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Report

The Origin of Centrosomes in Parthenogenetic Hymenopteran Insects

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Summary

A longstanding enigma has been the origin of maternal centrosomes that facilitate parthenogenetic development in Hymenopteran insects. In young embryos, hundreds of microtubule-organizing centers (MTOCs) are assembled completely from maternal components [1–3]. Two of these MTOCs join the female pronucleus to set up the first mitotic spindle in unfertilized embryos and drive their development [2]. These MTOCs appear to be canonical centrosomes because they contain γ-tubulin, CP190, and centrioles and they undergo duplication [1]. Here, we present evidence that these centrosomes originate from accessory nuclei (AN), organelles derived from the oocyte nuclear envelope [4–6]. In the parasitic wasps *Nasonia vitripennis* and *Muscidifurax uniraptor*, the position and number of AN in mature oocytes correspond to the position and number of maternal centrosomes in early embryos. These AN also contain high concentrations of γ-tubulin. In the honeybee, *Apis mellifera*, distinct γ-tubulin foci are present in each AN. Additionally, the Hymenopteran homolog of the *Drosophila* centrosomal protein Dgrip84 localizes on the outer surfaces of AN. These organelles disintegrate in the late oocyte, leaving behind small γ-tubulin foci, which likely seed the formation of maternal centrosomes. Accessory nuclei, therefore, may have played a significant role in the evolution of haplodiploidy in Hymenopteran insects.

Results and Discussion

To identify the cellular origin of maternal centrosomes, we performed an initial survey of cytoskeletal components in the developing oocytes of several Hymenopteran species. While staining the oocyte nucleus (germinal vesicle) by using an antibody against *Drosophila* lamin Dm0, we observed numerous lamin-positive spherical bodies in mid-stage oocytes of the parasitic wasps, *Nasonia vitripennis* (Figure 1A) and *Muscidifurax uniraptor* (Figure 1B). These cytoplasmic structures were also present in the oocytes of the other major Hymenopteran members, the honeybee, *Apis mellifera* (Figure 1C) and the ant, *Formica sp*. (Figure 1D). However, they were not found in the oocytes of *Drosophila melanogaster* (not shown) and the parthenogenetic *D. mercatorum* (Figure 1E), both members of the order Diptera. Given their presence in Hymenopteran oocytes, we conclude that these structures are the previously described accessory nuclei (AN), cytoplasmic organelles present in the oocytes of this prominent insect order [5–11]. Previous ultrastructural studies established that AN are devoid of DNA but contain nuclear factors typically found in Cajal bodies, such as snRNAs, snRNPs, Sm proteins, and nucleolar organizer proteins [6, 12]. This led to the suggestion that AN serve as transporters and storage sites for these factors [6, 12]. However, a conclusive function of AN has not been determined.

We examined the distribution of AN in *N. vitripennis* and *M. uniraptor* oocytes at different developmental stages by using both confocal and transmission electron microscopy (Figure 1F–1M). No AN are present in the earliest stages of oogenesis (Figures 1F and 1F′). However, in slightly older oocytes, small nuclear buds and a number of larger free AN (1–2 μM) form adjacent to the oocyte nucleus (Figures 1G and 1G′). As oogenesis progresses, AN bud from the oocyte nucleus, move toward the posterior pole, and become more numerous within the cytoplasm (Figures 1H and 1I). Electron micrographs confirmed the presence of AN in *N. vitripennis* oocytes (Figure 1K). In agreement with previous observations [6, 7, 12], several electron-dense inclusions are present within AN (Figure 1L). Their membrane consists of two layers similar to those of the membrane of the oocyte nucleus (Figure 1M). By late oogenesis, AN have increased to 8–10 μM in diameter and are evenly distributed around the perimeter of the oocyte cortex (Figure 1J). The positions of AN in the late oocyte correspond to the sites of maternally derived centrosomes, which become active at the cortex following oviposition [1, 2]. We found that late previtellogenic *M. uniraptor* oocytes contain 178–233 AN (average of 200.0 ± standard error of the mean [SEM] 5.6, n = 10). These numbers are similar to previous counts of 226–281 maternal centrosomes in newly laid *M. uniraptor* embryos [2]. These correlations in number and position raised the possibility of a functional link between AN and maternal centrosomes.

To explore this idea, we tested whether AN are associated with core centrosomal proteins. To do this, we identified several *Drosophila* antibodies that recognized different core centrosomal proteins in Hymenopterans. One antibody raised against the C terminus of *D. melanogaster* γ-tubulin (DrosC) appropriately recognizes a pair of centrosomes associated with each nucleus in fixed *N. vitripennis* embryos (Figures 2A and 2B). In western blots, this antibody recognizes a band corresponding to a protein size of 52 kDa in both *N. vitripennis* oocyte and embryo extracts (Figure 2C). This band approximately matches the predicted size of Hymenopteran γ-tubulin (50 kDa) as predicted on the basis of

