DOI: 10.1534/genetics.109.101709

A Proximal Centriole-Like Structure Is Present in Drosophila Spermatids and Can Serve as a Model to Study Centriole Duplication

Stephanie Blachon,*[,1] Xuyu Cai,*[,1] Kela A. Roberts,* Kevin Yang,* Andrey Polyanovsky,[†] Allen Church* and Tomer Avidor-Reiss*[,2]

**Department of Cell Biology, Harvard Medical School, Boston, Massachusetts 02115 and [†]Sechenov Institute, Russian Academy of Sciences, Saint Petersburg, 194223 Russia*

Manuscript received February 12, 2009
Accepted for publication March 10, 2009

ABSTRACT

Most animals have two centrioles in spermatids (the distal and proximal centrioles), but insect spermatids seem to contain only one centriole (Fuller 1993), which functionally resembles the distal centriole. Using fluorescent centriolar markers, we identified a structure near the fly distal centriole that is reminiscent of a proximal centriole (*i.e.*, **p**roximal **c**entriole-**l**ike, or PCL). We show that the PCL exhibits several features of daughter centrioles. First, a single PCL forms near the proximal segment of the older centriole. Second, the centriolar proteins SAS-6, Ana1, and Bld10p/Cep135 are in the PCL. Third, PCL formation depends on SAK/PLK4 and SAS-6. Using a genetic screen for PCL defect, we identified a mutation in the gene encoding the conserved centriolar protein POC1, which is part of the daughter centriole initiation site (Kilburn *et al.* 2007) in Tetrahymena. We conclude that the PCL resembles an early intermediate structure of a forming centriole, which may explain why no typical centriolar structure is observed under electron microscopy. We propose that, during the evolution of insects, the proximal centriole was simplified by eliminating the later steps in centriole assembly. The PCL may provide a unique model to study early steps of centriole formation.

THE centriole is a cylindrical structure rich in microtubules, which are organized in a ninefold symmetry. As the template of the ciliary axoneme, the centriole transmits its symmetry to the cilium. Dividing cells contain two centrosomes at the cell poles, each containing a pair of centrioles (mother and daughter centrioles) surrounded by a thick layer of pericentriolar material (PCM). Upon differentiation, the mother centriole of each pair becomes a basal body, which acts as a template for the cilium (Azimzadeh and Bornens 2007). The function of the daughter centriole is less clear. For example, in animal spermatids, the mother centriole, known as the distal centriole, becomes a basal body and gives rise to the sperm flagellum (Krioutchkova and Onishchenko 1999; Sathananthan *et al.* 2001). The daughter centriole in spermatids, known as the proximal centriole, is attached to the nucleus.

Unlike other animal groups, multiple ultrastructural studies of insect sperm find only one centriole that has the canonical structure of microtubules organized in a ninefold symmetry (Anderson 1967; Tates 1971; Tokuyasu 1975a,b). This centriole forms the flagellum and is therefore the homolog to the vertebrate distal centriole. During spermatid differentiation (Figure 2A), a structure, called the centriolar adjunct (CA), appears transiently around this centriole (Friedlander and Wahrman 1971; Tates 1971; Tokuyasu 1975a,b; Wilson *et al.* 1997). The centriolar adjunct is a very dynamic structure, which shrinks during spermatid differentiation to form a collar around the distal centriole and then disappears (Tates 1971; Wilson *et al.* 1997). Studies using light microscopy found that γ-tubulin localizes and redistributes around the centriole in a way similar to the CA (Wilson *et al.* 1997), suggesting that it may serve as its marker. Earlier electron microscopy (EM) studies describe the appearance of a centriole inside the centriolar adjunct after it obtains the collar-shape structure (Anderson 1967; Phillips 1970). But this structure is amorphous and does not exhibit the morphological features of a centriole. Interestingly, the earliest intermediate observed during centriole formation is described as an amorphous structure (Anderson 1967; Dippell 1968; Sorokin 1968; Allen 1969).

Centriole duplication provides the cell with a mechanism for tightly controlling the number of centrosomes and cilia. In most cells, the centriole duplicates once per cell cycle and a single new centriole is formed in the vicinity of each mother centriole. The mechanism ensuring that only one daughter centriole forms in the vicinity of the mother centriole is not known (Strnad

Supporting information is available online at http://www.genetics.org/cgi/content/full/genetics.109.101709/DC1.

[1]These authors contributed equally to this work.

[2]*Corresponding author:* Department of Cell Biology, Harvard Medical School, 250 Longwood Ave., Boston, MA 02115.
E-mail: tomer_avidor-reiss@hms.harvard.edu

and Gonczy 2008). Two major limiting factors hinder the investigation of this process: (1) the difficulty of distinguishing between the mother centriole and the forming daughter centriole and (2) the short time that it takes for the process to reach completion, which in turn hinders the identification of intermediates. Few model systems are currently available for studying this process (Pelletier *et al.* 2006; Kleylein-Sohn *et al.* 2007).

Here, we demonstrate that fly spermatids contain a novel structure that is labeled by centriolar proteins and that forms in the vicinity of the proximal end of the mother centriole. Because it is reminiscent of the vertebrate proximal centriole but no morphological signatures of a centriole have been observed, we propose to call it **p**roximal **c**entriole-**l**ike (PCL). While studying the pan-centriolar protein Ana1, we found that it labeled the PCL. The PCL forms before γ-tubulin is redistributed as a collar, showing that it is a distinct entity. We then found that the formation of the PCL depends on the proteins SAK/PLK4 and SAS-6, which are essential early in daughter centriole formation, but not on SAS-4, which in worms is required later in the process. These observations indicate that the PCL represents an early intermediate structure in centriole formation. We also tested the involvement of the centriolar protein Bld10p/Cep135, which was found in Chlamydomonas and humans to be a component of the centriole cartwheel and wall (Hiraki *et al.* 2007; Kleylein-Sohn *et al.* 2007). We found that Bld10p is recruited to the PCL only later in the process and is not required for PCL formation. We performed a genetic screen finding that the Drosophila ortholog of POC1 is essential for the formation of normal PCL. POC1 was identified previously in a proteomic screen as a centriolar protein and is localized to the early intermediate structure in centriole/basal body formation (Keller *et al.* 2005, 2008; Kilburn *et al.* 2007). We propose to use PCL formation as a model to study the molecular pathway for centriole initiation. Our results suggest that POC1, like PLK4 and SAS-6, plays an important role early in centriole formation whereas Bld10p function is required later as SAS-4 is.

MATERIALS AND METHODS

Mutants and transgenic fly constructs: The generation of *asl*, *ana1*, *sas-6*, and *bld10* fused with GFP or tdTomato was described previously (Blachon *et al.* 2008). SAS-4-GFP was done in a similar way. The *sas-4* gene, including 2-kb upstream elements immediately adjacent to the predicted initiator methionine and up to the stop codon, was cloned into the p{UAST} vector using *Eco*RI and *Not*I. GFP was fused at the C terminus between *Not*I and *Xba*I. $plk4^{c06612}$, $sas\text{-}6^{c02901}$, $sas\text{-}4^{S2214}$, and cnn^{HK21} mutant flies were obtained from the Bloomington Stock Center. $bld10^{c04199}$ and $poc1^{c06059}$ mutant flies were obtained from Exelixis collection (Thibault *et al.* 2004). $Ana1^{mecB}$ mutant flies were identified in a genetic screen of a collection of ~600 potential mechanosensory mutants (*pmm*) (Avidor-Reiss *et al.* 2004).

Tissue processing for electron microscopy: EM analysis of macrochaetae and fly testis were done as previously described (Eberl *et al.* 2000; Avidor-Reiss *et al.* 2004).

Immunofluorescence staining and imaging: Embryos aged between 0 and 3 hr were collected on grape agar plates. They were dechorionated and fixed according to Rothwell and Sullivan (2000). Whole-mount preparations of pupa or adult testis were imaged in PBS containing 1 μg/ml DAPI using phase-contrast light microscopy. For staining, dark pupa or adult testes were dissected in PBS and fixed 5 min in formaldehyde (3.7% in PBS). After squashing the tissue, the coverslip was removed using liquid nitrogen and slides were placed in methanol for 2 min. After washing in PBS, the preparations were permeabilized with PBS 0.1% TritonX-100 for 10 min and saturated with PBS, 1% BSA, and TritonX-100 0.1% for 45 min. Antibody staining with mouse anti-γ-tubulin (1:200; GTU88, Sigma) or with anti-Cnn (1:200; Megraw and Kaufman 2000) was performed for 1 hr at room temperature followed by three washes with PBS. Secondary antibody rhodamine goat anti-mouse or rhodamine goat anti-rabbit (1:200; Jackson Immunoresearch) was applied for 1 hr at room temperature. DAPI (at 1 μg/ml; D9564, Sigma) was used to stain DNA. The slides were then mounted in mounting media (PBS, 50% glycerol, 0.5% *N*-propyl-gallate) and examined using a Leica TCS SP5 scanning confocal microscope. Images were processed using Adobe Photoshop. Third instar larva brains were treated in the same way as in the testis, and the primary antibodies used were rabbit anti-GFP (1:200; Fitzgerald Industries), mouse anti-γ-tubulin (Sigma), and rat anti-α-tubulin (1:200; Chemicon). The corresponding secondary antibodies were used at 1:200 dilution, FITC goat anti-rabbit, rhodamine goat anti-mouse, and Cy5 donkey anti-rat (Jackson Immunoresearch). Anti-Ana1 (1191, 1:200, homemade) immunostaining was performed according to Varmark *et al.* (2007). We used rhodamine goat anti-rabbit or FITC goat anti-rabbit (1:200, Jackson Immunoresearch) as secondary antibodies. Basal body-length measurements were made using the Leica LAS AF software, and statistical analysis was done with Excel.

Antibody: Rabbit anti-peptide antibodies (1191) to Ana1 were generated against the peptide CSSTTASSPERRPRKSR by Immunology Consultants Laboratory (Newberg, OR) (Figure 1, A and E).

Genetic screening: A total of 150 fly lines from the Exelixis collection (Thibault *et al.* 2004) and 150 fly lines from the Zucker collection (Koundakjian *et al.* 2004) were crossed to a fly line containing w; Cyo/Sco; MKRS/TM6B. Males with w; +/Cyo; mutant/TM6B were selected and crossed to w; Ana1-GFP; MKRS/TM6B. Siblings with w; Ana1-GFP/Cyo; mutant/TM6B from this cross were crossed with each other to establish a stable stock. Testis from homozygote mutants were dissected as described above and screened for the absence of PCL using light microscopy.

