The Genetics and Cell Biology of *Wolbachia*-Host Interactions

Laura R. Serbus, Catharina Casper-Lindley, Frédéric Landmann, and William Sullivan

Molecular, Cell and Developmental Biology, University of California, Santa Cruz, California 95064; email: sullivan@biology.ucsc.edu

Annu. Rev. Genet. 2008. 42:683–707

First published online as a Review in Advance on August 19, 2008

The *Annual Review of Genetics* is online at genet.annualreviews.org

This article's doi: 10.1146/annurev.genet.41.110306.130354

0066-4197/08/1201-0683$20.00

Key Words

Wolbachia, *Drosophila*, transmission, cytoplasmic incompatibility, genomics

Abstract

Wolbachia are gram-negative bacteria that are widespread in nature, carried by the majority of insect species as well as some mites, crustaceans, and filarial nematodes. *Wolbachia* can range from parasitic to symbiotic, depending upon the interaction with the host species. The success of *Wolbachia* is attributed to efficient maternal transmission and manipulations of host reproduction that favor infected females, such as sperm-egg cytoplasmic incompatibility (CI). Much remains unknown about the mechanistic basis for *Wolbachia*-host interactions. Here we summarize the current understanding of *Wolbachia* interaction with insect hosts, with a focus on *Drosophila*. The areas of discussion include *Wolbachia* transmission in oogenesis, *Wolbachia* distribution in spermatogenesis, induction and rescue of the CI phenotype, *Wolbachia* genomics, and *Wolbachia*-membrane interactions.

INTRODUCTION

Cytoplasmic incompatibility (CI): embryonic lethality that results from mating *Wolbachia*-infected males to uninfected females

GSC: germline stem cell

Wolbachia are alpha-Proteobacteria that are harbored by insects, filarial nematodes, crustaceans, and mites (17, 65, 119, 130). Originally discovered in the ovaries of mosquitoes by Hertig and Wolbach in 1924, they have since been detected in every insect order (63). It was recently estimated that up to 66% of all insects species carry *Wolbachia* (65). The success of *Wolbachia* is thought to be due to efficient transmission through the female germline and through its manipulation of host reproduction to selectively favor infected females. This can be accomplished via induction of a number of host phenotypes such as sperm-egg cytoplasmic incompatibility (CI), parthenogenesis, feminization of males, and male-killing (63, 135, 140). In addition to vertical transmission, evidence is mounting that horizontal *Wolbachia* transmission is more common than previously thought (28, 68, 69, 149). Finally, both theoretical and experimental evidence suggests that in some instances *Wolbachia* increase host fitness (66, 116, 147).

Despite considerable progress in understanding the ecology and population genetics of *Wolbachia*, the cellular and molecular basis of *Wolbachia*-host interactions remain largely unknown. In this review, we describe the current understanding of the interactions between host and *Wolbachia* proteins in *Drosophila*. The specific topic areas include the cellular basis for *Wolbachia* transmission through the female germline, factors that influence *Wolbachia* distribution in the male germline, and current mechanistic insights into CI. Finally we discuss insights from the recent sequencing of the *Wolbachia* genome on molecular interactions between *Wolbachia* and host.

WOLBACHIA TRANSMISSION IN THE FEMALE GERMLINE

Wolbachia are transmitted predominantly through the female germline. Transmission rates measured for *Drosophila melanogaster* and *Drosophila simulans* are similar at about 97% efficiency in the field, and 100% in the laboratory (66, 67, 143). *Wolbachia* are excluded from the mature sperm and consequently transmission rates through the male germline are on the order of 2% (21, 35, 66, 143). In contrast, *Wolbachia* are present in the female germline throughout adulthood, ensuring high-fidelity *Wolbachia* transmission to the eggs produced by infected females. In addition, *Wolbachia* can repress sterile mutations and apoptotic pathways of the host germline (39, 40, 104, 134), which effectively preserves the *Wolbachia* transmission conduit and sets the stage for a symbiosis between *Wolbachia* and host organisms. Here we present an overview of maternal *Wolbachia* transmission in *Drosophila*, with a focus on how *Wolbachia* distribution in the female germline and early embryogenesis relates to its transmission.

Wolbachia Localization in Early *Drosophila* Oogenesis

Wolbachia are present in the female germline stem cells. *Drosophila* ovaries consist of a bundle of sheathed structures referred to as ovarioles (75). The distal tip region of each ovariole is referred to as the germarium, and this area is known to contain 2–3 germline stem cells (GSCs). As each GSC divides, it gives rise to an identical GSC and a differentiating germline cell referred to as the cystoblast. *Wolbachia* are visible within germline stem cells of *Wolbachia*-infected *Drosophila* (**Figure 1*a***). During stem cell mitosis, the bacteria are partitioned between the self-renewing stem cell and differentiating cystoblast. This allows for retention of *Wolbachia* in the germline stem cell as well as passage of *Wolbachia* into the differentiating daughter cell. The mechanism underlying this distribution of *Wolbachia* between the stem cell and cystoblast is not known.

As *Wolbachia*-bearing cystoblasts progress through the germarium, they become converted into *Wolbachia*-infected egg chambers. The cystoblast undergoes four rounds of mitosis with incomplete cytokinesis (75). This results in a cyst of 16 interconnected germline

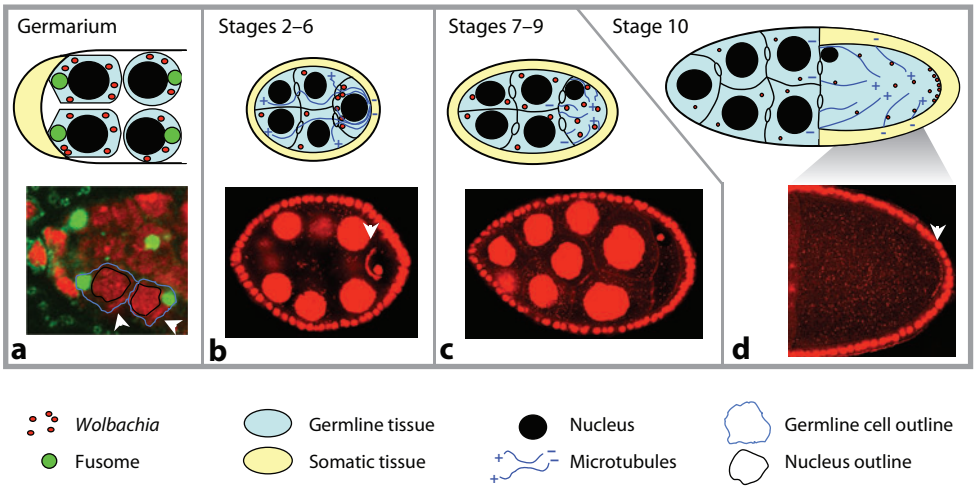

Figure 1

Wolbachia localization in *Drosophila melanogaster* oogenesis. All graphics are oriented with the posterior pole toward the right. Top row: schematic drawings of *Wolbachia* in *Drosophila* oogenesis. Bottom row: confocal microscope imaging of *Wolbachia*-infected ovaries. Propidium iodide labeling in red indicates host DNA as large circles, and *Wolbachia* as small puncta (49, 128). Groups of *Wolbachia* are indicated by arrowheads. (*a*) *Wolbachia* are present in stem cells and daughter cystoblasts. (*b*) *Wolbachia* concentrate at the oocyte anterior in early oogenesis. (*c*) *Wolbachia* distribution becomes homogeneous during mid-oogenesis. (*d*) A subset of *Wolbachia* become concentrated at the oocyte posterior cortex during late oogenesis.

cells linked by cytoplasmic bridges referred to as ring canals. *Wolbachia* are evenly distributed throughout all cells of the cyst (49). The cyst then contacts a group of somatic follicle stem cells in the germarium. The somatic follicle cell niche carries high levels of *Wolbachia*, and it may act as a secondary site for transmitting *Wolbachia* into germline cyst cells (54). As the germline cells pass through the follicle stem cell zone, the cyst becomes encapsulated by a layer of follicle cells. After this point, the encapsulated cysts are referred to as egg chambers (75).

