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Aster self-organization at meiosis: a conserved mechanism in insect parthenogenesis?

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Abstract

Unfertilized eggs usually lack maternal centrosomes and cannot develop without sperm contribution. However, several insect species lay eggs that develop to adulthood as unfertilized in the absence of a preexisting centrosome. We report that the oocyte of the parthenogenetic viviparous pea aphid *Acyrthosiphon pisum* is able to self-organize microtubule-based asters, which in turn interact with the female chromatin to form the first mitotic spindle. This mode of reproduction provides a good system to investigate how the oocyte can assemble new centrosomes and how their number can be exactly monitored. We propose that the cooperative interaction of motor proteins and randomly nucleated surface microtubules could lead to the formation of aster-like structures in the absence of pre-existing centrosomes. Recruitment of material along the microtubules might contribute to the accumulation of pericentriolar material and centriole precursors at the focus of the asters, thus leading to the formation of true centrosomes. The appearance of microtubule asters at the surface of activated oocytes could represent a possible common mechanism for centrosome formation during insect parthenogenesis.

Keywords: Parthenogenesis; Asters; Centrosome inheritance; Spindle formation; Aphids

Introduction

The proper inheritance of sister chromosomes to cell daughters at the completion of mitosis depends on the correct organization and functioning of the spindle machinery. The centrosome, the primary microtubule organizing centre (MTOC) of the cell, is the key organelle of spindle assembly. The centrosome is a peculiar non-membranous organelle defined by the presence of two orthogonally arranged centrioles that are surrounded by a cloud of pericentriolar material. Besides its ability to organize bipolar spindles and other microtubular arrays, the centrosome function is also required during progression from G1 to S phases of the cell cycle (Hinchcliffe et al., 2001; Khodjakov and Rieder, 2001) and at the final stages of cytokinesis (Piel et al., 2001).

While the primary function of the pericentriolar material is to nucleate and organize polarized microtubule arrays by means of γ-tubulin components (Oakley, 2000), the role of centrioles is still an opened question. Centriole disassembly provoked by the application of antibodies against polyglutamylated tubulin results in the dispersion of the pericentriolar material (Bobinnec et al., 1998); this strongly suggests a function of centrioles as organizers of the centrosome by recruitment of nucleating factors and structural proteins into a discrete focus.

In diploid cells, the number of centrosomes has to be highly regulated since the presence of more than two centrosomes usually provokes multipolar spindles responsible for unequal distribution of chromosomes to daughter cells (Sluder and Nordberg, 2004). The centrosomes have to duplicate once every cell cycle, and again, this process seems to be strictly dependent on intrinsic cell-cycle-coupled replication mechanisms of the centrioles (Sluder and Hinchcliffe, 2000). Centriole replication is more efficient in the presence of a preexisting centriole acting

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as template, even if centriole formation can occur without a pre-exiting one (Marshall, 2001).

During gametogenesis, sperm cells inherit one fully developed copy of a centriole, whereas the oocytes lose the centriole, but still contain the molecular components of the pericentriolar material. After fertilization, the male gamete supplies the first centriole that recruits the maternal pericentriolar material to organize a hybrid centrosome that nucleates microtubule arrays and is able to reproduce (Schatten, 1994). Thus, the external supply of the centriole appears to be a limiting factor for the zygotic development in animal cells. Activated unfertilized *Xenopus* eggs can, indeed, successfully develop only after the microinjection of a centrosome (Maller et al., 1976). A problem appears for parthenogenetic development, which does not require male contribution to embryo development. This phenomenon is common in invertebrates, but is also found in vertebrates, where about 25 species are known to reproduce by constant thelytoky (White, 1973).

Parthenogenetic eggs, which are naturally depleted of centrosomes, provided a useful experimental system in which to study centriole/centrosome formation. Some studies that have addressed centrosome inheritance in parthenogenetic *Drosophila* (Riparbelli and Callaini, 2003) and hymenopteran (Riparbelli et al., 1998; Tram and Sullivan, 2000) species have provided evidences for centrosome-dependent pathways for mitotic spindle assembly in unfertilized eggs. These analyses have revealed that the formation of the first zygotic spindle during parthenogenetic development depended on self-assembled centrosomes that appear during anaphase of the first meiosis after egg activation. To investigate whether the process of de novo centrosome formation could be triggered by downstream events following egg activation, we examined centrosome inheritance in the pea aphid *Acyrthosiphon pisum* that develops parthenogenetically from oocytes that lack a canonical activation process and have a modified meiosis.

Aphids are a widespread group of heterometabolous insects of great economic importance. Sexual and parthenogenetic embryonic developments have been described in several aphid species (Blackman, 1987; Miura et al., 2003; and references therein). The embryo of most of the parthenogenetic aphid species does not develop within a large yolky egg, as usual in insects, but in a small ovarian follicle. The developing follicle lies below the apical germarium at the tip of a telotrophic meroistic ovariole and moves down as a new oocyte separates from the germarium. Thus, individual ovarioles contain different developmental stage embryos that are laid by mother as nymph (Fig. 1). Aged embryos which are still in the ovariole already contain developing parthenogenetic oocytes and embryos at various stages of development: this is the so-called telescoping of generations. In addition to the peculiar parthenogenetic

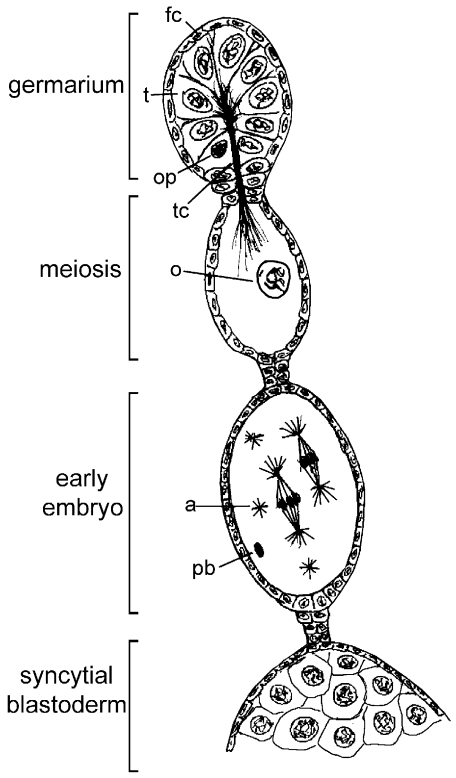

Fig. 1. Interpretative drawing of germarium and first ovarian follicles. fc, follicle cells; t, trophocytes; op, presumptive oocyte; tc, trophic cord; o, oocyte in growth phase; a, aster; pb, polar body.

