Available online at www.sciencedirect.com
SCIENCE DIRECT®
Microbes and Infection 5 (2003) 999–1011
Microbes and Infection
www.elsevier.com/locate/micinf

Review

Identification of *Wolbachia*–host interacting factors through cytological analysis

Uyen Tram *, Patrick M. Ferree, William Sullivan

Department of Molecular, Cellular and Developmental Biology, University of California, Santa Cruz, CA 95064, USA

Abstract

Manipulation of host reproduction and efficient maternal transmission have facilitated the global spread of *Wolbachia* through millions of insect species. Cytological studies of the most common *Wolbachia*–induced phenotype, cytoplasmic incompatibility (CI), demonstrate that *Wolbachia* induce CI by altering host cell cycle timing. Cytological analyses also suggest that microtubules and motor proteins may play a role in the maternal and somatic transmission of *Wolbachia*.

Keywords: Wolbachia; Cytoplasmic incompatibility; Cell cycle; Cytoskeleton

1. Introduction: the reproductive parasite *Wolbachia* is pervasive among insects worldwide

Bacteria of the genus *Wolbachia* are intracellular parasites of insects (reviewed in [1,2]), filarial nematodes (reviewed in [3]), crustaceans [4], and mites [5]. The founding member of this genus, *Wolbachia pipentis*, was first observed by Hertig and Wolbach [6] in the ovaries of the mosquito *Culex pipiens*. Since then, *Wolbachia* have been detected in most orders of insects and are believed to be the most pervasive endosymbiont, infecting 15–76% of all insect species worldwide [7–9]. The successful spread of *Wolbachia* is due in part to their extraordinary ability to alter host development, sex determination, and reproduction to their own advantage. Similarly to other bacteria in the order Rickettsiales [10], *Wolbachia* are obligate intracellular endosymbionts of eukaryotes. *Wolbachia* are most closely related to bacteria in the genera *Ehrlichia* and *Rickettsia*, both of which include arthropod-borne parasites that cause disease in mammals [11–13]. Unlike *Ehrlichia* and *Rickettsia*, *Wolbachia* do not infect mammals and are characterized by their ability to manipulate host reproduction [14].

It is currently unclear how many species of *Wolbachia* exist, and species designation remains an area of debate in the field. At present *Wolbachia* strains are classified into six subdivisions, designated A through F, based on nucleotide sequence of the cell cycle gene *ftsZ* [15,16] and 16S rDNA [13,16]. *Wolbachia* groups A and B infect arthropods, groups C and D infect nematodes, group E infects springtails, and group F infects termites [13,15–17]. With a few exceptions [18,19], *Wolbachia* groups A and B are not essential for their insect hosts and alter insect reproduction in a variety of ways (discussed below). Both *Wolbachia* groups A and B infect a wide range of insects, and many insects are simultaneously infected with both A and B *Wolbachia*. *Wolbachia* groups A and B do not appear to specialize in parasitizing a particular host species. For example, the parasitic wasp *Leptopilina heterotoma* and its fruitfly host *Drosophila simulans* are infected with the same *Wolbachia* strain [20]. *D. simulans* residing in different regions of the world, however, may be infected with unrelated strains of *Wolbachia* [21–26]. In addition, phylogenies of arthropod *Wolbachia* are not congruent with phylogenies of their hosts, suggesting that *Wolbachia* may be transmitted horizontally between hosts [11,15].

Wolbachia groups C and D have been found to infect only one family of nematodes, the Onchocercidae or filarial nematodes that cause disease in mammals (reviewed in [3]). Unlike *Wolbachia* that infect arthropods, *Wolbachia* that infect nematodes appear to specialize in the species of filarial nematode they infect and are essential for their hosts. For example, *Dirofilaria immitis* obtained from Italy, Spain, Cuba, and the United States all are infected with the same

* Corresponding author. Present address: Department of Microbiology, The Ohio State University, 417 Bioscience Building, 484 West 12th Avenue, Columbus, OH 43210-1292, USA. Tel.: +1-614-292-2301; fax: +1-614-292-8120.
E-mail address: tram@biology.ucsc.edu (U. Tram).

doi:10.1016/S1286-4579(03)00192-8

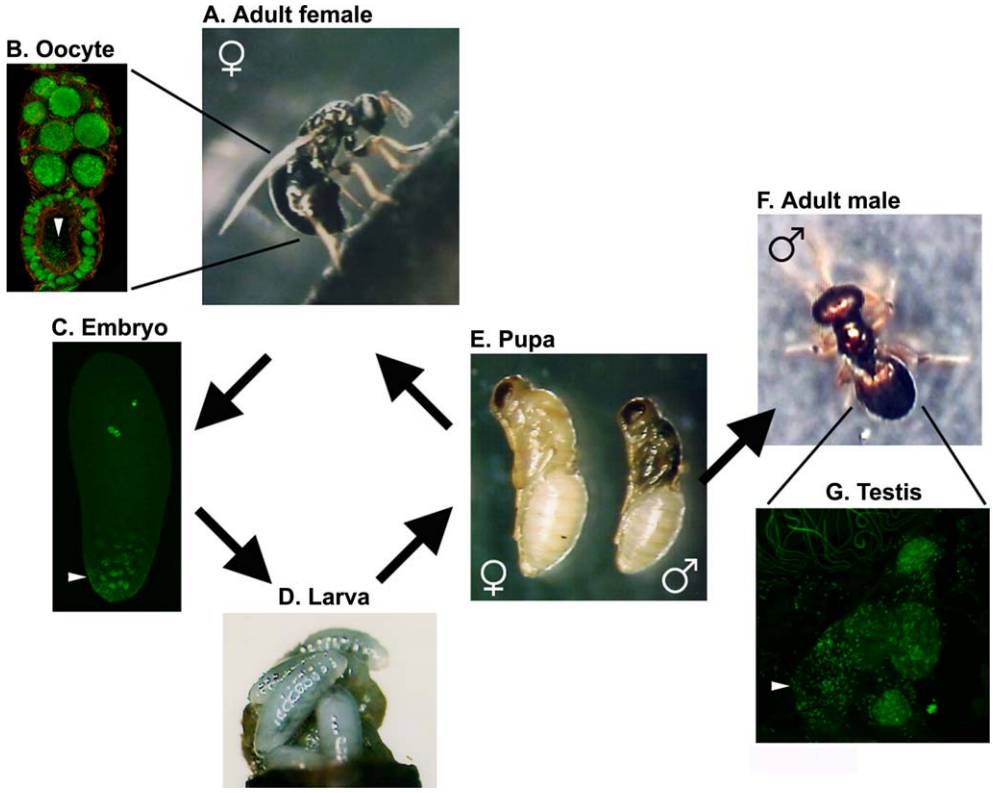

Fig. 1. *Wolbachia*'s maternal transmission illustrated in the parasitic wasp *N. vitripennis*. (A) In the adult female, *Wolbachia* are found in the ovaries. (B) In individual developing oocytes, *Wolbachia* are found in both the nurse cells and the developing oocyte (arrowhead). This oocyte was fixed and stained for DNA (green) and actin (red). (C) In *Nasonia* embryos, *Wolbachia* localize to the posterior pole (arrowhead), where the precursor cells for eggs/sperm are born. This embryo was fixed and stained for DNA. (D) Detailed cellular analysis of *Wolbachia* localization in *Nasonia* larvae is not available. However, PCR analysis of *Drosophila* and *C. cautella* reveal that *Wolbachia* are present in somatic tissues [33], and immunofluorescent analysis shows that *Wolbachia* indeed are present in *Drosophila* larval testes [51]. (E) Detailed cellular analysis of *Wolbachia* during pupal development is not available. However, cellular analysis of *Drosophila* pupae shows that *Wolbachia* are in the testes [51]. (F) In adult males, *Wolbachia* are present in the testes (arrowhead, G). *Wolbachia* are absent from mature sperm and thus are very rarely paternally transmitted.

strain of *Wolbachia* (see [27]). Removal of *Wolbachia* has detrimental effects on host development, reproduction, and survival. The effects *Wolbachia* groups E and F have on their hosts are not currently known [13,16]. In this review, we will focus solely on interactions between A and B *Wolbachia* and their insect hosts.

In insects, *Wolbachia* localize in the reproductive tissues and are efficiently transmitted through the female germline (Fig. 1), where they can induce parthenogenesis, male killing, and feminization (reviewed in [1,2]). In all of these cases, *Wolbachia* induce a female-biased sex ratio. Given their maternal inheritance, a distortion of the sex ratio towards female is advantageous for *Wolbachia*. In one instance, *Wolbachia* has become essential for normal oogenesis; in the parasitic wasp *Asorbara tabida* Nees, females cured of their *Wolbachia* infection are unable to produce eggs [18]. It is not known what role *Wolbachia* are playing in the process of oogenesis in *A. tabida*. A recent report showed that *Wolbachia* can suppress defects in oogenesis caused by specific alleles of *Sex-lethal* in *Drosophila melanogaster* [28]. *Sex-lethal* is the key gene controlling somatic sex determination and is essential for oogenesis in *D. melanogaster* (reviewed in [29]). This suggests that *Wolbachia* may be interacting with the pathways involved in oocyte development, and it provides a means to examine this obligatory relationship at a molecular level.

Wolbachia are also present in many somatic tissues [30–33] but little is known about the phenotypic consequences of this localization. The clearest example of *Wolbachia*'s somatic localization influencing host fitness is the virulent strain *popcorn*, which over-replicates in the nervous tissues and causes premature death in its host *Drosophila* [32,34].