After the germarium stages of oogenesis, *Wolbachia* become asymmetrically distributed throughout the germline cells of the egg chamber. These asymmetries appear to be strongly influenced by the cytoskeleton of the egg chamber. The posterior germline cell will become the oocyte, whereas the other 15 germ cells will become nurse cells (75). The oocyte notably has a microtubule organizing center (MTOC) at its posterior pole, which is rich in centrioles and microtubule minus ends (89, 139). Microtubules radiate from the oocyte posterior to extend their plus ends anteriorly through the ring canals into the nurse cells (139). This is thought to promote transport of components from nurse cells into the oocyte via minus end-directed motors such as cytoplasmic dynein (82, 87, 100). During stages 3–6 of oogenesis, while this microtubule arrangement is detected, *Wolbachia* bacteria become concentrated at the oocyte anterior cortex (**Figure 1*b***). This localization pattern has been reported both for the *wMel Wolbachia* strain carried by *D. melanogaster* as well as for *wRi Wolbachia* carried in *D. simulans* (49, 128). Experiments in *D. melanogaster* indicate that anterior *Wolbachia* concentration is dependent upon microtubules and cytoplasmic dynein (49). One explanation for this is that *Wolbachia* are carried into the oocyte from the nurse cells as a cargo of cytoplasmic dynein.

Wolbachia Localization in Mid- to Late *Drosophila* Oogenesis

Wolbachia localization shifts when the oocyte undergoes extensive cytoskeletal

Oogenesis: the process of oocyte development that occurs in the ovary

Microtubule organizing center (MTOC): an intracellular site marked by concentration of centrosomes and microtubule minus ends

Microtubule: a long filament with inherent structural polarity that acts as a scaffold for cargo transport by motor proteins

***wMel*:** the *Wolbachia* strain endogenous to *D. melanogaster*

***wRi*:** the primary *Wolbachia* strain endogenous to *D. simulans*

rearrangements in mid-oogenesis. During stage 6 of oogenesis, further polarization of the oocyte is triggered by epidermal growth factor receptor-based signaling between the oocyte and neighboring follicle cells (60, 101, 126). The immediate outcome of this signaling is a dramatic reorganization of the oocyte microtubule network in stage 7. The posterior MTOC disassembles and microtubules are reorganized such that the minus ends now emanate from the anterior cortex with the plus ends orienting toward the posterior of the oocyte (**Figure 1*c***) (19, 20, 33, 34, 81, 108, 139). During this reorganization, the oocyte nucleus migrates from the oocyte posterior to an anterior-dorsal position (75, 105). *Wolbachia* localization adjusts as well, transitioning from anterior concentration to a homogenous cytoplasmic distribution in the oocyte from stages 6–9 (49, 128). This localization shift in mid-oogenesis is consistent between *wMel* and *wRi Wolbachia* strains.

Wolbachia re-establish an asymmetrical distribution in late oogenesis. During stages 8–10A of *D. melanogaster* oogenesis, when the net direction of microtubule plus ends is toward the posterior pole, the polarity determinant *oskar* mRNA and numerous other factors also become enriched at the posterior cortex to form the pole plasm (47, 74, 88). Microtubules and the plus end-directed motor kinesin-1 are needed to transport *oskar* mRNA to the posterior cortex, and thus are integral to assembly of the pole plasm (19, 20, 29, 33, 108, 127). During late stage 9 and stage 10A, a subset of *wMel Wolbachia* also concentrate at the oocyte posterior pole (**Figure 1*d***) (128, 144). Drug inhibition tests and genetic disruptions indicate that microtubules, kinesin-1, and *oskar*-seeded pole plasm are necessary for proper posterior concentration of *wMel* (128). The data are consistent with a two-step mechanism for posterior *Wolbachia* enrichment: kinesin-1-mediated transport of *Wolbachia* along microtubules toward the oocyte posterior, followed by pole plasm-mediated anchorage of *Wolbachia* to the posterior cortex (128). Furthermore, the shift from dynein-dependent anterior localization in early oogenesis to kinesin-dependent posterior localization in late oogenesis raises the possibility that *Wolbachia* alternately engage dynein and kinesin during oogenesis.

How *Wolbachia* Localization Relates to Transmission

Posterior *Wolbachia* localization in late oogenesis ultimately promotes its germline-based transmission in embryos. In *D. melanogaster*, oocyte development is concluded by collapse of the nurse cells and expansion of the oocyte to fill the entire egg chamber, resulting in a completed egg (75). After the egg is fertilized, the embryo undergoes rapid cycles of nuclear division to give rise to hundreds of nuclei within a shared cytoplasm (2). The nuclei migrate out to the periphery of the embryo to become associated with the cortex. Nuclei that reach the posterior region are surrounded by pole plasm, which establishes a germline fate within the posterior nuclei. Plasma membranes then invaginate around all cortical nuclei to cellularize them, beginning first with the posterior germline zone (2). Throughout completion of oogenesis and early embryogenesis, *Wolbachia* maintain a pole plasm-dependent enrichment at the posterior cortex (62, 79, 128, 144). This positions *Wolbachia* at the site of the future germline prior to cellularization, promoting subsequent envelopment of *Wolbachia* by the germline cells. Posterior *Wolbachia* localization in late oogenesis has also been observed in several mosquito and hymenopteran host species (18, 40, 136, 140, 156, 160), suggesting that posterior localization is successful as a *Wolbachia* transmission strategy.

Posterior *Wolbachia* concentration in late oogenesis is not universal, however. The *wRi Wolbachia* strain endogenous to *D. simulans* remains homogeneously distributed throughout the ooplasm rather than concentrating at the oocyte posterior pole in late stage 9 and stage 10. This is surprising as *D. simulans* oocytes exhibit posterior localization of Oskar in stages 9–10 (L.R.S. & W.S., unpublished data), similar to *D. melanogaster* (90). This suggests that microtubule orientation, posteriorly

directed transport and pole plasm assembly are conserved in late oogenesis between the host species. To understand the basis for localization differences between *wRi* in *D. simulans* and *wMel* in *D. melanogaster*, staining experiments were performed to examine *wMel*-infected *D. simulans* oocytes (106, 128). Stage 9–10A *D. simulans* oocytes exhibited posterior *wMel* localization, confirming that *D. simulans* oocytes are competent to support such a localization pattern (128). This result also suggested that posteriorly localizing *Wolbachia* strains like *wMel* have intrinsic factors that promote posterior localization, perhaps by tethering *Wolbachia* to host pole plasm. In contrast, *Wolbachia* strains such as *wRi* may not produce the intrinsic factors necessary to establish a posterior localization pattern.

An alternative means by which *Wolbachia* can integrate into the germline is via association with embryonic nuclei (25, 79, 140). A small portion of nuclei in the early embryo are destined to reach the embryo posterior pole, which ensures that the subset of *Wolbachia* associated with those nuclei will become integrated into posterior germline cells. *wRi Wolbachia* appear to rely upon this nuclear-association strategy for incorporation into pole cells (25). Although the high *wRi* transmission rate suggests that the nuclear-association approach is successful, a potential drawback is that it requires a high bacterial titer in the embryo. As only ~3% of embryonic nuclei are destined to become posterior germline cells (2), a correspondingly small fraction of *wRi Wolbachia* would be expected to become transmitted by using this strategy, and thus a high bacterial titer would be necessary to ensure that germline incorporation is achieved. Consistent with this, the titer of *wRi* in *D. simulans* embryos is approximately sixfold greater than *wMel* in *D. melanogaster* embryos (144). This elevated *wRi* concentration may also aid the nuclear-association transmission strategy by buffering detrimental influences on *Wolbachia* titer such as adverse changes in host crowding, host age, host genetic background, and the availability of food (25, 46, 66, 76, 78, 94, 99, 106, 115, 144, 151).

Cellular analyses of *Wolbachia* localization/transmission mechanisms raise questions about the ancestral behaviors of *Wolbachia* in vivo. For example, was posterior localization the preeminent transmission strategy employed by ancestral *Wolbachia*, or did divergent *Wolbachia* strains independently adopt this localization strategy? If posterior localization was the ancestral pattern for *Wolbachia* distribution, it is possible that high-titer strains such as *wRi* in *D. simulans* lost this localization capability because the host permitted high replication levels and posterior localization became unnecessary for robust transmission. Conversely, if posterior *Wolbachia* localization evolved later, it may have arisen due to selfish competition between bacteria. If no other fitness variables are in play, a bacterial subset that localizes robustly to the posterior pole would be expected to outcompete non-localizing bacteria and eventually become the predominant variety. Mitochondria have been suggested to exhibit similar competitive behaviors (9). Studies of *Wolbachia* localization patterns as related to different host organisms will shed light on these fascinating issues in the future.