Molecular biology: For DNA sequencing, DNA was extracted from whole-body flies using Promega Wizard SV genomic DNA purification system. DNA samples were then amplified by PCR and purified using the QIAGEN gel extraction kit. DNA sequencing was done by Biopolymers Facility at Harvard Medical School. For mRNA experiments, RNA was extracted from whole-body flies using the Promega SV total RNA isolation system. cDNA sample was then generated by RT–PCR with oligo(dT). Primers used in the experiments were as follows: Actin, 5′-GCTGCTCTGGTCGTTGAC-3′ and 5′-CGCGATTT GACCGACTACC-3′; poc1, 5′-CCGCATCCTAGACCTGC-3′ and 5′-GGATGTCGAACTGCCTTCGC-3′.

RESULTS AND DISCUSSION

Ana1 is a pan-centriolar protein involved in centriole/cilium formation: In a screen for genes involved in centriole/cilium formation, we identified *mechanosensory*

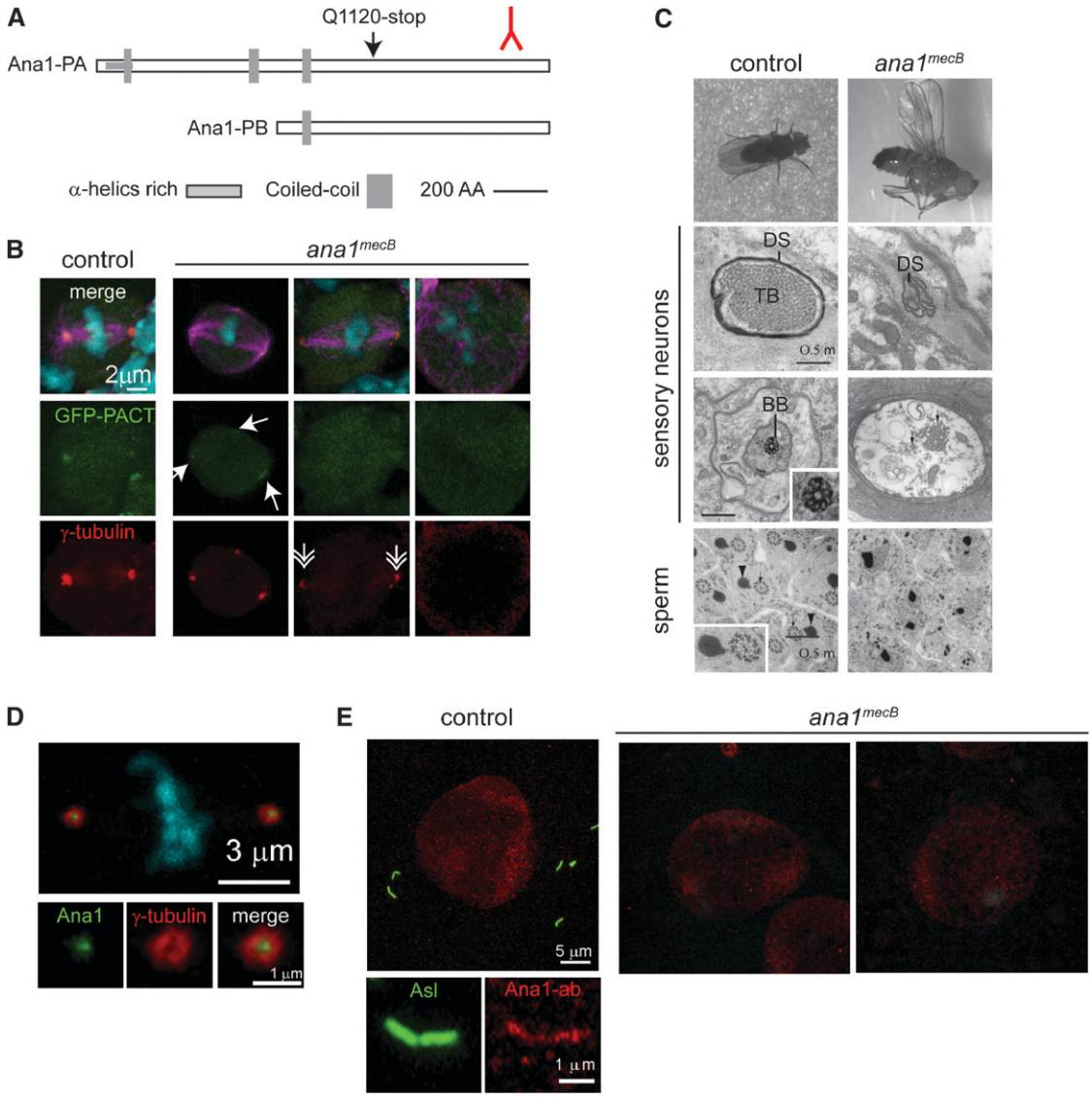

Figure 1.—Ana1 is a centriolar protein required for centriole and cilia formation. (A) Predicted structure of two isoforms (PA and PB) of the Ana1 protein. Red indicates the recognition site of the antibody in the C-terminal part of the protein. The *ana1*^{mecB} mutation results in a C-to-T transition at position 3358 (relative to ATG), which changes the Q 1120 codon to a stop codon. (B) Centrosomal labeling is reduced in *ana1*^{mecB} mutants. In the control, GFP–PACT (Martinez-Campos *et al.* 2004) labels the centriole and γ-tubulin the PCM of mitotic cells from larva brain. In *ana1*^{mecB}, only 5/25 cells show foci stained by both γ-tubulin and GFP–PACT (arrows), the majority of the cells (18/25) have only γ-tubulin foci, which look smaller than those of the control (double arrows). Few cells (2/25) show a total absence of centrosomal staining. (C) *Ana1*^{mecB} mutants (grown in 18°) present all the characteristics of ciliary mutants. Flies are uncoordinated, their legs are crossed, and their wings extend upward. EM cross sections of mechanosensory neurons in the control show the tubular body (TB) surrounded by the dendritic sheath (DS). Control images were previously published in Blachon *et al.* (2008). In *ana1*^{mecB}, the tubular body is absent but the dentritic sheath is present. At the base of the cilium, basal body (BB) is observed in the control whereas it is absent in *ana1*^{mecB}. Similarly, in sperm tails no axoneme is detected in *ana1*^{mecB} whereas in the control they are clearly visible (arrows) sitting near the mitochondria derivatives (arrowheads). (D) In embryos, Ana1-GFP localizes to the centriole surrounded by γ-tubulin. (E) Before meiosis, each spermatocyte contains four giant centrioles arranged by pairs as a V-shape. In primary spermatocytes expressing the centrosomal protein Asl-GFP (Varmark *et al.* 2007; Blachon *et al.* 2008), the anti-Ana1 antibody labels the V-shaped giant centrioles. Some nonspecific signal is detected in the nucleus, but in the *ana1*^{mecB} mutant no giant centriole labeling is detected.

mutant B (*mecB*), a mutant of Ana1 (Avidor-Reiss *et al.* 2004). We found that the *ana1*^{mecB} mutation changes C to T at position 3368 in the gene CG6631 (Figure 1A), leading to an early stop codon. Blast analysis finds that insect Ana1 bears significant similarity in its N terminus to two related vertebrate genes, KIAA1731 and Ddc8. KIAA1731 was previously identified as a candidate centriolar protein in a proteomic study for human centrosomes (Andersen *et al.* 2003), and Ddc8 is a gene expressed during spermatogenesis (Catalano *et al.* 1997). Fly Ana1 was previously identified by Goshima *et al.* (2007) in an RNA interference (RNAi) screen in S2

cells. RNAi depletion of Ana1 results in a decrease of SAS-6 at the spindle poles (Goshima *et al.* 2007). Consistent with this is our observation of a reduction of centrosome labeling by GFP–pericentrin/AKAP450 centrosomal targeting (PACT) and γ-tubulin in neuroblasts of *ana1*^{mecB} mutants (Figure 1B). In addition, *ana1*^{mecB} mutants exhibit all the features of a ciliary mutant (Figure 1C): flies are uncoordinated due to the absence of cilia in mechanosensory neurons. Additionally, axonemes are missing in sperm tails and the sperm is immotile. These results suggest that Ana1 has a role in centriole and cilium formation.

We made a GFP fusion of the Ana1 protein and confirmed its specific localization to the centriole in embryos (Figure 1D), which is consistent with Goshima's results that localize it to S2 cell centrioles. In addition, we have shown previously that Ana1 is a component of mature basal bodies in spermatids and sensory neurons (Blachon *et al.* 2008), suggesting that Ana1 is a core component of the centriole. We took advantage of the ubiquity and specificity of Ana1 labeling of the centriole to use it as a pan-centriolar marker.

We also generated an antibody against the C terminus of Ana1 and checked its specificity using the *ana1*^{mecB} mutant. As expected, in primary spermatocytes, the antibody labels the giant centriole stained by the centrosomal protein Asl-GFP (Varmark *et al.* 2007; Blachon *et al.* 2008); however, no centriolar staining was detected in *ana1*^{mecB} spermatocytes (Figure 1E).

Ana1 labels a novel structure that associates with the sperm basal body and is distinct from the centriolar adjunct: In fly spermatids, the centriole elongates to form the basal body, which is the base for the flagellum axoneme (Figure 2A). The basal body in this system is basically a giant centriole that measures 2.6 μm and therefore is easily observed using light microscopy. This system lends itself to cytological analysis as distinct stages in centriole biogenesis are associated with morphological changes of the cell and are organized in chronological order along the testes (Tates 1971; Cenci *et al.* 1994) (Figure 2A). While studying the localization of centriolar protein Ana1 using the Ana1-GFP reporter, we noted that it labels a novel structure in spermatids. We use phase-contrast imaging of live or fixed preparations to precisely identify the stage of spermatid development. Early spermatids (or onion stage, S13) are recognized as round cells containing a white, round nucleus (N) and dark, round mitochondria derivatives (M) of similar size (Figure 2B, left). In these cells, Ana1-GFP labels the giant centriole (also called the basal body) uniformly all along its length. Intermediate spermatids (S15) have short protrusions at one side and a white, round nucleus (Figure 2B, middle). In intermediate spermatids, the Ana1-GFP labeling of the basal body is no longer uniform and a single bulge appears on its proximal surface. Late spermatids are organized in a tight bundle of elongated cells and at that time the bulge disappears and a distinct Ana-1-labeled structure appears more toward the center of the giant centriole (Figure 2B, right). We measured the length of the fluorescence signal and found that the structure width is 0.44 ± 0.06 μm ($N = 34$), which is similar to the width of the giant centriole, 0.54 ± 0.04 μm ($N = 41$). We conclude that this novel structure first appears on the proximal surface of the giant centriole and later migrates more distally from the giant centriole during spermatid differentiation.