Wolbachia also manipulate male hosts to their reproductive advantage. The most common effect of *Wolbachia* infection in the male germline is a form of conditional male sterility called cytoplasmic incompatibility (CI) (reviewed in [2,35,36]). *Wolbachia*-induced CI arises when an infected male mates with an uninfected female (Fig. 2). This cross results in loss of genetic material from the male pronucleus. Depending on the insect host, CI produces either high rates of embryonic mortality or the production of excess males in some haplodiploid insects [20,21,37–42]. However, if infected males mate with females infected with the same strain of *Wolbachia*, normal frequencies of male and female progeny are produced [20,21,37–39,41–43]. Furthermore, infected females are fully fertile when mated with uninfected males. Therefore, an infected female has a tremendous selec-

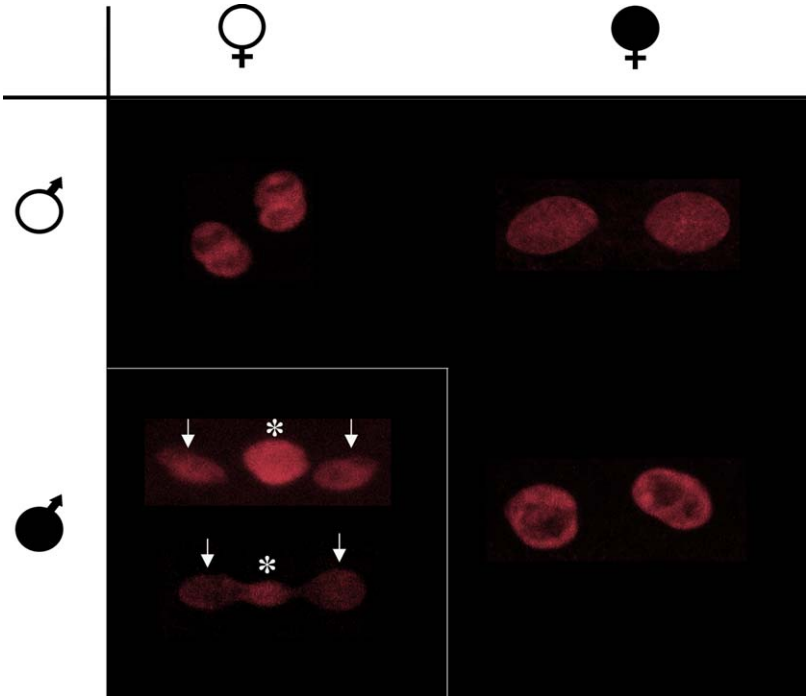

Fig. 2. *Wolbachia*-induced CI results in loss of the paternal chromosomes during the first mitotic division. CI results when a male infected with *Wolbachia* (filled symbol) mates with an uninfected female (open symbol). During the first mitotic division, the improperly condensed paternal chromosomes either (top) fail to segregate (asterisk), producing two haploid nuclei (arrows), or (bottom) segregate abnormally, producing a chromatin bridge (asterisk) and aneuploid nuclei (arrow). In haplodiploids, the former produces haploid male progeny while the latter results in embryonic lethality. In diplodiploid species, both result in lethality.

tive advantage over uninfected females in a *Wolbachia*-infected population because she can successfully breed with both uninfected and infected males. Despite this, *Wolbachia* are not found in all individuals of a population. This may be due to imperfect maternal transmission or to subtle but significant effects of *Wolbachia* on host fitness [44].

CI results in loss of paternal chromosomes during embryogenesis [37,45–48]. In diplodiploid insects such as mosquitoes and flies, this results in embryonic death [21,38,46,49]. In haplodiploid insects such as wasps, in which haploid eggs normally develop into males, CI embryos either die or develop into fertile males. Death is the more common CI phenotype among haplodiploid insects. Initially examined in the jewel wasp *Nasonia vitripennis*, it was concluded that CI produces haploid embryos that develop into males [37,39,45]. However, examination of additional haplodiploid insects revealed that *N. vitripennis* is unique. In two closely related species, *N. longicornis* and *N. giraulti*, CI results in embryonic death [42]. Furthermore, CI in the parasitic wasp *L. heterotoma* also results in embryonic death [20,41]. It has been proposed that CI-induced male development results from complete loss of the paternal chromosomes while CI-induced lethality results from partial loss [20,40–42].

These observations raise a number of questions concerning the interaction of *Wolbachia* with their hosts. What is the mechanism by which *Wolbachia* produce CI, and why is CI suppressed when the female as well as the male is infected? What are the cellular mechanisms by which *Wolbachia* are maternally and somatically transmitted? In this review, we will focus on insights that cytological analyses in *Drosophila* and *Nasonia* have provided towards understanding the molecular basis of *Wolbachia*-induced CI and *Wolbachia* transmission.

2. An abundance of *Wolbachia* in the testes is required to induce CI

Since *Wolbachia* are found in the testes but are absent from mature sperm, it has been hypothesized that *Wolbachia* induce CI by modifying sperm [50,51]. Therefore, to understand how *Wolbachia* induce CI, it is essential to determine when and how *Wolbachia* interact with developing sperm. This issue has been addressed in the most detail in *Drosophila*, where spermatogenesis is well understood [50–55]. In *Drosophila*, sperm develop in bundles of 64 (sperm cyst) (Fig. 3) (reviewed in [56]). Each sperm bundle originates from a single founder gonial cell (gonialblast). The gonialblast undergoes four rounds of mitosis to produce a 16-cell cyst and then meiosis to produce a cyst of 64 spermatids, which are interconnected by cytoplasmic bridges. Following meiosis, the spermatids undergo extensive morphological changes, including chromosome and nuclear condensation and extension of a tail to form motile sperm. At the end of this maturation period, excess cytoplasm is discarded in a waste bag, and 64 individual sperm are liberated from the cyst. Throughout spermatogenesis, each sperm bundle is sur-

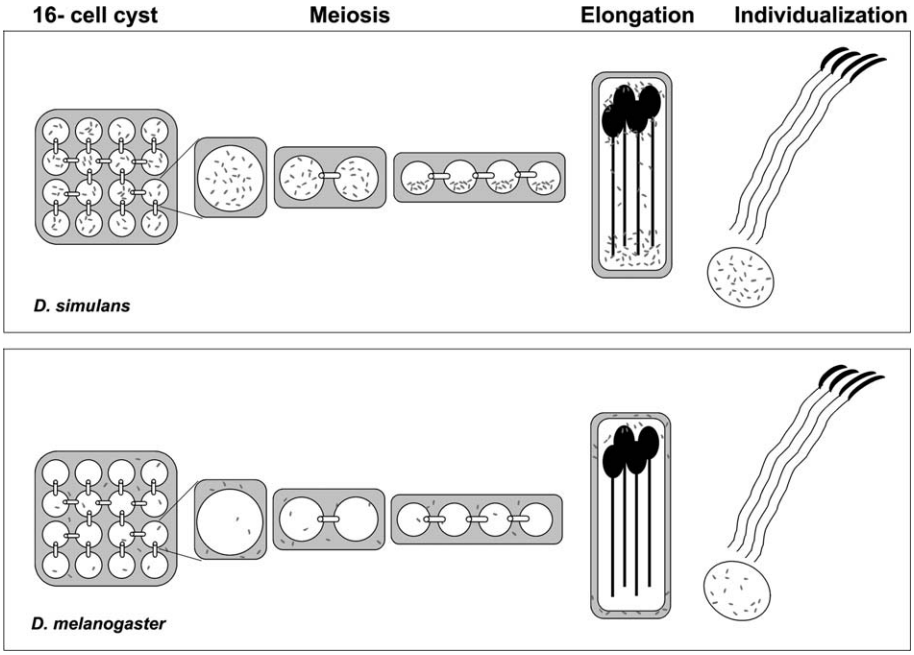

Fig. 3. *Wolbachia* replication and distribution during spermatogenesis are markedly different in *D. simulans*, a strong CI inducer, and *D. melanogaster*, a weak CI inducer [51]. This figure summarizes the comparative study described in [51]. Throughout spermatogenesis, sperm arising from the same founder cell remain interconnected by ring canals (white tubes). At the 16-cell stage, *Wolbachia* (small rods) are abundant and localize in the spermatocytes (white circles) rather than in the somatically derived cyst cell (gray box) in *D. simulans*. In contrast, *Wolbachia* are scarce and are mostly found in the cyst cells in *D. melanogaster*. Prior to entering meiosis, the spermatocytes grow. For clarity, only one of the 16 spermatocytes is depicted. During this period, *Wolbachia* replication is observed in *D. simulans* but not in *D. melanogaster*. During meiosis, *Wolbachia* do not replicate in either host. During these two periods, *Wolbachia* are distributed throughout the cytoplasm in both hosts. At the end of meiosis, *Wolbachia* aggregate at the distal side of the spermatocytes, away from the spermatocyte nuclei, in *D. simulans* but not in *D. melanogaster*. During elongation, *Wolbachia* undergo another period of replication and show three regions of localization: at the distal end of the cyst, and at the basal and apical ends of the nuclei (black ovals) in *D. simulans*. In contrast, *Wolbachia* replication is low, and strong localization at the distal end of cyst and around the nuclei is absent in *D. melanogaster* at this stage. During the individualization stage, all *Wolbachia* are shed from the mature sperm along with excess cytoplasm into the waste bag in both hosts.

rounded by two somatically derived cyst cells, which are essential for male fertility but whose function remains undefined.

Insights into *Wolbachia*-induced CI have been obtained by comparing *Wolbachia* behavior during spermatogenesis in *D. simulans*, a strong CI inducer, and in *D. melanogaster*, a weak CI inducer (summarized in Fig. 3). *Wolbachia* density in the testes and the number of infected sperm cysts are correlated with the strength of CI [50–52,55]. *Wolbachia* are more abundant, and more sperm cysts are infected in *D. simulans* than in *D. melanogaster* [50–52,55]. Furthermore, as males age, the level of *Wolbachia* infection and the rate at which males induce CI decrease [50–52]. These observations likely explain the dramatic age effect observed in *D. melanogaster* in which strong potential CI effects are detected only in 1-d-old males [57].