Spermatogenesis: the process of sperm development that occurs within the testis

WOLBACHIA IN SPERMATOGENESIS

Wolbachia concentrate in the germline of both sexes. As described above, concentration in the female germline is essential for efficient maternal transmission. Although not transmitted through the male germline, *Wolbachia* are distributed throughout cyst of postmeiotic spermatids and are eliminated only during the final stages of sperm maturation. There is evidence that this concentration and distribution contributes to induction of CI.

Wolbachia Distribution and Levels in Spermatogenesis

Little is known about the cellular mechanisms that mediate *Wolbachia* distribution during spermatogenesis. The patterns of mitotic division in spermatogenesis and oogenesis share a number

of features and thus *Wolbachia* may rely on mechanisms similar to those described above for oogenesis. Spermatogenesis occurs within a membranous tube, the testis, with germline stem cells residing at the apical tip and mature sperm positioned distally (57). Similar to oogenesis, 2 to 3 male GSCs surround a cluster of somatically derived cells referred to as the Hub that serve as a niche. The mitotic divisions of GSCs are oriented such that one daughter remains associated with the Hub, and the other is released distally to form the primary gonialblast cell. Little is known about the abundance and segregation patterns of *Wolbachia* in the male GSC. However, studies of the *Drosophila* gonialblast reveal *Wolbachia* are present at low levels, 4 to 5 per cell (**Figure 2*a***) (117).

Wolbachia assume an uneven distribution as spermatogenesis proceeds. The new gonialblast becomes surrounded by two somatically derived cyst cells, and this group of cells is thereafter referred to as a cyst. Within the cyst, the gonialblast undergoes four rounds of mitotic division with incomplete cytokinesis resulting 16 spermatagonia cells, connected by cytoplasmic ring canals, surrounded by lengthening somatic cyst cells (56). As the gonial divisions occur, *Wolbachia* segregate among the spermatagonial cells via unknown mechanisms (**Figure 2*b***) (117). *Wolbachia* do not exhibit a close association with centrosomes or

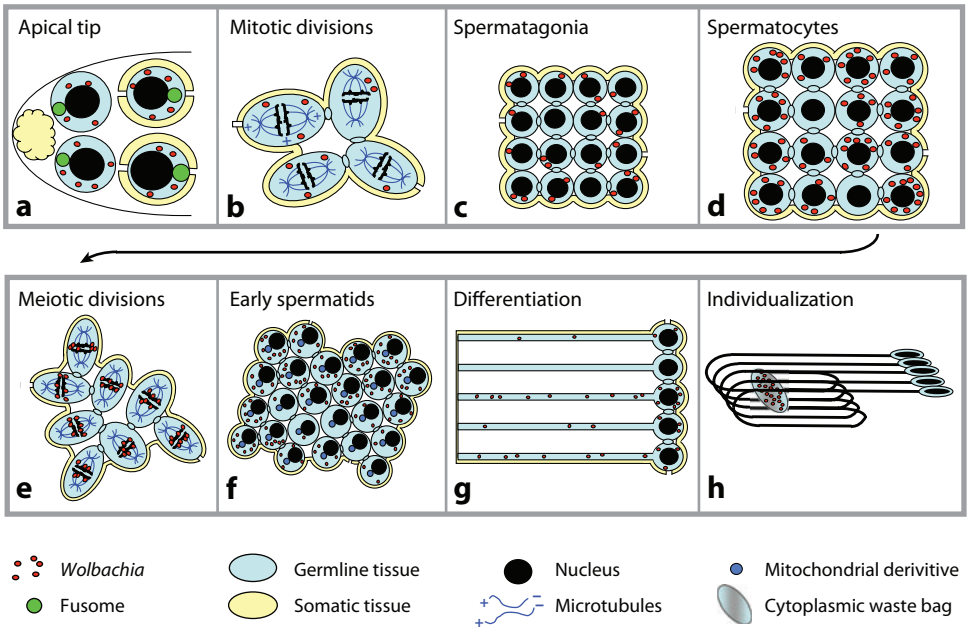

Figure 2

Wolbachia localization in *Drosophila* spermatogenesis. All drawings are oriented with the distally end of the testis toward the right. (*a*) *Wolbachia* may or may not be present within the germline stem cells. The stem cells divide to give rise to daughter gonialblast cells that contain *Wolbachia*. (*b–c*) The gonialblast divides mitotically to give rise to 16 interconnected spermatagonial cells with unevenly partitioned *Wolbachia*. (*d*) The spermatagonia become rapidly growing spermatocytes that contain proliferating *Wolbachia*. (*e–f*) The spermatocytes then undergo meiosis to create a 64-cell cyst of interconnected spermatids. (*e*) During meiosis, *Wolbachia* concentrate on the metaphase plate and segregate evenly to the spindle poles by telophase, (*f*) Upon completion of meiosis, many cells of the 64-cell spermatid cyst contain *Wolbachia*. (*g–h*) The *Wolbachia*-containing spermatids undergo differentiation and individualization. (*h*) During individualization, excess cytoplasmic components including *Wolbachia* are removed from the sperm.

microtubules to facilitate segregation, nor is there evidence to support transport of *Wolbachia* between spermatagonia via the ring canals. Numerous cysts have been found that contain a mix of infected and uninfected spermatagonia (**Figure 2*c***) (117). This indicates that segregation of *Wolbachia* to the daughter cells during these divisions is imperfect. Further support for this conclusion comes from the observation that even in completely infected cysts, there is extensive variation in the cell-to-cell *Wolbachia* concentration (35, 36, 117).

Wolbachia levels increase after the spermatagonia transition into a spermatocyte state. During this period, the spermatocytes enter a period of growth and gene expression. As the spermatocytes grow, *Wolbachia* levels increase to some 30 to 50 per cell, presumably through replication (**Figure 2*d***) (36, 117). It is also possible that the increased levels of *Wolbachia* infection may be the result of intercellular recruitment from neighboring *Wolbachia*-infected somatic cells. This latter idea is supported by in vivo documentation that *Wolbachia* can be transmitted from somatic to germline cells in the ovary (54).

Distinct from oogenesis, the 16 spermatocytes undergo two rounds of meiotic divisions with incomplete cytokinesis to produce a cyst of 64 interconnected spermatids. It does not appear that *Wolbachia* replicate during these divisions as the overall number of *Wolbachia* per cyst remains constant (36). However, *Wolbachia* do exhibit a close association with microtubules during meiotic divisions of spermatocytes (**Figure 2*e***). As the cells enter metaphase, *Wolbachia* concentrate at the equator where there is a high concentration of the microtubule plus ends (117). During late anaphase, *Wolbachia* segregate toward the poles to form two groups, ensuring each daughter cell receives equal numbers of bacteria (**Figure 2*f***). By telophase, *Wolbachia* are concentrated at the MTOC associated with each daughter nucleus. As *Wolbachia* maintain a close association with microtubules and move in the minus end direction, this suggests that *Wolbachia* movement from the equator to the poles relies on the minus-end motor protein dynein. Perhaps *Wolbachia* use plus-end motors such as kinesin to concentrate at the equator during metaphase and minus-end motors such as dynein to concentrate at the poles during anaphase and telophase. Thus, while segregation of *Wolbachia* in spermatagonia is imprecise during the mitotic divisions, *Wolbachia* appear to make use of more precise microtubule-based segregation mechanisms during the following meiotic divisions of spermatocytes.

Wolbachia are removed from the spermatids as spermatogenesis draws to a close. The 64 interconnected spermatids resulting from meiosis undergo an extraordinary morphological transformation that includes extensive mitochondrial fusion, elongation of the basal body-derived axoneme, and a decrease in nuclear volume (**Figure 2*g***) (56). Core histones are removed from the spermatid DNA and are replaced with protamines, which are thought to facilitate tighter packaging of DNA into the spermhead (63). In conjunction with protamine deposition, the spherical spermhead and nucleus change to an elongated spear shape. In addition, the spermatids then undergo an actin-based process, referred to as individualization, that sequesters the ring canals, cytoplasm, miscellaneous organelles, and *Wolbachia* into a waste bag at the distal tip of the sperm tail (**Figure 2*b***) (21, 35, 56). This results in elimination of *Wolbachia* from the entire sperm.