We then studied the relationship between this novel structure and the PCM marker γ-tubulin. Although no direct demonstration has been done using immuno-EM on γ-tubulin during spermatogenesis, γ-tubulin staining shows a remarkably close correlation with the morphogenesis of CA, therefore it is accepted that it most likely represents the centriolar adjunct (Wilson *et al.* 1997; Callaini *et al.* 1999; Baker *et al.* 2004; Texada *et al.* 2008). In early spermatids, γ-tubulin labels the vicinity of the giant centriole along most of its length (Figure 2C). As the spermatids continue to differentiate, γ-tubulin labeling gets shorter and is shaped into a collar that surrounds the centriole on one side (Figure 2C). The open side of the γ-tubulin collar faces the Ana1-GFP-labeled focus; clearly, the two signals label two different structures. Later, γ-tubulin fades away while Ana1-GFP staining is maintained (Figure 2C). This demonstrates that Ana1-GFP labels a novel structure that is clearly distinct from the centriolar adjunct. Since this structure forms near the proximal side of the giant centriole, is labeled by a centriolar protein, and is distinct from the centriolar adjunct, it presumably represents the fly equivalent of the vertebrate proximal centriole, which is why we called this proximal centriole-like (PCL).

In electron microscopy, the centriolar adjunct appears very dark, which possibly hinders the capacity to distinguish structures that are closely associated with it (Anderson 1967; Tates 1971; Tokuyasu 1975a,b). In an attempt to visualize the PCL using electron microscopy, we performed serial electron microscopy of fixed tissue in late spermatids when the centriolar adjunct is absent (Figure S1 and Figure S2). We have observed only one centriolar structure, the basal body, suggesting that the PCL is hard to detect structurally.

Strong overexpression of centriolar proteins generate ectopic structures (Kleylein-Sohn *et al.* 2007; Peel *et al.* 2007; Rodrigues-Martins *et al.* 2007; Strnad *et al.* 2007). To minimize this effect, Ana1 and other centriolar proteins used in this study are driven by their own promoters. Each of the centriolar protein GFP fusion constructs studied in this work show a strict specificity to centriolar structures. Additionally, to ensure that the formation of the PCL is not a consequence of the expression of Ana1-GFP, we used the antibody against Ana1. As expected, the antibody recognizes the centriole and the PCL in testes from flies expressing Ana1-GFP (Figure 2D). Importantly, in spermatids, which do not

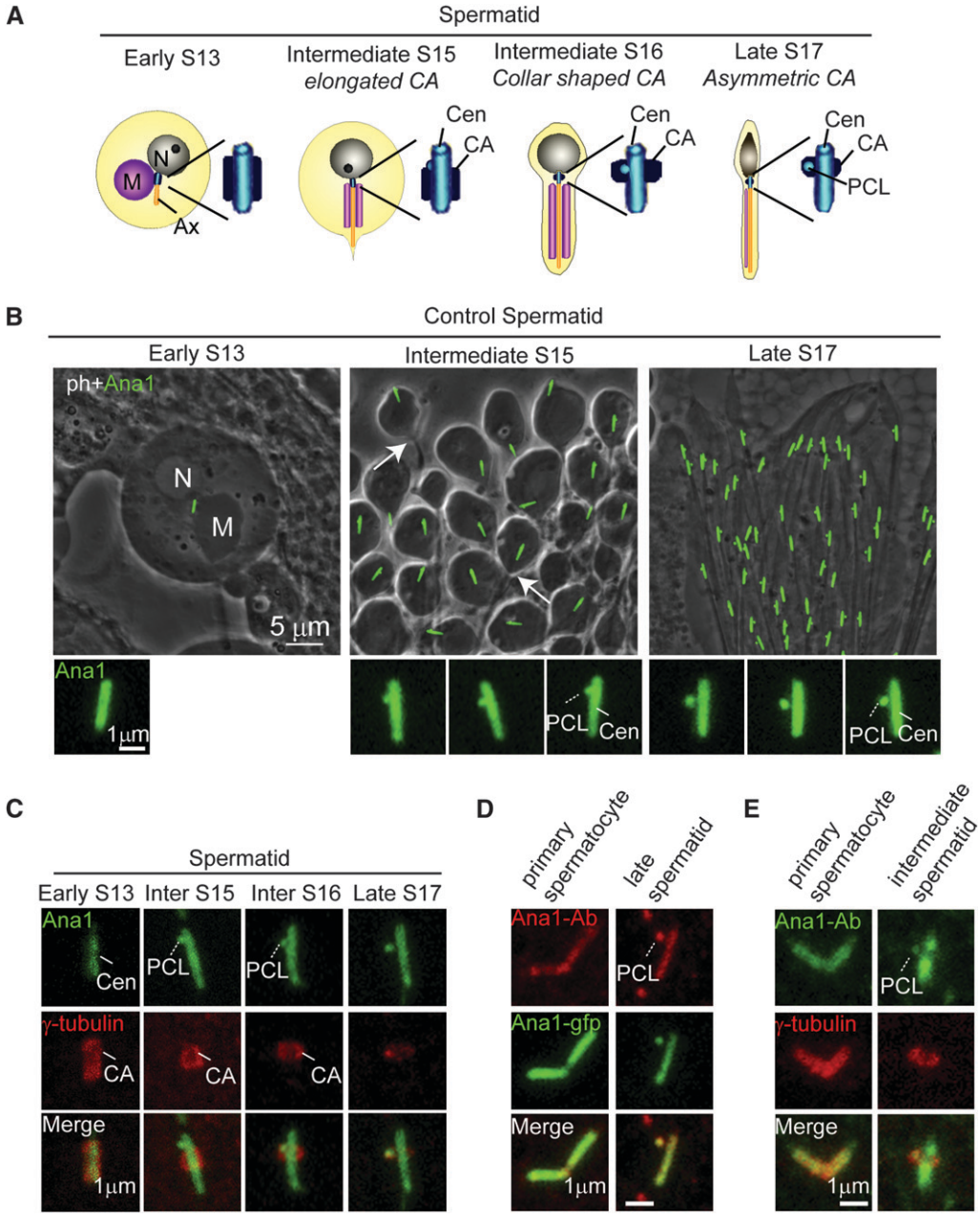

FIGURE 2.—Ana1 labels a novel structure appearing near the mother centriole in spermatids. (A) Diagram depicting the different stages of spermatid development based on the observations of TATES (1971). (M, mitochondria; N, nucleus; Ax, axoneme). The basal body or giant centriole (Cen) is surrounded by the centriolar adjunct (CA) and, near it, we can follow the formation of the PCL. (B) We use phase-contrast pictures (unfixed testis) to determine the spermatid stage. The onion stage (stage S13) is characterized by a round nucleus (N) of the same size as the mitochondrial derivatives (M). The cell body of intermediate spermatids (stages 15 and 16) elongates, forming short protrusions (arrows), but the nucleus remains round. In late spermatid development (stage 17), the nucleus becomes oval. Ana1-GFP labels the giant centriole (Cen), and in intermediate spermatids a bulge forms on one side and becomes individualized as PCL in late spermatid development. (C) Staining with anti-γ-tubulin antibody shows that the PCL labeled by Ana1 is an entity different from the γ-tubulin collar that is reminiscent of the centriolar adjunct (CA). (D) Antibody against Ana1 labels the V-shape pair of giant centrioles in primary spermatocytes (left) and the giant centriole and PCL in spermatids (right) in flies expressing Ana1-GFP. (E) In wild-type primary spermatocytes, anti-Ana1 antibody stains the endogenous protein in the giant centrioles and colocalizes with γ-tubulin staining (left). In spermatids, the antibody labels the PCL, demonstrating that its formation is not due to centriolar protein overexpression (right).

overexpress any centriolar proteins, the Ana1 antibody recognizes the PCL found near the centriole that is surrounded by the γ-tubulin collar (Figure 2E).

Altogether, these results indicate that Ana1 is a component of a novel centriolar structure forming near the giant centriole. In vertebrates, the distal and proximal centrioles are mother and daughter centrioles, respectively (KRIOUTCHKOVA and ONISHCHENKO 1999). In numerous animals including fish, starfish, annelids, and mollusks, the proximal centriole is smaller than the distal centriole (KRIOUTCHKOVA and ONISHCHENKO 1999). The proximal centriole-like structure resembles a daughter centriole as it exhibits two features that are universally shared when daughter centrioles are formed: it associates with the vicinity of the proximal end of the mother centriole and its number is restricted to one. As a result, PCL formation likely depends on the same molecular mechanism that triggers daughter centriole formation.