Wolbachia density and strength of CI are correlated with timing of *Wolbachia* replication [51,55]. In *D. simulans*, *Wolbachia* have two periods of significant replication: during the mitotic divisions when the spermatocytes are growing and during sperm elongation and maturation [51]. In comparison, *Wolbachia* replication in *D. melanogaster* testes is not significant during the mitotic divisions, and the growth period subsequent to meiosis is delayed relative to *D. simulans* [51].

Wolbachia's specific cellular localization within the sperm cyst also correlates with CI induction (Fig. 3) [51,53,54]. During the mitotic growth period, *Wolbachia* are found throughout the cytoplasm in both *D. simulans* and *D. melanogaster*, with the significant difference that *Wolbachia* are in the sperm bundle in *D. simulans* and are mostly in the somatic cyst cells in *D. melanogaster* [51,53,54]. At the end of meiosis, *Wolbachia* aggregate at the distal end of the spermatocyte, away from the nucleus in *D. simulans* [51]. During elongation, *Wolbachia* localize at the distal end of the sperm cyst, near the growing sperm tail, and adjacent to the sperm nuclei in *D. simulans* [51,55]. In *D. melanogaster*, *Wolbachia* do not show a strong localization at the distal end of the cyst or near the growing tail [51,55]. The significance of this difference in subcellular localization is not known.

Wolbachia replication and localization within the sperm cyst are influenced by host nuclear background [53–55]. For example, the *Wolbachia* strain from *D. simulans* Queensland (*wAu*), which normally has a low infection level, has a significantly higher level of infection when transferred into the *D. simulans* Riverside nuclear background [55]. When transferred into *D. simulans*, *D. melanogaster Wolbachia* (*wmel* or *popcorn*) show strong localization with the sperm and are able to induce CI [53,54]. These studies indicate that the host may play a role in determining whether CI is expressed by

controlling *Wolbachia* replication and localization [53–55]. *Wolbachia*, however, must also possess the genetic machinery to induce CI [54,55]. For example, the *Wolbachia* strain *wCof* localizes within the sperm bundle but does not induce CI in *D. simulans* [54].

While these studies have described conditions in the testes that are important for *Wolbachia* to induce CI, the fundamental question remains: how do *Wolbachia* induce CI? Another approach to identify the molecular mechanism of CI is to characterize how sperm from *Wolbachia*-infected males malfunction in early embryos from CI crosses.

3. Cytological analyses of *Wolbachia*-induced CI suggest host cell cycle regulators are targeted

CI results from inappropriate interactions between the sperm and egg [37,45–48,58–61]. In CI embryos, the paternal chromosomes are improperly condensed and are not accurately segregated during the first mitosis. In contrast, the maternal chromosomes condense and segregate normally. This results in embryos with a complete maternal chromosome complement but with a reduced or absent paternal chromosome complement [45–47]. CI embryos from mosquitoes (*C. pipiens* and *Aedes polinesiensis*), flies (*Drosophila*), and wasps (*N. vitripennis*) all exhibit these same defects, suggesting that *Wolbachia* may be disrupting conserved host targets.

Detailed cytological analyses of the first mitotic division in *D. simulans* and *N. vitripennis* CI embryos strongly support the hypothesis that *Wolbachia* are manipulating host proteins involved in cell cycle regulation [45–48]. Events leading up to the apposition of male and female pronuclei occur normally in both *Drosophila* and *Nasonia* CI embryos [46–48]. As in control embryos, sperm entry and its conversion to a functional male pronucleus occur as the egg completes meiosis. As these events are similar in both *Drosophila* and *Nasonia*, the description that follows applies to both unless otherwise specified. After entry, the condensed, needle-shaped (*Drosophila*) or rod-shaped (*Nasonia*) sperm nucleus is transformed into a spherical pronucleus. Normally, this transformation is accompanied by the formation of a new pronuclear envelope composed of maternally supplied products and the replacement of sperm specific histones with maternally supplied histones [62]. Although the sperm undergoes a shape change in CI embryos, it has not been tested directly whether formation of the pronuclear envelope and repackaging of the chromosomes occur normally. These two events are important, as they are required for DNA replication ([63]; reviewed in [64]). Simultaneously, the egg completes meiosis to produce three haploid polar bodies and a haploid female pronucleus.

In preparation for the first mitosis, the male and female pronuclei migrate toward each other. This results in juxtaposition, but not fusion, of the pronuclei [47,65–68]. As a result, the maternal and paternal chromosomes do not mingle until the end of telophase. In *D. melanogaster*, it has been demonstrated that remnants of the pronuclear envelopes maintain separation between the maternal and paternal chromosome sets during metaphase [47]. As a result, the metaphase spindle that forms during the first nuclear division is composed of two physically distinct bundles of microtubules, each controlling the alignment and segregation of its own set of maternal or paternal chromosomes. This unique spindle is called a gonomeric spindle and is common in insects, including *Nasonia* ([47,65–68]; reviewed in [69]). It is therefore essential that the male and female pronuclei enter and exit mitosis synchronously to successfully produce daughter nuclei that contain both maternal and paternal chromosomes.

Indeed, coordination between the maternal and paternal pronuclei is disrupted in *D. simulans* and *N. vitripennis* CI embryos [47,48]. Entry into and exit from mitosis are regulated by the activation and inactivation, respectively, of cyclin-dependent kinase 1 (CDK1) (reviewed in [70,71]). Using an antibody against phospho-histone H3 (PH3), which specifically labels nuclei containing active CDK1 [72–74], it was demonstrated that the male and female pronuclei enter and exit mitosis asynchronously in *N. vitripennis* CI embryos [48]. In control embryos, CDK1 is active from prophase through anaphase. CDK1 is activated in both pronuclei at prophase and is completely inactive by telophase. In contrast, CDK1 activation at prophase occurs in only one of the two pronuclei in CI embryos. By prometaphase, both pronuclei contain active CDK1. CDK1 inactivation begins at anaphase and is completed in only one of the two pronuclei by telophase. Since it has been shown genetically that it is the male chromosomes that are discarded during CI [37], it is likely that it is the male pronucleus that enters and exits mitosis late relative to the female pronucleus.

In *N. vitripennis*, loss of coordination between the male and female pronuclei is also evident with regard to nuclear envelope breakdown (NEB) [48], an event signaling entry into mitosis [75]. NEB of the male and female pronuclei occurs synchronously in control embryos but asynchronously in CI embryos [48]. There is a gap of 31–120 s between NEB of the male pronucleus and that of the female pronucleus in CI embryos.

Chromosome condensation is asynchronous in both *Drosophila* and *Nasonia* CI embryos [47,48]. Whereas condensed chromosomes are visible in both the male and female pronuclei at the end of prophase in control embryos, only one set of chromosomes is condensed in CI embryos while the other remains diffuse. Even at metaphase, only one set of chromosomes is clearly condensed. At anaphase/telophase, only one set of chromosomes segregates while the other lags at the metaphase plate. It is likely that it is the male chromosomes that are delayed in initiating condensation and are not segregating because it is the male chromosomes that are lost in CI embryos. *N. vitripennis* CI embryos express maternal but not paternal markers [37]. In *D. simulans* CI embryos, the Y chromosome is never seen among the developing haploid embryos [47].

These cytological observations provide important clues regarding the molecular mechanisms of *Wolbachia*-induced CI. The earliest defect described in CI embryos is delayed entry into mitosis [48]. This observation suggests that *Wolbachia* are targeting proteins that directly or indirectly regulate the cell cycle. There are two distinct mechanisms by which *Wolbachia* can delay entry into mitosis: direct inhibition of the enzymatic machinery that drives cells into mitosis or indirectly through activation of cell cycle checkpoints. In response to improperly executed events, checkpoints delay progression of the cell cycle (reviewed in [76]). With respect to the first alternative, many bacteria produce toxins that disrupt the eukaryotic cell cycle machinery (reviewed in [77]). For example, *Actinobacillus actinomycetemcomitans* produces gapstatin, a toxin that causes cells to arrest in G2 [78]. It has been hypothesized that gapstatin induces this arrest by downregulating cyclin B1 expression [79]. Association of cyclin B1 with CDK1 is an important regulatory step in driving a cell into mitosis (reviewed in [70,71]). Similarly, *Wolbachia* may produce a toxin that inhibits cyclin B1 specifically in the male pronucleus and causes its delayed entry into mitosis. This is a reasonable model given that cyclin B1 and CDK1 activity are locally regulated in the syncytial *D. melanogaster* embryo [73,80]. Even within a single spindle, CDK1 activity is detected in a gradient [73].

Bacteria can also delay the host cell cycle by producing toxins that activate cell cycle checkpoints. For example, many Gram-negative bacteria produce cytolethal distending toxin (CDT), which causes their host to arrest in G2 or early mitosis (reviewed in [81,82]). As CDT has homology to DNase1, one hypothesis is that CDT causes DNA damage, which induces the G2/M checkpoint, resulting in arrest of the cell cycle at G2 [82–84]. By analogy, *Wolbachia* may produce a toxin that causes DNA damage or slows down DNA replication in the male pronucleus, which results in the activation of a G2 checkpoint and delayed entry into mitosis.

Studies examining the relative timing of NEB of the male and female pronuclei in sea urchin embryos provide support for this model [85]. By treating either eggs or sperm with a DNA cross-linking agent, the S-phase checkpoint could be separately activated in either the male or female pronucleus. When only the paternal DNA is cross-linked, breakdown of the male pronucleus is substantially delayed relative to the female pronucleus. Conversely, when the maternal DNA is cross-linked, breakdown of the female pronucleus is delayed relative to the male pronucleus. This experimental system provides an excellent illustration of how in principle *Wolbachia* may act at the cellular level to induce CI.