(T)EM: (transmission) electron microscopy

Functional Consequences

Although key events through spermatid elongation proceed normally in *Wolbachia*-infected testis, light microscopy and EM analysis reveal a number of disruptions later in the maturation process (117). In elongated sperm, the nuclei are normally positioned apically. However, in sperm derived from infected testis, the nuclei will occasionally exhibit improper basal positions. In addition, TEM analyses of infected testes revealed fused sperm, misoriented axonemes, two axonemes per spermatid, and failed individualization. Significantly, some of

WISS: *Wolbachia*-infected spermatocyte/spermatid

SCR: sperm chromatin remodeling

these phenotypes, such as nuclear mispositioning, were observed in sperm derived from uninfected cysts, suggesting a *Wolbachia*-supplied diffusible factor is responsible. These observations provide a cellular explanation for reduced fertility observed in *Wolbachia*-infected males (37, 132).

A defining feature of *Wolbachia* is that the bacteria are often highly concentrated in the spermatocytes and spermatids but absent from the mature sperm. Although *Wolbachia* produce only subtle effects on sperm development and morphology, they produce dramatic effects on chromosome organization in the male pronucleus (see below). These observations led to the idea that *Wolbachia* exert an effect during spermatogenesis when high concentrations of *Wolbachia* are positioned closely to the paternal chromosomes. Evidence for this model derives from studies using a series of *Wolbachia*-infected *Drosophila* lines in which CI expression ranges from strong to weak (36). Clark et al. found a strong correlation between the strength of CI and the number of infected spermatocytes and spermatids. This is known as the *Wolbachia* Infected Spermatocyte/Spermatid (WISS) hypothesis. According to this hypothesis, all CI-expressing strains will have infected spermatocytes/spermatids ($WISS^+$ cysts). While the close proximity of *Wolbachia* to host DNA may maximize the CI effect in some cases, other studies indicate that cyst (spermatocyte/spermatid) infection is not required for CI induction. For example, studies in *Culex pipiens* find little correlation between bacterial density and CI strength (45). In addition, Riparbelli et al. (117) recently demonstrated that *D. simulans* males produce large numbers of sperm from uninfected cysts, which was surprising as nearly all sperm from infected *D. simulans* males induce CI. These observations suggest a model in which *Wolbachia* produce a diffusible CI-inducing factor that can spread from infected to uninfected cells throughout the testis (117). It remains to be seen whether the CI-inducing factor is the same factor that affects sperm tail morphology as described above.

CYTOPLASMIC INCOMPATIBILITY

Wolbachia exhibit an extraordinary ability to alter host reproduction to selectively favor infected females, thus facilitating their maternal transmission. *Wolbachia* use a variety of mechanisms to achieve this, of which CI is the most prevalent (135). CI is a form of conditional male sterility that occurs when infected males mate with uninfected females. High mortality rates are observed from the embryos derived from these crosses. First discovered in *C. pipiens* (59, 77), CI has since been documented in every insect order (63). The most intriguing feature of CI is that infected females suppress embryonic lethality associated with CI. Because infected females are also fully fertile when mated with uninfected males, infected females enjoy a selective advantage over uninfected females. Thus CI rapidly drives *Wolbachia* through insect populations.

From Mature Sperm to Male Pronucleus in Normal and CI Embryos

CI produces dramatic defects in the first mitotic division, but the key toward understanding the molecular basis of CI is to identify its earlier developmental manifestations. When the sperm initially enters the egg, the plasma membrane and nuclear envelope are immediately removed. The mechanism involved in nuclear envelope removal is unclear as the envelope lacks lamin and nuclear pores (56). At this time, protamines are removed from the paternal DNA, and Sperm Chromatin Remodeling (SCR) is ensured by replication-independent de novo nucleosome assembly using maternally supplied core histones and the histone variant H3.3 (85). In somatic cells, histone H3.3 is deposited in a replication-independent manner and is believed to play an essential role in transcriptional regulation (1). Concomitant with the SCR, the male pronucleus again acquires a spherical shape and a maternally supplied nuclear envelope containing lamins and nuclear pores. When the nuclear repackaging is

complete, paternal chromatin replicates and condenses in preparation for the first mitotic division. The paternal and maternal pronuclei migrate toward one another in a manner dependent upon microtubules and microtubule motor proteins (133b). Upon pronuclei apposition, the nuclear envelope breaks down, a spindle forms and the chromosomes separate at anaphase to create two diploid daughter nuclei.

In CI embryos, the first mitotic division following fertilization is severely disrupted. The first cytological studies to describe this phenotype were performed in the parasitic wasp, *Nasonia vitripennis*, reporting paternal "tangled chromatin" next to the properly condensed female chromosomes during metaphase of the first mitotic division (120). Other studies confirmed this phenotype and attributed it to defects in paternal chromosome condensation (18). Later work further indicated that the improperly condensed paternal chromosomes become fragmented during the first mitotic division, with some of the fragments becoming incorporated into daughter nuclei (112, 141). Similar to *N. vitripennis*, analyses of CI in *D. melanogaster and D. simulans* revealed that the male pronuclear DNA was diffuse during pronuclear apposition. Distinct from *N. vitripennis*, however, extensive chromosome bridging was visible during anaphase (24, 80, 141). Chromosome bridging in anaphase is a hallmark of damaged or incompletely replicated DNA, presumably due to failed separation of sister chromosomes in the unreplicated regions. One interpretation of these collected observations is that paternal chromatin is damaged in CI embryos, leading to defects in chromatin condensation and segregation.

Additional CI-related effects on the male pronucleus have been reported as well. Studies in *Nasonia* demonstrate a delay in nuclear envelope breakdown and Cdk1 (cyclin-dependent kinase 1) activation in the male pronucleus relative to the female pronucleus (142). Significantly, this delay occurs during prophase prior to the DNA condensation defects described above. Studies of cell cycle checkpoint mutants in early *Drosophila* embryos demonstrate that defects in cell cycle timing result in condensation defects (158). These results implicate cell cycle disruption as a contributor to paternal DNA condensation defects in CI embryos. Perhaps a direct disruption of cell cycle regulators leads to delayed Cdk1 activation in the male pronucleus. Another possibility is that Cdk1 activation is prevented by checkpoints that control entry into mitosis. It is well established that DNA damage inhibits mitotic entry by triggering the DNA damage checkpoint (32). Perhaps damaged paternal chromatin induces this checkpoint, resulting in delayed paternal nuclear envelope breakdown and chromosome condensation. Notably, such damage to paternal chromatin would also have to be reversible, as CI is suppressed by infected females (see below).

Cdk1: cyclin-dependent kinase 1

CI embryos also exhibit abnormal centrosome behavior. Immediately following fertilization, the sperm basal body transforms into a bona fide centrosome (59b, 133b). The nascent centrosome duplicates and during pronuclear apposition, the daughter centrosomes migrate away from one another to form the poles of the first mitotic spindle. As with DNA replication, it is critical that the centrosome undergo one round of duplication with each nuclear cycle to ensure the generation of a bipolar mitotic spindle. In *D. simulans* CI embryos, the cytoplasm contains a large excess of centrosomes unassociated with the maternal and paternal pronuclei (24, 80). Excess centrosomes have also been found in embryos lacking specific mitotic kinases (114), suggesting that *Wolbachia*-induced CI may disrupt mitotic kinases that coordinate the cytoplasmic-driven centrosome replication cycle with the nuclear-driven mitotic cycle. In addition to excess centrosomes, barrel-shaped spindles lacking centrosomes are often observed in CI embryos, similar to centrosomeless spindles observed in other studies (38). Thus it is possible that male pronuclei in CI embryos are disrupted in their association with centrosomes, unlike the tight centrosome-nuclear association normally observed in the early *Drosophila* embryonic

divisions (118). In accord with this observation is the finding that the *Drosophila* sperm tail prematurely disassociates from the male pronucleus in CI embryos (80). Significantly, defects in chromosome condensation and delays in mitotic progression have been shown to result in the disassociation of centrosomes from the nucleus (138). Thus the centrosome attachment defect observed in CI embryos may be a direct consequence of cell cycle timing and chromosome condensation defects described above.