mode of development that involves viviparity, to allow rapid colonization of new host plants, aphid embryos restore diploidy by a modified meiosis in which the oocyte undergoes a single maturation division in absence of meiotic recombination and crossing-over (Blackman, 1987). The single maturation division of the diploid chromosomes that results in the casting out of a diploid polar body is, therefore, accomplished by a mitotic-like process. This system, in which many embryos are produced in a short time could provide interesting insight to the mechanism the cell uses to rapidly and efficiently assemble mitotic spindles in the absence of pre-existing centrosomes. Some aphid genotypes developed a peculiar adaptation to environment changes since a single genotype is able to multiply either by parthenogenesis or by sexual reproduction. This is the reproductive polyphenism (Tagu et al., 2004). This system allows the direct comparison of the process of centrosome inheritance and bipolar spindle formation in parthenogenetic and fertilized aphid oocytes. Herein, we have shown that the cytoplasm of the parthenogenetic oocyte is capable of forming, as meiosis resumed, self-organized microtubule asters not associated with chromatin. Two of such asters interact with the prophase nucleus and organize the first mitotic spindle. The presence of more than two cortical asters did not provoke multipolar spindles and chromosome distribution was not perturbed.

Materials and methods

Stocks

Several strains of *Acyrthosiphon pisum* (Harris) were used for that study. Parthenogenetic females from the holocyclic genotype YR2 were reared on *Vicia fabae* (Ramos et al., 2003) under long photoperiod condition (16-h light/8-h dark cycles, 18°C). Adult females were collected and used for dissection of ovarioles. Eggs were obtained from the fertilization of sexual females from the holocyclic clone L2–17 with males from the androcyclic clone L4–13. Both genotypes were isolated in France from alfalfa plants in 2002 and kept in collection at Inra, Rennes (H. Frantz and J.C. Simon, unpublished). Briefly, sexual females and males were obtained by induction of parthenogenetic females under short photoperiod (12-h light/12-h dark cycles, 18°C). About 80 females and males were reared on the same plant for 16 h and 100 oviposited eggs were collected after 1 h, 2–4 h, and 5 h.

Reagents

A mouse monoclonal anti-β-tubulin (Boehringer Mannheim, UK) was used at a 1:200 dilution; a rat monoclonal YL1/2 directed against tyrosinated α-tubulin (Harlan Sera-Lab, England) at a dilution of 1:20; a mouse anti-βγ-tubulin monoclonal antibody (Sigma, St. Louis, MO) at 1:100; Goat anti-mouse, anti-rat or anti-rabbit secondary antibodies coupled to fluorescein or rhodamine (Cappel, West Chester, PA) were used at 1:600 dilution. DNA was stained with propidium iodide, Hoechst 33258 (Sigma) or TOTO-3 iodide (Molecular Probes, Europe, BV). Bovine serum albumin (BSA) and ribonuclease A (RNAse) were obtained from Sigma.

Fluorescence preparations

Fertilized eggs obtained from sexually reproducing females were dechorionated in a 50% bleach solution for 2–3 min and rinsed in distilled water. Their vitelline envelope was removed with a solution of 50% heptane and methanol and the eggs were then fixed in cold methanol. Ovarioles were dissected from parthenogenetic females and fixed 10 min in cold methanol. After fixation, the eggs and the ovarioles were washed in phosphate-buffered saline (PBS) and incubated for 1 h in PBS containing 0.1% bovine serum albumin (BSA). For simultaneous localization of microtubules and γ-tubulin, the ovarioles were incubated overnight at 4°C with the anti-γ-tubulin antibody, then the YL1/2 antibody was added and the incubation proceeded for 2 h at room temperature. After washing in PBS-BSA, the embryos were incubated for 1 h with the appropriate secondary antibodies. Controls of the secondary antibodies alone were done for all staining. For simultaneous tubulin and DNA staining, the eggs were incubated for 4–5 h at room temperature, or overnight, in the anti-β-tubulin antibody. After washing in PBS-BSA, the eggs were then incubated in the goat anti-mouse antibody to which 1 mg/ml RNAse was added. After washing in PBS the eggs were incubated 30 min in 1 μg/ml propidium iodide. Eggs were mounted in small drops of 90% glycerol containing 2.5% n-propyl-gallate.

Confocal microscopy

Digital optical sections of whole mount eggs and ovarioles were examined using a Leica TCS 4D laser scanning confocal microscope equipped with an argon-krypton Laser and coupled to a Leica DMRBE microscope equipped with 63× PL Apo 1.4 objectives (Leica Lasertechnik, Heidelberg). For double-stained samples, the images of the two fluorochrome distributions were recorded separately by averaging 8–16 scans of a single optical section to improve the signal/noise ratio. Images of chromosomes, microtubules, and centrosomes collected at several focal planes were superimposed, merged into a single file and imported into Adobe Photoshop to adjust size and contrast.

Results

Meiosis in parthenogenetic oocytes

Aphids have telotrophic meroistic ovarioles in which the nurse cells, or throphocytes, are located in a common region at the tip of the ovariole, the germarium (Fig. 1). In parthenogenetic viviparous females, the embryos develop within the ovaries and a range of serial developmental stages can be distinguished (Figs. 1 and 2A). The oocyte differentiated in the posterior region of the germarium and was easily recognizable from the sister nurse cells and the other presumptive oocytes by its more condensed chromatin (Fig. 2B). During the growth phase, the oocyte was gradually displaced out of the germarium, becoming enclosed by a layer of follicle cells (Fig. 2C). The extending follicle layer squeezed the oocyte cytoplasm, thus, separating the germarium from the oocyte chamber (Fig. 2D). The continuity between the germarium and the oocyte was maintained through a trophic cord, a specialized structure, mainly composed of microtubule bundles that ensures nutritive support to the early developing embryo. These different observations are in agreement to what is usually described in different aphid species (Blackman, 1987).