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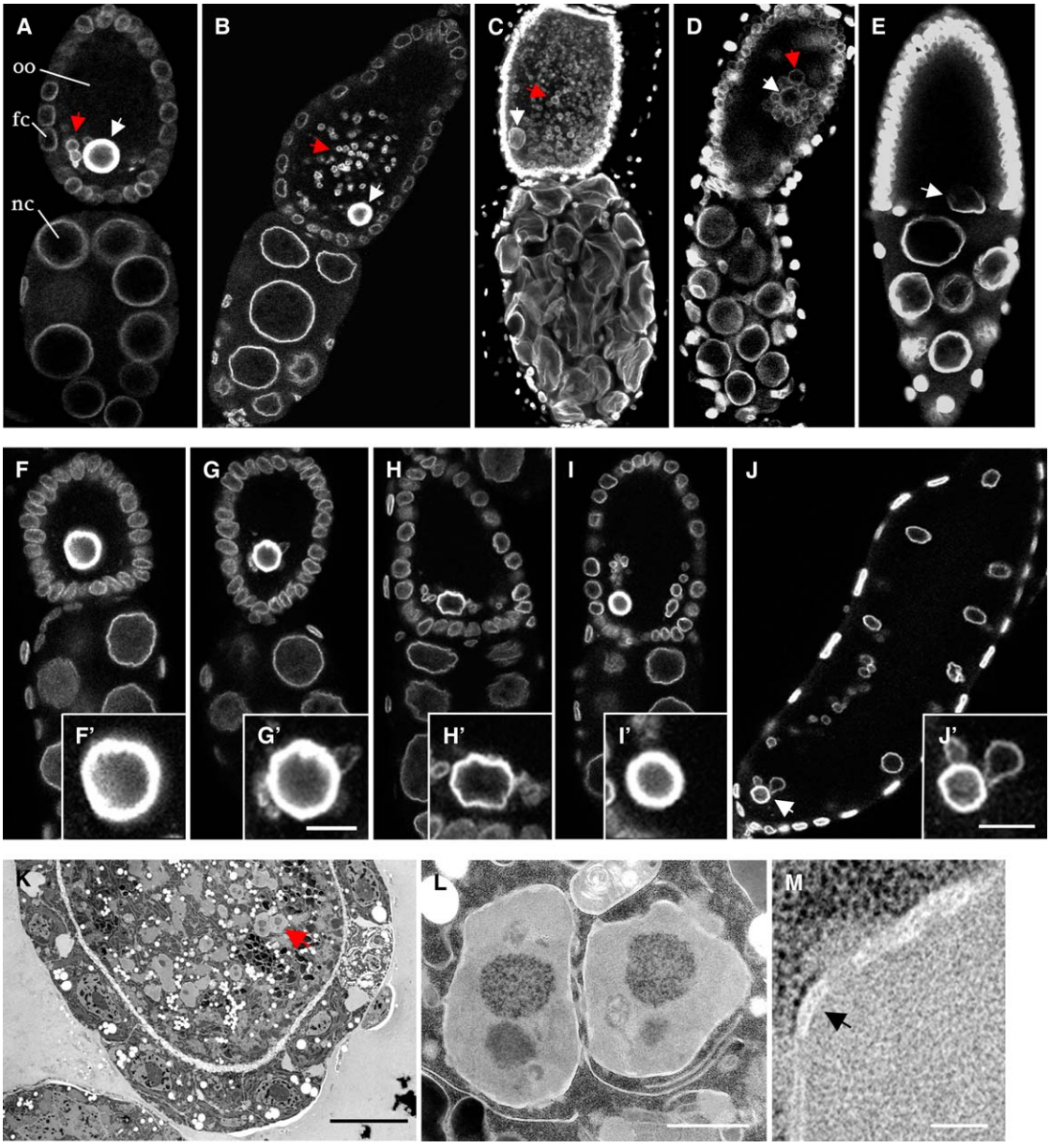

Figure 1. AN Are Derived from the Oocyte Nuclear Envelope in Hymenopteran Insects

(A–J′) Lamin is shown in grayscale. (A–E) A series of mid-stage egg chambers from the Hymenopterans (A) *N. vitripennis*, (B) *M. uniraptor*, (C) *A. mellifera*, and (D) *Formica sp*. (E) A mid-stage egg chamber from the parthenogenetic Dipteran *D. mercatorum*. Oocytes are oriented toward the top (labeled "oo" in [A]) and are surrounded by follicle cells (labeled "fc" in [A]), whereas nurse cells are oriented toward the bottom (labeled "nc" in [A]). Red arrows indicate AN, and white arrows indicate the oocyte nucleus (germinal vesicle). (F–J) Egg chambers from *N. vitripennis* are shown at successive stages of oogenesis. AN are not present in the earliest oocytes (F) but appear as buds along the nuclear envelope of the germinal vesicle in subsequent stages (G). By mid oogenesis, AN become more numerous and move outward from the oocyte nucleus (H). AN move toward the posterior pole as oogenesis progresses (I). By late oogenesis, AN have become distributed around the oocyte perimeter (J). Arrow in (J) indicates the oocyte nucleus. Nurse-cell remnants are not shown. High magnifications (F′–J′) show AN budding off of the oocyte nucleus. Scale bar equals 5 μm in (G′) and 10 μm in (J′).