A puzzling aspect of the CI phenotype is that the defects are not caused in the embryo directly by *Wolbachia* themselves, as *Wolbachia* are excluded from the mature sperm (21, 35). This indicates that *Wolbachia* must disrupt sperm development in some way that is not manifest until after fertilization occurs. One possibility is that *Wolbachia* load a diffusible factor into the sperm that then causes the CI phenotype in early embryogenesis, perhaps by activating a cell-cycle checkpoint. An alternative possibility is that *Wolbachia* leaves a molecular imprint on the paternal chromatin that disrupts cell-cycle events in the male pronucleus. An elegant study by Presgraves et al. distinguished between these possibilities using gynogenetic *Drosophila* females (111). These females produce diploid eggs that rely on extranuclear paternal factors for normal development, but do not actually require a male pronucleus. Crosses of gynogenetic females to *Wolbachia*-infected males resulted in viable progeny carrying maternally derived chromosomes, as opposed to CI-induced embryonic mortality. This result suggests that CI is not caused by diffusible factors loaded into the sperm. Rather, CI must ultimately be due to *Wolbachia*-induced impairment of the male pronucleus, likely related to the integrity of paternal DNA.

Unique Properties of the First Insect Mitotic Spindle Determines Outcome of CI

During the first mitotic division after pronuclear apposition in insects, the maternal and paternal chromosome complements remain separated in distinct regions of the metaphase plate (24). Although they share common spindle poles, the spindle itself is bifurcated with the distinct microtubule arrays interacting with the two sets of chromosomes. This separation between chromosome complements is in part due to the fact that the two paternal and maternal chromosome complements reside in separate nuclei and the nuclear envelope only partially breaks down, thus leaving significant amounts of intact nuclear envelope acting as a physical barrier between the maternal and paternal chromosome complements. Thus during anaphase, the two chromosome sets remain separate and only comingle during telophase. This spindle organization, known as the gonomeric spindle, is unique to the first mitotic division in insects (24, 73).

In *D. simulans* CI embryos, the maternal chromosome set enters the metaphase/anaphase transition while the improperly condensed paternal chromosome remnants fail to segregate and remain arrested in metaphase (**Figure 3**) (24). As described above, this arrest is likely the result of activation of the spindle assembly checkpoint. In all other cells examined, activation of the spindle assembly checkpoint prevents segregation of the entire chromosome complement. However, in CI embryos, one half of the gonomeric spindle remained arrested while the other half entered anaphase. This indicates that with respect to the spindle checkpoint, the paternal and maternal chromosome complements are regulated independently even though they share a common spindle.

The independent control of the metaphase/anaphase transition in the maternal and paternal halves of the first mitotic spindle in insects readily explains the different developmental outcomes of CI in both haplo-diploid and diplo-diploid insects. For example, in three closely related haplo-diploids within the *Nasonia* genus, CI results in embryonic lethality in *N. longicornis* and *N. giraulti*, whereas CI leads to conversion to all male progeny in *N. vitripennis* (**Figure 3**) (11). Cytological analysis of the first mitotic division in these three species reveals that embryonic lethality results

from abnormal segregation of paternal chromatin during the first mitosis producing aneuploid daughter nuclei. In contrast, the male progeny in *N. vitripennis* result from the fact that CI-induced paternal defects are so severe that the paternal chromosomes do not enter anaphase, presumably because of activation of the spindle assembly checkpoint. However, because the two halves of the bifurcated spindle function independently with respect to the metaphase anaphase transition, the maternal chromosomes segregate normally, producing viable haploid daughter mitotic products (**Figure 3**). These embryos ultimately develop into haploid males.

Thus when less dramatic chromosome packaging defects fail to activate the spindle checkpoint, this would allow improper chromosome segregation and inviable aneuploid nuclei to result. However, when CI produces severe defects in paternal chromosome condensation, the spindle checkpoint is activated ultimately resulting in haploid male progeny. In strict diploid insect species haploid embryos are not viable, and CI results only in embryonic lethality.

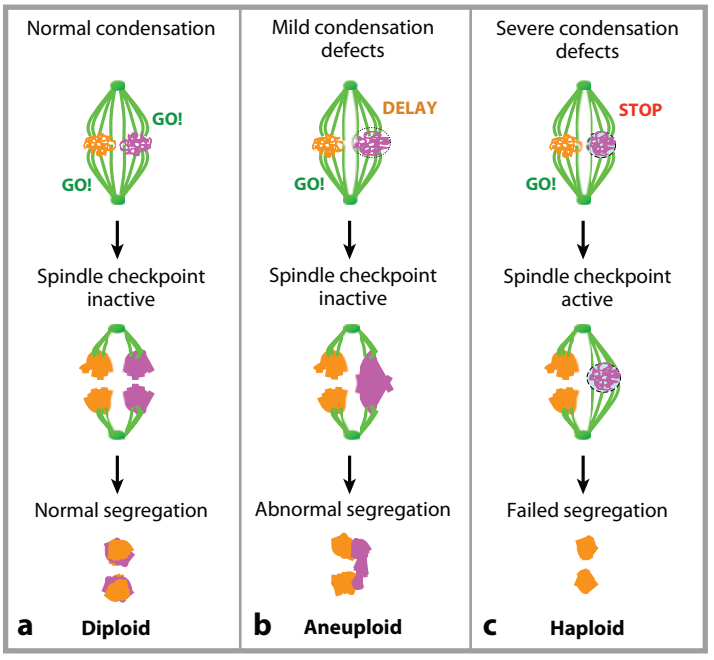

Figure 3

Developmental outcomes of CI may depend on whether the spindle-checkpoint is activated. (*a*) In the gonomeric spindle, the paternal and maternal chromosome complements behave independently with respect to checkpoint activation. (*b*) If CI produces only minor defects in chromosome condensation, the spindle checkpoint is not activated and the paternal chromosomes segregate abnormally to produce in aneuploid daughter nuclei, resulting in inviable embryos. (*c*) If CI produces extensive defects in paternal chromosome condensation, the spindle checkpoint is activated. This results in haploid daughter nuclei containing only maternal chromosomes. In *Hymemoptera*, this results in viable male progeny.

Mechanisms of Rescue

A critical feature of CI-induced embryonic lethality is that this phenotype can be suppressed if the infected males mate with females carrying the same strain of *Wolbachia* (148). As described above, while the maternal chromosomes segregate normally, the paternal chromosomes either fail to segregate or undergo extensive bridging during anaphase in CI embryos. However, if the embryos are also infected with *Wolbachia*, the male pronucleus and the paternal chromosome complement condense and segregate normally during anaphase, resulting in the production of viable embryos (24, 80, 112, 142). This phenomenon in which eggs derived from infected mothers suppress the CI-induced defects in paternal chromosome condensation and segregation is known as Rescue. CI and Rescue have been formalized into a Modification/Rescue (Mod/Resc) model (107). *Wolbachia*-based Mod occurs in the sperm and disrupts the paternal chromosome set during sperm differentiation, resulting in CI. The presence of *Wolbachia* in the female counters (Resc) these effects on the paternal chromosomes. Thus each strain of *Wolbachia* can be functionally characterized with respect to Mod and Resc. It appears that $Mod^+/Resc^+$ is the most common form, capable of both inducing and rescuing CI, but all combinations of + and – have been observed (30, 95, 148, 159).

A number of models have been proposed to explain the basis of Rescue. All of the models share the feature that maternally supplied *Wolbachia* condition the egg cytoplasm such that the CI-induced paternal chromosome condensation and segregation defects are suppressed. These models fall into three classes (**Figure 4**).