When the oocyte has reached its full growth, the nucleus moved to the periphery of the cytoplasm and its chromatin condensed (Fig. 3A). A bipolar anastral spindle then organized around the chromosomes that were aligned in the metaphase plate (Fig. 3B). The meiotic apparatus, parallel to the oocyte surface, was organized by microtubule bundles that were focused to opposite poles (Fig. 3B). As meiosis progressed, the metaphase spindle rotated and its longitudinal

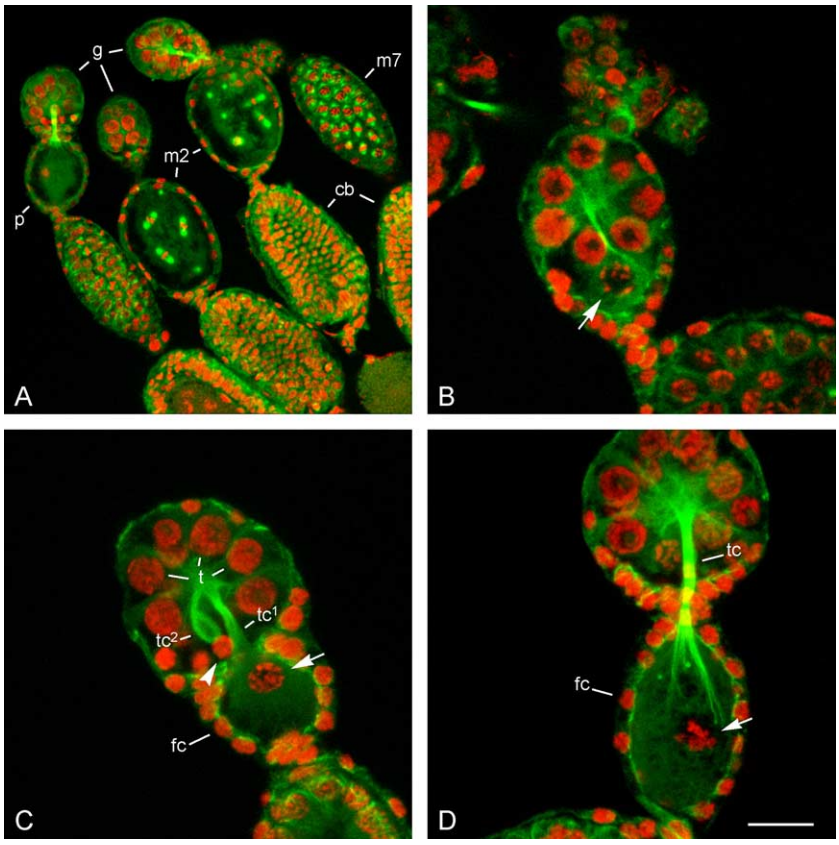

Fig. 2. Oocyte development in the parthenogenetic aphid *Acyrthosiphon pisum*. Microtubules (green) are revealed by staining with antibodies against β-tubulin and DNA (red) is visualized with propidium iodide. (A) The ovarioles contain embryos at different stages of development: g, germarium; p, prophase of the first meiosis; m2, second mitosis; m7, seventh mitosis: at this time the nuclei have lost their synchrony in division and prophase and metaphase spindles were found in the anterior and in the posterior region of the embryo, respectively; cb, cellular blastoderm. (B) Detail of a germarium with the differentiated oocyte (arrow). (C) The oocyte (arrow), pushed out of the germarium, is surrounded by follicle cells (fc) and is connected to trophocytes (t) by a trophic cord (tc^1); another oocyte (arrowhead) linked by a smaller trophic cord (tc^2) is selected within the germarium. (D) When the oocyte is separated from the germarium, the trophic cord (tc) that supports the growth phase extends until the nucleus (arrow). Scale bar: 80 μm in A, 20 μm in B–D.

axis became orthogonally oriented to the oocyte surface. By anaphase, the sister chromosomes separated and moved to the opposite poles of the barrel-shaped anastral spindle (Fig. 3C). At the end of telophase, most of the spindle microtubules disappeared and only a small mid-body persisted between the diploid sets of chromosomes (Fig. 3D). The putative zygotic pronucleus was found slightly deeper in the cytoplasm, whereas the diploid complement just beneath the oocyte surface was the polar body.

Self-organization of cortical microtubules into asters

In the case of parthenogenetic oocytes, in the absence of a sperm centriole, a centrosome-mediated microtubule nucleation is not expected. Surprisingly, as soon as the meiotic metaphase spindle forms, from 1 to 3 small asters not associated with chromatin became evident in the oocyte cytoplasm (Fig. 3B). These structures were never detected during earlier stages of meiosis, although a dense microtubule network was present within the peripheral oocyte cytoplasm. Thus, the process of aster assembly could be closely related to downstream events triggering meiotic resumption. Optical sectioning revealed that asters were enriched at the periphery of the oocyte, whereas the inner cytoplasm lacked these structures. Since asters were unevenly distributed within the whole periphery of the oocyte, the organization of the microtubules in astral arrays seems to be an intrinsic property of the whole cortex.

To determine if microtubule organizing centers (MTOCs) were absent in parthenogenetic oocytes, they were stained with an anti-γ-tubulin antibody, a core component of the microtubule nucleation machinery (Oakley and Oakley, 1989). No detectable γ-tubulin aggregates were found within the cytoplasm of the growing prophase oocytes, nor were there any accumulations of this protein at the poles of the meiotic metaphase or anaphase spindles (not shown). Likewise, no γ-tubulin staining was found at the poles of the tapered spindle and within the cytoplasm of the metaphase arrested oocyte obtained from dioic females. Therefore, spindle morphogenesis during female meiosis of both parthenogenetic and dioic strains was not dependent on MTOC function.