(K–M) Transmission electron micrographs of AN in a mid-stage *N. vitripennis* oocyte. (K) The anterior region of the oocyte is shown. Red arrow indicates a pair of AN. (L) High-magnification image shows electron-dense inclusions within AN. (M) High magnification shows two layers of AN membrane (black arrow). Scale bar equals 10 μm in (K), 1 μm in (L), and 150 nm in (M).

A. mellifera genome sequence. These results indicate that this antibody recognizes Hymenopteran γ-tubulin.

Using this antibody, we found that in *N. vitripennis* and *M. uniraptor* oocytes, AN contain high levels of γ-tubulin (Figures 2D–2I, *M. uniraptor* not shown). Additionally, the oocyte nucleus, the follicle cells surrounding the oocyte, and the nuclei of nurse cells adjacent to the oocyte also contain high levels of γ-tubulin (Figures 2E and 2F). However, the nuclei of more distal nurse cells are consistently devoid of this protein (Figures 2E and 2F), further suggesting that these staining patterns likely reflect γ-tubulin and are not artifacts of

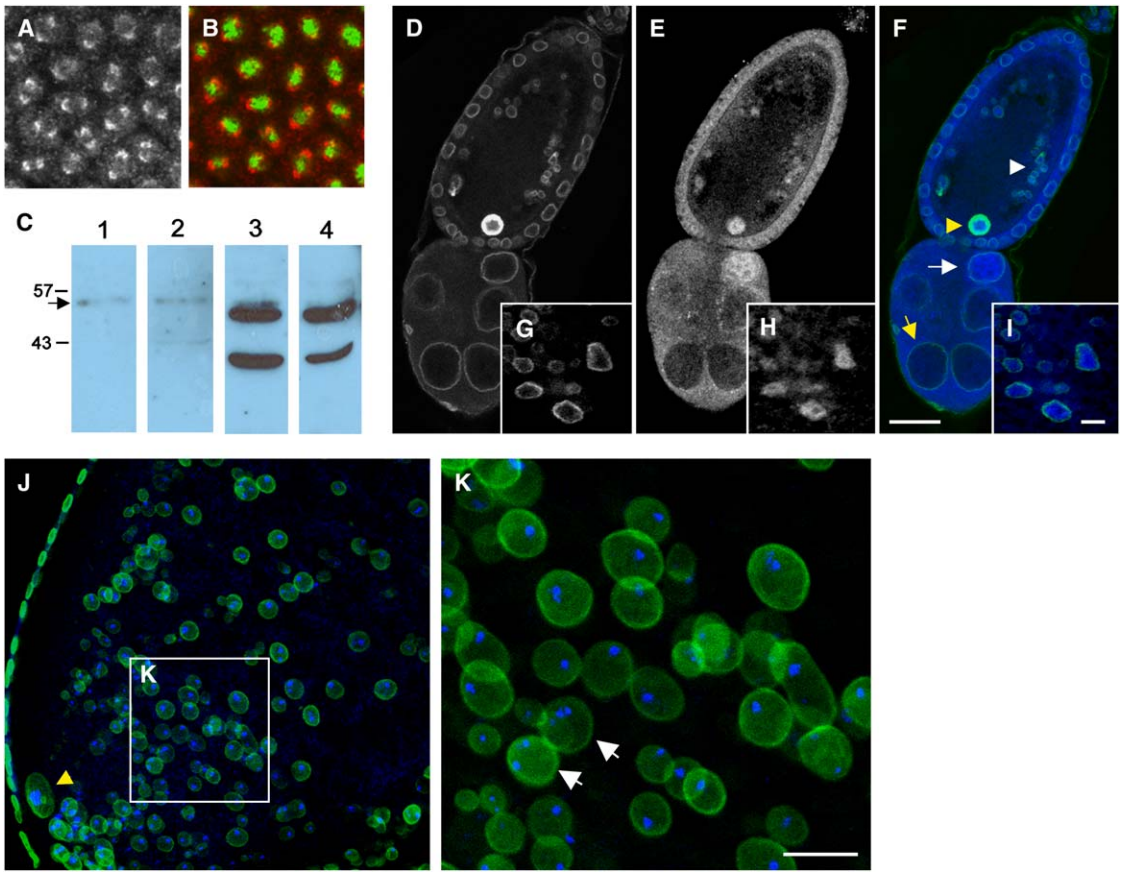

Figure 2. AN in *N. vitripennis* and *A. mellifera* Oocytes Contain High Concentrations of γ-Tubulin

(A and B) The DrosC anti-γ-tubulin antibody stains centrosomes in the *N. vitripennis* early embryo. Centrosomes are shown in grayscale in (A) and red in (B), and DNA (Oligreen) is shown in green (B).

(C) A western blot shows that the DrosC antibody recognizes a band of 52 kDa in extracts from *N. vitripennis* oocytes (lane 1) and early embryos (lane 2). Control extracts from *D. melanogaster* oocytes (lane 3) and early embryos (lane 4) are shown. Positions corresponding to size markers of 43 kDa and 57 kDa are indicated.