Proximal centriole-like structure formation is mediated by a conserved molecular pathway: Recently, studies in *Caenorhabditis elegans* using RNAi established

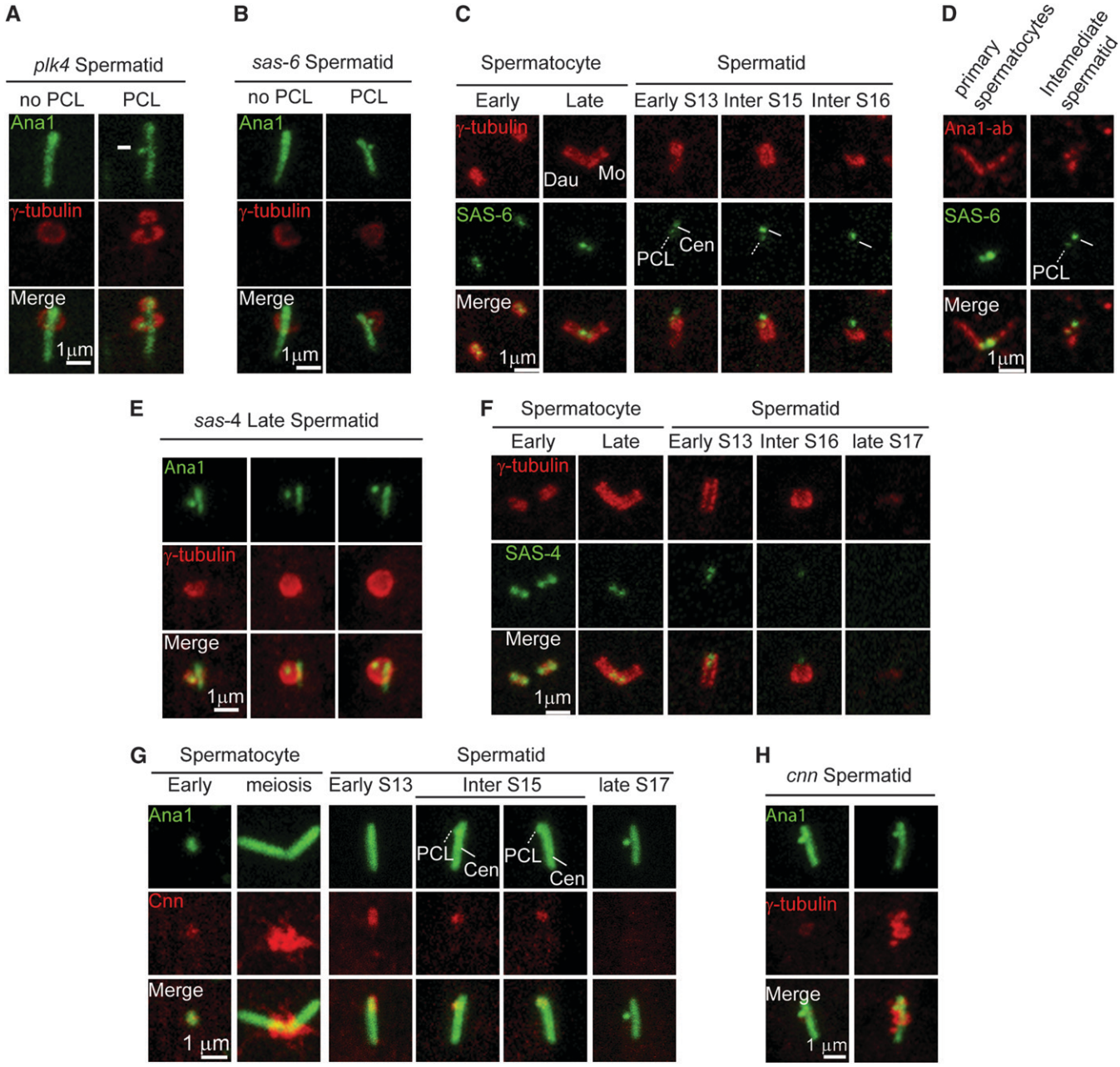

FIGURE 3.—PCL formation depends on early players in centriole duplication. (A and B) PCL formation is impaired in *plk4* and *sas-6* mutants (for quantification see Table 1). (C) In primary spermatocytes, we can distinguish the daughter centriole (Dau) sitting perpendicularly to the mother centriole (Mo) (based on the γ-tubulin staining). SAS-6-GFP localizes to the proximal part of both mother (Mo) and daughter (Dau) giant centrioles in spermatocytes (left) but is enriched in the daughter. In spermatids, SAS-6-GFP localizes to the proximal side of the giant centriole (Cen) and in addition a second dot, probably in the PCL, appears (right). (D) SAS-6-GFP is localized to the proximal part of the V-shaped pair of giant centrioles in primary spermatocytes (left) stained with anti-Ana1 antibody. In spermatids (right), the staining with anti-Ana1 antibody confirms the colocalization of the SAS-6-GFP second dot with the PCL. (E) We follow the fate of the maternally contributed centriole (for explanation see BLACHON *et al.* 2008) in *sas-4* mutants. In *sas-4*, centrioles are shorter but it does not affect PCL formation, demonstrating that SAS-4 is not required for PCL formation. (F) Like SAS-6-GFP, SAS-4-GFP is localized to the proximal part of the giant centriole in primary spermatocytes. In early spermatids, SAS-4-GFP is still present at the proximal part of the centriole and a second dot can be distinguished. Later, the SAS-4-GFP signal decreased and no SAS4-GFP was detected in the PCL. (G) The PCM protein Cnn shows a similar localization to γ-tubulin (BLACHON *et al.* 2008) in meiosis. However, after meiosis, it is restricted to the proximal part of the giant centriole in early spermatids; subsequently, it is colocalized transiently with the forming PCL in intermediate spermatids. Later, it disappears in late spermatid development. (H) In *cnn* mutant spermatids, we still observe the PCL labeled by Ana1-GFP, but the γ-tubulin pattern is disrupted. Some spermatids show a very faint or an absence of γ-tubulin labeling (left) whereas others have abnormal localization of the γ-tubulin collars (right).

TABLE 1

Quantification of centriole duplication failure in *plk4* and *sas-6* mutants

	PCL (%)	No PCL (%)	*N*
Control (*plk4*/TM6B)	90.2	9.8	516
plk4 mutants	21.4	78.6	42
	28.1	71.9	57
	20.9	79.1	67
Control (*sas-6*/TM6B)	93.4	6.6	682
sas-6 mutants	31.4	68.6	373
	33.2	66.8	196

Consistent with previously published partial effect on centriole formation (Bettencourt-Dias *et al.* 2005; Rodrigues-Martins *et al.* 2007), we observed a high percentage of spermatids with no PCL in *plk4* and *sas-6* mutants. For each experiment, heterozygotes flies were counted as a control. *N* is the number of spermatids that were counted.

the chronology of a module of proteins responsible for the formation of the daughter centriole (Delattre *et al.* 2006; Pelletier *et al.* 2006). For a recent review, see Strnad and Gonczy (2008). The consensus resulting from these studies indicates that a Zyg-1/PLK4 family of kinases is required for the early recruitment of SAS-6 and SAS-5. Electron tomography made it possible to show that centriole assembly in *C. elegans* starts with the formation of a central tube that depends on SAS-6 and SAS-5, followed by the assembly of a microtubule wall under the control of SAS-4 (Pelletier *et al.* 2006). In addition, it was found recently that, while SAS-6 incorporates into a stable structure early on, SAS-4 remains in dynamic equilibrium until later when it is stably incorporated in a step requiring γ-tubulin and mediating centriole wall assembly (Dammermann *et al.* 2008).

This module of proteins is conserved in other organisms; for example, in humans the homolog of Zyg1, the kinase SAK/PLK4, is required early on in centriole formation (Kleylein-Sohn *et al.* 2007). The role of SAS-6 in centriole duplication has been confirmed in humans, and in addition it is found localized in the centriole cartwheel in humans (Kleylein-Sohn *et al.* 2007), in Chlamydomonas (Nakazawa *et al.* 2007), and in Tetrahymena (Kilburn *et al.* 2007). Finally, SAS-4 has two homologs in humans; one of them, CPAP, is a centriolar protein that is able to bind microtubules (Hung *et al.* 2000; Hsu *et al.* 2008). This fits with a model where, in a first step, early players such as SAK/PLK4 and SAS-6 build the centriole core, and at a later time SAS-4, via its interaction with microtubules, assembles the centriolar wall. As in other model organisms, Drosophila SAK/PLK4, SAS-6, and SAS-4 are essential for centriole formation (Bettencourt-Dias *et al.* 2005; Basto *et al.* 2006; Rodrigues-Martins *et al.* 2007).

To test our hypothesis on the nature of the PCL, we studied the phenotypes of available mutants in Drosophila—*plk4/sak*, *sas-6*, and *sas-4*—and followed the localization of the corresponding proteins. In flies, the hypomorphic mutation of $plk4^{c06612}$ contains a *piggyBac* insertion near the end of the gene that affects centriole duplication. It was reported that, in this mutant, 72% of the neuroblast cells have fewer than two centrioles (Bettencourt-Dias *et al.* 2005). Similarly, a *piggyBac* insertion in the second exon of the *sas-6* gene leads to a decrease of centriole number, and 82% of neuroblast cells have fewer than two centrioles (Rodrigues-Martins *et al.* 2007). Consistent with this, using Ana1-GFP as a reporter, we noted that $plk4^{c06612}$ and $sas\text{-}6^{c02901}$ have a reduced number of centrioles in spermatocytes (data not shown). More importantly, as expected from our hypothesis, similar reduction in PCL formation is observed in $plk4^{c06612}$ and $sas\text{-}6^{c02901}$ spermatids (Figure 3, A and B, and Table 1).

Examination of the localization of SAS-6 tagged with GFP that shows a specific localization to the centriole in neuroblast cells (Blachon *et al.* 2008) demonstrates that, in early spermatocytes, SAS-6-GFP labels the center of the centrosome and that, in late spermatocytes after centriole elongation, only the proximal end of the centriole is labeled (Figure 3C). This localization is different from previous research, which reported that SAS-6-GFP also labeled the distal end of the centriole (Rodrigues-Martins *et al.* 2007), which is likely to be due to the strong ubiquitin promoter used to drive SAS-6-GFP expression in previous studies. In spermatids, SAS-6-GFP maintains its labeling at the proximal end of the giant centriole (Figure 3C). However, we noted that, in addition to this signal, a second SAS-6-GFP focus appears in early spermatids and remains in intermediate spermatids. To determine whether this focus corresponds to the PCL, we stained the testes with Ana1 antibody (Figure 3D). We found that the second SAS-6-GFP focus colocalizes with the Ana1-labeled PCL in intermediate spermatids. This result confirms that the PCL is labeled by SAS-6, which is consistent with the role of SAS-6 in PCL formation.

