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Centrosome Duplication and Nematodes: Recent Insights from an Old Relationship

Review

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Centrosome duplication is required for proper cell division, and centriole formation is a key step in this process. This review discusses recent studies in *C. elegans* that have identified five core proteins required for centriole formation, thus shedding light into the mechanisms underlying centrosome duplication in nematodes and beyond.

Centrosomes and nematodes have a long-standing relationship. Although centrosomes were initially spotted by Flemming in a fresh-water mussel (Flemming, 1875), the pioneering studies of Van Beneden and Boveri were carried out in embryos of the parasitic nematode *Parascaris equorum* (Boveri, 1887; Van Beneden and Neyt, 1887). Thereafter, the focus of centrosome research shifted to other experimental systems for well over a century. It is only in recent years that *Caenorhabditis elegans* has brought back the nematode phylum to the limelight of centrosome biology. In this review, we discuss how these studies in *C. elegans* identified five proteins, the kinase ZYG-1 as well as the coiled-coil proteins SAS-4, SAS-5, SAS-6, and SPD-2, that are required for the process of centriole formation, which is key for duplication of the entire centrosome.

Centriole Formation: At the Heart of the Centrosome Duplication Cycle

A pair of centrioles constitutes the core of the centrosome. Centrioles are barrel-shaped microtubule-based structures that, in vertebrate cells, are ~175 nm in diameter and ~400 nm in length (Chretien et al., 1997; Kuriyama and Borisy, 1981; Paintrand et al., 1992; Vorobjev and Chentsov, 1982). Centrioles are composed of stable microtubule arrays organized in a 9-fold radial symmetry, a structure also found in basal bodies, with which centrioles can interconvert, for example, in ciliated epithelial cells (reviewed in Mogensen, 2004). Centriolar microtubule arrays usually consist of triplet microtubules, although doublets or singlets are present in some species (reviewed in Delattre and Gönczy, 2004). Moreover, mature centrioles in vertebrate cells harbor conspicuous appendages that are thought to be important for microtubule anchoring (Chretien et al., 1997; Mogensen et al., 2000). The pair of centrioles is embedded in the pericentriolar material (PCM), thus, forming a centrosome. Whereas the function of many PCM proteins remains to be elucidated, an important PCM constituent is the γ-TuRC complex (Gunawardane et al., 2000). As this complex promotes microtubule nucleation, the centrosome is the principal microtubule organizing center (MTOC) of most animal cells. Consequently, it is required for microtubule-based cellular processes, such as polarized secretion or cell division.

Centrosome number is carefully coordinated with other cell cycle events. In proliferating cells, there is a single centrosome early in the cell cycle, and this centrosome duplicates during S phase to yield two centrosomes before M phase. The two centrosomes lead to bipolar spindle assembly during mitosis, which ensures faithful segregation of sister chromatids to daughter cells, which also each inherit one centrosome. The centrosome duplication cycle has been described by ultrastructural analysis conducted primarily in vertebrate tissue culture cells (Chretien et al., 1997; Kuriyama and Borisy, 1981; Paintrand et al., 1992; Vorobjev and Chentsov, 1982). First, the two centrioles lose their characteristic orthogonal arrangement and move slightly away from each other. Second, a small procentriole forms perpendicular to each original centriole, which is often referred to as the mother centriole. Subsequently, the procentriole elongates and forms a complete centriole, which is often referred to as the daughter centriole. Next, the PCM splits, and the two resulting centrosomes, each containing one mother and one daughter centriole, separate. While the above-described sequence is typical, formation of centrioles differs in some cases, including in early mouse embryos or in tissue culture cells following laser ablation of the centrosome; in these cases, centrioles form de novo in the absence of a preexisting one (Khodjakov et al., 2002; La Terra et al., 2005; Szollosi et al., 1972). However, in most proliferating cells, the canonical centrosome duplication cycle prevails, with assembly of a procentriole and a daughter centriole (hereafter referred to collectively as "centriole formation") being central to this cycle.

In contrast to the wealth of ultrastructural information, the mechanisms governing the centrosome duplication cycle remain incompletely understood. Work in frog egg extracts and vertebrate cells indicates that the cyclin-dependent kinase Cdk2 plays a central role in coupling the onset of DNA replication with that of centrosome duplication (Hinchcliffe et al., 1999; Matsumoto et al., 1999; Meraldi et al., 1999). Whether a cyclin-dependent kinase similarly ensures this coupling in *C. elegans* embryos is not known. In vertebrate cells, several Cdk2 substrates, including nucleophosmin and CP110 (Chen et al., 2002; Okuda et al., 2000), have been proposed to be central to this coupling. Kinases, including Mps1, as well as the Polo-like kinases Plk1 and Plk2, also appear to be important, as interfering with their activity by using siRNAs or dominant-negative contstructs prevents centriole formation in vertebrate cells (Fisk and Winey, 2001; Liu and Erikson, 2002; Warnke et al., 2004).