To investigate if the formation of the asters might be due to a microtubule nucleation process, we asked if γ-tubulin was

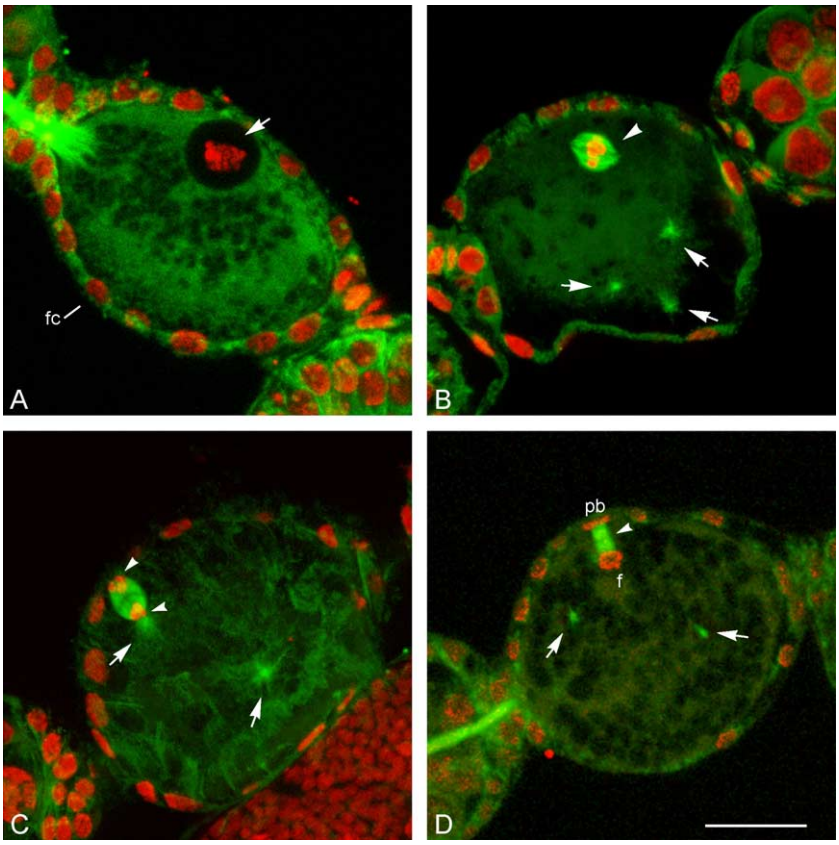

Fig. 3. First meiosis in the parthenogenetic oocyte. Projected series of optical sections of oocytes stained with antibodies against β-tubulin (green) and propidium iodide (red). (A) Prophase: no microtubules are associated with the condensed chromosomes (arrow), nor microtubule organizing centers are observed within the cytoplasm of the maturing oocyte. (B) Metaphase: the bipolar spindle is anastral (arrowhead) and oriented parallel to the oocyte surface. (C) Anaphase: the sister chromosomes (arrowheads) migrate to the opposite poles of the meiotic spindle that is orthogonal to the surface. (D) Telophase: a small midbody (arrowhead) separates two set of diploid chromosomes, the more interior becomes the female pronucleus (f), the other will be the polar body (pb). asters (arrows) are seen at the oocyte surface starting from metaphase; they played no role in meiotic spindle assembly and are not associated with the spindle poles. fc, follicle cells. Scale bar, 10 μm.

present within them. Immunofluorescence analysis revealed, indeed, that this protein was localized at the focus of larger asters at the end of meiosis and in both spindle poles and asters during early mitoses (Fig. 4A). Remarkably, this protein was barely detectable or absent within the asters seen during metaphase/anaphase progression of the first meiosis (not shown).

Counting of asters from resumption of meiosis to fourth mitosis, corresponding to the stage where these structures were still easily distinguishable, was performed. In oocytes

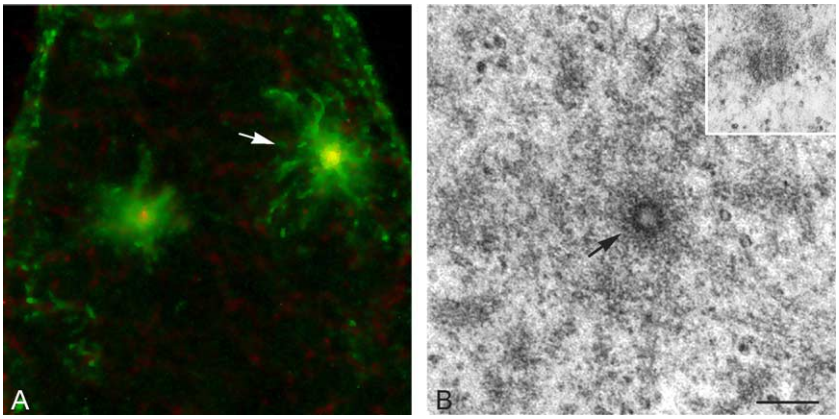

Fig. 4. Asters contain true centrosomes. (A) Double labeling with antibodies against γ-(yellow) and α-tubulin (green) reveals a focus of centrosomal material (arrow) within the asters. (B) Transmission electron microscope observations show the presence of centrioles (arrow) through the focus of asters; inset, longitudinal section of a centriole from another aster. Scale bar: 10 μm in A, 0.7 μm in B.

(n = 19), from early metaphase to the end of telophase of the first meiosis, the number of the asters ranged from 1 to 4. During prophase of the first mitotic division cycle, we scored from 3 to 8 asters (n embryos = 11). Embryos ranging from the second to fourth mitotic division (n = 15) had from 2 to 4 asters. Therefore, the number of the asters roughly doubled at the beginning of the first mitosis and decreased or remained constant afterwards. To assess whether the number of asters at prophase might be the result of rapid self-assembly or duplication processes, early prophase embryos were stained with antibodies against β- and γ-tubulin in order to label both microtubules and centrosomal material. Immunolabeling revealed that asters had a single focus for microtubules. No closely spaced aster pairs, indicative of a recent process of centrosome replication, were observed. These observations suggest that asters did not duplicate but rather were all formed de novo. However, duplication of asters could not be completely ruled out since a short window of time following the end of meiosis could escape our analysis. Because centrioles are known as the key elements for centrosome organization, their presence were checked within the focus of the asters. Centrioles were apparently lacking from asters during earlier stages of meiosis, but they were found within both spindle poles and cytoplasmic asters during the first nuclear division cycle (Fig. 4B).