4. *Wolbachia* do not disrupt paternal centrosome function

In addition to the paternal genome, sperm contribute the basal body. Immediately following fertilization, the basal body is transformed into the zygotic centrosome, a factor essential for the development of diploid embryos (reviewed in [86]). While it is clear that *Wolbachia* influence paternal chromosome function, *Wolbachia* do not appear to disable centrosome function during the first mitosis of CI embryos in *D. simulans* and *N. vitripennis* [46–48,61]. Sperm from *Wolbachia*-infected males are able to nucleate microtubule asters and to organize a functional mitotic spindle capable of segregating chromosomes [46–48]. In *N. vitripennis*, *Wolbachia*–induced CI produces males that have paternal rather than maternal centrosomes. [48,68]. Normally, males inherit maternally derived centrosomes and females inherit paternally derived centrosomes [68]. That CI produces this unusual combination of a male embryo with paternal centrosomes further indicates that *Wolbachia* do not interfere with centrosome function in *N. vitripennis*.

Centrosome behavior deteriorates, however, in *D. simulans* CI embryos after cycle 1 [46,49]. In many embryos, the centrosomes detach from nuclei and from mitotic spindles. In addition, these embryos exhibit other mitotic defects, including aberrantly shaped mitotic spindles, highly condensed chromosomes that appear arrested in metaphase, and DNA bodies of various sizes [46,49]. Interestingly, in contrast to control embryos, the sperm tail, which also originates from the sperm basal body, becomes detached in many CI embryos [46]. Normally the sperm tail wholly enters the egg and remains attached to a nucleus throughout development [87]. Furthermore, CI embryos with detached sperm tails exhibit more severe mitotic defects. Thus, while the sperm-contributed centrosome appears to behave normally during the first mitotic division, it degenerates thereafter. These effects, however, may be secondary consequences of defects in chromosome segregation [88].

5. *Wolbachia* exploit a unique aspect of insect development to induce CI

Understanding the molecular mechanism of *Wolbachia*-induced CI requires an appreciation of the unique gonomeric spindle found in many insect embryos at nuclear cycle 1 (reviewed in [69]). A striking feature of this spindle is that it is composed of two physically distinct bundles of microtubules: one bundle holds the paternal chromosomes, while the other holds the maternal chromosomes. It was discovered in *D. simulans* CI embryos that one half of the gonomeric spindle, and its chromosome complement, can enter anaphase independently of the other [47]. The properly condensed chromosome set entered anaphase, while the under-condensed set was delayed in entering anaphase. One interpretation of this observation is that the spindle checkpoint, which monitors the metaphase to anaphase transition (reviewed in [89]), operates independently in the two halves of the gonomeric spindle. This checkpoint monitors the state of the kinetochore, a proteinaceous complex on the chromosome that interacts with spindle microtubules. Specifically, it detects microtubule occupancy of the kinetochore and/or

tension on the kinetochore due to bipolar microtubule attachment. Once sister chromosomes are bipolarly attached and under tension, the spindle checkpoint is inactivated and anaphase is initiated. This checkpoint is extremely sensitive; a single unattached kinetochore is sufficient to prevent the metaphase to anaphase transition of the entire chromosome complement. The mechanism by which a single wayward kinetochore causes the arrest of the entire spindle remains unclear, but appears to involve a diffusible signal (reviewed in [89]).

Given these constraints on the metaphase to anaphase transition, it is intriguing that the maternal chromosomes can enter anaphase, while the paternal chromosomes remain on the metaphase plate in CI embryos [47,48]. One interpretation of this observation is that the wait anaphase signal acts locally in its half spindle and prevents anaphase in only the paternal half of the spindle (Fig. 4). Although it is not known if the spindle checkpoint can work differently on chromosomes that are sharing a gonomeric spindle, it has been shown in vertebrate cells that have been fused to contain two independent spindles that a mono-oriented chromosome in one spindle can delay anaphase in its own spindle and not delay anaphase in the neighboring spindle [90]. This result demonstrates that proximity and a common cytoplasm are not sufficient for transmission of the wait anaphase signal.

These observations provide insight into the puzzling observation that CI produces very different developmental outcomes in haplodiploid hosts. Depending on the host, CI results in either embryonic mortality or male development. CI in *N. vitripennis* results in the production of all male progeny, while in two closely related species, *N. giraulti* and *N. longicornis*, CI results in embryonic lethality [42]. One possible mechanism for these different phenotypes is whether the CI-induced paternal chromosome defects are severe enough to maintain activation of the spindle checkpoint of the paternal half of the spindle (Fig. 4). For example, in *N. vitripennis*, CI-induced defects may prevent microtubules from attaching to the kinetochores of the paternal chromosomes. Consequently, the spindle checkpoint remains activated in the paternal, but not the maternal, half of the gonomeric spindle. This results in all male progeny inheriting only maternal chromosomes. In contrast, the CI-induced defects on the paternal chromosome complements in *N. giraulti* and *N. longicornis* may be less severe and fail to maintain activation of the spindle checkpoint. Consequently, abnormally processed paternal chromosomes as well as maternal chromosomes segregate at anaphase. This produces aneuploid nuclei and eventually embryonic death. In diplodiploid insects, the severity of the chromosome defects does not influence the CI phenotype, as both haploid and aneuploid embryos die. *Drosophila*, *Culex*, and *Aedes* CI embryos develop to varying degrees but eventually die due to haploidy, aneuploidy, or mitotic defects [46,47,49,58,59]. Embryos that die early in development are aneuploid, while those that develop further are haploid [47].

The phenotypic consequence of CI may also depend on *Wolbachia* strain. In the parasitoid *L. heterotoma*, CI results in embryonic death if the male is infected with three strains of *Wolbachia* but allows embryonic development if the male is infected with only one strain of *Wolbachia* [41]. One interpretation of this observation is that the titer of the *Wolbachia* strain responsible for inducing CI is lower in triply infected males than in singly infected males [41]. As a result, sperm from triply infected males would have fewer modifications, which would fail to maintain the spindle checkpoint and would produce aneuploid embryos that die. In singly infected males, modifications of the paternal chromosomes would be more severe because the titer of the CI-inducing *Wolbachia* strain is higher [41]. As a result, the spindle checkpoint remains on in the paternal half of the spindle, only maternal chromosomes segregate, and haploid embryos develop successfully.

6. *Wolbachia* in the egg can suppress CI

CI is not expressed when infected males mate with females infected with the same strain of *Wolbachia* [20–22,37–39,41–43]. *Wolbachia* present in the egg rescue or suppress the defects responsible for inducing CI. Consistent with the model that *Wolbachia* induce CI by altering progression of the cell cycle, coordination between the male and female pronuclei is restored in embryos derived from a mating between two infected parents in *N. vitripennis* [48]. CDK1 activation and inactivation, NEB, and chromosome condensation all occur synchronously in these embryos. Rescue of CI defects could occur either through correcting the timing defects in the male pronucleus or through slowing the timing of the female pronucleus so that it is synchronous with the slowed male pronucleus. Although both mechanisms are possible, we favor the latter because it requires only that *Wolbachia* induce similar cell cycle timing defects in male and female pronuclei.

The presence of *Wolbachia* in the egg at the time of fertilization is not required to rescue CI defects [43]. *Nasonia* females treated with tetracycline produce mature eggs that are *Wolbachia*-free by day 5 post-treatment. For an additional 3–4 d, treated females continue to produce mature eggs that are free of *Wolbachia* but that are compatible with sperm from infected males. This suggests that the *Wolbachia* factor or action required for rescue has been preloaded or has occurred in the oocyte prior to *Wolbachia* elimination from the developing oocyte. The observation that the *Wolbachia*-free eggs remain compatible with sperm from infected males for several days also suggests that *Wolbachia* modification of the oocyte occurs during early oogenesis because it takes several days to produce a mature egg. The critical time and location for *Wolbachia* action in the developing oocyte is still unknown. Thus, it will be important to examine in detail *Wolbachia*'s behavior during oogenesis to understand the mechanism of rescue.