While these findings shed some light on the coupling

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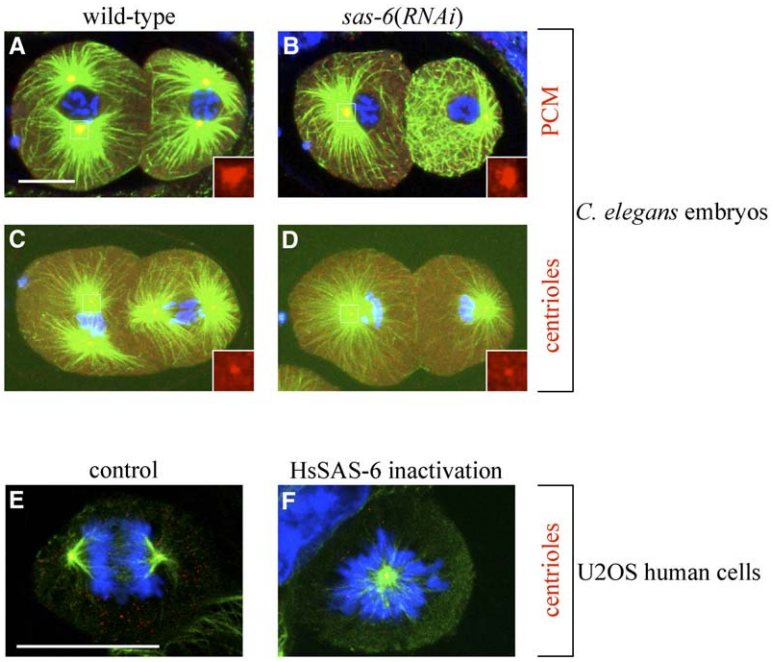

Figure 1. Centrosomes in *C. elegans* Embryos and Human Cells

(A–D) Centrosomal proteins in (A and C) wild-type or (B and D) *sas-6(RNAi)* two-cell-stage *C. elegans* embryos. Embryos stained with antibodies against the (A and B) PCM protein TAC-1 or the (C and D) centriolar protein SAS-4 (both red), as well as α-tubulin (green); DNA is shown in blue. In this figure, as well as in Figure 3, insets are 3-fold magnified views of one centrosome and scale bars represent 10 μm.

(E and F) Human U2OS osteosarcoma cells in mitosis that were transfected with siRNAs against (E) lamin B or (F) HsSAS-6 and stained 96 hr later with antibodies against the centriolar protein C-Nap1 (red) and α-tubulin (green); DNA is shown in blue.

between cell cycle progression and centrosome duplication, knowledge about the structural basis of centriole formation remains scarce. This is due in part to the paucity of proteins identified as being directly required for this step of the duplication cycle. One interesting player in this respect is the divergent tubulin isoform ϵ-tubulin, which localizes to the appendages of mature centrioles, and whose immunodepletion from frog egg extracts likely prevents centriole formation (Chang et al., 2003). In *Chlamydomonas reinhardtii*, analysis by electron microscopy established that basal body structure is perturbed in the absence of ϵ-tubulin (Dupuis-Williams et al., 2002). Another protein family of interest is that of the calcium binding protein centrin. RNAi-mediated inactivation of centrin-2 prevents centriole formation in HeLa cells (Salisbury et al., 2002), whereas mutations in the *Saccharomyces cerevisiae* centrin homolog Cdc31p prevent duplication of the spindle pole body, the MTOC of yeast cells (Baum et al., 1986). Cdc31p binds a protein called Sfi1p, and Sfi1p/Cdc31p may form filaments conferring elastic properties to replicating spindle pole bodies (Kilmartin, 2003). There is a human Sfi1p homolog, but it remains to be determined whether it interacts with centrin-2 and is needed for centriole formation.

The above-described findings notwithstanding, the mechanisms governing centriole formation remain poorly understood. There are a number of important open questions. What ensures that each mother centriole gives rise to only one daughter centriole at each cell cycle? Cell fusion experiments indicate that human G2 cells possess a centrosome-intrisic mechanism that prevents formation of additional centrosomes, but the underlying molecular tenets remain to be identified (Wong and Stearns, 2003). What determines the invariable location on the mother centriole from which the procentriole grows? Perhaps there is a seed that, albeit not apparent by electron microscopy, serves as a mark for procentriole formation. And, what limits the extent of centriolar growth? Centrioles can form in as little as 4 min in *Spisula solidissima* egg extracts (Palazzo et al., 1992), and microtubule polymerization rates should enable centrioles to reach several microns given the duration of S phase in most cell types. Nevertheless, daughter centriole growth stops when the size of the mother centriole is reached. How is this achieved? While the recent findings in *C. elegans* have not yet addressed these important questions, they have led to the identification of five proteins that may eventually provide some of the answers.

Analyzing Centrosome Duplication in *C. elegans* Embryos

Two main concerns have been evoked when considering whether findings that pertain to centriole formation in *C. elegans* are generally applicable (discussed in Azimsadeh and Bornens, 2004). First, centrioles in *C. elegans* embryos are atypical in several aspects: they are ~200 nm long and thus shorter than those in vertebrate cells, they are comprised of microtubule singlets, and they do not exhibit prominent appendages (O'Toole et al., 2003). Such structural divergences may be accompanied by variations in the mechanisms underlying centriole formation. A second concern is that several proteins important for centriole formation in other species, including ϵ-tubulin and centrin, are not present in the *C. elegans* proteome.