Rescue: suppression of cytoplasmic incompatibility that occurs when both male and female mates are *Wolbachia*-infected

Resc: *Wolbachia*-rescued

Mod: *Wolbachia*-modified

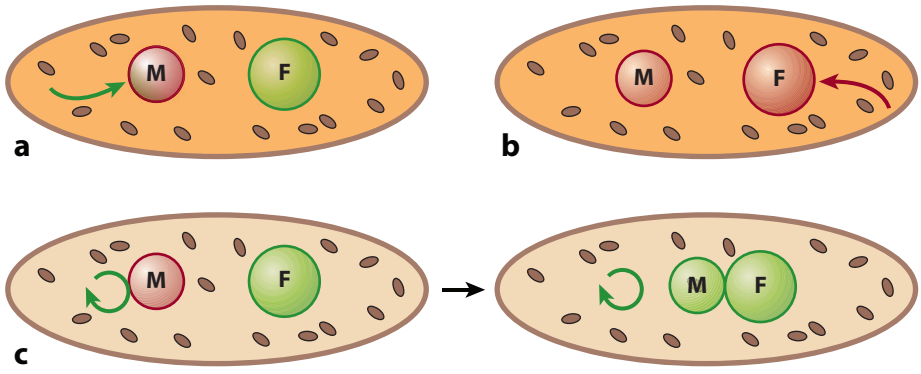

Figure 4

Models for rescue of CI. The first mitotic division in *Drosophila* embryos involves three distinct cell cycles: the nuclear cycles of the male (M) and female (F) pronuclei and the cycling of cytoplasmic mediated events such as spindle assembly. A key issue in understanding the mechanism of rescue is identifying which of these cycles is initially targeted. Possible Rescue scenarios include: (*a*) CI-induced defects are corrected in the male pronucleus. (*b*) The female pronucleus is modified in order to restore the pronuclei synchrony. (*c*) Cytoplasm-driven mitotic events are slowed, giving the male pronucleus sufficient time to correct CI-induced defects.

In the first class, the cytoplasm directly influences the state of the male pronucleus to correct the CI-induced defects (**Figure 4*a***). One scenario known as the lock and key model suggests that *Wolbachia* produce a factor that binds a component of the male pronucleus, and maternally provided *Wolbachia* produce a second component that removes the first component. In an alternative titration-restitution model, *Wolbachia* removes an essential component from the male pronucleus and maternal *Wolbachia* restore this factor (107).

In a second class of Rescue models, Rescue occurs through compensating changes in the female pronucleus (**Figure 4*b***). A specific version of this is known as the mistiming model in which the CI-induced embryonic lethality is caused by the developmental mistiming between the paternal and maternal pronuclei. Rescue is achieved by compensatory changes in either the male or female pronucleus that restores developmental timing between the two pronuclei. This model arose in part from studies in sea urchin embryos demonstrating that the relative cell-cycle timing in the male and female pronuclei could be controlled by selective checkpoint activation by individually exposing the sperm or eggs to a DNA cross-linking agent (131). Evidence that similar mechanisms may be operating in CI embryos comes from the finding that Cdk1 activation and nuclear envelope breakdown are specifically delayed relative to the female pronucleus in CI embryos (142). However, cell cycle synchrony between the two pronuclei is restored in Rescue crosses.

Finally, in the third class of Rescue models, maternal rescue of *Wolbachia* may occur through targeting cytoplasmic cell-cycle events (**Figure 4*c***). For example, the CI lethality may result from an asynchrony between the modified paternal pronucleus and the maternal cytoplasm. Experiments in *Xenopus* embryos demonstrate that the mitotic cycle of the cytoplasm not only functions independently of the nuclei, but is dominant in driving the nuclear cycles. For example, even after the male pronucleus is removed, oscillations in the cytoplasmic mitotic cycles still continue (146). More significantly, placing interphase nuclei in mitotic cytoplasmic extracts prematurely drives DNA synthesis and chromosome condensation. Thus CI may be the result of a specific slowing of events in the male pronucleus and Rescue may result from compensatory slowing of

cytoplasm-driven mitotic events such as spindle assembly.

Cytological studies have provided insight into the timing of Rescue. As described above, Rescue suppresses the CI-induced condensation and segregation defects. Subsequent studies demonstrated that Rescue also restores synchrony with respect to nuclear envelope breakdown and Cdk1 activation (142). However, these studies do not distinguish between a compensatory slowing of the female pronucleus or a restoration of timing of Cdk1 activation in the male pronucleus. Consequently, the cytological studies above cannot distinguish between the models outlined above. One method of distinguishing between the models is to assay the developmental competence of the male pronucleus in the CI crosses. With respect to the lock and key and titration models, CI renders the male pronucleus incapable of normal development. In contrast, the timing models suggest that the male pronucleus is capable of normal development; however, it is out of synch with the female pronucleus, and this asynchrony ultimately causes CI. To probe for the developmental competency of the male pronucleus, the ability of CI crosses to support androgenetic development was assayed (50). Mutations that disrupt pronuclear fusion result in the production of androgenetic adult *Drosophila*, i.e., adults that developed solely from the male pronucleus. *Wolbachia*-induced CI dramatically reduces the frequency of androgenetic progeny produced, arguing against models in which the embryonic lethality is due to an asynchrony between the female and male pronuclei. Rather, these results are in accord with models in which CI targeting of the male pronucleus renders it incapable of normal mitotic progression.

Wolbachia and Host Genotype Influence on CI and Rescue

As host and *Wolbachia* coevolve, both are likely to influence *Wolbachia-host* interactions such as in the context of CI and Rescue. Examples of host- and *Wolbachia*-based effects on CI have been analyzed in *Wolbachia* transfection studies. It is well documented that CI properties of a *Wolbachia* strain change according to their host fly (15). One host in particular, *D. simulans*, seems to be permissive for a strong CI-inducing phenotype (15, 93, 106, 159). For instance, the *wTei* and *wMelPop Wolbachia* strains, which do not cause CI in their endogenous hosts, induce a robust CI phenotype in *D. simulans* hosts (96, 159). It is unclear why those strains induce such strong CI in *D. simulans*, as there is no obvious feature consistently shared by *wTei* and *wMelPop* with regard to titer or localization in *D. simulans* testes (96, 159). Reciprocally, *Wolbachia* exhibit substantial influence on the CI phenotype as well. For example, unlike the *wTei* and *wMelPop* strains described above, the *wMa* and *wAu Wolbachia* strains do not induce CI in either their endogenous host or within the context of *D. simulans* (159). This indicates that an active *Wolbachia* contribution is critical for CI induction.

Rescue of CI appears to rely on both *Wolbachia* and host. It has been shown in mosquitoes that certain *Wolbachia* have the capability of rescuing CI (42, 129). For example, *C. quinquefasciatus* mosquitoes cured of *wPel* and then transformed with *wBei Wolbachia* are enabled to rescue *wBei*- and *wPel*-induced CI (129). Extensive work in *D. simulans* similarly indicates that many *Wolbachia* strains transferred into the same host background are enabled to rescue CI modifications induced by their own strain and often other *Wolbachia* strains as well (summarized in 159). However this is not universally the case, with a recent example that the *wTei Wolbachia* strain was not fully able to rescue its own CI modification in *D. simulans* (159). These observations indicate that contributions are required from both host and *Wolbachia* in order to facilitate Rescue, and that some of these Rescue mechanisms are shared among *Wolbachia* strains.

GENOMIC INSIGHTS INTO *WOLBACHIA*-HOST INTERACTIONS

Wolbachia genomics has progressed to a point where it can begin to inform us about

molecular interactions between *Wolbachia* and its host. The entire genomes of arthropod and nematode-infecting *Wolbachia* strains have been sequenced, and alleles of selected genes have been sequenced from a large number of *Wolbachia* strains. Arthropod and nematode *Wolbachia* have genome sizes ranging from 1.2 to 1.6 Mb and 0.9 to 1.1 Mb, respectively (51, 155). The genome of the *wMel Wolbachia* strain of *D. melanogaster* is moderately streamlined, yet it contains a high frequency of mobile/repetitive elements, comprising up to 14% of the genome. This finding indicates that although *wMel* possesses intact recombination and repair mechanisms, natural selection may be inefficient (155). An alterative explanation is that *wMel* experiences frequent population bottlenecks (15, 155).

Here, we focus on the genomics of arthropod *Wolbachia*. Similar to other obligate intracellular bacteria, arthropod *Wolbachia* have diminished metabolic capacity, although capabilities remain for synthesizing nucleotide triphosphates, including ATP (48, 51, 155). Genomic analyses have also focused on Wsp, a variable outer-membrane protein, as well as on highly abundant ankyrin-repeat containing proteins and WO phage-encoded genes. In the absence of genetic tools to modify *Wolbachia*, comparative analyses have focused on relating naturally occuring genetic differences to the various reproductive changes that bacteria cause in their hosts.