(D–I) A previtellogenic *N. vitripennis* egg chamber is shown (D–F), with high magnifications of AN (G–I). (D and G) Lamin appears in grayscale. (E and H) γ-tubulin is shown in grayscale. (F and I) Lamin is green and γ-tubulin is blue. (F) White arrowhead indicates a group of AN, yellow arrowhead indicates the oocyte nucleus, white arrow points to a nurse-cell nucleus adjacent to the oocyte, and yellow arrow indicates a nurse-cell nucleus distal to the oocyte.

(J and K) γ-tubulin is organized into distinct foci in AN in an *A. mellifera* oocyte. The anterior of the oocyte is shown. Lamin is green and γ-tubulin is blue. Yellow arrowhead in (J) indicates the oocyte nucleus. (K) High magnification of a group of AN in (J). White arrows indicate AN containing double foci of γ-tubulin.

Scale bar equals 20 μm in (F), 5 μm in (I), and 15 μm in (K).

immuno-cross-reactivity with other nuclear components. In late *A. mellifera* oocytes, AN exhibit striking foci of γ-tubulin (Figures 2J and 2K). γ-tubulin also concentrates into a single large brightly stained focus within the oocyte nucleus (Figure 2J), reminiscent of γ-tubulin enrichment within the *N. vitripennis* oocyte nucleus.

We also identified an antibody that recognizes the Hymenopteran homolog of the *Drosophila* core centrosomal protein, Dgrip84 [13]. Homologs of this protein are known to form a complex with γ-tubulin in several organisms and are believed to play a general role in the microtubule-nucleating ability of γ-tubulin [13–17]. In fixed *N. vitripennis* embryos, this antibody exhibits a perinuclear staining with slight enrichments at opposite sides of each nucleus, likely the positions of the centrosomes (Figure 3A). This staining pattern matches that of the microtubule network in *N. vitripennis* (Figure 3B) and *M. uniraptor* embryos [1]. In western blots, the anti-Dgrip84 antibody recognizes a protein of approximately 70 kDa in both *N. vitripennis* oocyte and embryo extracts (Figure 3E). This band corresponds in size to the predicted Hymenopteran homolog (70 kDa), as determined from *A. mellifera* genome sequence.

Stainings of fixed *N. vitripennis* and *M. uniraptor* oocytes with the anti-Dgrip84 antibody revealed that the Dgrip84 homolog is also associated with AN (Figures 3F–3I). However, in contrast to γ-tubulin, which is concentrated within AN, the Dgrip84 homolog is organized as puncta that localize primarily on the outer surfaces of these organelles rather than within them (Figures 3G–3I). The majority of these puncta are associated with AN membrane, although some appeared free within the oocyte cytoplasm.

To further test the relationship between AN and maternal centrosomes, we examined freshly laid *N. vitripennis* embryos for the presence of AN. In all embryos analyzed

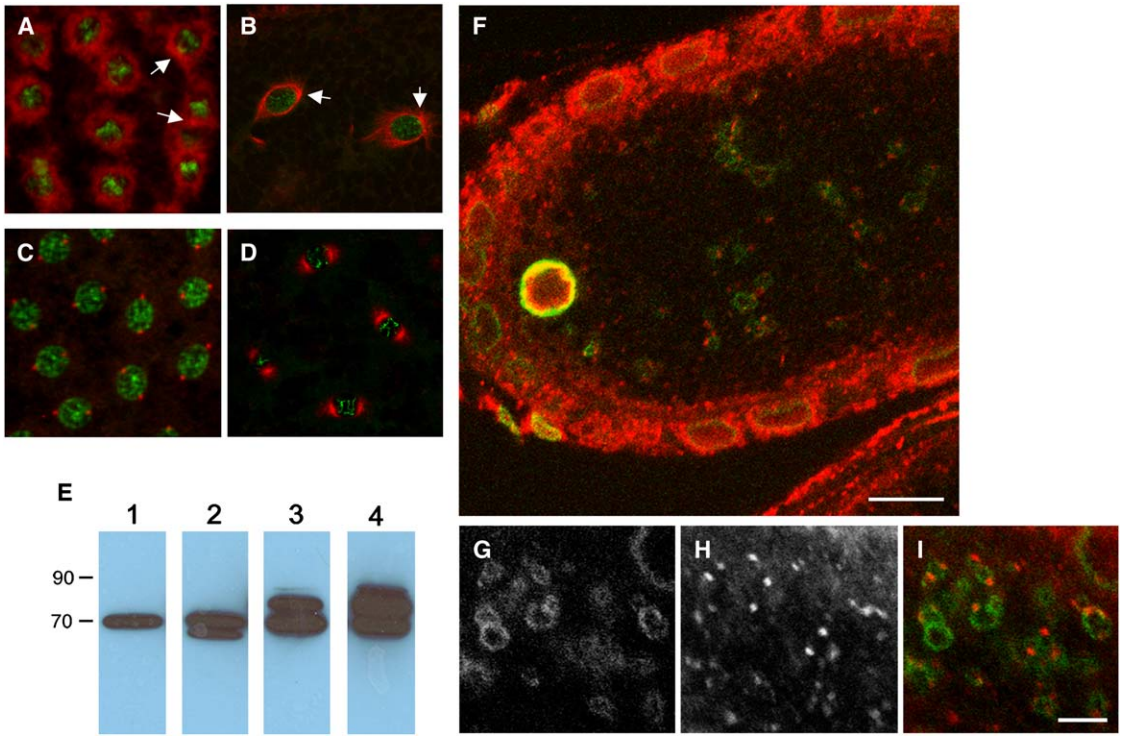

Figure 3. The Hymenopteran Homolog of Dgrip84 Localizes on the Outer Surfaces of AN