Despite these considerations, *C. elegans* has emerged in recent years as an attractive model system in which to identify components essential for centriole formation. The one-cell-stage embryo is ~50 μm long, which allows for an analysis of centrosome duplication in live

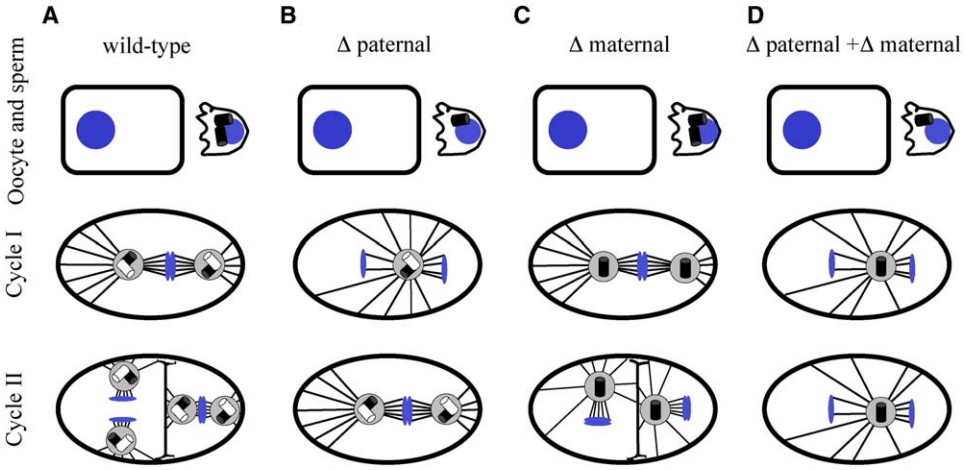

Figure 2. Differential Parental Contribution to Centriole Formation in *C. elegans* Embryos

(A–D) Schematic representation of oocyte and sperm (top), as well as of embryos during mitosis of the first (middle) or second (bottom) cell cycle. Shown are (A) wild-type or mutant animals (*zyg-1* or *sas-5* mutants) lacking (B) paternal function, (C) maternal function, or (D) both paternal and maternal function. Centrioles present in each centrosome are shown schematically: black barrel, mother centriole; white barrel, daughter centriole. The PCM are shown as gray disks, chromosomes are shown in blue, and microtubules are shown as black lines. Note that a daughter centriole formed in the first cell cycle becomes a mother centriole in the second cell cycle. (A) Wild-type. Two centrioles are contributed paternally. In the first cell cycle, these two centrioles split and a daughter centriole forms next to each of them, generating two centrosomes, each with a mother and a daughter centriole. At the second cell cycle, centrosome duplication results in the generation of two centrosomes in each blastomere, each with a mother and a daughter centriole. (B) Paternal inactivation of centriole formation using mutant males crossed to wild-type hermaphrodites. Only one centriole is contributed paternally. While a daughter centriole is formed in the first cell cycle, a single centrosome, containing a mother and a daughter centriole, assembles. At the second cell cycle, the regular duplication cycle ensues, generating two centrosomes, each with a mother and a daughter centriole. Note that the presence of one centriole, as opposed to none, in *zyg-1* or *sas-5* mutant sperm indicates that only one round of centriole formation failed during spermatogenesis. (C) Maternal inactivation of centriole formation using wild-type males crossed to mutant hermaphrodites. Two centrioles are contributed paternally. In the first cell cycle, these two centrioles split and recruit PCM despite the absence of daughter centriole formation. In the second cell cycle, the single centriole in each PCM results in assembly of a monopolar spindle. (D) Dual paternal and maternal inactivation of centriole formation using mutant hermaphrodites. Only one centriole is contributed paternally, and no centriole is formed in the resulting embryo, either in the first or the second cell cycle.

specimens with high spatial and temporal resolution. In contrast to what is seen in vertebrate cells, there is a clear size difference between centrioles and the PCM in *C. elegans* embryos, enabling one to readily determine by immunofluorescence whether a protein localizes to centrioles or the PCM (compare Figures 1A and 1C). Importantly, powerful genetic and functional genomic tools are available in *C. elegans*. In particular, RNAi-mediated inactivation permits the systematic identification of genes required for cell division processes. Genes required specifically for centrosome duplication can be identified, because interference with S phase progression results in phenotypic manifestations that are distinguishable from centrosome duplication failure. In addition, cell cycle checkpoints are relaxed in early *C. elegans* embryos (Brauchle et al., 2003; Encalada et al., 2005), such that phenotypes that may not be detected in other systems due to a block in cell cycle progression can be uncovered in the nematode. Furthermore, as in embryos of many species, early blastomeres in *C. elegans* oscillate between S phase and M phase, with the entire cell cycle lasting ~10–15 min (Edgar and McGhee, 1988). Consequently, it is plausible that centrosome duplication occurs without the regulatory mechanisms present in more complex cell cycles. As a result, proteins uncovered as being essential for centriole formation in *C. elegans* embryos may identify core components of this process.

The ZYG-1 Kinase: How Dad and Mom Contribute to Centriole Formation in *C. elegans* Embryos

ZYG-1 was the first protein identified as being essential for centriole formation in *C. elegans*, and its analysis yielded key insights for understanding the role of subsequently identified components. Conditional alleles of *zyg-1* (for zygote defective: embryonic lethal) indicated that the gene is required in proliferating tissues (Hirsh and Vanderslice, 1976; O'Connell et al., 1998). However, it was the analysis of *zyg-1* in the early embryo that revealed its requirement for centriole formation (O'Connell et al., 2001). As in other metazoans, there is a dual parental contribution to forming the centrosome in the wild-type one-cell-stage *C. elegans* embryo. The sperm provides a pair of centrioles stripped of PCM components, whereas the oocyte is devoid of centrioles but provides an abundant source of PCM proteins. After fertilization, the paternally contributed centrioles recruit the PCM from maternal stores, thus reconstituting one centrosome in the zygote. This single centrosome undergoes duplication during S phase, yielding two centrosomes, each containing one mother and one daughter centriole, which assemble a bipolar spindle during mitosis (Figure 2A).