Wolbachia Metabolism and Intracellular Localization

Intracellular *Wolbachia* localization may relate to metabolic requirements of the bacteria. Depending on the cell type, *Wolbachia* are found closely associated with either microtubules or the cell cortex. *Wolbachia* are associated with the MTOC in early *Drosophila* embryos (25, 79). *Wolbachia* also associate with the meiotic spindle in spermatogenesis, segregating to the poles in a manner analogous to chromosomes (117). While these associations ensure that *Wolbachia* segregate with both daughter nuclei, any additional functional significance of this association is unknown. In the early oogenesis, a dramatic microtubule-dependent, anterior cortical association is observed (49, 128), also with unclear functional significance. Genomic analysis indicates that *Wolbachia* have diminished capacity for membrane metabolism, and have specifically lost most of the machinery necessary for producing lipopolysaccharide, the major component of the *Wolbachia* outer membrane (155). Thus *Wolbachia* must rely on host factors for additional membrane required for replication. Perhaps microtubule-dependent localization enables replicating *Wolbachia* to reach intracellular sites that facilitate membrane addition. For example, the MTOC is a hub for endosomal membrane traffic shuttling vesicles to the plasma membrane (92).

Wolbachia Surface Protein

Wsp (*Wolbachia* surface protein) was originally identified as an abundant *Wolbachia*-derived protein present in fly ovaries and testis. It is a low-molecular-weight protein of approximately 22 kDa, exhibiting extensive strain-to-strain variability in size (16, 124). Biochemical analysis demonstrates a strong membrane association, and selective detergent extractions indicate that Wsp is specifically associated with the outer bacterial membrane (16). In accord with this conclusion, Wsp contains a conserved gram-negative secretion signal sequence. Sequence analysis reveals Wsp exists as a single-copy gene and shares homology with *Rickettsiae* outer-membrane proteins (16).

Wsp belongs to a large family of surface proteins, known as pfam0617, primarily defined by antibody recognition (102). Other members of the pfam01617 superfamily include the outer-membrane proteins (OMPs) of *Anaplasma* and *Ehrlichia* that trigger antigenic reactions in their mammalian hosts and are differentially expressed during the bacteria's life cycle and in changing environments (53, 83, 84, 102). Pfam01617 proteins belong to a larger group (CL0193) of beta-barrel protein families with varying numbers of beta

strands. This group includes OmpA, which has been implicated in the effective bacterial invasion of host cells (97). Also included are the opacity proteins (Opa) of *Neisseria*, which allow bacteria adherence to host cells and mediate bacterial aggregation through interaction with lipopolysaccarides (8). Other OMP functions include the formation of outer-membrane channels that allow for passive and active transport (26). These similarities suggest that Wsp may have a comparable role in either *Wolbachia* infection, proliferation, or pathogenicity.

Sequence analysis from the five *Wolbachia* supergroups reveals that Wsp consists of four hypervariable regions (HVRs) separated by conserved regions (4). These HVRs have a high rate of intra- and intergenic recombination. HVRs evolve at a faster rate than other genes in the arthropod *Wolbachia* genome and have undergone strong positive selection (4, 5, 72). It may be that HVR-interacting host factors are driving this selection.

Wsp may function to inhibit apoptosis of host cells. Polymorphonuclear (PMN) cells in mammals are the first responders against invading bacteria and act through bacterial phagocytosis. Many bacteria defend themselves against this innate immune response by inhibiting apoptosis of the phagocytosing host cells (110). Significantly, in vitro studies demonstrate that Wsp inhibits apoptosis of PMN cells (7). Wsp also has been shown to function as a ligand of TLR2, a receptor that controls apoptosis of PMNs and other types of host cells (52).

An additional example of a possible role in apoptosis inhibition by Wsp is from the parasitoid wasp, *Asobara tabida*. *A. tabida* is a striking exception to the finding that *Wolbachia* is facultative in insects. Oogenesis is severely disrupted when female wasps are cured of *Wolbachia* (40, 41). Recent work has demonstrated a dramatic increase in apoptosis of nurse cells in the absence of *Wolbachia* (104). Consequently, the associated oocytes fail to progress past mid-oogenesis. Studies in *Drosophila* demonstrate that regulation of nurse cell apoptosis is an important feature of normal oogenesis (61). The specific *Wolbachia* factors influencing apoptosis in insects have not been identified but given the results described above, Wsp is an excellent candidate.

Ankyrin Repeat Proteins

Ank protein: a protein containing multiple ankyrin-repeats

The *wMel* genome contains 23 proteins with ankyrin repeats (Ank proteins) (155). Eight of these proteins are encoded in phage regions. A similarly high number of Ank proteins was found in other arthropod-infecting *Wolbachia* strains: 34 in *wAna* (48), and 54 in *wPip* (43). The symbiotic *Wolbachia* strain of the nematode *Brugia*, *wBm*, has only 9 such proteins (51). This correlation suggests that Ank proteins are instrumental in the parasitic lifestyle of arthropod-borne *Wolbachia*.

Ank proteins are of great interest in the analysis of host-pathogen relationships because they are involved in a diversity of protein-protein interactions (98). Ankyrin repeats consist of 33 amino-acid sequences and are the most abundant repeat motif found in sequenced genomes. The number of repeats in Ank proteins varies greatly, and the binding specificity does not lie within the ankyrin motif itself but in associated surface residues, explaining the diversity of interacting proteins such as intracellular adaptors, regulators, oncogenes, transcriptional regulators, cyclin-dependent kinase inhibitors, and others (98). Most Ank proteins are found in eukaryotes, but they occur in prokaryotes as well. Viruses and bacteria are thought to have acquired them by horizontal gene transfer (13).

The *wMel* host *D. melanogaster* expresses cell-cycle regulating factors that are Ank proteins, such as Plutonium (113) and Notch (64). Since *Wolbachia*-induced CI causes an asynchrony in Cdk1 activation in female and male pronuclei (142), one intriguing idea is that *Wolbachia* Ank proteins may influence cell-cycle timing during CI expression. Several studies have been conducted to investigate the extent to which *Wolbachia*-encoded Ank proteins may influence CI (43, 70, 129). Although there are some correlations between Ank variants and CI,

it is clear that additional *Wolbachia* encoded factors influence CI.

WO Phage

Wolbachia strains that infect arthropods contain a varying number of WO prophages (91, 155), in contrast to the strictly mutualistic *Wolbachia* strains in filarial nematodes that do not contain phage DNA (51). The sequenced *wMel* and *wRiverside Wolbachia* strains from *Drosophila melanogaster* and *D. simulans* harbor two divergent prophage families, the active WO-B and the dormant WO-A. The presence of lytic *Wolbachia* viruses was verified by electron microscopy inside the bacteria (10, 154) and outside the bacteria cells (10). Approximately 90% of all *Wolbachia* strains examined to date carry WO-B bacteriophages (12, 31). Phylogenies of *Wolbachia*, WO-B, and arthropods are not congruent, indicating that WO-B is undergoing horizontal transfer and recombination, which is unusual for phages in endosymbiont bacteria (12, 31, 44, 58, 91).

Much effort has been made to determine if *Wolbachia*-induced changes in arthropod reproduction (especially CI) are related to phage-encoded gene products. A putative virulence-related gene, VrlC, has been found among the WO-encoded genes (55). Williams et al. observed that CI-inducing characteristics can be transferred from infected to uninfected hosts by microinjecting egg cytoplasm that was filtered through a membrane with 0.23 μm pores, suggesting that a virus-size or smaller particle can carry the transferable characteristics (150). The heritability of these CI transformations was not tested, however, and the experiments have not yet been confirmed in other species. This leaves open whether the CI characteristics were indeed transferred by a virus or other small factors.

A number of studies were undertaken to explore the relationship between the presence of active WO phage and CI. To address this issue, WO phylogeny and CI characteristics were examined for correlation in *C. pipiens*. These hosts are infected with a wide variety of *Wolbachia* strains that have complex compatibility patterns (43). Specific phage sequences such as the minor capsid proteins (*orf7* and *orf2*) (42, 44, 58, 123) were used to differentiate between up to 40 phage variants. These studies found no strong correlation between phage phylogeny and the CI type in the host, however. Host reproductive defects such as CI, male killing, and parthenogenesis-inducing phenotypes were distributed throughout the entire examined phage phylogeny (58). Significantly, those reproductive effects were observed even in phage-free *Wolbachia* strains (58). Only one case of a weak correlation was found between the WO-encoded gene *gp15*, a putative type-IV secreted protein, and CI expression (44). In summary, the lytic WO-B phage does not appear to directly confer host-altering properties to their *Wolbachia* strain. Genes that are encoded by the dormant WO-A have not yet been examined extensively.

