Centrosome inheritance: **A central ‘in-egg-ma’ solved?**

Timothy L. Karr

The mechanism of centrosome inheritance during parthenogenetic development has long been an outstanding mystery of cell biology. New observations of centrosome inheritance in hymenopterans have provided our first direct insights into this enigmatic process.

Address: Department of Organismal Biology and Anatomy,
1027 East 57th Street, Chicago, Illinois 60637, USA.

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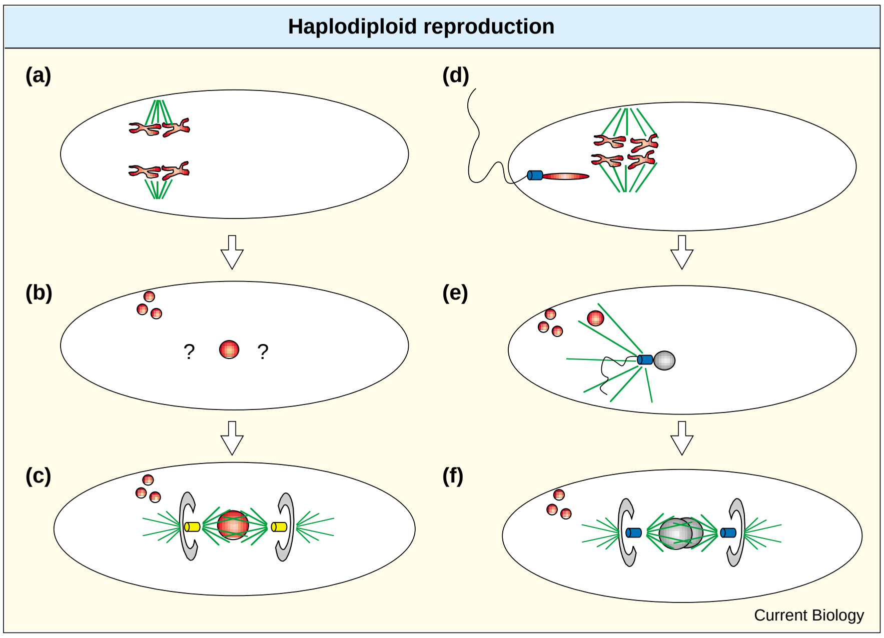
Sexually reproducing organisms cannot exist without the proper transmission of genetic information contained within the chromosomes. Another vitally important cellular structure, the centriole, must also be properly transmitted during reproduction. The centriole, first recognized by Boveri [1] as a separate “independent cell organ” over a century ago, has been described as a “central enigma of cell biology” [2]. This diminutive and enigmatic structure has come to be recognized as arguably the singular most important extra-nuclear structure within the cell, the integrity and function of which are crucial either directly or indirectly — through its microtubule organizing capacity — in almost every aspect of cellular life. It has also become clear over the past decade that centrosome numbers must be carefully monitored and controlled — supernumerary centrosomes can have devastating consequences leading to improper chromosome separation and cell death (for a review of centrosome biology see [3]).

Microtubule organization in a cell is under the control of the centrosome, also known as the microtubule organizing center (MTOC). The centrosome is defined as the centriole and surrounding ‘peri-centriolar material’. In order to ensure species continuity, a functional centrosome must thus be inherited each generation. In sexually reproducing organisms, the centriole is most often inherited from the sperm, and a functional centrosome arises in the egg following fertilization [4]. It is thought that the egg cytoplasm contains the peri-centriolar material — or at least the raw materials to produce it — and that the sperm-derived centriole and peri-centriolar material form a functional centrosome after fertilization (see Figure 1). Centrosome inheritance is thus biparental, with each parent providing essential elements.

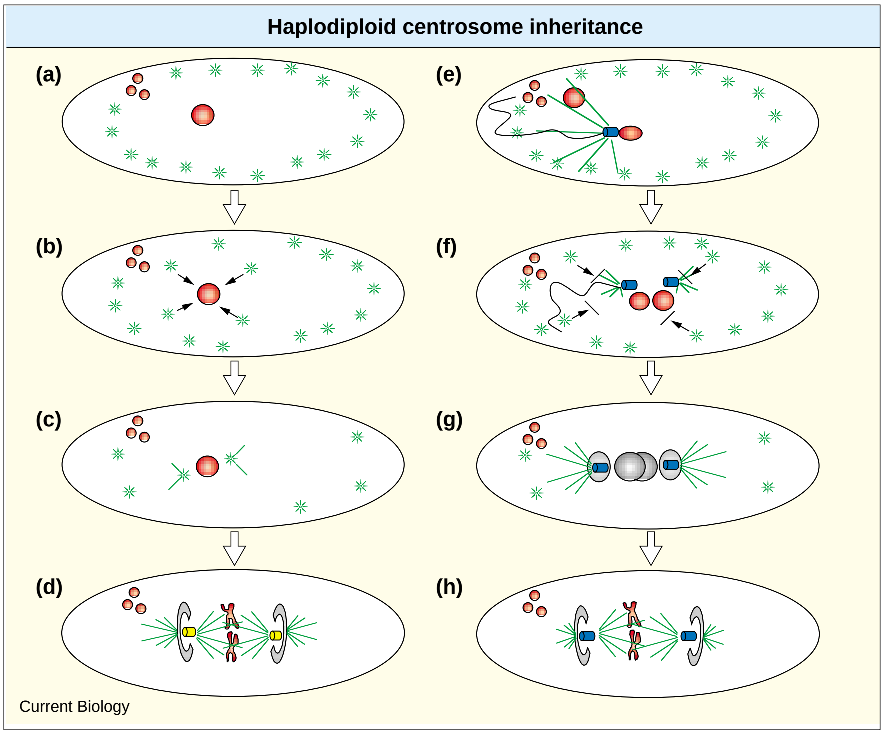
This model of sexual reproduction does not, however, explain the mode of centrosome inheritance during parthenogenesis. In the haplodiploid reproduction exhibited