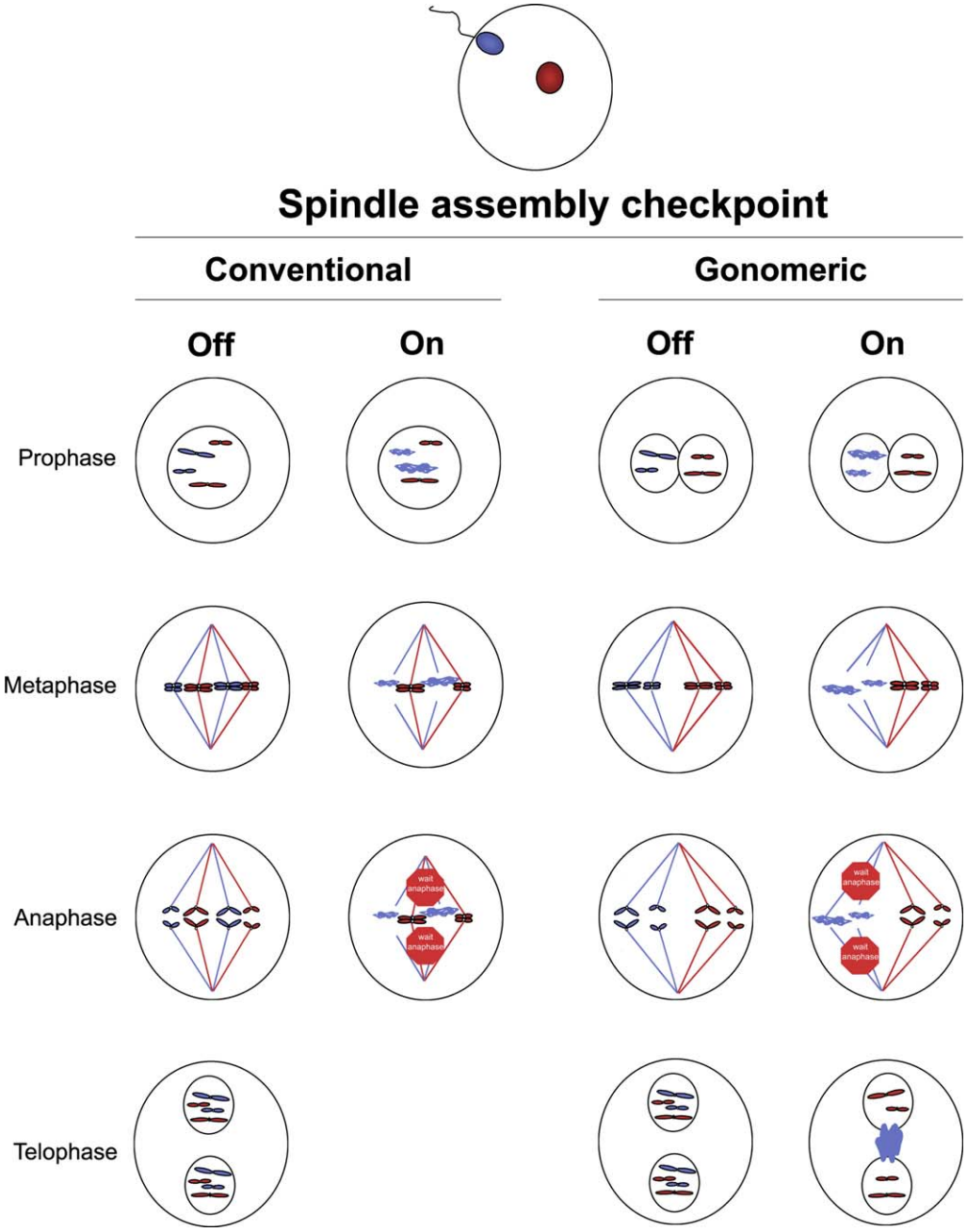

Fig. 4. *Wolbachia*-induced CI phenotype is dependent on the unique structure of the gonomeric spindle, in which the spindle assembly checkpoint may act independently. Unlike a conventional spindle (first column), the gonomeric spindle is composed of two independent bundles of microtubules, each regulating its own set of paternal (blue) or maternal (red) chromosomes (third column). This unique structure forms because the nuclear envelopes that contain the maternal and paternal chromosomes do not completely break down when the two pronuclei meet, preventing the two sets of chromosomes from mixing prior to entry into mitosis [67]. Thus, when the spindle forms, the maternal and paternal chromosome sets reside in separate regions; one bundle of microtubules (blue lines) controls the paternal chromosomes, while a separate bundle (red lines) controls the maternal chromosomes. The maternal and paternal chromosomes mix for the first time at telophase, unlike in the conventional spindle, where the chromosomes mix at prophase. In CI embryos, the paternal chromosomes are not properly condensed at prophase (fourth column). With a conventional spindle, kinetochores on the paternal chromosomes would send a "wait anaphase" signal (red octagon), which would result in the spindle assembly checkpoint remaining on and preventing anaphase, until all the chromosomes are attached (second column). With the gonomeric spindle, in contrast, the wait anaphase signal from the paternal chromosomes causes the spindle assembly checkpoint to remain on and prevents anaphase only in the paternal half of the spindle.

7. The role of the cytoskeleton in the maternal transmission of *Wolbachia*

The presence of *Wolbachia* in the oocyte not only allows for the suppression of CI, but also is the basis for efficient vertical transmission. How *Wolbachia* become preferentially concentrated in the oocyte is unknown. However, it is likely that they rely on the same processes by which other cytoplasmic components are deposited into the oocyte. The process of oogenesis has been worked out primarily in *D. melano-*

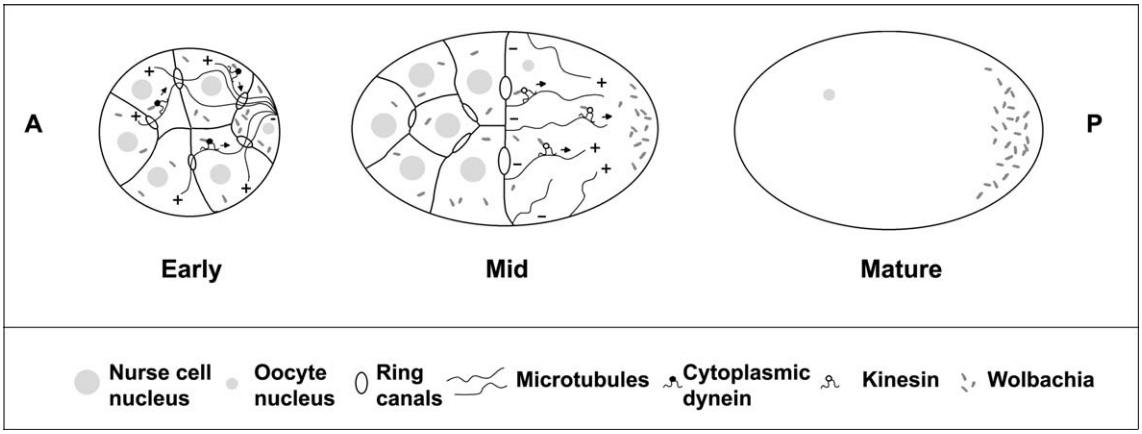

Fig. 5. Movement of *Wolbachia* into the oocyte and their localization to the posterior pole may depend on the microtubule network. An egg chamber is composed of one oocyte and 15 nurse cells (fewer are shown here) that are interconnected by ring canals. During early oogenesis, *Wolbachia* may use minus-end motor proteins to move along microtubules into the oocyte. At this time, microtubules are nucleated from a single MTOC located at the posterior pole of the oocyte. Minus ends (–) of microtubules originate from the MTOC, and the plus ends (+) project through the ring canals into the nurse cells. During mid oogenesis, microtubules rearrange so that they are nucleated from the anteriolateral cortex of the oocyte. Following this microtubule rearrangement, *Wolbachia* may use plus-end motor proteins to localize to the posterior pole. *Wolbachia* remain localized at the posterior pole through late oogenesis and in the mature egg.

gaster (for a full review see [91,92]). In *D. melanogaster, N. vitripennis*, and many other insects, oogenesis is meroistic, in which the developing eggs belong to a syncytium of sister germ cells. In this section, we highlight several cytoskeletal components and developmental events of oogenesis that may be important for the successful maternal inheritance of *Wolbachia*.

In early oogenesis, a single cystoblast, the precursor to the mature oocyte, undergoes four rounds of mitosis with incomplete cytokinesis. This produces a cluster of 16 cells connected through a series of cytoplasmic bridges known as ring canals. One of the two cells that possess four ring canals becomes the oocyte, while the remaining 15 become supporting nurse cells. Concomitant with oocyte differentiation, a network of microtubules becomes organized from a single microtubule-organizing center (MTOC) located at the posterior end of the future oocyte [93]. These microtubules extend outward through the ring canals and into the nurse cells. During early oogenesis, this microtubule network guides specific mRNA and maternal factors into the oocyte from the nurse cells.

During the early stages of oogenesis, *Wolbachia* may use the microtubule network to enter the oocyte (Fig. 5). *Wolbachia* have been observed throughout the nurse cell/oocyte complex in many insect orders [6,30,31,94–97]. As oogenesis proceeds, *Wolbachia* must localize in the oocyte to ensure successful inheritance. In *Aphytis lignanensis* and *A. yanonensis* (Hymenoptera), *Wolbachia* are concentrated in the nurse cells and also localize at the anterior end of the oocyte, near the nurse cell/oocyte border during early stages of oogenesis [97]. Based on this observation, it was proposed that *Wolbachia* divide in the nurse cells and passively diffuse from the nurse cells into the oocyte [97]. Alternatively, *Wolbachia* may rely on motor proteins and microtubules for active transport into the oocyte. This is consistent with *Wolbachia*'s microtubule-dependent association with the MTOC in the early syncytial embryo [22,98,99]. Whether passive or active, movement of *Wolbachia* into the oocyte must occur through the ring canals, which connect the oocyte and nurse cells. During early oogenesis, ring canals are 0.5 μm in diameter; by late oogenesis, they expand to 5.5 μm [100]. Since the diameter of *Wolbachia* is 0.25–0.5 μm [101], it is possible that they pass through the ring canals even during the earliest stages of oogenesis. However, it is likely that movement occurs later in oogenesis, when the ring canal diameter is greater.

Wolbachia tightly localize to the posterior pole in mature Hymenopteran oocytes [39,45,97]. In *D. melanogaster* and *D. simulans*, *Wolbachia* are evenly distributed beneath the cortex of the oocyte, with only a two-fold increase at the posterior pole [22,102]. The components and cellular processes mediating the posterior localization of *Wolbachia* are not known. However, this localization, like the movement of *Wolbachia* into the oocyte, also may be dependent on interactions with motor proteins and microtubules.

Proteins and mRNAs that specify the development of germ cells localize to the posterior pole. In *D. melanogaster*, localization of these determinants occurs after a major rearrangement of the microtubule network in the oocyte during mid-oogenesis. Instead of being organized from a single MTOC at the posterior pole, microtubules become nucleated along the anteriolateral cortex, with their plus ends directed toward the posterior pole [92,103]. Following this microtubule reorganization, oskar mRNA, a primary morphogenic determinant of germ plasm and abdominal development, is localized at the posterior pole of the oocyte [104]. To reach the posterior pole, oskar mRNA is transported along microtubules by the plus-end motor protein, kinesin [105]. *Wolbachia* also may rely on plus-end-directed motor proteins to concentrate at the posterior pole.