(A and B) Nuclei in early *N. vitripennis* embryos. Red is anti-Dgrip84 in (A) and microtubules in (B). Green in (A) and (B) is DNA. White arrows in (A) and (B) indicate the likely positions of centrosomes. (C and D) Nuclei in early *D. melanogaster* embryos are shown. Red is anti-Dgrip84 in (C) and microtubules in (D). Green in (C) and (D) is DNA. (E) A western blot indicating that anti-Dgrip84 recognizes a 70 kDa band in extracts from *N. vitripennis* oocytes (lane 1) and early embryos (lane 2). Control extracts from *D. melanogaster* oocytes (lane 3) and early embryos (lane 4) are shown. Positions corresponding to size markers of 70 kDa and 90 kDa are labeled. The anterior region of a late previtellogenic oocyte is shown (F), with high-power magnifications of AN (G–I). (F and I) Anti-Dgrip84 is shown in red, and lamin is shown in green. (G) Lamin appears in grayscale. (H) Anti-Ggrip84 is shown in grayscale.
Scale bar equals 10 μm in (F) and (I).

(n = 75), anti-lamin highlighted the nuclear envelope of the single maternal pronucleus in unfertilized embryos (not shown) or both maternal and paternal pronuclei in fertilized embryos (Figure 4A). However, AN were not present in the majority of these embryos (73/75) (Figure 4A). In two embryos, remnants of AN were observed (Figure 4B). EM analysis confirmed the absence of AN in embryos of the same age (not shown). Staining a subset of young embryos with anti-α-tubulin revealed the presence of multiple cytoplasmic asters, indicating that maternal centrosomes become active by the first mitotic division (Figure 4C). These results suggest that AN degenerate sometime between late oogenesis and early embryogenesis—just before maternal centrosome activity begins. To confirm this interpretation, we analyzed AN and γ-tubulin in late vitellogenic eggs. In the majority of these eggs, AN had either begun to break down (Figure 4D) or were completely absent (not shown). In the former case, remnants of AN were often observed adjacent to small foci of γ-tubulin in the oocyte cytoplasm (Figure 4D).

Taken together, these results suggest that maternal centrosomes in Hymenopteran embryos originate from AN. These organelles contain high concentrations of γ-tubulin, which in late honeybee oocytes are organized into distinct foci. Previous studies showed that γ-tubulin is required not only for microtubule nucleation [18–20], but also for centrosome assembly. Ablation of centrosomes in vertebrate somatic cells results in the accumulation of γ-tubulin and other core centrosomal proteins in nuclear-envelope invaginations [21]. These events represent the first steps in de novo centrosome assembly, occurring before the appearance of new centrioles [21]. Other studies indicate that γ-tubulin plays an essential role in the formation of centrioles [22], basal bodies [23], and other microtubule organizing centers [24, 25]. In light of these findings, our observations suggest that AN facilitate centrosome formation by sequestering and perhaps concentrating γ-tubulin. We speculate that breakdown of AN membrane during late oogenesis exposes concentrated γ-tubulin to other core centrosomal proteins present in the oocyte cytoplasm, initiating the formation of maternal centrosomes. Our finding that the Hymenopteran homolog of Dgrip84 localizes on the outer surfaces of AN but is not present within them supports this idea. Furthermore, previous studies have established that additional centrosome-associated proteins are not visible in the oocyte cytoplasm until maternal centrosomes become active, suggesting that their addition is a later step in this process [1]. That AN membrane has nuclear-envelope characteristics (i.e., double membrane and nuclear pores) [4–6] may allow for the possibility of cell-cycle-regulated control of maternal centrosome formation following germinal-vesicle

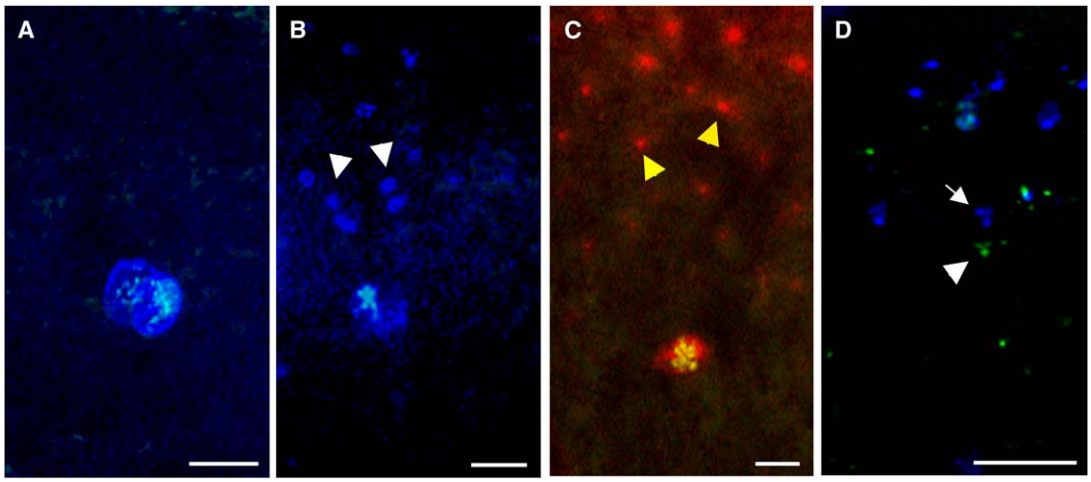

Figure 4. AN Break Down during Late Oogenesis and before Maternal Centrosome Formation in *N. vitripennis*