If paternal *zyg-1* function is lacking (Figure 2B), centriole formation fails during spermatogenesis, and mature sperm contain a single centriole. After fertilization, a daughter centriole forms during the first cell cy-

cle because the oocyte provides normal *zyg-1* function. This pair of centrioles recruits PCM components, but a single centrosome is present at mitosis, resulting in monopolar spindle assembly and cell division failure. During the second cell cycle, centrosome duplication occurs normally, resulting in bipolar spindle assembly, but embryos are tetraploid and eventually die. If maternal *zyg-1* function is lacking (Figure 2C), the sperm provides a pair of centrioles as in the wild-type. The two paternally contributed centrioles split, but centriole formation does not occur in the absence of *zyg-1* function. Nevertheless, each single centriole recruits PCM components, allowing for bipolar spindle assembly in the one-cell stage. The phenotype becomes apparent only at the two-cell stage, when the absence of centriole formation results in monopolar spindle assembly in each blastomere. If both paternal and maternal *zyg-1* function are lacking (Figure 2D), a single centriole is contributed, and no further centrioles are formed in the embryo, leading to monopolar spindle assembly during both the first and second cell cycles. Thus, *zyg-1* has a dual paternal and maternal requirement to ensure proper centriole formation in the embryo. These results mirror classical work in sea urchin embryos establishing that the reproductive capacity of centrosomes correlates with the presence of a pair of centrioles at the onset of the duplication cycle (Mazia et al., 1960; Sluder and Rieder, 1985).

ZYG-1 is a divergent kinase that cannot be placed clearly into one of the known kinase families (O'Connell et al., 2001) (Figure 4A). However, it may be that a kinase required for centriole formation in other organisms, such as human Mps1, Plk1, or Plk2, is functionally related to ZYG-1. In *C. elegans*, ZYG-1 was reported to be present at centrioles during mitosis, which immediately precedes S phase and the onset of centriole formation in these embryonic blastomeres (Leidel and Gönczy, 2003; O'Connell et al., 2001). However, another study found ZYG-1 to be present at centrioles throughout the cell cycle, a result reported to be confirmed with GFP-ZYG-1 transgenic animals (Dammermann et al., 2004). Recombinant ZYG-1 can autophosphorylate in vitro, but in vivo substrates have yet to be identified (O'Connell et al., 2001).

SAS-4 and SAS-6: Coiled-Coil Proteins Stably Incorporated into Centrioles

Whereas *zyg-1* was discovered through forward genetic screens, identification of the other genes known to be required for centriole formation in *C. elegans* benefited from comprehensive RNAi-based reverse genetic screens. As the first metazoan with a sequenced genome, and as the one in which RNAi was discovered in animals, *C. elegans* lent itself particularly well to RNAi-based functional genomic screens. The vast majority (~98%) of predicted genes have been subjected to RNAi to test their requirement for cell division processes in the early embryo by using time-lapse differential interference contrast (DIC) microscopy (Sönnichsen et al., 2005).

Both *sas-4* and *sas-6* (for spindle assembly) were identified in large-scale RNAi-based screens. In *sas-4*(*RNAi*) or *sas-6*(*RNAi*) embryos, a bipolar spindle assembles at the one-cell stage, but a monopolar spindle assembles in each blastomere at the two-cell stage (Dammermann et al., 2004; Kirkham et al., 2003; Leidel et al., 2005; Leidel and Gönczy, 2003). This phenotype is identical to that of embryos lacking *zyg-1* strictly maternally, which may be expected given that sperm is not affected by using standard RNAi conditions. Serial-section electron microscopy confirmed that each spindle pole contains a single centriole in *sas-4*(*RNAi*) and *sas-6*(*RNAi*) embryos (Dammermann et al., 2004; Kirkham et al., 2003). Thus, like *zyg-1*, *sas-4* and *sas-6* are required for centriole formation. SAS-4 and SAS-6 are both coiled-coil proteins (Figure 4A) enriched in a tiny dot at the center of centrosomes throughout the cell cycle, suggestive of centriolar localization (Dammermann et al., 2004; Kirkham et al., 2003; Leidel et al., 2005; Leidel and Gönczy, 2003) (Figures 1C and 1D). That this is the case was demonstrated by immunoelectron microscopy (Dammermann et al., 2004; Kirkham et al., 2003). Insights into the dynamics of centriolar recruitment were gained through two lines of experiments conducted with transgenic animals expressing GFP-SAS-4 or GFP-SAS-6. First, fluorescence recovery after photobleaching (FRAP) experiments were performed. In these experiments, the centriolar signal was photobleached at different time points in one-cell-stage embryos, and fluorescence recovery at centrioles was assayed by using confocal time-lapse microscopy (Leidel et al., 2005; Leidel and Gönczy, 2003). These experiments established that, irrespective of when photobleaching takes place, centriolar recovery of both GFP-SAS-4 and GFP-SAS-6 initiates shortly after cytokinesis. Therefore, centriolar recruitment of both SAS-4 and SAS-6 occurs once per cell cycle and initiates at around the time of the onset of daughter centriole formation.