Figure 1



Haplodiploid development in hymenopterans. (a–c) Haploid development. (a) Oviposition of unfertilized egg activate meiosis (green, microtubules; red, chromosomes). (b) Completion of meiosis results in the formation of three polar bodies (small pink circles) and a female pronucleus (red circle). The female pronucleus acquires centrosomes by an unknown mechanism (question marks), resulting in (c) a functional mitotic spindle containing centrioles (yellow, to distinguish them from those arising from the sperm basal body in fertilized egg) and pericentriolar material (gray semicircles). (d–f) Diploid development. (d) Fertilization by sperm (nucleus, red; basal body, solid blue cylinder) activates the egg to complete meiosis. (e) A centriole is generated from the sperm basal body (blue cylinder) and sperm-astral microtubules (small green lines) interact with the female pronucleus. (f) Following centriole duplication and pronuclear migration, the first spindle forms around the diploid nuclei and initiates the zygotic developmental program. At some point during this time, each centriole becomes invested with an amorphous pericentriolar material.

Figure 2



Centrosome inheritance in haploid (a–d) or diploid (e–h) development. (a) Female pronuclear formation is accompanied by the formation within the cortical cytoplasm of numerous foci of microtubule assembly (green asterisks). (b) Some, but not all, cytoplasmic asters approach the nucleus (arrows). (c) Exactly two asters associate with the nucleus and presumably acquire pericentriolar material (gray stippling). (d) Maternally inherited centrosomes (centrioles, yellow cylinders; pericentriolar material, gray semicircles) form and nucleate microtubules in anticipation of the first zygotic division. (e–h) Sperm entrance and completion of meiosis generates a male and female pronucleus (stippled red oval and circle, respectively). At the same time, cytoplasmic asters form. (f) The sperm centriole (blue cylinder) duplicates and associates with the female pronucleus as they approach each other. Concurrently, cytoplasmic asters advance towards the pronuclei but do not successfully attach. (g) As the pronuclei appose one another the centrioles become invested with pericentriolar material (gray stippling) and begin to nucleate microtubules concurrent with the disappearance of cytoplasmic asters. (h) Completion of DNA synthesis, chromosome formation and mitotic spindle assembly result in the first diploid mitotic division.

by the hymenopterans, for example, the sex of the offspring is determined by the fertilization pattern. Haploid males develop from unfertilized eggs and diploid females develop from fertilized eggs (Figure 1), so a functional centrosome must be established in both fertilized and unfertilized eggs. In a fertilized egg, the sperm basal body becomes the centriole, which organizes a functional centrosome during the completion of meiosis and pronuclear migration and fusion. An unfertilized egg must complete meiosis, select the pronucleus from the four products of meiosis and then form a functional centrosome before the first haploid mitotic division.

If centrosomes are present in the haploid zygote, they must be inherited from the egg. But because centrioles are not associated with the meiotic divisions in the egg, it has remained unclear how, when and where the centrioles arise during male haploid development. Until now just a single report on the mode of centrosome inheritance in hymenopterans has appeared in the literature. Riparbelli *et al.* [5] investigated centrosome formation during haploid development in the parasitic wasp *Muscidifurax uniraptor*. They actually followed diploid development of unfertilized eggs, as they used a strain of *M. uniraptor* infected by the endosymbiont *Wolbachia*, which causes diploidization of unfertilized eggs. The precise relationship of the results to haplodiploid development is thus an open question, but Riparbelli *et al.* [5] were the first to suggest that cytoplasmic centrosome formation occurs after meiosis in unfertilized *M. uniraptor* eggs. They also showed that the egg cytoplasm contains centrioles, and that these appeared to be at the foci where cytoplasmic centrosomes formed. Because fixed samples were used, this provocative and important study could not fully address the dynamics of centrosome inheritance during haplodiploid development.