(A–C) High magnifications of pronuclei in early *N. vitripennis* embryos. (A and B) Lamin is blue and DNA is green. (A) Juxtaposed paternal and maternal pronuclei. (B) A single maternal pronucleus. White arrowheads indicate AN remnants. (C) Microtubules are red and DNA is yellow. A single nucleus in prometaphase of the first mitotic cycle is shown. Yellow arrowheads indicate maternal centrosomes.

(D) High magnification of AN remnants in a late vitellogenic oocyte from *N. vitripennis*. The oocyte nucleus is not shown. Lamin is green and γ-tubulin is blue. White arrowhead indicates AN remnants, and white arrow indicates foci of γ-tubulin.

Scale bar equals 20 μm in (A–D).

breakdown. This idea is supported by previous findings that maternal centrosome formation occurs only after germinal vesicle breakdown has occurred in the oocytes of *M. uniraptor* [1] and the ribbon worm *Cerebratulus lacteus* [26]. Additionally, nuclear pores may allow the selective import of γ-tubulin into AN from the surrounding cytoplasm.

Our finding that γ-tubulin and the Dgrip84 homolog associate with AN membrane in *N. vitripennis* oocytes suggests a close relationship between centrosomal proteins and membrane. This relationship is further supported by the fact that in the early *N. vitripennis* embryo, the Dgrip84 homolog and microtubules colocalize around the periphery of the nuclear envelope, instead of primarily around centrosomes. Similar patterns of microtubule nucleation from membrane have been found in other organisms. For example, in higher plants, microtubules are nucleated from the surface of the nuclear envelope [27, 28], where γ-tubulin and at least one other centrosomal protein localize [28, 29]. In the acentrosomal ommatidia cone cells and oocytes of *D. melanogaster*, microtubules are nucleated from the plasma membrane [30, 31]. These observations together illustrate the potential for membrane as a substrate for microtubule nucleation. It remains to be determined, however, how this process is regulated.

Regarding their role in centrosome formation, AN appear to be analogous to structures called multivesicular aggregates (MVA) in mouse blastomeres [32]. In mice, centrosomes are assembled entirely from maternal components present in MVA [32, 33]. Two of these structures, composed of γ-tubulin, pericentrin, and small (~25 μm) vesicles containing electron-dense particles, form during late oogenesis near the blastomere cortex [32]. Concomitant with germinal vesicle breakdown, MVA undergo fragmentation into smaller subunits, a few of which eventually acquire microtubule-nucleating ability [32]. Unlike AN, MVA are not surrounded by membrane, and currently it is not understood how γ-tubulin and other pericentriolar materials accumulate within them. It is possible that, similar to AN membrane, the small vesicles in MVA function to sequester centrosomal proteins for controlled release at the appropriate developmental time. Future studies aimed at the identification of proteins within these vesicles and within Hymenopteran AN will likely provide further insights into the mechanism of maternal and de novo centrosome formation.

Some non-Hymenopteran insects, such as *D. mercatorum*, reproduce parthenogenetically [34] but do not contain AN (Figure 1E). These species have evolved other mechanisms to produce maternal centrosomes. Such is the case for the pea aphid, whose maternal centrosomes may be formed with the help of microtubules and associated motor proteins [3]. Conversely, AN may serve additional developmental functions, because they have been found in several diplodiploid organisms [4, 35–37] whose centrosomes are derived from both maternal and paternal materials [38, 39]. Given that other nuclear-associated proteins are present in AN, the role of these organelles in concentrating maternal γ-tubulin in Hymenopterans may represent the adaptation of a general structure for an additional, more specific function. Additionally, it is intriguing to speculate that the nuclear factors previously found in AN may be involved in centrosome formation. Whatever the case, the successful exploitation of these organelles as facilitators of maternal centrosome formation has likely played a significant part in the evolution of haplodiploidy in this prominent insect order.

Experimental Procedures

Egg chambers were dissected and fixed as described [40]. For embryo collection, virgin *N. vitripennis* females were allowed to oviposit into host Sarcophaga pupae for 90 min. Embryos were removed

from host pupae, fixed in 100% methanol, rehydrated, and lanced to allow antibody permeation. Primary antibodies were incubated overnight at 4ºC at the following dilutions: mouse anti-lamin at 1:50 [41], rabbit anti-maternal γ-tubulin at 1:400 and rabbit anti-Dgrip84 at 1:500 (both gifts from Yixian Zheng), and mouse anti-α-tubulin (Sigma) at 1:500. Following three washes in 1× PBTA, samples were incubated at room temperature for 1 hr with Alexa 488- or Alexa 633-coupled anti-mouse or anti-rabbit antibodies (1:150) (Molecular Probes). For visualizing DNA, samples were placed in Oligreen (Molecular Probes) at 1:750 for 15 min before mounting. All images were obtained on a Leica DM IRB confocal microscope and processed with Adobe Photoshop 7.0.