In a second type of experiment, hermaphrodites expressing GFP-SAS-4 or GFP-SAS-6 were crossed with wild-type males that contributed unlabeled sperm (Kirkham et al., 2003; Leidel et al., 2005; Leidel and Gönczy, 2003) (Figure 3A). By examining the distribution of centriolar markers and of GFP in the resulting telophase one-cell-stage embryos, at which point mother and daughter centrioles split and are distant enough to be resolved as single entities, one can assess whether the fusion protein has been incorporated into one or both centrioles during the first duplication cycle. For GFP-SAS-4, such marked mating experiments demonstrated that the protein is recruited to just one centriole, and analogous analysis of two-cell-stage embryos established that this corresponds to the daughter centriole (Kirkham et al., 2003; Leidel and Gönczy, 2003) (Figure 3A). Thus, SAS-4 is incorporated into the forming centriole and remains stably associated with it thereafter (Figure 4B, top). Importantly, these findings established that GFP-SAS-4 incorporation can be utilized as an in vivo assay of centriole formation (Dammermann et al., 2004; Delattre et al., 2004; Leidel et al., 2005) (Figure 3B). SAS-4 is the second component known to behave in this manner, with α/β-tubulin dimers, the principal building block of centrioles, being the first (Kochanski and Borisy, 1990). More recently, centriolar recruitment coincident with centriole formation has been reported for a GFP fusion with a

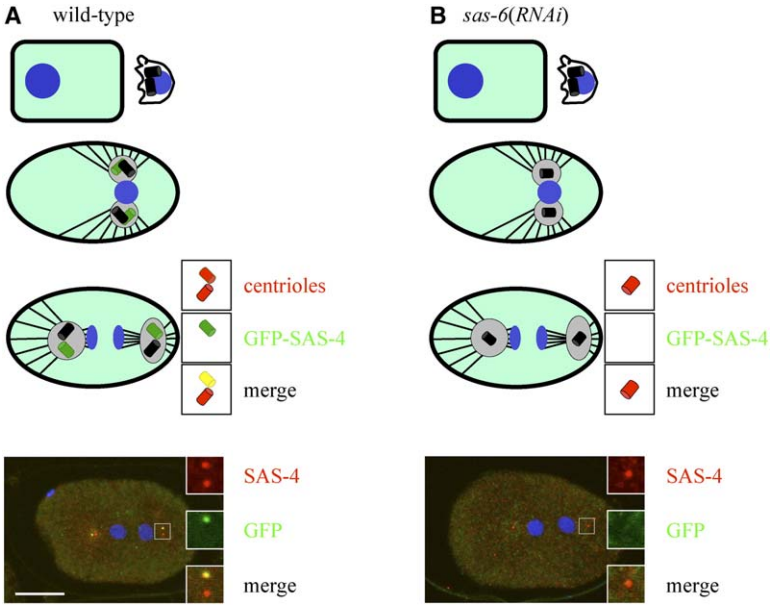

Figure 3. GFP-SAS-4 Incorporation Assay

(A and B) Marked mating experiments using GFP-SAS-4 incorporation as an assay for centriole formation in (A) wild-type or (B) *sas-6(RNAi)* embryos. On the top is an illustration of oocytes, sperm, and embryos utilized in this assay; PCM, microtubules, and DNA are represented as in Figure 2. Black barrel, mother centriole; green barrel, daughter centriole that incorporated GFP-SAS-4. On the bottom are actual embryos from such an experiment, stained with antibodies against SAS-4 (red) and GFP-SAS-4 (green); DNA is shown in blue. The panels with actual embryos are reprinted by permission from Nature Cell Biology (7:115–125) copyright (2005) MacMillan Publishers Ltd. (A) In the wild-type background, the sperm provides unlabeled centrioles. Soon after fertilization, a daughter centriole forms next to each mother centriole. When centrioles split at telophase, at the onset of the next round of centrosome duplication, the two centrioles can be distinguished as separate entities by immunofluorescence. In this example, the upper centriole is a daughter centriole, since it has incorporated GFP-SAS-4 during the first cell cycle. (B) In the *sas-6(RNAi)* background, the sperm also provides unlabeled centrioles, but no daughter centriole forms after fertilization.

domain of the *Drosophila* pericentrin-like protein (D-PLP) (Martinez-Campos et al., 2004). However, whether such kinetics extend to full-length PLP remains to be determined. Furthermore, cell division is not impaired in *d-plp* likely null mutants, indicating that the gene is not required for centriole formation.