Asters increased dimensions at the end of meiosis and their microtubules followed a cell cycle-dependent dynamic: they elongated during prophase and shortened during the following metaphase. To assay whether the ability to assemble asters was specific to the parthenogenetic oocytes or occurred also in the fertilized eggs, oocytes obtained by dioic females were analyzed. Meiosis resumed in these eggs and the presumptive female pronucleus formed at the end of telophase II; asters were never seen in these eggs. The only visible aster within the fertilized eggs is associated with the male pronucleus and is organized by the sperm provided basal body (Fig. 5A).

Centrosome-dependent spindle formation

Fertilized eggs build their first mitotic spindle forming a centrosome from the sperm provided basal body. At the end of meiosis, the male and female haploid pronuclei moved along the microtubular tracks of the large sperm aster. Gradually, the distance between the pronuclei decreased and, once they were in contact, a bipolar array of microtubules started to organize between them (Fig. 5B). In parthenogenetic eggs, the end of meiosis was marked by the disappearance of the meiotic spindle and by the increase in size of the asters, which were constituted by microtubules that not only formed a dense network at the egg surface but also elongated in the internal cytoplasm. Because the condensing female chromatin was eccentrically positioned within the egg cytoplasm, it could preferentially interact with the microtubules radiating from the nearest aster. Serial optical sections showed, indeed, that despite the presence of several asters at the oocyte surface, only one was in contact with the female pronucleus (Figs. 6A, B). When a 3-D reconstructed image obtained by projecting a Z-series stack was performed during early prophase, we observed that only one aster interacted with the female pronucleus, even if a second aster was located at the opposite egg surface (Fig. 6C). On the basis of these findings, only monopolar spindles could be formed in the early parthenogenetic zygote. However, we usually found normal bipolar spindles, whereas monopolar spindles were only occasionally (3/103) observed in parthenogenetic eggs scored during the first mitosis.

Since the formation of a bipolar spindle is mainly due to the nucleation of antiparallel microtubules from two opposite centrosomes that had migrated along the nuclear envelope, the female pronucleus in parthenogenetic eggs probably had to be in contact with a second aster to undergo proper mitotic progression. Whether the formation of a bipolar spindle in parthenogenetic eggs depended on an earlier event of centrosome duplication or was the result of the association of two independently assembled asters is still unclear. During

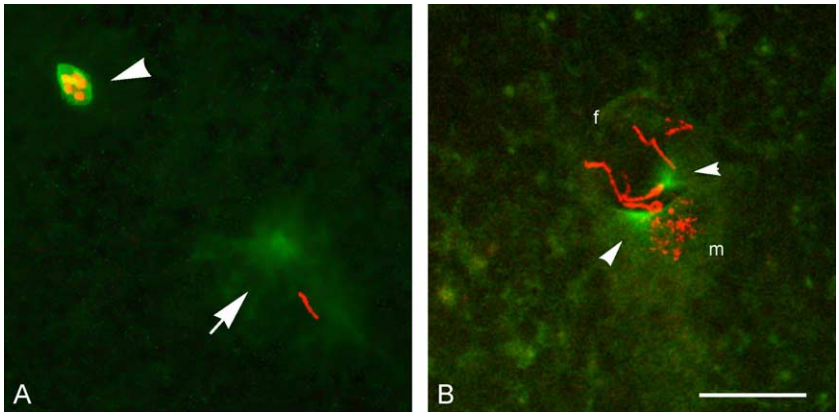

Fig. 5. Meiosis and pronuclear fusion in fertilized eggs collected after 1 and 4 h. Microtubules are stained green and DNA is red. (A) Metaphase of the first meiosis: the meiotic spindle (arrowhead) is anastral and a large aster is associated with the sperm head (arrow). (B) Pronuclear fusion: when the parental complements are in contact a bipolar array of microtubules nucleated by two distinct centrosomes (arrowheads) starts to organize among them; the female chromatin (f) is more condensed than the male one (m). Scale bar, 10 μm.