It was previously shown that, in $sas\text{-}4^{s2214}$ flies, centriole numbers are reduced in the development of the fly until they are completely missing in adult flies (Basto *et al.* 2006). Since in $sas\text{-}4^{s2214}$ flies adult spermatids lack new centrioles, we analyzed the formation of the PCL at the vicinity of maternally contributed centrioles. This method (Blachon *et al.* 2008) is based on following the fate of centrioles built during embryonic development using the wild-type maternal proteins accumulated in the egg (maternal contribution). After the maternal proteins are depleted, we can study this preassembled centriole in the mutant context. We noted that $sas\text{-}4^{s2214}$ centrioles are abnormally short (1.1 ± 0.3 μm, $N = 11$, *vs.* 2.3 ± 0.4 μm, $N = 41$, for wild type; Blachon *et al.* 2008) (Figure 3E). This result is consistent with a previous report that showed that the partial inactivation of SAS-4 in *C. elegans* leads to the formation of smaller centrioles with an abnormal

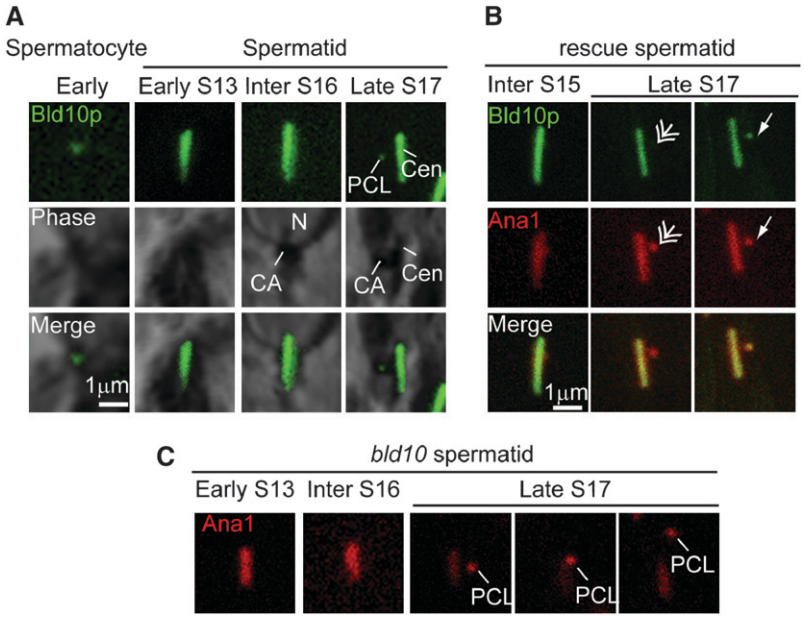

FIGURE 4.—Bld10p is recruited late to the PCL and is not required for its formation. (A) Bld10p-GFP labels the centriole in spermatocytes (left) and the giant centriole (Cen) in spermatids (right). Only later, in late spermatids, is Bld10p recruited to the PCL. The centriolar adjunct (CA) and the nucleus (N) are visible in phase-contrast pictures (middle). (B) Colocalization of Bld10p-GFP with Ana1-tdTomato confirms that Bld10p localizes to the PCL only later (arrow). The double arrowhead points to the PCL labeled by Ana1-tdTomato and not yet by Bld10p-GFP. To maximize the signal, Bld10p-GFP was expressed in a *bld10* mutant context (rescue flies). (C) In the *bld10* mutant, centrioles are shorter but PCL formation is not affected, consistent with the role of Bld10p later in the process.

structure (KIRKHAM *et al.* 2003). Together, this suggests that SAS-4 has a role in centriole stability. Despite the fact that the giant centriole is abnormal, the PCL is present in spermatids (Figure 3E). This result suggests that SAS-4 is not involved in PCL formation and that PCL formation can take place even if the mother centriole integrity is compromised.

To determine whether SAS-4 plays a nonessential role, we analyzed SAS-4-GFP localization. We found that, like SAS-6-GFP, SAS-4-GFP in spermatocytes and spermatids labels the proximal end of the centriole. Again, this is different from previous reports that used a strong ubiquitin promoter to drive SAS-4 expression (BASTO *et al.* 2006). In early spermatids (S13), similarly to SAS-6-GFP, SAS-4-GFP shows two dots. However, in intermediate and late spermatids, SAS-4-GFP does not label the PCL (Figure 3F), supporting the observation that SAS-4 is not essential for PCL formation. Consistent with results in worms, it seems that, while SAS-4 and SAS-6 are recruited together to an early structure during the PCL formation, only SAS-6, and not SAS-4, is essential at that time (PELLETIER *et al.* 2006; DAMMERMANN *et al.* 2008). SAS-4 in worms is essential later for the formation of the centriole wall (DAMMERMANN *et al.* 2008), leading us to hypothesize that the PCL might not have a microtubule-containing wall, which explains why it is so hard to find it using electron microscopy.

Most of the more established markers of centrosomes in flies are components of the PCM and are not essential for centriole duplication (KELLOGG *et al.* 1995; MEGRAW *et al.* 1999; BUTCHER *et al.* 2004). We have tested the localization of one of these proteins: centrosomin (Cnn), which has been identified as a PCM protein (LI and KAUFMAN 1996). In early spermatocytes, Cnn is localized to the centriole and, like γ-tubulin (BLACHON *et al.* 2008), it is reorganized to the PCM during meiosis (Figure 3G, left). In early spermatids, Cnn is localized at the extremities of the centriole with some enrichment at the proximal part. In intermediate spermatids when the PCL starts to form, Cnn colocalizes with the bulge (Figure 3G, right) and later disappears. It has recently been shown that Cnn is required for the connection between the centriole and the PCM (LUCAS and RAFF 2007); consequently, in *cnn* mutants, PCM recruitment to the centriole is impaired and centrioles segregate abnormally. Since Cnn colocalizes with the forming PCL, we wondered if PCL formation would be impaired in *cnn* mutants. We found that in *cnn* spermatids the PCL forms (Figure 3H); however, the γ-tubulin collars were largely reduced and almost absent (Figure 3H, left) or showed a totally abnormal localization (Figure 3H, right). These results demonstrate that, as in normal daughter centriole formation, Cnn is dispensable for PCL formation. We propose that, similar to its role in recruiting the PCM, Cnn localization to the forming PCL is required for the proper localization of the γ-tubulin collar.

Bld10p is recruited late to the PCL but is not required for its formation: The above findings suggest that the PCL is accessible for genetic analysis. Therefore, we first tested whether this system could be used to determine the involvement of known centriolar protein in the initiation of the daughter centriole. Bld10p/Cep135 is a centriolar protein that was found in Chlamydomonas to be localized just internally to the wall of microtubules. In human cells, it was shown to be a component of the centriole wall (HIRAKI *et al.* 2007; KLEYLEIN-SOHN *et al.* 2007). For this reason, it is possible that Bld10p acts between SAS-6 and SAS-4 in the centriole assembly pathway. To test this, we examined the localization of a Bld10p-GFP reporter (BLACHON *et al.* 2008). We found that Bld10p-GFP labels the centriole in early spermatocytes and labels them along their length after elonga-

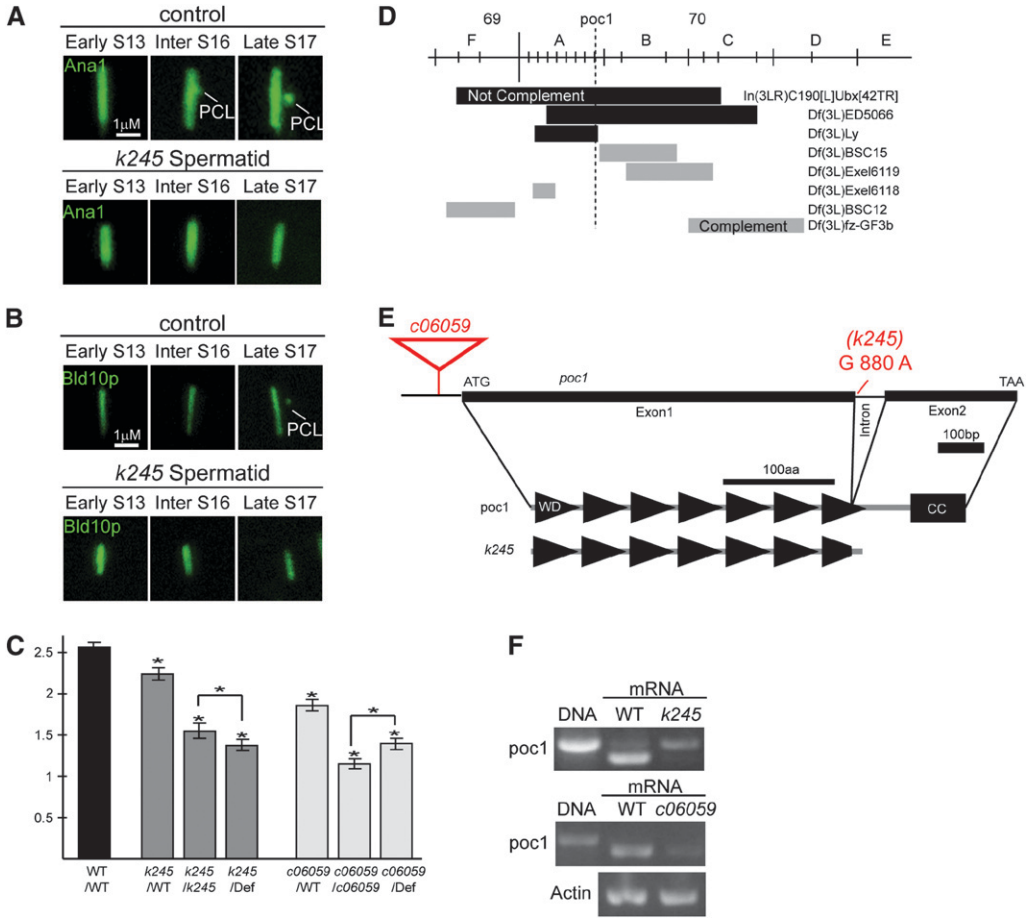

FIGURE 5.—POC1, a conserved centriolar protein, is involved in PCL formation. (A) A forward genetic screen identified a new mutation, *k245*, that impairs the formation of the PCL as judged by Ana1-GFP labeling. (B) Absence of a normal PCL in *k245* is confirmed using Bld10p-GFP. (C) Statistical analysis of the basal body length on *poc1* alleles. The deficiency used is Df(3L)Ly. Each experiment was performed on three different flies and 60 basal bodies were measured. The columns marked by a star (*) are statistically different from the control; additional pairs were also found significantly different on the basis of $P < 0.01$ using Student's *t*-test. (D) Complementation test with several deletions shows that *k245* maps to an interval containing the gene CG10191. Solid bars: deletions that did not complement the *k245* phenotype; shaded bars: deletions that did complement *k245*. (E) CG10191 encodes the conserved centriolar protein POC1. *k245* mutation changes a G to an A at position 880 (in red), which is predicted to eliminate a splicing site. $poc1^{c06059}$ contains an insertion in poc1 promoter (in red). POC1 protein is composed of 7 WD domains known to form a β-propeller structure and contains a coiled-coil domain in its C terminus. The *k245* mutation is predicted to induce a frameshift resulting in a truncated protein containing only the WD domains. (F) RT–PCR on mRNA shows that in wild-type flies mRNA is spliced to give a smaller product, which is absent in $poc1^{k245}$ flies, confirming that the *k245* mutation eliminates a splicing site. $poc1^{c06059}$ shows a significantly lower amount of mRNA than wild type. Actin mRNA is shown as a control for loading.

tion (Figure 4A). This localization is maintained in spermatids. In addition, Bld10p-GFP labels the PCL; however, this labeling appears later in spermatid differentiation when the PCL is more distal and farther away from the centriole. To directly observe the sequential order of Ana1 and Bld10p in centriole assembly, we generated flies expressing both Bld10p-GFP and Ana1-tdTomato in a *dbld10* mutant background as a mean to maximize the Bld10p signal (Figure 4B). We confirmed that Bld10p localizes to the PCL only after Ana1 is already there, demonstrating that Bld10p is recruited later in PCL formation (Figure 4B).