As recently published in *Current Biology*, Tram and Sullivan [6] have now determined the mode of centrosome inheritance in the hymenopteran wasp *Nasonia vitripennis* by direct observation of living eggs. Working with Bruce Alberts and his colleagues, Sullivan [7] pioneered techniques for making real-time observations of early developmental events in the fruitfly *Drosophila*, by confocal microscopy of embryos labelled with fluorescently tagged tubulin and DNA-specific dyes. These techniques have now been used to observe centrosomal and nuclear behavior during fertilization and haplodiploid development in *N. vitripennis* [6]. The results have profound implications for future studies of centrosome structure, function and inheritance. Perhaps even more importantly for those interested in the developmental and evolutionary origins of this enigmatic cell organelle, the results could provide a framework for future evolutionary hypotheses.

The results reported by Tram and Sullivan [6] make three significant advances in our understanding of centrosome inheritance. First, they found that meiosis is indistinguishable in fertilized and unfertilized eggs. The formation and migration of the pronucleus are thus independent of the sperm. Second, they show that the acquisition of functional centrosomes in an unfertilized egg is a highly dynamic process, involving the entire cortical cytoplasm. In an unfertilized egg, after activation during egg oviposition and the completion of meiosis, the female pronucleus migrates inwards and acquires two functional centrosomes. Third, their real-time analysis clearly shows that, in a fertilized egg, only the paternal centrosome is inherited, even though the pool of maternal centrosomes form and migrate towards the male and female pronuclei. Female eggs thus inherit paternally derived centrosomes, while the centrosomes of male eggs are inherited maternally (Figure 2). Below I shall highlight just some of the many implications of these results, and speculate on future directions. For example, even the seemingly simple observation that meiosis and female pronuclear maturation are indistinguishable in fertilized and unfertilized eggs clearly shows that the sperm cannot have any obligate specialised effect at this stage in this organism.

It is clear from the results of Tram and Sullivan [6] that, although numerous centrosomes appear in the cortical cytoplasm in an unfertilized egg, only two ‘win’ what appears to be a cytoplasmic race to the female pronucleus (Figure 2). Assuming that all centrosomes in the cortical region are capable of nuclear attachment and subsequent zygotic centrosome activity, a form of ‘centrosome competition’ between these structures may occur. Observations of cortical centrosome behavior in fertilized eggs have also suggested the existence of mechanisms that actively exclude female centrosomes from the zygote nucleus. Tram and Sullivan [6] suggest a temporal mechanism of exclusion — sperm centrioles appear to organize a functional centrosome and interact with the female pronucleus at the same time as, or just before, cortical centrosome migration. Presumably the paternally derived centrosomes, once established, can prevent maternal centrosomes from approaching and interacting with the forming zygotic nucleus. Because cortical centrosomes are not observed after the first zygotic division, there is presumably another, independent mechanism for suppressing centrosome activity. These results clearly establish the existence of mechanisms that regulate centrosome appearance, migration and disappearance during this critical stage of development. It will be of great interest to define the molecular basis of these mechanisms and their relationship to the overall picture of reproduction in hymenopterans and other organisms.

These remarkable results clearly define the general outline of the process, but they also raise more questions than they answer — as all useful science should. For example, how is cortical centrosome formation regulated? Tram and Sullivan [6] suggest, by analogy to non-insect systems, that the pH and calcium changes known to accompany egg activation may be involved. Do other insects that reproduce sexually exhibit similar transient maternal centrosome formation during fertilization? In this regard, it will be of interest to investigate centrosome formation in a *Drosophila* strain capable of gynogenetic development — the formation of diploid females from eggs fertilized by sperm capable of fertilization but not zygote formation. For example, the *gyn 2-3* strain of *Drosophila* produces diploid females when fertilized by non-functional sperm [8], and this may begin a search for maternal centrosomes. That this search might be fruitful is suggested by two studies of centrosome-like structures in the *Drosophila* embryo. Fertilized eggs from *giant nucleus* mothers display abortive mitoses because of defects in DNA replication, and foci of microtubules were seen to form in the cytoplasm of these eggs [9]. In a second study [10], egg lethality induced by the symbiotic microbe *Wolbachia pipientis* also resulted in the appearance of anucleate centrosome-like structures. If these observations are verified, then the power of *Drosophila* genetics can be brought to bear on this intriguing problem.

The occurrence of both paternal and maternal centrosomes within a common cytoplasm could have important consequences for our understanding of the evolutionary forces involved. How common is the potential for maternal centrosome formation in other organisms? For example, do eggs of obligate sexually reproducing organisms contain maternal centrioles? Is there inter-specific and intra-specific variation in centrosome number? Are there conditions under which maternal centrosomes outcompete the paternal centrosome for the zygote? What happens during blastoderm formation, where cortical centrosomes might be waiting to replace the paternal ones? In the spirit of speculation, might the egg act as the venue for a centrosomal battleground of inter-sexual conflict? The resolution of such a conflict might explain the present day patterns of centrosome inheritance, where each parent provides the crucial elements to the centrosome. Armed with the tools given by Tram and Sullivan [6], answers to these and other questions could provide new perspectives for an evolutionary analysis of centrosome origins and history, and perhaps someday provide a more definitive solution to this central ‘in-egg-ma’.

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