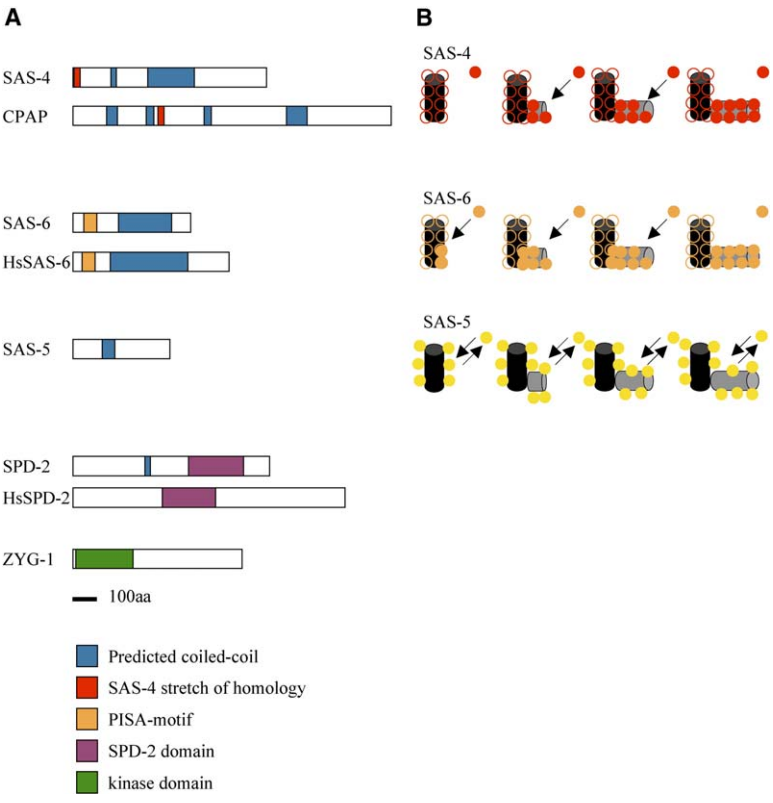

Figure 4. Structure and Dynamics of Proteins Required for Centriole Formation in *C. elegans*

(A) Schematic representation of proteins required for daughter centriole formation in *C. elegans* embryos. Predicted domains and motifs are represented by colored boxes. Note that the likely full-length human SPD-2 corresponds to Cep 192 (Andersen et al., 2003), and whereas SPD-2 is represented here according to NCBI entry Bab13395.1, its N-terminus may extend further and contain another coiled-coil motif (L. Pelletier and B. Habermann, personal communication).

(B) Recruitment dynamics to centrioles of SAS-4, SAS-6, and SAS-5. Successive steps of centriole formation are depicted, from a single mother centriole (left) to a fully formed centriole pair (right). Black barrel, mother centriole; gray barrel, daughter centriole. Proteins incorporated into centrioles in previous cell cycles are shown as open circles; those recruited in the current cell cycle are shown as filled circles. SAS-4 is incorporated strictly into the forming daughter centriole (top). SAS-6 is recruited initially to the mother centriole, and then into the forming daughter centriole (middle). SAS-5 shuttles between the cytoplasm and both centrioles throughout the cell cycle (bottom). Note that whereas SAS-5 targets centrioles even after formation of the daughter centriole, this is not the case for SAS-4 or SAS-6. Note also that for clarity SAS-5 and SAS-6 are represented separately, even though they physically interact (see text).

Marked mating experiments conducted with GFP-SAS-6 established that both centrioles are GFP positive in telophase one-cell-stage embryos, with one typically labeled more strongly than the other (Leidel et al., 2005). Although other explanations can be envisaged, this suggests that GFP-SAS-6 is recruited to the mother and the daughter centriole at each cell cycle. Compatible with a recruitment to the mother centriole, GFP-SAS-6 is present at centrioles in *sas-4*(*RNAi*) embryos (Leidel et al., 2005), in which daughter centrioles do not form (Kirkham et al., 2003). Interestingly, newly recruited GFP-SAS-6 is no longer present at centrioles during mitosis in *sas-4*(*RNAi*) embryos (Leidel et al., 2005). Taken together, these findings suggest that SAS-6 is first recruited to the mother centriole and then incorporated into the daughter centriole that emanates from it (Figure 4B, middle), with the second phase being affected by the absence of *sas-4*.

Toward Understanding the Mechanism of Action of SAS-4 and SAS-6

It has been proposed that centriolar SAS-4 directly or indirectly controls PCM size (Kirkham et al., 2003). Upon incomplete RNAi-mediated inactivation of *sas-4*, two-cell-stage embryos can assemble asymmetric spindles in which one spindle pole is smaller than normal and harbors a partially formed centriole. As there is a correlation between the levels of SAS-4 and of γ-tubulin at such smaller spindle poles, centriolar levels of SAS-4 may control PCM size (Kirkham et al., 2003). Alternatively, this correlation could reflect the fact that centriole size, not centriolar SAS-4, controls PCM size (see also Wong and Stearns, 2003). Compatible with this view, asymmetric spindles are also observed upon incomplete RNAi-mediated inactivation of *zyg-1*, *sas-6*, or *sas-5*, indicating that the correlation is not exclusive to SAS-4 (Delattre et al., 2004; Leidel et al., 2005). Moreover, experiments in vertebrate cells established that impairment of centrioles after injection of antibodies against polyglutamylated tubulin also results in PCM dispersal (Bobinnec et al., 1998). Overall, it appears likely that the size of the centriole determines that of the PCM in *C. elegans* embryos, with SAS-4 controlling PCM size through its role in setting centriole size.

An understanding of the mechanism of action of SAS-4 may be aided by the analysis of a putative distant human relative, CPAP (Centrosomal P4.1-Associated Protein) (Leidel and Gönczy, 2003). Besides both being large proteins with coiled-coil motifs, the resemblance between SAS-4 and CPAP is restricted to a short stretch of ~30 mostly charged amino acids (Figure 4A), leaving doubts as to whether they are truly related. Interestingly, however, CPAP is present at centrosomes, as judged by antibody staining and mass-spectrometric analysis (Andersen et al., 2003; Hung et al., 2000). CPAP interacts with γ-tubulin and harbors a ~120 amino acid fragment that encompasses the short stretch of homology with SAS-4 and that can bind α/β-tubulin dimers, as well as destabilize microtubules (Hung et al., 2000; Hung et al., 2004). These findings raise the possibility that SAS-4 somehow modulates microtubule behavior during centriole formation. Whether this is the case is an open question, especially since it is not known whether CPAP is required for centrosome duplication in human cells.