To test for the role of Bld10p in initiating daughter centriole formation, from the Exelixis collection we obtained the mutant allele $dbld10^{e04199}$ (THIBAULT *et al.* 2004), which has an insertion in the middle of the gene resulting in Bld10p truncation, and most likely represents a severe loss of function. We analyzed flies that are heterozygous for this allele over the chromosomal deletion Df(3L)ED218, which covers this gene. We found that males are sterile, have shorter centrioles than the control, and occasionally have mislocalized PCLs (Figure 4C). Consistent with the late recruitment of Bld10p to the PCL, Bld10p is not essential for its formation. This indicates that Bld10p acts after the initiation of PCL formation.

Centriolar markers of the giant centriole and PCL disappear in mature sperm: Sperm centrosomes are known to undergo a process in which they lose many of their markers; this process is known as centrosome reduction (SCHATTEN 1994). In flies it was reported that centrosomal proteins such as γ-tubulin, Cnn, centrin, and CP190 are not found in mature sperm centrosomes and centrioles (WILSON *et al.* 1997; CALLAINI *et al.* 1999). To test what happened to the PCL at the final stages of spermatogenesis, we analyzed mature sperm for PCL markers. We were unable to detect any of the markers that we tested (Figure S3). As a consequence, until now, it was not possible to follow the fate of the PCL after fertilization to investigate its function.

A forward genetic screen identified a new player in PCL formation—POC1: Next we were interested in testing if we could use forward genetics in this system to identify novel centriolar mutants involved in the initiation of daughter centriole formation. Mutations that specifically affect the centriole and cilia, such as *plk4*, *sas-6*, and *sas-4*, develop into adult flies (Bettencourt-Dias *et al.* 2005; Basto *et al.* 2006; Rodrigues-Martins *et al.* 2007). Therefore, we expected that mutations that specifically affect the PCL would not cause lethality early in development. We screened 150 fly lines randomly selected from the Exelixis collection (Thibault *et al.* 2004) and 150 lines from the Zuker collection (Koundakjian *et al.* 2004) that either had a ciliary/centriolar phenotype producing uncoordinated flies or had a known mutation in a candidate ciliary gene (Avidor-Reiss *et al.* 2004). To observe the centriole and the PCL, we introduced the Ana1-GFP reporter into the genetic background of each of these mutants. A single mutation found in the Zuker collection, named *k245*, had a complete loss of Ana1-GFP (Figure 5A) and Bld10p-GFP (Figure 5B) labeling of the PCL. Other mutants from the Zuker collection had clear PCL labeling, indicating that the lack of PCL is due to a particular mutation in the *k245* line (data not shown). In addition to the absence of PCL labeling, *k245* had a shorter centriole in spermatocytes (Figure 5C) but was able to generate viable and fertile flies.

Positional cloning found that *k245* mapped to an interval containing 10 genes (Figure 5D). A single gene in the interval, CG10191, was identified previously as a candidate ciliary gene in a bioinformatics screen (Avidor-Reiss *et al.* 2004) and was later reported in centriolar proteomic studies to be the ortholog of Chlamydomonas and Tetrahymena: **p**roteome **o**f **c**entrioles 1, or POC1 (Keller *et al.* 2005; Kilburn *et al.* 2007). We found that a *piggyBac* insertion allele (*c06059*) generated by Exelixis (Thibault *et al.* 2004) in the predicted gene CG10191 failed to complement *k245* (Figure 5E), demonstrating that *k245* is a *poc1* allele. POC1 is a 391-amino-acid protein composed of a highly conserved N-terminal domain with 7 WD repeats (amino acids 1–300) and a less conserved C terminus of 90 amino acids that contains a coiled-coil domain of 42 amino acids at its end (Figure 5E). Seven WD repeats are known to form a β-propeller structure (Neer and Smith 1996), and this domain is encoded by the first exon that is separated from the rest of the protein by a single intron (Figure 5E). We sequenced $poc1^{k245}$ and found that it contains the mutation-changing nucleotide G880A (relative to ATG), which eliminates the predicted 5′ intron splice site (Figure 5E). This was confirmed by RT–PCR of mRNA from $poc1^{k245}$ and wild-type control flies (Figure 5F). Therefore, the $poc1^{k245}$ is predicted to result in a frameshift after the first exon and to generate a truncated protein consisting of only the WD β-propeller structure. $poc1^{c06059}$ has a *piggyBac* insertion in the promoter of the gene (67 bases upstream to the ATG) (Figure 5E) that results in a reduced mRNA level (Figure 5F). $poc1^{c06059}$, like $dpoc1^{k245}$, had centrioles that were shorter than normal in spermatocytes (Figure 5C) and was able to generate viable and fertile flies.

The identification of POC1 as a protein essential for PCL formation suggests that POC1 plays a central role in centriole formation. This is consistent with the localization study in Tetrahymena, which shows that POC1 associates with the site of daughter centriole assembly (Kilburn *et al.* 2007). In addition, a very recent study shows that, in Chlamydomonas, POC1 localizes to the forming basal body (pro-basal body) and that, in human cells, RNAi against POC1 reduces centriole overduplication (Keller *et al.* 2008). This demonstrates that studying PCL formation can serve to identify novel mutation in conserved centriolar genes.

Conclusion: We have found that topological, molecular, and genetic criteria point to the presence of a daughter centriole in fly spermatids. By analogy with other animal groups, the PCL is likely the fly counterpart of the proximal centriole found in vertebrates. PCL formation depends on SAK/PLK4 and SAS-6, which are proteins that act early in centriole duplication, but is independent of proteins such as SAS-4 or Bld10p, which act later. This indicates that the PCL represents an early step of the centriole formation that has stopped in its development. Unlike the vertebrate procentriole, the PCL did not conserve the distinctive structure of mature centrioles, suggesting that its unknown function does not require maturation in spermatids. Consequently, the PCL is an easily accessible model for studying genetically the early steps of centriole formation.

We thank the Harvard Medical School EM facility for their help with electron microscopy, J. W. Raff for the GFP-PACT flies, and T. C. Kaufman for Cnn antibodies. This work was supported partially by a grant from the Hellman Family Fund Program. Stephanie Blachon was supported partially by a Fondation pour la Recherche Medicale grant. Andrey Polyanovsky was supported by the Russian Foundation for Basic Research grant no. 07-04-01127.

LITERATURE CITED

Allen, R. D., 1969 The morphogenesis of basal bodies and accessory structures of the cortex of the ciliated protozoan Tetrahymena pyriformis. J. Cell Biol. **40:** 716–733.

Andersen, J. S., C. J. Wilkinson, T. Mayor, P. Mortensen, E. A. Nigg *et al.*, 2003 Proteomic characterization of the human centrosome by protein correlation profiling. Nature **426:** 570–574.

Anderson, W. A., 1967 Cytodifferentiation of spermatozoa in Drosophila melanogaster: the effect of elevated temperature on spermiogenesis. Mol. Gen. Genet. **99:** 257–273.

Avidor-Reiss, T., A. M. Maer, E. Koundakjian, A. Polyanovsky, T. Keil *et al.*, 2004 Decoding cilia function: defining specialized genes required for compartmentalized cilia biogenesis. Cell **117:** 527–539.

Azimzadeh, J., and M. Bornens, 2007 Structure and duplication of the centrosome. J. Cell Sci. **120:** 2139–2142.

Baker, J. D., S. Adhikarakunnathu and M. J. Kernan, 2004 Mechanosensory-defective, male-sterile unc mutants identify a novel basal body protein required for ciliogenesis in Drosophila. Development **131:** 3411–3422.

Basto, R., J. Lau, T. Vinogradova, A. Gardiol, C. G. Woods *et al.*, 2006 Flies without centrioles. Cell **125:** 1375–1386.

Bettencourt-Dias, M., A. Rodrigues-Martins, L. Carpenter, M. Riparbelli, L. Lehmann *et al.*, 2005 SAK/PLK4 is required for centriole duplication and flagella development. Curr. Biol. **15:** 2199–2207.

Blachon, S., J. Gopalakrishnan, Y. Omori, A. Polyanovsky, A. Church *et al.*, 2008 Drosophila Asterless and vertebrate Cep152 are orthologs essential for centriole duplication. Genetics **180:** 2081–2094.

Butcher, R. D., S. Chodagam, R. Basto, J. G. Wakefield, D. S. Henderson *et al.*, 2004 The Drosophila centrosome-associated protein CP190 is essential for viability but not for cell division. J. Cell Sci **117:** 1191–1199.

Callaini, G., M. G. Riparbelli and R. Dallai, 1999 Centrosome inheritance in insects: fertilization and parthenogenesis. Biol. Cell **91:** 355–366.

Catalano, R. D., M. Vlad and R. C. Kennedy, 1997 Differential display to identify and isolate novel genes expressed during spermatogenesis. Mol. Hum. Reprod. **3:** 215–221.

Cenci, G., S. Bonaccorsi, C. Pisano, F. Verni and M. Gatti, 1994 Chromatin and microtubule organization during premeiotic, meiotic and early postmeiotic stages of Drosophila melanogaster spermatogenesis. J. Cell Sci. **107**(Pt. 12): 3521–3534.

Dammermann, A., P. S. Maddox, A. Desai and K. Oegema, 2008 SAS-4 is recruited to a dynamic structure in newly forming centrioles that is stabilized by the gamma-tubulin-mediated addition of centriolar microtubules. J. Cell Biol. **180:** 771–785.

