figure 1.

The molecular pathway of centriole assembly is conserved between *C. elegans* and humans. (A) The ultrastructurally-defined steps of the centriole assembly pathway in *C. elegans* embryos are shown at the top, and the centriole assembly factors required at various steps of this process are shown below. Initially, SAS-4 (blue) is not stably associated with the forming centriole but the addition of centriole microtubules by γ-tubulin, and possibly other PCM components, stabilizes SAS-4 (black). A similar mechanism appear to operate in human cells where the ZYG-1-related kinase Plk4 acts upstream of SAS-4 (CPAP) and SAS-6 orthologs. Cross-sectional views of centrioles in *C. elegans* (B) and vertebrates (C) are shown. The *C. elegans* centriole contains a central tube surrounded by singlet microtubules (green) while human cells possess microtubule triplets but lack a central tube.

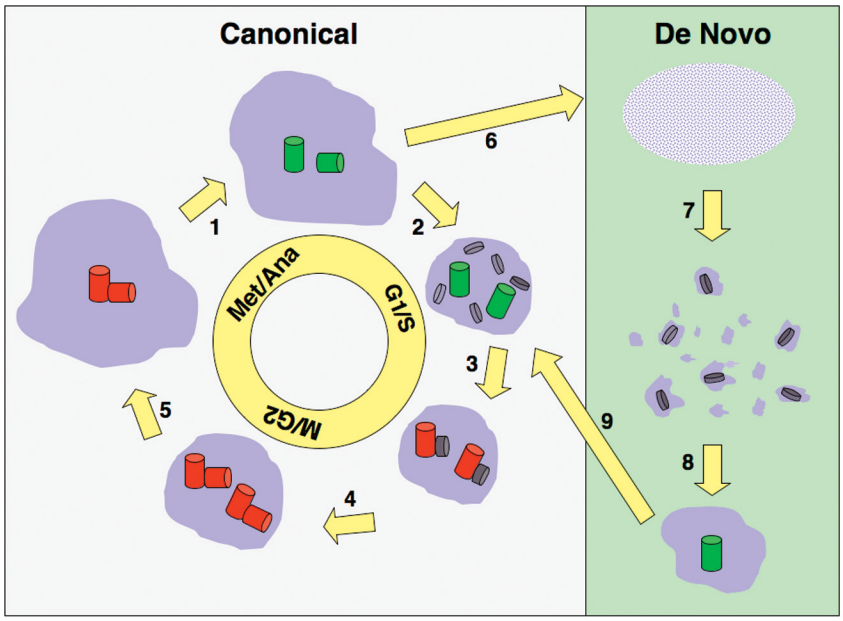

Figure 2.

Canonical and de novo centriole assembly. In the canonical pathway, (1) separase is activated at the metaphase-to-anaphase transition and drives the disengagement of mother and daughter centrioles thereby licensing them for replication (green shading). (2) During S phase, centriole precursors (grey disks) form in the PCM (purple). The extent of the PCM is reduced during interphase, which might serve to limit the number of precursors that form. (3) This initial phase of centriole assembly ends when a single precursor engages each of the mother centrioles. Upon engagement, each mother centriole loses its license (red shading) and engagement with additional precursors is blocked. (4) Engagement stabilizes the precursor and allows the assembly process to complete. (5) Segregation of centriole pairs to the spindle poles re-establishes the original number of centrioles per cell. In cases where centriole are lost or damaged, centrioles may be regenerated via the de novo pathway. (6) Loss of centrioles results in dispersion of the PCM. (7) The PCM randomly aggregates in the cytoplasm whereupon precursors can form. (8) Some of these precursors complete assembly leading to a random number of centrioles. Formation of just a single centriole can aggregate the PCM shutting off further de novo assembly. These centrioles then re-enter the canonical pathway.