Recent work suggests that during mid oogenesis, kinesin transports dynein toward microtubule plus-ends, at the pos-

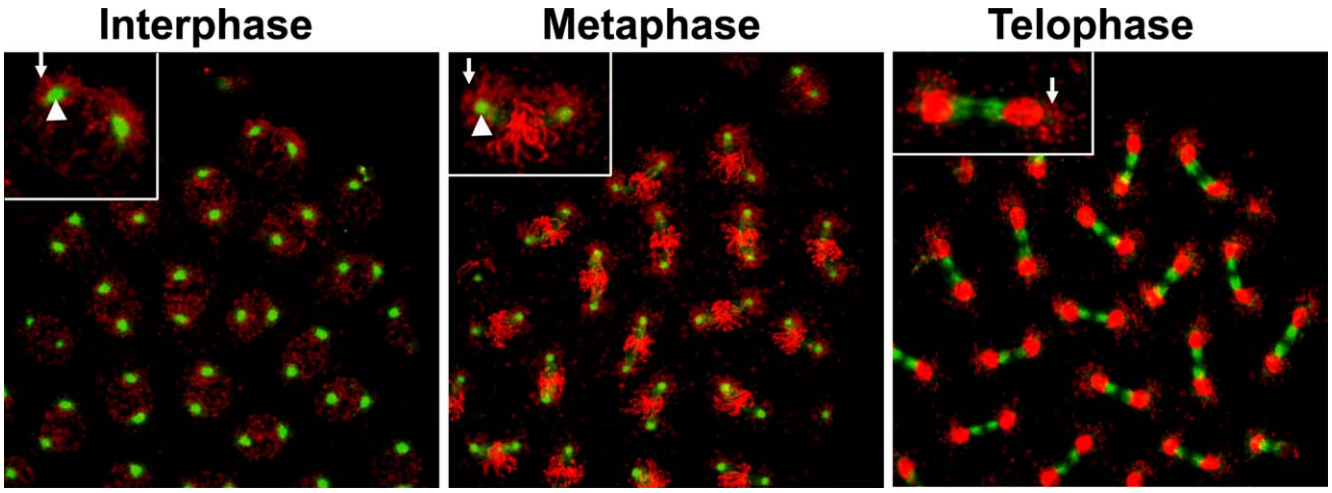

Fig. 6. *Wolbachia* localize to the centrosome during embryogenesis in *N. vitripennis*. Embryos from a female infected with W*olbachia*, fixed and stained for DNA (red) and tubulin (green). Inset, *Wolbachia* (arrow) can be seen clustered near the centrosomes (arrowhead) throughout the cell cycle.

terior pole of the oocyte, so that dynein may then carry cargo toward microtubule minus-ends, at the anterior pole [106]. These findings raise the possibility that *Wolbachia* may depend on both motors for its maternal inheritance. First, *Wolbachia* may associate with dynein to move into the oocyte along microtubules. Once in the oocyte, *Wolbachia* may be co-transported with dynein by kinesin to the posterior pole. After reaching the posterior pole, *Wolbachia* may either associate with components of germ plasm or become anchored to the oocyte cortex. Localization at the posterior pole increases the probability that *Wolbachia* will become stably incorporated into the germline of the next generation [59].

During late oogenesis, cytoplasm from the nurse cells is pumped through the ring canals into the oocyte. This process, termed cytoplasmic dumping, accounts for the bulk of the increase in oocyte volume. Cytoplasmic dumping requires contraction of actin filaments beneath the nurse cell cortex (reviewed in [107]). It is likely that *Wolbachia* remaining in the nurse cells at this time will be "dumped" into the oocyte along with host organelles and other cytoplasmic constituents. Together, microtubule-mediated transport and cytoplasmic dumping allow for efficient accumulation of *Wolbachia* in the oocyte.

8. Centrosomes, microtubules, and the movement of *Wolbachia* in the embryo

Wolbachia localize at the centrosome during embryogenesis in *D. melanogaster* [98], *D. simulans* [22,99], *Muscidifurax uniraptor* [108], and *N. vitripennis* (Fig. 6). Centrosomes are the mitotic organizing center in the embryo. During interphase, long, stable microtubule arrays emanate from a pair of nuclear-associated sister centrosomes. During prophase, sister centrosomes separate and migrate along the nuclear envelope to form the poles of the mitotic spindle. The geometry of the spindle dictates that each daughter nucleus inherits a complete set of chromosomes and a single centrosome. Therefore, by localizing at the centrosomes, *Wolbachia* ensure their efficient transmission to daughter nuclei [98,99,109]. In *D. simulans*, it has been shown that *Wolbachia's* centrosome localization requires microtubules, and this association may be mediated by minus-end-directed molecular motors such as dynein [98]. *Wolbachia* may also rely on microtubules and molecular motors to move and distribute themselves within the embryo [110]. In *Drosophila*, *Wolbachia* are randomly distributed throughout the embryo. To increase the probability of being associated with a nucleus destined to become a germ cell, *Wolbachia* might migrate on astral microtubules, between neighboring nuclei toward the posterior end of the embryo, where germ cells are formed [110].

Wolbachia may also associate with centrosomes to meet their demand for host membrane. *Wolbachia* are encompassed by a host-derived plasma membrane [96,98,111]. The source of this membrane is unknown. One possibility is the recycling endosome, which is also closely associated with the centrosome. The recycling endosome is a key trafficking component responsible for delivering vesicles to the plasma membrane, Golgi, and other endosomal compartments [112]. *Wolbachia* may intercept delivery and induce fusion of these vesicles with their outer host membrane.

9. Conclusions

Cytological analysis has played a pivotal role in identifying molecular interactions between many pathogens and their hosts. For example, the discovery and our current understanding of actin-based movement of the intracellular pathogen *Listeria monocytogenes* relied heavily on microscopy (reviewed in [113]). It is likely that detailed analysis of the cell biology of *Wolbachia*-induced CI, maternal transmission, and centrosome-based somatic transmission will provide insights into the molecular basis of these phenomena. Studies devoted to *Wolbachia*/host cell biology are key to this

emerging field because they highlight outstanding issues and guide future work. Cytological analyses have already demonstrated that *Wolbachia* induce CI by altering the host cell cycle timing. In addition, they suggest that microtubules and motor proteins are likely to play an important role in many aspects of *Wolbachia* transmission and host interactions.

Acknowledgements

We thank Harriet Harris, Anne Royou, Kurt Fredrick, Elise D. Ferree, and Carol Koyama for helpful discussions and critical reading of the manuscript. This work was sponsored by grants from the NSF and NIH.