Western blots were performed according to methodology as described [42].

N. vitripennis oocytes were prepared for standard transmission EM (TEM) by high-pressure freezing and freeze substitution as described previously [43]. Fixed oocytes were embedded in Eponate 12/Araldite (Ted Pella). Thirty-five to fifty nanometer sections were collected on an Ultracut T microtome (Leica) and picked up on copper grids coated with 0.3%–0.5% formvar (Electron Microscopy Sciences). Sections were post stained for 5 min in 2% uranyl acetate/70% methanol and 4 min in 0.5% lead citrate. Samples were visualized on a Philips 410 LS transmission electron microscope.

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References

1. Riparbelli, M.G., Stouthamer, R., Dallai, R., and Callaini, G. (1998). Microtubule organization during the early development of the parthenogenetic egg of the hymenopteran *Muscidifurax uniraptor*. Dev. Biol. *195*, 89–99.
2. Tram, U., and Sullivan, W. (2000). Reciprocal inheritance of centrosomes in the parthenogenetic hymenopteran *Nasonia vitripennnis*. Curr. Biol. *10*, 1413–1419.
3. Riparbelli, M.G., Tagu, D., Bonhomme, J., and Callaini, G. (2005). Aster self-organization at meiosis: A conserved mechanism in insect parthenogenesis? Dev. Biol. *278*, 220–230.
4. Meyer, G.F., Skoloff, S., Wolf, B.E., and Brand, B. (1979). Accessory nuclei (nuclear membrane balloons) in the oocytes of dipteran Phryne. Chromosoma *75*, 89–99.
5. Bilinski, S.M. (1991). Are accessory nuclei involved in the establishment of developmental gradients in hymenopteran oocytes? Ultrastructural studies. Rouxs Arch. Dev. Biol. *199*, 423–426.
6. Jaglarz, M.K., Bilinski, S.M., and Kloc, M. (2005). Assembly and breakdown of Cajal bodies in accessory nuclei of Hymenoptera. Differentiation *73*, 99–108.
7. Hopkins, C.R. (1964). The histochemistry and fine structure of the accessory nuclei in the oocyte of *Bombus terrestris*. Quarterly Journal of Microscopic Science *105*, 475–480.
8. Cassidy, J.D., and King, R.C. (1972). Ovarian development in *Habrobracon juglandis* (Ashmead) (Hymenoptera: Braconidae). I. The origin and differentiation of the oocyte-nurse cell complex. Biol. Bull. *143*, 483–505.
9. Bilinski, S.M., Klag, J., and Kubrakiewicz, J. (1995). Subcortical microtubule network separates the periplasm from the endoplasm and is responsible for maintaining the position of accessory nuclei in hymenopteran oocytes. Rouxs Arch. Dev. Biol. *205*, 54–61.
10. Bilinski, S.M., Zawadzka, M., and Bunning, J. (1995). Visualization of accessory nuclei from oocytes of the sawfly, *Athalia rosae* (Hymenoptera: Tenthridinidae) by a spreading technique. Folia Histochem. Cytobiol. *33*, 197–200.
11. Jablonska, A., and Bilinski, S.M. (2001). Structure of ovarioles in adult queens and workers of the common wasp, *Vespula germanica* (Hymenoptera: Vespidae). Folia Biol. (Krakow) *49*, 191–198.
12. Bilinski, S.M., and Kloc, M. (2002). Accessory nuclei revisited: The translocation of snRNPs from the germinal vesicle to the periphery of the future embryo. Chromosoma *111*, 62–68.
13. Oegama, K., Wiese, C., Martin, O.C., Milligan, R.A., Iwamatsu, A., Mitchison, T.J., and Zheng, Y. (1999). Characterization of two related Drosophila γ-tubulin complexes that differ in their ability to nucleate microtubules. J. Cell Biol. *144*, 721–733.
14. Knop, M., Pereira, G., Geissler, S., Grein, K., and Schiebel, E. (1997). The spindle pole body component Spc97p interacts with the γ-tubulin of *Saccharomyces cerevisiae* and functions in microtubule organization and spindle pole body duplication. EMBO J. *16*, 1550–1564.
15. Martin, O.C., Gunawardane, R.N., Iwamatsu, A., and Zheng, Y. (1998). Xgrip109: A γ-tubulin-associated protein with an essential role in gamma-tubulin ring complex (γTuRC) assembly and centrosome function. J. Cell Biol. *141*, 675–687.
16. Murphy, S.M., Urbani, L., and Stearns, T. (1998). The mammalian γ-tubulin complex contains homologues of the yeast spindle pole body components spc97p and spc98p. J. Cell Biol. *141*, 663–674.
17. Tassin, A.M., Celati, C., Moudjou, M., and Bornens, M. (1998). Characterization of the human homologue of the yeast spc98p and its association with γ-tubulin. J. Cell Biol. *141*, 689–701.
18. Oakley, B.R., Oakley, C.E., Yoon, Y., and Jung, M.K. (1990). γ-tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. Cell *61*, 1289–1301.
19. Joshi, H.C., Palacios, M.J., McNamara, L., and Cleveland, D.W. (1992). γ-Tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. Nature *356*, 80–83.
20. Stearns, T., and Kirschner, M. (1994). In vitro reconstitution of centrosome assembly and function: The central role of γ-tubulin. Cell *76*, 623–650.
21. Khodjakov, A., Rieder, C.L., Sluder, G., Cassels, G., Sibon, O., and Wang, C.L. (2002). De novo formation of centrosomes in vertebrate cells arrested during S phase. J. Cell Biol. *158*, 1171–1181.
22. Dammermann, A., et al. (2004). Centriole assembly requires both centriolar and pericentriolar material proteins. Dev. Cell *7*, 815–829.
23. Ruiz, F., Beisson, J., Rossier, J., and Dupuis-Williams, P. (1999). Basal body duplication in paramecium requires gamma-tubulin. Curr. Biol. *9*, 43–46.
24. Shang, Y., Li, B., and Gorovsky, M.A. (2002). *Tetrahymena thermophila* contains a conventional gamma-tubulin that is differentially required for the maintenance of different microtubule-organizing centers. J. Cell Biol. *158*, 1195–1206.
25. Shin, M.R., and Kim, N.H. (2003). Maternal gamma (γ)-tubulin is involved in microtubule reorganization during bovine fertilization and parthenogenesis. Mol. Reprod. Dev. *64*, 438–445.
26. Yatsu, N. (1905). The formation of centrosomes in enucleated egg-fragments. J. Exp. Zool. *2*, 287–312.
27. Lambert, A.-M. (1993). Microtubule-organizing centers in higher plants. Curr. Opin. Cell Biol. *5*, 116–122.
28. Stoppin, V., Vantard, M., Schmit, A.-C., and Lambert, A.-M. (1994). Isolated plant nuclei nucleate microtubule assembly: The nuclear surface in higher plants has centrosome-like activity. Plant Cell *6*, 1099–1106.
29. Liu, B., Marc, J., Joshi, H.C., and Palevitz, B.A. (1993). A γ-tubulin related protein associated with the microtubule arrays of higher plants in a cell-cycle dependent manner. J. Cell Sci. *104*, 1217–1228.
30. Mogensen, M.M., Tucker, J.B., and Baggaley, T.B. (1993). Multiple plasma membrane-associated MTOC systems in the