Delattre, M., C. Canard and P. Gonczy, 2006 Sequential protein recruitment in C. elegans centriole formation. Curr. Biol. **16:** 1844–1849.

Dippell, R. V., 1968 The development of basal bodies in paramecium. Proc. Natl. Acad. Sci. USA **61:** 461–468.

Eberl, D. F., R. W. Hardy and M. J. Kernan, 2000 Genetically similar transduction mechanisms for touch and hearing in Drosophila. J. Neurosci. **20:** 5981–5988.

Friedlander, M., and J. Wahrman, 1971 The number of centrioles in insect sperm: a study in two kinds of differentiating silkworm spermatids. J. Morphol. **134:** 383–397.

Fuller, M. T., 1993 *Spermatogenesis.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Goshima, G., R. Wollman, S. S. Goodwin, N. Zhang, J. M. Scholey *et al.*, 2007 Genes required for mitotic spindle assembly in Drosophila S2 cells. Science **316:** 417–421.

Hiraki, M., Y. Nakazawa, R. Kamiya and M. Hirono, 2007 Bld10p constitutes the cartwheel-spoke tip and stabilizes the 9-fold symmetry of the centriole. Curr. Biol. **17:** 1778–1783.

Hsu, W. B., L. Y. Hung, C. J. Tang, C. L. Su, Y. Chang *et al.*, 2008 Functional characterization of the microtubule-binding and -destabilizing domains of CPAP and d-SAS-4. Exp. Cell Res. **314:** 2591–2602.

Hung, L. Y., C. J. Tang and T. K. Tang, 2000 Protein 4.1 R-135 interacts with a novel centrosomal protein (CPAP) which is associated with the gamma-tubulin complex. Mol. Cell. Biol. **20:** 7813–7825.

Keller, L. C., E. P. Romijn, I. Zamora, J. R. Yates, III and W. F. Marshall, 2005 Proteomic analysis of isolated Chlamydomonas centrioles reveals orthologs of ciliary-disease genes. Curr. Biol. **15:** 1090–1098.

Keller, L. C., S. Geimer, E. Romijn, J. Yates, III, I. Zamora *et al.*, 2008 Molecular architecture of the centriole proteome: the conserved WD40 domain protein POC1 is required for centriole duplication and length control. Mol. Biol. Cell **20:** 1150–1166.

Kellogg, D. R., K. Oegema, J. Raff, K. Schneider and B. M. Alberts, 1995 CP60: a microtubule-associated protein that is localized to the centrosome in a cell cycle-specific manner. Mol. Biol. Cell **6:** 1673–1684.

Kilburn, C. L., C. G. Pearson, E. P. Romijn, J. B. Meehl, T. H. Giddings, Jr. *et al.*, 2007 New Tetrahymena basal body protein components identify basal body domain structure. J. Cell Biol. **178:** 905–912.

Kirkham, M., T. Muller-Reichert, K. Oegema, S. Grill and A. A. Hyman, 2003 SAS-4 is a C. elegans centriolar protein that controls centrosome size. Cell **112:** 575–587.

Kleylein-Sohn, J., J. Westendorf, M. Le Clech, R. Habedanck, Y. D. Stierhof *et al.*, 2007 Plk4-induced centriole biogenesis in human cells. Dev. Cell **13:** 190–202.

Koundakjian, E. J., D. M. Cowan, R. W. Hardy and A. H. Becker, 2004 The Zuker collection: a resource for the analysis of autosomal gene function in *Drosophila melanogaster.* Genetics **167:** 203–206.

Krioutchkova, M. M., and G. E. Onishchenko, 1999 Structural and functional characteristics of the centrosome in gametogenesis and early embryogenesis of animals. Int. Rev. Cytol. **185:** 107–156.

Li, K., and T. C. Kaufman, 1996 The homeotic target gene centrosomin encodes an essential centrosomal component. Cell **85:** 585–596.

Lucas, E. P., and J. W. Raff, 2007 Maintaining the proper connection between the centrioles and the pericentriolar matrix requires Drosophila centrosomin. J. Cell Biol. **178:** 725–732.

Martinez-Campos, M., R. Basto, J. Baker, M. Kernan and J. W. Raff, 2004 The Drosophila pericentrin-like protein is essential for cilia/flagella function, but appears to be dispensable for mitosis. J. Cell Biol. **165:** 673–683.

Megraw, T. L., and T. C. Kaufman, 2000 The centrosome in Drosophila oocyte development. Curr. Top. Dev. Biol. **49:** 385–407.

Megraw, T. L., K. Li, L. R. Kao and T. C. Kaufman, 1999 The centrosomin protein is required for centrosome assembly and function during cleavage in Drosophila. Development **126:** 2829–2839.

Nakazawa, Y., M. Hiraki, R. Kamiya and M. Hirono, 2007 SAS-6 is a cartwheel protein that establishes the 9-fold symmetry of the centriole. Curr. Biol. **17:** 2169–2174.

Neer, E. J., and T. F. Smith, 1996 G protein heterodimers: new structures propel new questions. Cell **84:** 175–178.

Peel, N., N. R. Stevens, R. Basto and J. W. Raff, 2007 Overexpressing centriole-replication proteins in vivo induces centriole overduplication and de novo formation. Curr. Biol. **17:** 834–843.

Pelletier, L., E. O'Toole, A. Schwager, A. A. Hyman and T. Muller-Reichert, 2006 Centriole assembly in Caenorhabditis elegans. Nature **444:** 619–623.

Phillips, D. M., 1970 Insect sperm: their structure and morphogenesis. J. Cell Biol. **44:** 243–277.

Rodrigues-Martins, A., M. Bettencourt-Dias, M. Riparbelli, C. Ferreira, I. Ferreira *et al.*, 2007 DSAS-6 organizes a tube-like centriole precursor, and its absence suggests modularity in centriole assembly. Curr. Biol. **17:** 1465–1472.

Rothwell, W. F., and W. Sullivan, 2000 Fluorescent analysis of Drosophila embryos, pp. 141–157 in *Drosophila Protocols*, edited by W. Sullivan, M. Ashburner and R. Scott Hawley. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sathananthan, A. H., W. D. Ratnasooriya, P. K. de Silva and J. Menezes, 2001 Characterization of human gamete centrosomes for assisted reproduction. Ital. J. Anat. Embryol. **106:** 61–73.

Schatten, G., 1994 The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization. Dev. Biol. **165:** 299–335.

Sorokin, S. P., 1968 Reconstructions of centriole formation and ciliogenesis in mammalian lungs. J. Cell Sci. **3:** 207–230.

Strnad, P., and P. Gonczy, 2008 Mechanisms of procentriole formation. Trends Cell Biol. **18:** 389–396.

Strnad, P., S. Leidel, T. Vinogradova, U. Euteneuer, A. Khodjakov *et al.*, 2007 Regulated HsSAS-6 levels ensure formation of a single procentriole per centriole during the centrosome duplication cycle. Dev. Cell **13:** 203–213.

Tates, A. D., 1971 *Cytodifferentiation During Spermatogenesis in Drosophila melanogaster: An Electron Microscope Study.* Rijksuniversiteit de Leiden, Leiden, Netherlands.

Texada, M. J., R. A. Simonette, C. B. Johnson, W. J. Deery and K. M. Beckingham, 2008 Yuri gagarin is required for actin, tubulin and basal body functions in Drosophila spermatogenesis. J. Cell Sci. **121:** 1926–1936.

Thibault, S. T., M. A. Singer, W. Y. Miyazaki, B. Milash, N. A. Dompe *et al.*, 2004 A complementary transposon tool kit for

Drosophila melanogaster using P and piggyBac. Nat. Genet. **36:** 283–287.

Tokuyasu, K. T., 1975a Dynamics of spermiogenesis in Drosophila melanogaster. V. Head-tail alignment. J. Ultrastruct. Res. **50:** 117–129.

Tokuyasu, K. T., 1975b Dynamics of spermiogenesis in Drosophila melanogaster. VI. Significance of "onion" nebenkern formation. J. Ultrastruct. Res. **53:** 93–112.

Varmark, H., S. Llamazares, E. Rebollo, B. Lange, J. Reina *et al.*, 2007 Asterless is a centriolar protein required for centrosome function and embryo development in Drosophila. Curr. Biol. **17:** 1735–1745.

Wilson, P. G., Y. Zheng, C. E. Oakley, B. R. Oakley, G. G. Borisy *et al.*, 1997 Differential expression of two gamma-tubulin isoforms during gametogenesis and development in Drosophila. Dev. Biol. **184:** 207–221.

Communicating editor: M. Johnston

GENETICS

Supporting Information

http://www.genetics.org/cgi/content/full/genetics.109.101709/DC1

A Proximal Centriole-Like Structure Is Present in Drosophila Spermatids and Can Serve as a Model to Study Centriole Duplication

Stephanie Blachon, Xuyu Cai, Kela A. Roberts, Kevin Yang, Andrey Polyanovsky, Allen Church and Tomer Avidor-Reiss