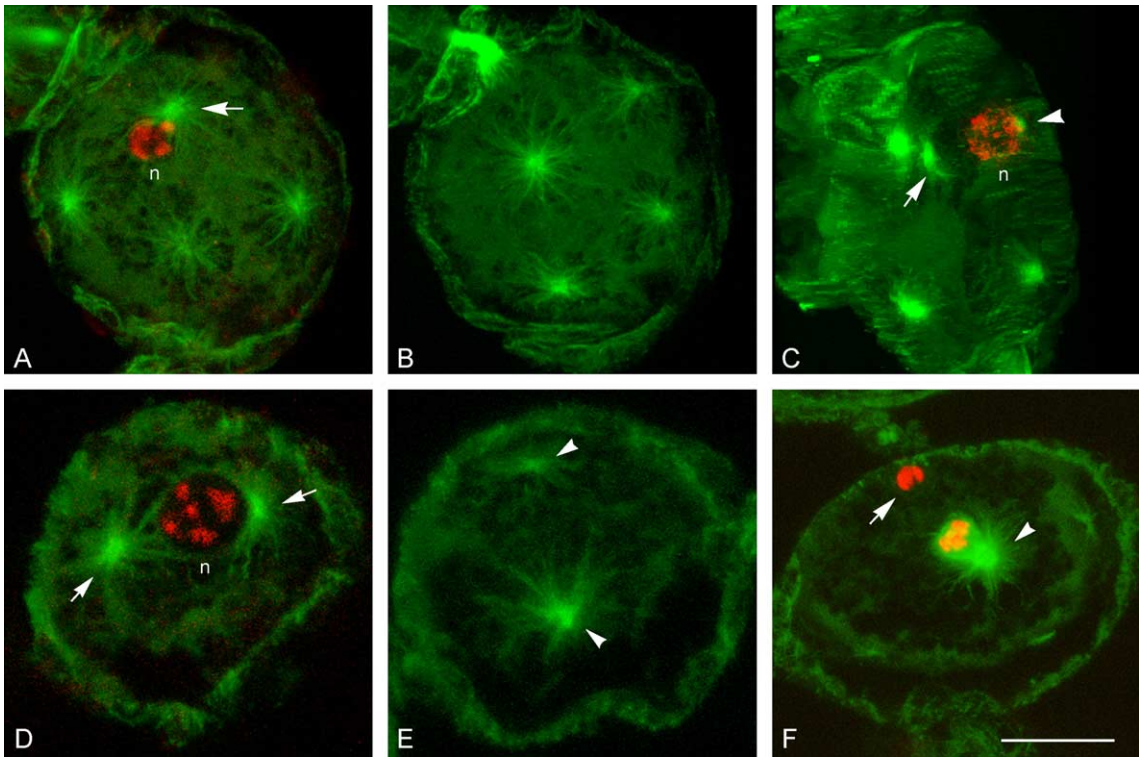

Fig. 6. Formation of the first mitotic spindle in parthenogenetic eggs. Merged images show microtubules (green) and DNA (red). Projections of three consecutive optical sections at 3-μm interval both taken at the top (A) and at the bottom (B) of the ovarian follicle: the early prophase nucleus (n) is associated with only one aster (arrow). (C) Projected series of ten optical sections at 3-μm intervals tilted to view the ovarian chamber laterally: five asters are present at the egg surface, two on the left, three on the right of the picture; note that the prophase nucleus (n) is associated with only one aster on the right (arrowhead), although at the opposite egg surface, an aster has elongated microtubules directed toward the nucleus itself (arrow). (D, E) Optical sections within an egg taken at opposite levels at 15 μm interval: at one side of the egg, two differently positioned asters (arrows) interact with the prophase nucleus (n), whereas the opposite side has only free asters (arrowheads). (F) A bipolar monoastral spindle that can support chromosome congression at metaphase is formed in the presence of only one aster (arrowhead); the arrow points to the polar body. Scale bar, 10 μm.

centrosome duplication, the separation of the centrosomal material that preceded centrosome duplication is usually accompanied by an enlargement of the spindle pole. In the case of parthenogenetic development in aphids, the enlargement of the focus of the asters was never observed, and no close aster pairs associated with the nucleus were detected during the first mitosis. We did observed aster pairs at one side of the nuclei but in late stages of development, during early prophase of the second mitosis. Thus, the centrosomes that nucleated the astral arrays of microtubules were duplicated at this time. However, these observations do not rule out that the process of centrosome duplication at the first mitosis might be so rapid to escape from the samples analyzed ($n = 91$).

The hypothesis that the bipolar zygotic spindle in parthenogenetic eggs is formed from two distinct centrosome-based asters is suggested by two lines of evidence. First, we found that during the first mitosis, prophase nuclei could interact with two asters that were positioned at different distance from the nucleus. One of the aster was in close contact with the nucleus, whereas the other was far, but its microtubules extended to the nuclear envelope (Figs. 6C, D). Second, when monoastral bipolar spindles were observed during metaphase of the first mitosis (Fig. 6E), the only aster found in the cytoplasm was at one pole of the spindle. This indicates that one aster led to the formation of one astral pole alone that was unable to replicate. At the end of prophase, two asters were seen to interact with the zygotic nucleus (Fig. 7A) and a regular symmetric bipolar spindle that progressed through the first mitosis was assembled (Fig. 7B). The number of cytoplasmic asters did not increase during the first mitosis, suggesting that the centrosomes they contained were unable to replicate (Figs. 7A–D). Later, during prophase of the second mitosis, the centrosomes associated with the nuclei were competent to duplicate (Fig. 8A), as indicated by the close aster pairs, and moved to the opposite poles of the nuclear envelope to organize a bipolar spindle (Fig. 8B). Cytoplasmic asters did not duplicate and were clearly seen at the surface of the parthenogenetic embryo until third and fourth mitoses (Figs. 8C, D).

Discussion

The centrosome plays a crucial role in the assembly of the bipolar spindle that drives the correct segregation of the genetic material and directs many of the microtubule-based processes within the cell (Doxsey, 2001). Thus, the cell has

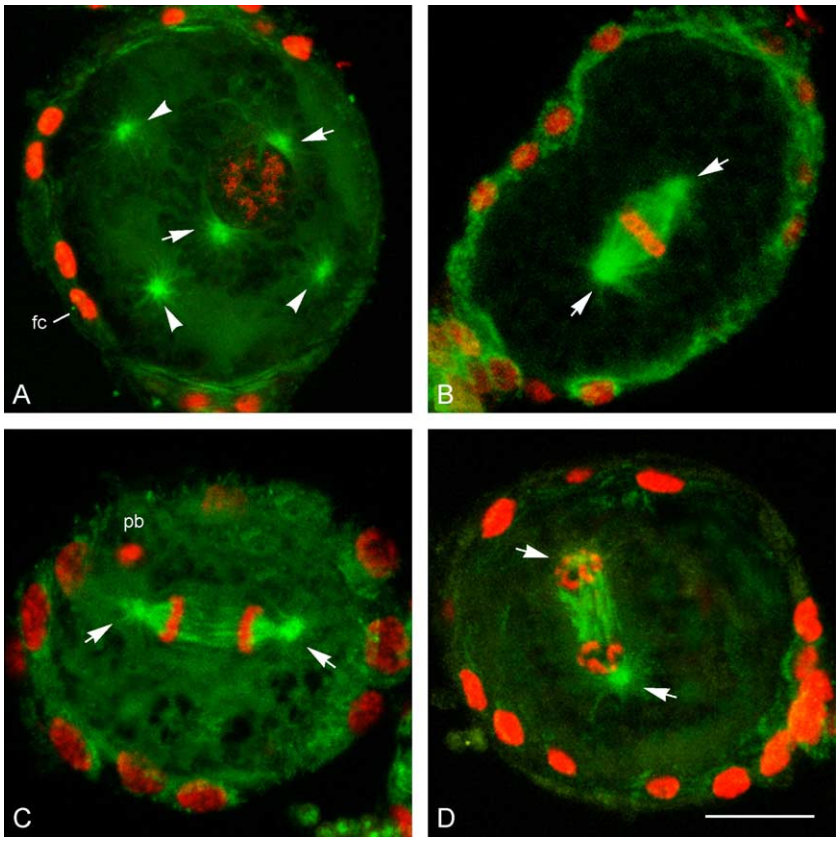

Fig. 7. First mitosis in parthenogenetic eggs. Microtubules are stained with antibodies against β-tubulin (green) and propidium iodide (red). (A) Prophase. (B) Metaphase. (C) Anaphase. (D) Telophase. Note that when only the focal plane of the mitotic spindle is represented (B, C, D), only the aster microtubules associated with the spindle poles are visible (arrows), whereas when the z-stack series is comprehensive of the surface asters are also visible (arrowheads in A). pb, polar body; fc, follicle cells. Scale bar, 10 μm.