References

[1] S.L. O'Neill, A.A. Hoffmann, J.H. Werren (Eds.), Influential Passengers: Inherited Microorganisms and Arthropod Reproduction, Oxford University Press, Oxford, 1997.
[2] R. Stouthamer, J.A.J. Breeuwer, G.D.D. Hurst, *Wolbachia pipientis*: Microbial manipulator of arthropod reproduction, Annu. Rev. Microbiol 53 (1999) 71–102.
[3] C. Bandi, A.J. Trees, N.W. Brattig, *Wolbachia* in filarial nematodes: evolutionary aspects and implications for the pathogenesis and treatment of filarial diseases, Vet. Parasitol 98 (2001) 215–238.
[4] R. Cordaux, A. Michel-Salzat, D. Bouchon, *Wolbachia* infection in crustaceans: novel hosts and potential routes for horizontal transmission, J. Evol. Biol. 14 (2001) 237–243.
[5] J.A.J. Breeuwer, G. Jacobs, *Wolbachia*: intracellular manipulators of mite reproduction, Exp. Appl. Acarol. 20 (1996) 421–434.
[6] M. Hertig, S.B. Wolbach, Studies on rickettsia-like microorganisms in insects, J. Med. Res. 44 (1924) 329–374.
[7] J.H. Werren, D. Windsor, L. Guo, Distribution of *Wolbachia* among neotropical arthropods, Proc. R. Soc. Lond. B 262 (1995) 197–204.
[8] J.H. Werren, D.M. Windsor, *Wolbachia* infection frequencies in insects: evidence of a global equilibrium?, Proc. R. Soc. Lond. B 267 (2000) 1277–1285.
[9] A. Jeyaprakash, M.A. Hoy, Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species, Insect Mol. Biol. 9 (2000) 393–405.
[10] G. Weisburg, M.E. Dobson, J.E. Samuel, G.A. Dasch, L.P. Mallavia, O. Baca, L. Mandelco, J.E. Sechrest, E. Weiss, C.R. Woese, Phylogenetic diversity of the Rickettsiae, J. Bacteriol 171 (1989) 4202–4206.
[11] S.L. O'Neill, R. Giordano, A.M.E. Colbert, T.L. Karr, H.M. Robertson, 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects, Proc. Natl. Acad. Sci. USA 89 (1992) 2699–2702.
[12] J.A.J. Breeuwer, R. Stouthamer, S.M. Barns, D.A. Pelletier, W.G. Weisburg, J.H. Werren, Phylogeny of cytoplasmic incompatibility microorganisms in the parasitoid wasp genus *Nasonia* (Hymenoptera: Pteromalidae) based on 16S ribosomal DNA sequences, Insect Mol. Biol. 1 (1992) 25–36.
[13] T.T.M. Vandekerckhove, S. Watteyne, A. Willems, J.G. Swings, J. Mertens, M. Gillis, Phylogenetic analysis of 16S rRNA of the cytoplasmic bacterium *Wolbachia* from the novel host *Folsomia candida* (Hexapoda, Collembola) and its implications for wolbachial taxonomy, FEMS Microbiol. Lett. 180 (1999) 279–286.
[14] C.L. Anderson, T.L. Karr, Wolbachia: Evolutionary novelty in a rickettsial bacteria, BMC Evol. Biol. 1 (2001) 10.
[15] J.H. Werren, W. Zhang, L.R. Guo, Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods, Proc. R. Soc. Lond. B 261 (1995) 55–71.
[16] N. Lo, M. Casiraghi, E. Salati, C. Bazzocchi, C. Bandi, How many *Wolbachia* supergroups exist?, Mol. Biol. Evol. 19 (2002) 341–346.
[17] C. Bandi, T.J.C. Anderson, C. Genchi, M. Blaxter, Phylogeny of *Wolbachia* in filarial nematodes, Proc. R. Soc. Lond. B 265 (1998) 2407–2413.
[18] F. Dedeine, F. Vavre, F. Fleury, B. Loppin, M.E. Hochberg, M. Boulétreau, Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp, Proc. Natl. Acad. Sci. USA 98 (2001) 6247–6252.
[19] Y. Gottlieb, E. Zchori-Fein, Irreversible thelyokous reproduction in *Muscidifurax uniraptor*, Entomol. Exp. Appl. 100 (2001) 271–278.
[20] F. Vavre, F. Fleury, J. Varaldi, P. Fouillet, M. Boulétreau, Evidence for female mortality in *Wolbachia*-mediated cytoplasmic incompatibility in haplodiploid insects: epidemiologic and evolutionary consequences, Evolution 54 (2000) 191–200.
[21] A.A. Hoffmann, M. Turelli, G.M. Simmons, Unidirectional incompatibility between populations of *Drosophila simulans*, Evolution 40 (1986) 692–701.
[22] S.L. O'Neill, T.L. Karr, Bidirectional incompatibility between conspecific populations of *Drosophila simulans*, Nature 348 (1990) 178–180.
[23] H. Merçot, B. Llorente, M. Jacques, A. Atlan, C. Montchamp-Moreau, Variability within the Seychelles cytoplasmic incompatibility system in *Drosophila simulans*, Genetics 141 (1995) 1015–1023.
[24] H. Merçot, D. Poinsot, Rescuing *Wolbachia* have been overlooked and discovered on Mount Kilimanjaro, Nature 391 (1998) 853.
[25] W. Zhou, F. Rousset, S.L. O'Neill, Phylogeny and PCR-based classification of *Wolbachia* strains using *wsp* gene sequences, Proc. R. Soc. Lond. B 265 (1998) 509–515.
[26] S. Charlat, L.L. Chat, H. Merçot, Characterization of non-cytoplasmic incompatibility inducing *Wolbachia* in two continental African populations of *Drosophila simulans*, Heredity 90 (2003) 49–55.
[27] M.J. Taylor, A. Hoerauf, *Wolbachia* bacteria of filarial nematodes, Parasitol. Today 15 (1999) 437–442.
[28] D.J. Starr, T.W. Cline, A host-parasite interaction rescues *Drosophila* oogenesis defects, Nature 418 (2002) 76–79.
[29] T.W. Cline, B.J. Meyer, Vive la différence: males vs females in flies vs worms, Annu. Rev. Genet. 30 (1996) 637–702.
[30] R.C. King, Ovarian Development in *Drosophila melanogaster*, Academic Press, New York, NY, 1970.
[31] J.R. Byers, A. Wilkes, A rickettsia like microorganism in *Dahlbominus fuscipennis* (Zett.) (Hymenoptera, Eulophidae): observations on its occurrence and ultrastructure, Can. J. Zool. 48 (1970) 959–968.
[32] K.-T. Min, S. Benzer, *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death, Proc. Natl. Acad. Sci. USA 94 (1997) 10792–10796.
[33] S.L. Dobson, K. Bourtzis, H.R. Braig, B.F. Jones, W. Zhou, F. Rousset, S.L. O'Neill, *Wolbachia* infections are distributed throughout insect somatic and germ line tissues, Insect Biochem. Mol. Biol. 29 (1999) 153–160.
[34] E.A. McGraw, D.J. Merritt, J.N. Droller, S.L. O'Neill, *Wolbachia* density and virulence attenuation after transfer into a novel host, Proc. Natl. Acad. Sci. USA 99 (2002) 2918–2923.
[35] A.A. Hoffmann, M. Turelli, in: S.L. O'Neill, A.A. Hoffmann, J.H. Werren (Eds.), Influential Passengers: Inherited Microorganisms and Arthropod Reproduction, Oxford University Press, New York, 1997, pp. 42–80.
[36] D. Poinsot, S. Charlat, H. Merçot, On the mechanism of *Wolbachia*-induced cytoplasmic incompatibility: confronting the models with the facts, BioEssays 25 (2003) 259–265.
[37] S.L. Ryan, G.B. Saul, Post-fertilization effect of incompatibility factors in *Mormoniella*, Mol. Gen. Genet. 103 (1968) 29–36.
[38] J.H. Yen, A.R. Barr, New Hypothesis of the cause of cytoplasmic incompatibility in *Culex pipiens* L, Nature 232 (1971) 657–658.
[39] J.A.J. Breeuwer, J.H. Werren, Microorganism associated with chromosome destruction and reproductive isolation between two insect species, Nature 346 (1990) 558–560.

[40] J.A.J. Breeuwer, *Wolbachia* and cytoplasmic incompatibility in the spider mite *Tetranychus urticae* and *T. turkestani*, Heredity 79 (1997) 41–47.
[41] F. Vavre, F. Dedeine, M. Quillon, P. Fouillet, F. Fleury, M. Boulétreau, Within-species diversity of *Wolbachia*-induced cytoplasmic incompatibility in haplodiploid insects, Evolution 55 (2001) 1710–1714.
[42] S.R. Bordenstein, J.J. Uy, J.H. Werren, Host genotype determines cytoplasmic incompatibility type in the haplodiploid genus Nasonia, Genetics 164 (2003) 223–233.
[43] J.A.J. Breeuwer, J.H. Werren, Cytoplasmic incompatibility and bacterial density in *Nasonia vitripennis*, Genetics 135 (1993) 565–574.
[44] A.A. Hoffmann, M. Turelli, L.G. Harshman, Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*, Genetics 126 (1990) 933–948.
[45] K.M. Reed, J.H. Werren, Induction of paternal genome loss by the paternal-sex-ratio chromosome and cytoplasmic incompatibility bacteria (*Wolbachia*): a comparative study of early embryonic events, Mol. Reprod. Dev. 40 (1995) 408–418.
[46] C.W. Lassy, T.L. Karr, Cytological analysis of fertilization and early embryonic development in incompatible crosses of *Drosophila simulans*, Mech. Dev. 57 (1996) 47–58.
[47] G. Callaini, R. Dallai, M.G. Riparbelli, *Wolbachia*-induced delay of paternal chromatin condensation does not prevent maternal chromosomes from entering anaphase in incompatible crosses of *Drosophila simulans*, J. Cell Sci. 110 (1997) 271–280.
[48] U. Tram, W. Sullivan, Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility, Science 296 (2002) 1124–1126.
[49] G. Callaini, M.G. Riparbelli, R. Giordano, R. Dallai, Mitotic defects associated with cytoplasmic incompatibility in *Drosophila simulans*, J. Invertebr. Pathol. 67 (1996) 55–64.
[50] C. Bressac, F. Rousset, The reproductive incompatibility system in *Drosophila simulans*: Dapi-staining analysis of the *Wolbachia* symbionts in sperm cysts, J. Invertebr. Pathol. 61 (1993) 226–230.
[51] M.E. Clark, Z. Veneti, K. Bourtzis, T.L. Karr, The distribution and proliferation of the intracellular bacteria *Wolbachia* during spermatogenesis in *Drosophila*, Mech. Dev. 111 (2002) 3–15.
[52] K. C. Binnington, A. A. Hoffmann, *Wolbachia*-like organisms and cytoplasmic incompatibility in *Drosophila simulans*, J. Invertebr. Pathol. 54 (1989) 344–353.
[53] D. Poinsot, K. Bourtzis, G. Markakis, C. Savakis, H. Merçot, *Wolbachia* transfer from *Drosophila melanogaster* into *D. simulans*: host effect and cytoplasmic incompatibility relationships, Genetics 150 (1998) 227–237.
[54] E.A. McGraw, D.J. Merritt, J.N. Droller, S.L. O'Neill, *Wolbachia*-mediated sperm modification is dependent on the host genotype in *Drosophila*, Proc. R. Soc. Lond. B 268 (2001) 2565–2570.
[55] M.E. Clark, Z. Veneti, K. Bourtzis, T.L. Karr, *Wolbachia* distribution and cytoplasmic incompatibility during sperm development: the cyst as the basic cellular unit of CI expression, Mech. Dev. 120 (2003) 185–198.
[56] M.T. Fuller, Genetic control of cell proliferation and differentiation in *Drosophila* spermatogenesis, Semin. Cell Dev. Biol. 9 (1998) 433–444.
[57] K.T. Reynolds, A.A. Hoffmann, Male age host effects and the weak expression or non-expression of cytoplasmic incompatibility in *Drosophila* strains infected by maternallly transmitted *Wolbachia*, Genet. Res. 80 (2002) 79–87.
[58] E. Jost, Untersuchungen zur Inkompatibilität im *Culex pipiens*-Komplex, Roux Arch. Dev. Biol. 166 (1970) 173–188.
[59] J.D. Wright, A.R. Barr, *Wolbachia* and the normal and incompatible eggs of *Aedes polynesiensis* (Diptera: Culicidae), J. Invertebr. Pathol. 38 (1981) 409–418.
[60] S. Dobson, M. Tanouye, The paternal sex ratio chromosome induces chromosome loss independently of *Wolbachia* in the wasp *Nasonia vitripennis*, Dev. Genes Evol. 206 (1996) 207–217.
[61] D.C. Presgraves, A genetic test of the mechanism of *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila*, *Genetics* 154 (2000) 771–776.
[62] J. Liu, H. Lin, J.M. Lopez, M.F. Wolfner, Formation of the male pronuclear lamina in *Drosophila melanogaster*, Dev. Biol. 184 (1997) 187–196.
[63] B. Loppin, F. Berger, P. Couble, The *Drosophila* maternal gene sésame is required for sperm chromatin remodeling at fertilization, Chromosoma 110 (2001) 430–440.
[64] J. Liu, J.M. Lopez, M.F. Wolfner, Developmental modulation of the nuclear envelope, Curr. Top. Dev. Biol. 35 (1997) 47–70.
[65] A.F. Huettner, Maturation and fertilization in *Drosophila melanogaster*, J. Morphol. 39 (1924) 249–265.
[66] A.L. Bull, Stages of living embryos in the jewel wasp *Mormoniella* (*Nasonia) vitripennis* (*Walker*) (Hymenoptera: Pteromalidae), Int. J. Insect Morphol. Embryol. 11 (1982) 1–23.
[67] G. Callaini, M.G. Riparbelli, Fertilization in *Drosophila melanogaster*: centrosome inheritance and organization of the first mitotic spindle, Dev. Biol. 176 (1996) 199–208.
[68] U. Tram, W. Sullivan, Reciprocal inheritance of centrosomes in the parthenogenetic Hymenopteran *Nasonia vitripennis*, Curr. Biol. 10 (2000) 1413–1419.
[69] N. Kawamura, Fertilization and the first cleavage mitosis in insects, Develop. Growth Differ. 43 (2001) 343–349.
[70] W. Zachariae, K. Nasmyth, Whose end is destruction: cell division and the anaphase-promoting complex, Genes Dev. 13 (1999) 2039–2058.
[71] P.H. O'Farrell, Triggering the all-or-nothing switch into mitosis, Trends Cell Biol. 11 (2001) 512–519.
[72] M.J. Hendzel, Y. Wei, M.A. Mancini, A. Van Hooser, T. Ranalli, B.R. Brinkley, D.P. Bazett-Jones, C.D. Allis, Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation, Chromosoma 106 (1997) 348–360.
[73] T.T. Su, F. Sprenger, P.J. DiGregorio, S.D. Campbell, P.H. O'Farrell, Exit from mitosis in Drosophila syncytial embryos requires proteolysis and cyclin degradation, and is associated with localized dephosphorylation, Genes Dev. 12 (1998) 1495–1503.
[74] Y. Wei, C.A. Mizzen, R.G. Cook, M.A. Gorovsky, C.D. Allis, Phosphorylation of histone H3 at serine 10 is correlated with chromosome condensation during mitosis and meiosis in *Tetrahymena*, Proc. Natl. Acad. Sci. USA 95 (1998) 7480–7484.
[75] K.R. Yu, R.B. Saint, W. Sullivan, The Grapes checkpoint coordinates nuclear envelope breakdown and chromosome condensation, Nat. Cell Biol. 2 (2000) 609–615.
[76] L.H. Hartwell, T.A. Weinert, Checkpoints: controls that ensure the order of cell cycle events, Science 246 (1989) 629–634.
[77] B. Henderson, M. Wilson, J. Hyams, Cellular microbiology: cycling into the millennium, Trends Cell Biol. 8 (1998) 384–387.
[78] P. White, M. Wilson, S. Nair, A. Kirby, K. Reddi, B. Henderson, Characterization of an antiproliferative surface-associated protein from Actinobacillus actinomycetemcomitans which can be neutralized by sera from a proportion of patients with localized juvenile periodontitis, Infect. Immun. 63 (1995) 2612–2618.
[79] P.A. White, M. Patel, S. Nair, J. Ashmore, P. Galgut, M. Wilson, B. Henderson, I. Olsen, Control of the human cell cycle by a bacterial protein, gapstatin, Eur. J. Cell Biol. 77 (1998) 228–238.
[80] J.-Y. Huang, J.W. Raff, The disappearance of cyclin B at the end of mitosis is regulated spatially in *Drosophila* cells, EMBO J. 18 (1999) 2184–2195.
[81] C.L. Pickett, C.A. Whitehouse, The cytolethal distending toxin family, Trends Microbiol 7 (1999) 292–297.
[82] J. De Rycke, E. Oswald, Cytolethal distending toxin (CDT): a bacterial weapon to control host cell proliferation?, FEMS Microbiol. Lett. 203 (2001) 141–148.