DOI: 10.1534/genetics.109.101709

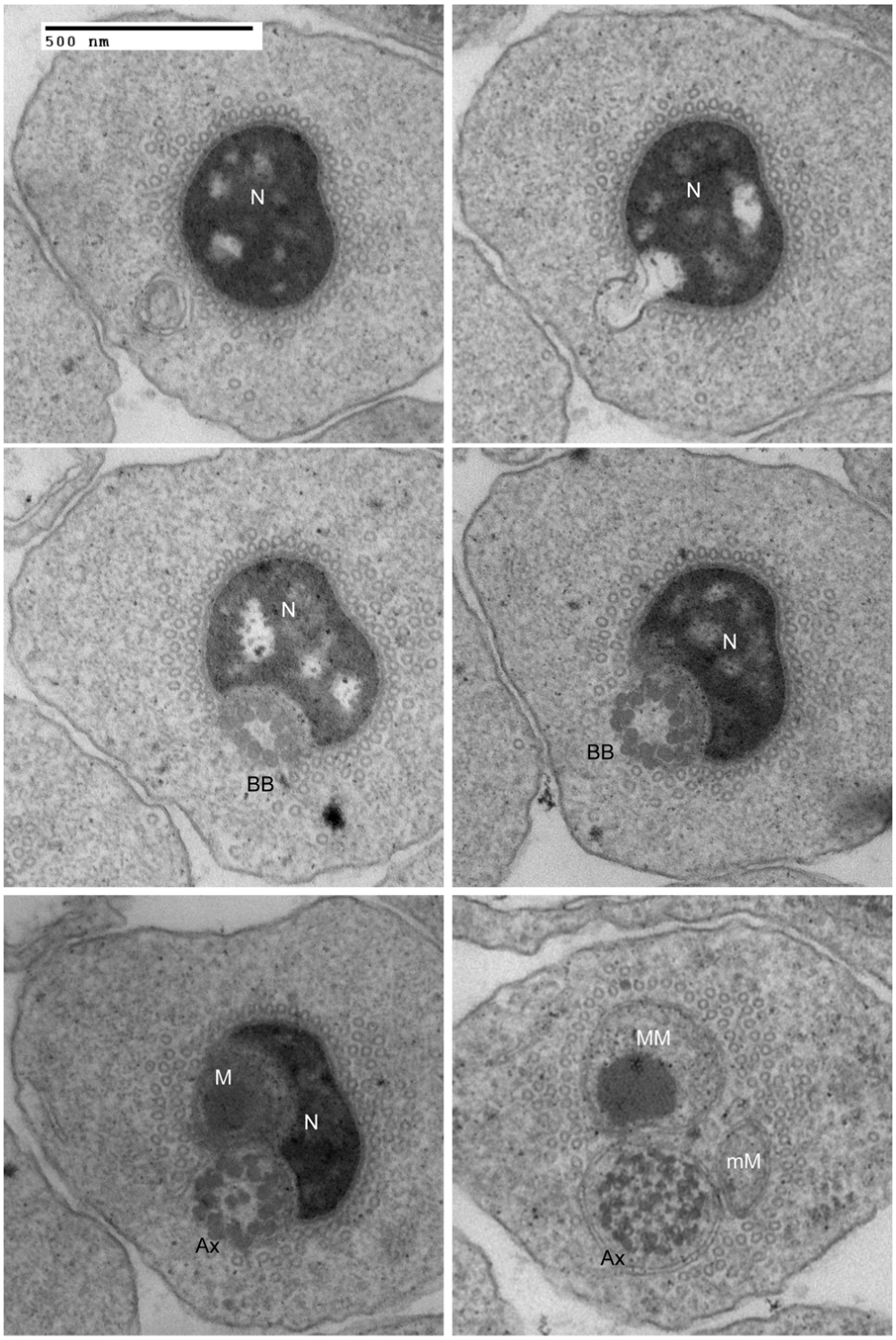

FIGURE S1.—A single centriolar structure is detected in late spermatids (stage 18). Representative sections (70 nm thick) from serial sections at the nucleus level (top two panels); the basal body lacking the 2 central tubule (middle two panels) and up to the axoneme that have 2 central tubules (bottom two panels). N, Nucleus; BB, basal body; M, mitochondria; MM, major mitochondria; mM, minor mitochondria; Ax, axoneme.

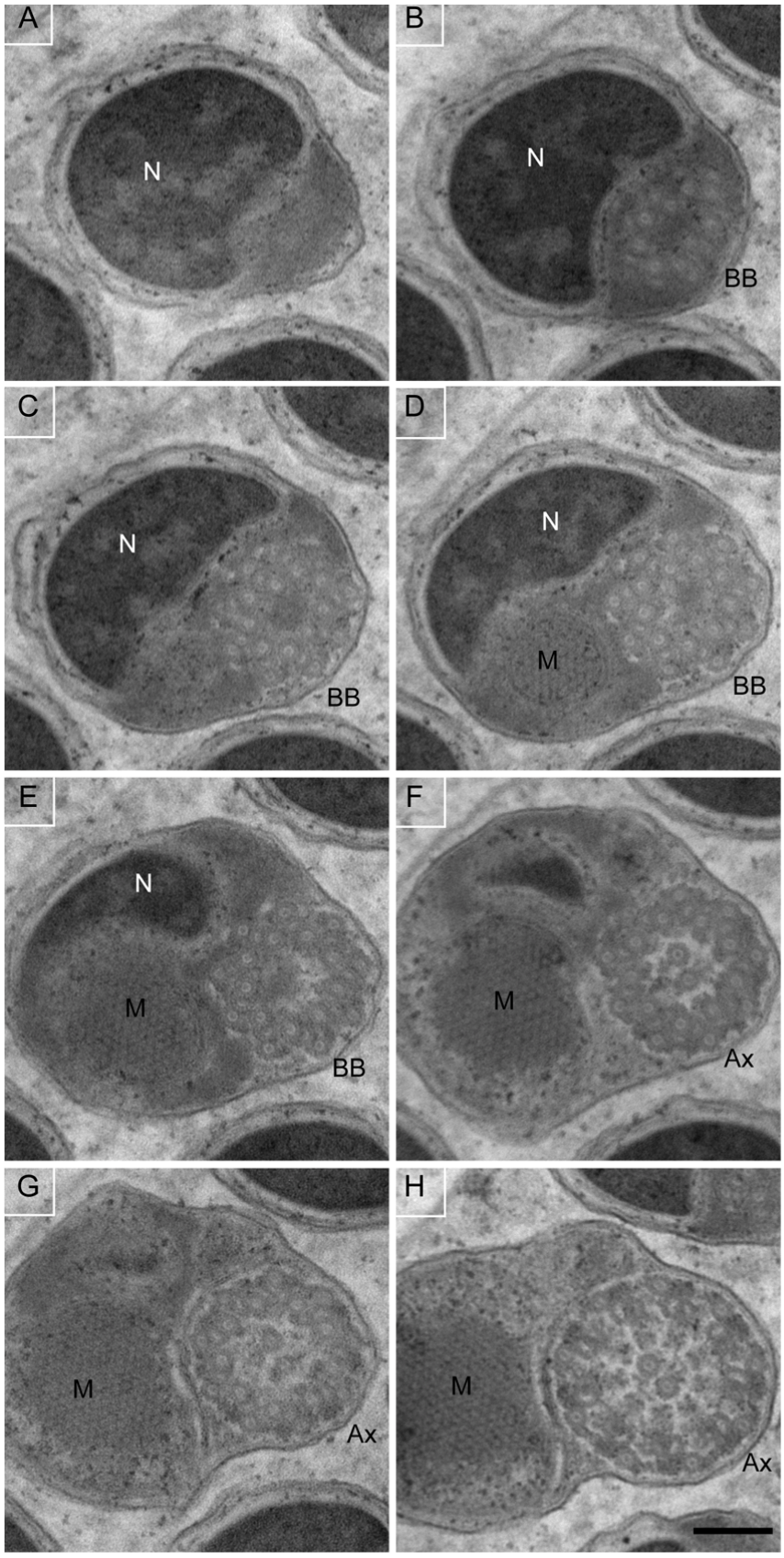

FIGURE S2.—A single centriolar structure is detected in mature sperm (stage 19). Representative sections from serial section: (A-E) the basal body level that lacks central pair of microtubule; (F-H) the axoneme level that has central pair of microtubule. Scale bar: 100 nM; N, Nucleus; BB, basal body; M, mitochondria; Ax, axoneme.

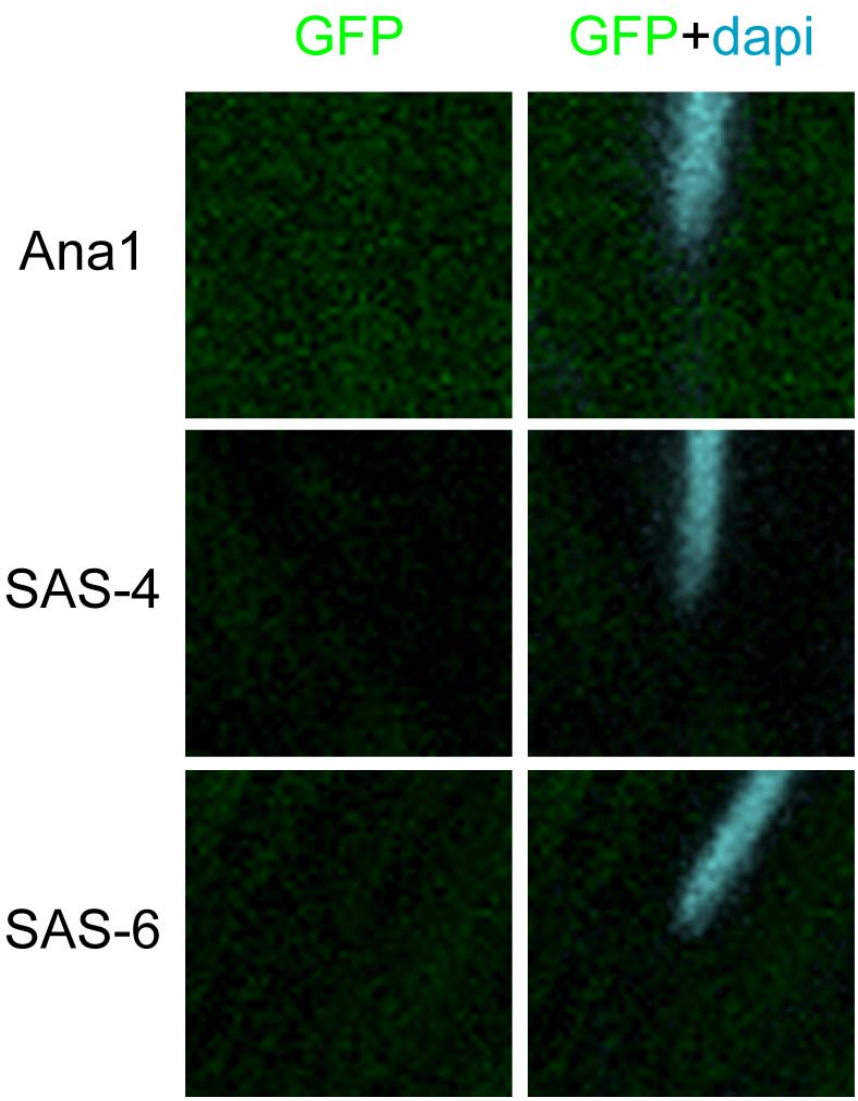

FIGURE S3.—Ana1-GFP, SAS-4-GFP, SAS-6-GFP are not detectable on basal body or PCL of mature sperm. None of the three markers we tested (Ana1-GFP, SAS-4-GFP, SAS-6-GFP) are labeling a dot or a line in the mature sperm. Dapi stained the condensed nucleus.