acentrosomal cone cells of Drosophila ommatidia. Eur. J. Cell Biol. *60*, 67–75.
31. Theurkauf, W.E., Smiley, S., Wong, M.L., and Alberts, B.M. (1992). Reorganization of the cytoskeleton during Drosophila oogenesis: Implications for axis specification and intercellular transport. Development *11*, 923–936.
32. Calarco, P.G. (2000). Centrosome precursors in the acentriolar mouse oocyte. Microsc. Res. Tech. *49*, 428–434.
33. Manandhar, G., Schatten, H., and Sutovsky, P. (2005). Centrosome reduction during gametogenesis and its significance. Biol. Reprod. *72*, 2–13.
34. Riparbelli, M.G., and Callaini, G. (2003). *Drosophila* parthenogenesis: A model for *de novo* centrosome assembly. Dev. Biol. *260*, 298–313.
35. Goldstein, P. (1981). Accessory nuclei in female *Ascaris suum*. J. Parasitol. *67*, 697–701.
36. Swiatek, P. (2005). Structure of the germinal vesicle during oogenesis in the leech *Glossiphonia Heteroclita* (Annelida, Hirudinea, Rhynchobdellida). J. Morphol. *263*, 330–339.
37. Kubrakiewics, J., Adamski, R.T., and Bilinski, S.M. (1991). Ultrastructural studies on accessory nuclei in developing oocytes of the crustacean, *Siphonophanes grubei*. Tissue Cell *23*, 903–907.
38. 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.
39. Callaini, G., Giovanna, M., and Dallai, R. (1999). Centrosome inheritance in insects: Fertilization and parthenogenesis. Biol. Cell. *91*, 355–366.
40. Verheyen, E., and Cooley, L. (1994). Looking at oogenesis. Methods Cell Biol. *44*, 545–561.
41. Riemer, D., Stuurman, N., Berrios, M., Hunter, C., Fisher, P.A., and Weber, K. (1995). Expression of *Drosophila* lamin C is developmentally regulated: Analogies with vertebrate A-type lamins. J. Cell Sci. *108*, 3189–3198.
42. Saxton, W.M., Porter, M.E., Cohn, S.A., Scholey, J.M., Raff, E.C., and McIntosh, J.R. (1988). *Drosophila* kinesin: Characterization of microtubule motility and ATPase. Proc. Natl. Acad. Sci. USA *85*, 1109–1113.
43. McDonald, K. (1994). Electron microscopy and EM immunocytochemistry. Methods Cell Biol. *44*, 411–444.