[83] C.A. Ellwell, L.A. Dreyfus, DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest, Mol. Microbiol 37 (2000) 952–963.
[84] M. Lara-Tejero, J.E. Galan, A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I–like protein, Science 290 (2000) 354–357.
[85] G. Sluder, E.A. Thompson, C.L. Rieder, F.J. Miller, Nuclear envelope breakdown is under nuclear not cytoplasmic control in sea urchin zygotes, J. Cell Biol. 129 (1995) 1447–1458.
[86] G. Schatten, The centrosome and its mode of inheritance: the reduction of the centrosome during gametogenesis and its restoration during fertilization, Dev. Biol. 165 (1994) 299–335.
[87] T.L. Karr, Intracellular sperm/egg interactions in *Drosophila*: a three-dimensional structural analysis of a paternal product in the developing egg, Mech. Dev. 34 (1991) 101–111.
[88] W. Sullivan, D.R. Daily, P. Fogarty, K.J. Yook, S. Pimpinelli, Delays in anaphase initiation occur in individual nuclei of the syncytial *Drosophila* embryo, Mol. Biol. Cell 4 (1993) 885–896.
[89] A. Musacchio, K.G. Hardwick, The spindle checkpoint: structural insights into dynamic signalling, Nat. Rev. Mol. Cell Bio. 3 (2002) 731–741.
[90] C.L. Rieder, A. Khodjakov, L.V. Paliulis, T.M. Fortier, R.W. Cole, G. Sluder, Mitosis in vertebrate somatic cells with two spindles: implications for the metaphase/anaphase transition checkpoint and cleavage, Proc. Natl. Acad. Sci. USA 94 (1997) 5107–5112.
[91] A.C. Spradling, in: M. Bate, A. Martinez Arias (Eds.), The Development of *Drosophila melanogaster*, Cold Spring Harbor Press, Plainview, NY, 1993, pp. 1–70.
[92] L. Cooley, W.E. Theurkauf, Cytoskeletal functions during *Drosophila* oogenesis, Science 266 (1994) 590–596.
[93] W.E. Theurkauf, Microtubules and cytoplasm organization during *Drosophila* oogenesis, Dev. Biol. 165 (1994) 352–360.
[94] J.H. Yen, A.R. Barr, in: R. Pal, M.J. Whitten (Eds.), The Use of Genetics in Insect Control, Elsevier, North Holland, 1974, pp. 97–118.
[95] M.S. Al-Khalifa, Symbionts in the follicle cells of the beetle *Aepopsis robinii* (Laboulbene) (Coleoptera:Carabidae), Int. J. Insect Morphol. Embryol. 14 (1985) 319–321.
[96] C. Louis, L. Nigro, Ultrastructural evidence of *Wolbachia* Rickettsiales in *Drosophila simulans* and their relationships with unidirectional cross-incompatibility, J. Invertebr. Pathol. 54 (1989) 39–44.
[97] E. Zchori-Fein, R.T. Roush, D. Rosen, Distribution of parthenogenesis-inducing symbionts in ovaries and eggs of *Aphytis* (Hymenoptera: Aphelinidae), Curr. Microbiol. 36 (1998) 1–8.
[98] G. Callaini, M.G. Riparbelli, R. Dallai, The distribution of cytoplasmic bacteria in the early *Drosophila* embryo is mediated by astral microtubules, J. Cell Sci. 107 (1994) 673–682.
[99] H. Kose, T.L. Karr, Organization of *Wolbachia pipientis* in the *Drosophila* fertilized egg and embryo revealed by an anti-Wolbachia monoclonal antibody, Mech. Dev. 51 (1995) 275–288.
[100] L.G. Tilney, M.S. Tilney, G.M. Guild, Formation of actin filament bundles in the ring canals of developing *Drosophila* follicles, J. Cell Biol. 133 (1996) 61–74.
[101] M. Hertig, The rickettsia, Wolbachia pipiens (gen. et sp.n.) and associated inclusions of the mosquito, Culex pipiens, Parasitology 28 (1936) 453–486.
[102] S.J. Hadfield, J.M. Axton, Germ cells colonized by endosymbiotic bacteria, Nature 402 (1999) 482.
[103] T. Hays, R. Karess, Swallowing dynein: a missing link in RNA localization?, Nat. Cell Biol. 2 (2000) E60–E62.
[104] J. Kim-Ha, J.L. Smith, P.M. MacDonald, *oskar* mRNA is localized to the posterior pole of the *Drosophila* oocyte, Cell 66 (1991) 23–35.
[105] R.P. Brendza, L.R. Serbus, J.B. Duffy, W.M. Saxton, A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein, Science 289 (2000) 2120–2122.
[106] J.E. Duncan, R. Warrior, The cytoplasmic Dynein and Kinesin motors have interdependent roles in patterning the *Drosophila* oocyte, Curr. Biol. 12 (2002) 1982–1991.
[107] D.N. Robinson, L. Cooley, Genetic analysis of the actin cytoskeleton in the Drosophila ovary, Annu. Rev. Cell Dev. Biol. 13 (1997) 147–170.
[108] M.G. Riparbelli, R. Stouthamer, R. Dallai, G. Callaini, Microtubule organization during the early development of the parthenogenetic egg of the hymenopteran *Muscidifurax uniraptor*, Dev. Biol. 195 (1998) 89–99.
[109] J.D. Wright, A.R. Barr, The ultrastructure and symbiotic relationships of *Wolbachia* of mosquitoes of the *Aedes scutellaris* group, J. Ultrastruct. Res. 72 (1980) 52–64.
[110] U. Tram, P.M. Ferree, T. Hays, W. Sullivan, In vivo analysis of *Wolbachia* during *Drosophila* embryogenesis, Mol. Biol. Cell 13 (Suppl) (2002) 472a–473a.
[111] A. Szollosi, A. Debec, Presence of rickettsias in haploid *Drosophila melanogaster* cell lines, Biol. Cellulaire 38 (1980) 129–134.
[112] J. Gruenberg, The endocytic pathway: a mosaic of domains, Nat. Rev. Mol. Cell Bio. 2 (2001) 721–730.
[113] K. Ireton, P. Cossart, Host-pathogen interactions during entry and actin-based movement of *Listeria monocytogenes*, Annu. Rev. Genet. 31 (1997) 113–138.