to carefully regulate the centrosome behavior to avoid supernumerary centrosomes and the formation of multipolar spindles that unequally distribute chromosomes to daughter cells (Delattre and Gonczy, 2004). It has been reported that centrosomes exhibiting aberrant features, including alteration in number, size, and shape can be induced in many malignant tumors through the misexpression of regulatory proteins (Sluder and Nordberg, 2004, and references therein). These abnormalities could be generated by centrosome replication defects, or by self-assembly of centrosomal material in the absence of centrioles.

Gametogenesis and fertilization are relevant examples in which the cell actively monitors the presence of only one functional centrosome. Echiurans, molluscs, and echinoderms silence the maternal centrosome after egg activation (Stephano and Gould, 1995; Uetake et al., 2002; Wu and Palazzo, 1999; Zhang et al., 2004), whereas insect oocytes lose their centrosomes early in oogenesis (Buning, 1994). The sperm does not contribute the centrosome during murine fertilization (Manandhar et al., 2000). Our findings that parthenogenetic development in aphids requires the presence of multiple centrosome-based asters in the same cytoplasm, represent a remarkable exception to the assumption that the centrosome number within a cell must be strictly monitored. Perhaps one of the most relevant aspects regarding parthenogenetic development in insects is that a cell, usually unable to develop beyond meiosis without a sperm basal body, can successfully divide by means of de novo-assembled centrosomes.

The mode of centrosome inheritance in parthenogenetic aphids is very efficient since among hundred early embryos only 1.9% (9/471) developed multipolar spindles. How, is the interaction of more than two centrosomes to the zygotic nucleus prevented in aphids? Hymenopteran eggs, which also develop parthenogenetically by means of spontaneously assembled asters, avoid abnormal spindle formation by a mechanism of temporal exclusion of supernumerary centrosomes (Tram and Sullivan, 2000). The first two centrosomes that contact the nuclear envelope of the zygotic nucleus become stabilized and prevent interaction of the remaining centrosomes. The basis of this mechanism is unknown. It is also unclear how multipolar spindles are prevented in the anterior half of the parthenogenetic *Drosophila mercatorum* egg where multiple centrosomes are presented (Riparbelli and Callaini, 2003).

In parthenogenetic aphids, the organization of the first zygotic spindle is presumably due to a stochastic event that requires the interaction of self-assembled asters with the female nucleus. Prevention of abnormal spindles presumably relies on the low number of asters that are evenly

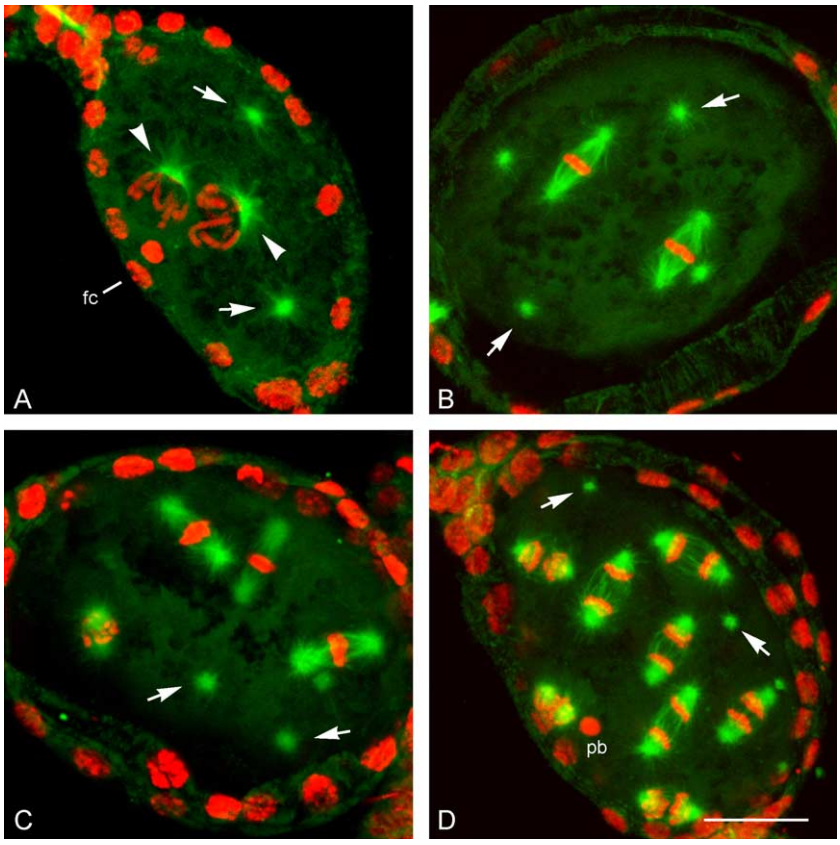

Fig. 8. Asters persist at the egg surface throughout later mitotic divisions. Projected series of optical sections of eggs stained with antibodies against β-tubulin (green) and propidium iodide (red). (A) Prophase of the second mitosis: centrosomes at one side of the nuclei have duplicated (arrowheads), whereas the asters (arrows) did not. (B) Metaphase of the second mitosis. (C) Metaphase of the third mitosis. (D) Anaphase of the fourth mitosis. Asters (arrows) do not disappear through earlier mitoses, although their number does not increase. pb, polar body; fc, follicle cells. Scale bar, 10 μm.

spaced in the cortical region of the ovarian follicle. At the end of meiosis, the microtubules of the asters have increased in length and formed a dense network that presumably avoids asters from lateral shifting, thus, ensuring their proper spacing and preventing their interaction. Only the asters that interact with the female nucleus can, in turn, move down from their initial cortical position toward the inside of the ovarian follicle, sliding along the nuclear envelope. Thus, only the nearest aster gains the correct interaction with the nucleus becoming the spindle poles, whereas the others are spatially excluded.