That a human homolog is required for centrosome duplication has been ascertained in the case of SAS-6. Bioinformatic analysis revealed that a SAS-6 homolog is present in species that have basal bodies or centrioles (Leidel et al., 2005). Members of this protein family have a similar domain composition: a coiled-coil motif toward the middle of the coding region and a novel ~50 amino acid region of homology toward the N terminus, dubbed the PISA motif (for present in SAS-6) (Figure 4A). HsSAS-6, the human family member, was identified as a probable centrosomal protein by mass-spectrometry (Andersen et al., 2003). Accordingly, GFP or YFP fused to full-length HsSAS-6 localize to centrosomes in tissue culture cells (Dammermann et al., 2004; Leidel et al., 2005). Importantly, siRNA-mediated inactivation of HsSAS-6 impairs the centrosome duplication cycle of U2OS cells, as judged by the presence of monopolar spindles in ~44% of mitotic cells (Leidel et al., 2005). Cells treated with siRNA against HsSAS-6 also exhibit signs of cell death and defective cytokinesis. The cytokinesis phenotype is reminiscent of that observed in vertebrate cells after centrosomes are removed (Khodjakov and Rieder, 2001) or the centriolar coiled-coil protein centriolin is inactivated with siRNA (Gromley et al., 2003). Such a cytokinesis phenotype is compatible with evidence indicating that centrioles are required for abscission (Piel et al., 2001). Overall, these findings establish that HsSAS-6 is required for centrosome duplication in human cells, although whether this requirement occurs at the step of centriole formation, as for *C. elegans* SAS-6, remains to be investigated.

One particularly intriguing result is that many cells overexpressing GFP-HsSAS-6 contain more than two units bearing the centriolar markers C-Nap1, centrin-3, and polyglutamylated tubulin (Leidel et al., 2005). It is unlikely that this results from a block in S phase or a failure in cytokinesis, because FACS analysis indicates that cell cycle progression is not altered and tetraploid figures are only scarcely observed. While ultrastructural analysis will be needed to ascertain whether these extra units are bona fide centrioles, the results to date raise the possibility that HsSAS-6 is rate limiting for centriole formation. As such, HsSAS-6 may be part of the mechanism that normally ensures that a single daughter centriole forms next to each mother centriole.

SAS-5: Getting Intimate with SAS-6

SAS-5 was identified through both forward and reverse genetic screens (Dammermann et al., 2004; Delattre et al., 2004). Analysis analogous to that conducted with *zyg-1* mutants demonstrated that *sas-5* also has both paternal and maternal requirements for centriole formation in the early embryo (Delattre et al., 2004) (Figure 2). Like SAS-4 and SAS-6, SAS-5 is a coiled-coil protein enriched at centrioles throughout the cell cycle (Dammermann et al., 2004; Delattre et al., 2004) (Figure 4A). However, in contrast to SAS-4 and SAS-6, FRAP analysis established that GFP-SAS-5 shuttles between both centrioles and the cytoplasm throughout the cell cycle, with a $t_{1/2}$ of ~40 s (Delattre et al., 2004) (Figure 4B, bottom). SAS-5 and SAS-6 physically interact in a yeast

two-hybrid assay (Leidel et al., 2005). This interaction is severely compromised when a SAS-5 protein corresponding to a *sas-5* mutant allele is utilized, suggesting that the interaction between SAS-5 and SAS-6 is of biological relevance. Compatible with this view, SAS-6 antibodies coimmunoprecipitate SAS-5 from wild-type *C. elegans* embryonic extracts (Leidel et al., 2005). Interestingly, the two proteins depend on one another for their centriolar localization (Leidel et al., 2005). As may be expected from the severely compromised physical interaction between the mutant SAS-5 protein and wild-type SAS-6, the SAS-5 mutant protein does not localize to centrioles in *C. elegans* embryos (Delattre et al., 2004). Interestingly, in addition, the presence of SAS-5 and SAS-6 at centrioles requires *zyg-1* function, making the two coiled-coil proteins candidate substrates for the ZYG-1 kinase (Delattre et al., 2004; Leidel et al., 2005).

How can the relationship between SAS-5 and SAS-6 be explained? One possibility is that SAS-5 serves as a carrier that brings SAS-6 to centrioles. While attractive, this scenario requires another step of regulation, without which GFP-SAS-6, just like GFP-SAS-5, should shuttle between centrioles and cytoplasm throughout the cell cycle. Perhaps unloading of SAS-6 at centrioles is regulated and can occur only at the onset of the centrosome duplication cycle. Regardless of the underlying mechanism, it is puzzling that, while the interaction between SAS-5 and SAS-6 appears to be important in *C. elegans*, no homolog of SAS-5 has been identified outside nematodes, despite SAS-6 family members being present. While it remains possible that functional SAS-5 homologs are not recognized based on their amino acid sequence alone, an alternative is that SAS-6 family members in other organisms may do without SAS-5—for instance, by being able to directly target to centrioles.

Overall, the available data with ZYG-1, SAS-4, SAS-6, and SAS-5 suggest a working model of centriole formation in *C. elegans* in which the kinase ZYG-1 is required for the ability of SAS-5/SAS-6 to target to centrioles. This enables SAS-6 recruitment to the mother centriole once per cell cycle, which is followed by incorporation of SAS-6 and SAS-4 into the emerging daughter centriole.