Parthenogenetic aphids silence the remnant supernumerary centrosomes that do not interact with chromatin, so they disappear by the first mitoses. It is unclear how these centrosomes are inactivated. One possibility is that centrosomes become more stable following interaction with chromatin. Alternatively, the centrioles might be unable to replicate in the cortical region in the absence of nuclear signals. This might prevent the reproduction and functionality of the cortical asters, in turn promoting their disappearance. By contrast, in parthenogenetic *Drosophila mercatorum* eggs, the supernumerary centrosomes are able to duplicate during the first mitoses and persist until later syncytial blastoderm stages, giving rise to errors in the correct positioning of the dividing nuclei, with consequent failure of development (Riparbelli and Callaini, 2003). When the inactivation of supernumerary centrosomes is incompletely executed, as it might be the case of the thychoparhenogenetic *Drosophila mercatorum*, the rate of embryo survival is very low even in these embryos that successfully restore diploidy. Supernumerary centrosome inactivation is, therefore, absolutely required to ensure successful parthenogenetic development in insects, and aphids represent good biological systems to study this phenomenon.

Asters can be organized in different systems by centrosome-based nucleation mechanisms or by centrosome-independent pathways (Hyman and Karsenti, 1998). The detection of a dense network of microtubules at the surface of the ovarian follicle in parthenogenetic aphids and the lack of cytoplasmic γ-tubulin aggregates, suggests a self-organization process of the asters independently from centrosomal components. It has been shown in several systems that oligomeric motor complexes have an important role in self-organization processes, by cross-linking and organizing randomly nucleated microtubules into aster-like structures (Nédèlec et al., 1997; Verde et al., 1991). The parthenogenetic signals might trigger specific processes involved in up-regulating motor protein activity that is down-regulated during normal development. Raising the concentrations of

kinesin and ncd motor complexes in vitro leads, indeed, to the formation of asters, with accumulation of the motors in the center (Nédèlec et al., 2003). The finding that newly laid unfertilized *Laborc*^{D} eggs, a dominant-negative dynein mutation, spontaneously assemble microtubule asters (Belecz et al., 2001), supports the possibility of intrinsic controls of motor protein activity. The asters that form in parthenogenetic insect oocytes could recruit centrosomal proteins and centriole subunits from the surrounding cytoplasm, leading to their accumulation at the center of the aster. This is consistent with the observation that the accumulation of pericentrin and γ-tubulin at the vertebrate centrosome is inhibited in the absence of tubulin or by microinjection of antibodies against cytoplasmic dynein (Young et al., 2000). Thus, the focus of the asters could represent a suitable environment in which the centrosome can organize.

Since unfertilized eggs from sexually reproducing aphids never assemble a dense network of cortical microtubules after activation, we speculate that one of the limiting factors that shift sexual to parthenogenetic development could be intrinsic mechanisms that control microtubule dynamics. Parthenogenetic development might, therefore, rely on the self-nucleation and/or stabilization of surface microtubules by activating stabilizing factors or/and inactivating destabilizing ones. The ovulation process that triggers the activation of the oocyte and its release from the metaphase block (Heifetz et al., 2001; Page and Orr-Weaver, 1997) could induce the nucleation of non-centrosomal microtubules in parthenogenetic eggs. Consistently, newly activated parthenogenetic eggs of the hymenopteran *Muscidifurax* have a dense network of surface microtubules (Riparbelli et al., 1998). A mechanism of activation by extrinsic factors, such as that in *Drosophila* and hymenopterans, is presumably not required in parthenogenetic aphids since their ovarian follicles undergo full embryonic development within the ovaries. The cortical network of microtubules could, therefore, form through the same intrinsic signals that drive meiotic resumption.

The question of whether a centrosome-independent pathway for spindle formation exists in unfertilized insect eggs is controversial. Studies on parthenogenetic development of *Drosophila mercatorum* (Riparbelli and Callaini, 2003) and some hymenopteran species (Riparbelli et al., 1998; Tram and Sullivan, 2000) agree that the early embryonic divisions are supported by centrosome-based spindles, although the organization of the first zygotic spindle required the interaction with asters that were organized during meiosis by a centrosome-independent pathway. By contrast, some observations on *Sciara* (de Saint Phalle and Sullivan, 1998) and *Bacillus* (Marescalchi et al., 2002) activated eggs suggested that mitotic spindles can be assembled during the early parthenogenetic development in the absence of functional centrosomes. The finding that the unfertilized insect egg can organize anastral bipolar spindle is not surprising, since *Drosophila polo* (Riparbelli et al., 2000) and *centrosomin* (Megraw et al., 1999) embryos also form tapered anastral bipolar spindles by sorting and focusing antiparallel microtubule arrays around mitotic chromosomes. However, these spindles are improperly spaced, nuclear migration is affected, and chromosome segregation is abnormal. Thus, mutant embryos have severe mitotic defects and do not develop. However, we cannot exclude that stick insects have different control mechanisms that enable embryonic development in the absence of true centrosomes.

The finding that distantly related insect species develop parthenogenetically by means of self-organized asters, that in turn recruit centriole precursors and pericentriolar material, indicates the existence of common pathways for centrosome assembly. This process presumably involves the overriding of intrinsic control points that prevent centrosome self-assembly in eggs from dioic species, thus avoiding attempt to develop in the absence of a male gamete. We suspect that the spontaneous organization of asters could be an evolutionary conserved process leading to parthenogenetic development. In some insect species, parthenogenesis firstly appears to be occasional and is based on the slow and inaccurate self-organization process of asters. This is presumably due to the incomplete working of the control mechanisms that monitor spontaneous aster organization in sexually reproducing eggs. The successful developmental rate increases by evolving speed and fidelity of the self-organization processes, consequently to the complete silencing of the control mechanisms. In the case of aphids, for which parthenogenesis is highly regulated and very efficient, the precise cellular mechanisms of meiosis regulation adapted during the evolution are part of the large success of these insects as plant pests.

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