PCM Proteins, Including SPD-2, Contribute to Centriole Formation

Whereas centrioles are required for PCM recruitment, PCM proteins conversely play a role in centriole formation in a number of systems. For instance, when centrosomes are destroyed with a laser microbeam in human cells held in S phase, centrioles form de novo ~24 hr thereafter from areas that resemble the PCM by electron microscopy and which are apparent already ~4 hr after laser irradiation (Khodjakov et al., 2002). Similarly, overexpression of a dominant-negative variant of the PCM protein AKAP450 interferes not only with the PCM, but also with centriole duplication in human cells (Keryer et al., 2003). Furthermore, the core PCM protein γ-tubulin is required for basal body duplication in *Paramecium* and *Tetrahymena* (Ruiz et al., 1999; Shang et al., 2002).

The PCM plays an important role in centriole formation in *C. elegans* embryos as well. Although initially overlooked due to the additional requirements for microtubule nucleation and spindle assembly, this role has been uncovered with the aid of the GFP-SAS-4 incorporation assay (Dammermann et al., 2004). After inactivation of either γ-tubulin or the PCM protein SPD-5, GFP-SAS-4 fails to be incorporated in ~50% of the centrosomes. Moreover, the levels of GFP-SAS-4 in the remaining ~50% are less than in the wild-type, suggesting that centrioles are only partially formed. By contrast, GFP-SAS-4 incorporation is not affected when later steps of PCM maturation are impaired, for instance by inactivating the Aurora kinase AIR-1. Thus, while not being essential, the PCM plays an important role in ensuring efficient centriole formation in *C. elegans* embryos (Dammermann et al., 2004). One possibility is that a local concentration of PCM proteins, and of the γ-TuRC complex in particular, aids nucleation of centriolar microtubules. Alternatively, the PCM may be important for recruiting proteins specifically required for centriole formation. For instance, this could be the case for γ-tubulin and SAS-4, if these two proteins undergo an interaction analogous to that of γ-tubulin and CPAP in human cells.

SPD-2 is unique among proteins essential for centriole formation in *C. elegans* because it is also required for PCM assembly (Kemp et al., 2004; Pelletier et al., 2004). The protein distribution mirrors this dual requirement, as SPD-2 is enriched in the PCM compared to the cytoplasm, but enriched even further at centrioles, and this throughout the cell cycle. SPD-2 is a coiled-coil protein that has distant homologs in other metazoan organisms, including a human protein that has also been identified in the mass-spectrometric analysis of the human centrosome (Figure 4A) (Andersen et al., 2003; Kemp et al., 2004; Pelletier et al., 2004). In *C. elegans*, it is formally possible that SPD-2 exerts its requirement for centriole formation in part through its role in PCM assembly. However, this is unlikely to be the sole reason, given that embryos lacking the PCM components γ-tubulin or SPD-5 exhibit some centriole formation, unless residual gene function present after their inactivation is sufficient to ensure partial centriole formation. Thus, it appears to be most plausible that SPD-2 plays a distinct role in centriole formation (Dammermann et al., 2004), and should therefore be considered along with the other four proteins as being directly required for this step of the duplication cycle.

On the Number of Genes Required for Centriole Formation in *C. elegans*

The work we have covered has led to the identification of five proteins essential for centriole formation in *C. elegans* embryos. The large-scale forward genetic and functional genomic screens did not uncover components other than ZYG-1, SAS-4, SAS-6, SAS-5, and SPD-2 as being essential for centriole formation. Therefore, few, if any, other components are expected to be identified through such maternal-effect screens relying on time-lapse DIC microscopy. However, some participating components may not have been identified in these screens for a number of reasons. First, an earlier requirement, for instance in meiotic cell cycle pro-

gression, might obscure a requirement for centriole formation. Second, as exemplified by γ-tubulin and SPD-5, embryos must, in some cases, be analyzed by using more refined assays such as GFP-SAS-4 incorporation to uncover partial requirements. Third, as RNAi-based screens only target the maternal contribution, proteins essential for centriole formation that are under strict paternal control would not be recognized in such screens. Fourth, such screens only target predicted genes, such that ORFs that have not been properly annotated, or unusual genetic elements such as noncoding RNAs, have not been tested. Moreover, properly annotated and conventional genes may have gone unnoticed if they are refractory to RNAi. Importantly, in addition, the centrosome duplication cycle in early embryos is likely to be simplified, as cell cycle progression is extremely rapid and gap phases are not present. Therefore, additional proteins are probably required later in development when cell cycles become more complex.

To conclude, while other proteins undoubtedly remain to be discovered, ZYG-1, SAS-4, SAS-6, SAS-5, and SPD-2 probably correspond to a core set of proteins essential for centriole formation in *C. elegans*. One of the obvious challenges at this juncture is to understand how these proteins function to ensure centriolar assembly. As some of these proteins appear to be conserved in other metazoan organisms, and as HsSAS-6 is required for centrosome duplication in human cells, it is reasonable to speculate that this core will be evolutionarily conserved in design. Therefore, the work in *C. elegans* has paved the way for a dissection of the mechanisms underlying duplication of the fascinating centrosome. More than 100 years after Van Beneden and Boveri used nematodes for their pioneering studies, fittingly another nematode is contributing to unravel this long-standing question in cell biology.

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