An alternative possibility is that WO phages attenuate the CI phenotype by reducing *Wolbachia* titer. Support for this model comes from the finding that high levels of phage are associated with low bacterial titer and low CI (10). These findings suggest that either higher numbers of lytic phages slow the bacteria replication rate, or that phage-mediated lysis decreases the bacteria titer.

So far, CI is the only functional aspect that has been analyzed with respect to *Wolbachia* phages. Given that a number of ankyrin-containing proteins are encoded on phage DNA and that no phages exist in the symbiotic nematode-infecting *Wolbachia* strains, it will be interesting to analyze a possible role of phage-encoded proteins in *Wolbachia* inheritance and proliferation in arthropods.

WOLBACHIA AND HOST MEMBRANE

Wolbachia reside within a membrane compartment, similar to other obligate intracellular bacteria (3, 6, 109). Numerous electron microscopy studies from *Drosophila* embryos show *Wolbachia* to be surrounded by three membrane layers, the outermost layer presumably derived from

membraneous organelles of the host cell (25, 41b, 86, 103, 133, 137, 153, 154, 157, 160). On rare occasions, *Wolbachia* appear to be surrounded by multiple layers of host membrane (23, 152). It is not yet clear what host organelle or organelles serve as the source of this membrane. In two electron microscopy studies, the *Wolbachia*-encompassing membrane appeared to be coated with particulate structures, suggesting that the membrane is derived from ribosome-studded endoplasmic reticulum (ER) (145, 152). If indeed *Wolbachia*-encompassing membrane compartments are derived from the ER, perhaps rapid turnover of the ER-derived membrane surface markers obscures the donor origin of the compartment. Consistent with this possibility, other types of intracellular bacteria have been shown to change the complement of protein markers associated with their surrounding host membranes such that they do not precisely resemble any endogenous host organelle (22, 121). These modifications are thought to disable targeting of membrane-encased bacteria to degradative lysosomal compartments, thereby allowing the bacteria to persist in the host cell. Perhaps *Wolbachia* employ similar persistence mechanisms.

Wolbachia face an interesting challenge in that they must retain the ability to access food, replicate at a sufficient rate, and maintain a presence in the germline while encased within a host-derived membrane compartment. Interactions between host proteins and *Wolbachia*-derived outer membrane proteins are likely to play a major role in these processes. With the proper array of associated of host factors, *Wolbachia* compartments would retain membrane fusion mechanisms similar to endogenous host organelles. Fusion with host organelles would provide *Wolbachia*-encompassing compartments with infusions of nutrients as well as new membrane to coat the *Wolbachia* as they replicate within the compartment. In addition, host proteins and/or *Wolbachia* factors secreted to the exterior surface of the membrane compartment may aid *Wolbachia* interaction with microtubule motor proteins. This would be inherently useful to *Wolbachia*, as their association with microtubules and microtubule motors appears strongly linked to their maternal transmission. It will be interesting to learn the identity of these key membrane proteins as future genetic and biochemical screens unfold.

ER: endoplasmic reticulum

SUMMARY POINTS

1. Efficient maternal germline inheritance requires that *Wolbachia* sequentially engage dynein and kinesin during oogenesis.
2. Posterior *Wolbachia* localization in late oogenesis may be due to interactions of *Wolbachia*-intrinsic factors with host pole plasm factors that specify the adult germline.
3. Current data favor models suggesting that rescue of CI is due to suppression of male pronuclear defects in *Wolbachia*-infected embryos.
4. The correlation between the strength of CI and the number of infected spermatocytes and spermatids has led to the WISS (*Wolbachia* Infected Spermatocyte/Spermatid) hypothesis. According to this hypothesis, all CI-expressing strains will have infected spermatocytes/spermatids (WISS+ cysts).
5. *Wolbachia* may synthesize diffusible factor that disrupts the sperm head to male pronucleus transformation during CI. Cytological consequences include delayed Cdk1 activation, delayed nuclear envelope breakdown and defects in chromosome condensation and segregation.

6. The mechanism of Rescue remains unclear, but current data favor either direct correction of CI-induced from the defects in the male pronucleus or a more indirect mechanism by compensating changes in the maternal cytoplasm.
7. Lytic phages harbored by *Wolbachia* appear to repress rather than enhance the CI phenotype.
8. Genomic analyses of Wsp and Ank proteins suggest that these factors have a substantial role in *Wolbachia*-host interactions.

FUTURE ISSUES

1. Further investigation is needed into how *Wolbachia* interact with the host cytoskeleton, germline determinants, and organelles at the molecular level. Also important will be to determine how *Wolbachia*-encompassing membranes are modified with respect to host- and *Wolbachia*-intrinsic proteins.
2. The molecular basis of CI and Rescue remains unresolved.
3. How CI and male-killing mechanisms relate to one another also awaits clarification. Two studies have demonstrated that CI-inducing *Wolbachia* strains switch to induction of male-killing when transferred into a new host (71, 125), suggesting a close overlap in their molecular underpinnings.
4. Genomic studies have revealed interesting targets for further investigation, such as Wsp and specific Ank proteins. Future steps include identifying host-interacting proteins and developing techniques to probe the function of these *Wolbachia*-encoded proteins.
5. How bacterial replication and titer are controlled in vivo is poorly understood. *Wolbachia* levels are quite variable between hosts and appear to be influenced by both host- and *Wolbachia*-intrinsic factors (94, 144). Identifying these factors will be an important area of future research. Understanding *Wolbachia*-host interactions at the molecular level will require the identification and characterization of the outer host-derived *Wolbachia* membrane.
6. Current studies of *Wolbachia*-host interactions must be expanded to include the development of *Wolbachia* culturing, transfection, and mutagenesis techniques, which will enable functional tests from the *Wolbachia* side as well.

DISCLOSURE STATEMENT

The authors are not aware of any biases, genetic or biochemical, that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

Funding for this work was provided by the National Institute of Health Ruth L. Kirschstein National Service Award (GM080192-02) and by the National Science Foundation (EF-0328363).

LITERATURE CITED

1. Ahmad K, Henikoff S. 2002. The histone variant H3.3 marks active chromatin by replication-independent nucleosome assembly. *Mol. Cell* 9:1191–200
2. Ashburner M. 1989. *Drosophila, a Laboratory Handbook*. New York: Cold Spring Harbor Lab. Press. 1331 pp.
3. Avakyan AA, Popov VL. 1984. Rickettsiaceae and Chlamydiaceae: comparative electron microscopic studies. *Acta Virol.* 28:159–73
4. Baldo L, Lo N, Werren JH. 2005. Mosaic nature of the *Wolbachia* surface protein. *J. Bacteriol.* 187:5406–18
5. Ballard JW. 2004. Sequential evolution of a symbiont inferred from the host: *Wolbachia* and *Drosophila simulans*. *Mol. Biol. Evol.* 21:428–42
6. Baumann P, Baumann L, Lai CY, Rouhbakhsh D, Moran NA, Clark MA. 1995. Genetics, physiology, and evolutionary relationships of the genus Buchnera: intracellular symbionts of aphids. *Annu. Rev. Microbiol.* 49:55–94
7. Bazzocchi C, Comazzi S, Santoni R, Bandi C, Genchi C, Mortarino M. 2007. *Wolbachia* surface protein (WSP) inhibits apoptosis in human neutrophils. *Parasite Immunol.* 29:73–79
8. Blake MS, Blake CM, Apicella MA, Mandrell RE. 1995. Gonococcal opacity: lectin-like interactions between Opa proteins and lipooligosaccharide. *Infect. Immun.* 63:1434–39
9. Boore JL. 1997. Transmission of mitochondrial DNA–playing favorites? *BioEssays* 19:751–53
10. Bordenstein SR, Marshall ML, Fry AJ, Kim U, Wernegreen JJ. 2006. The tripartite associations between bacteriophage, *Wolbachia*, and arthropods. *PLoS Pathog.* 2:e43
11. Bordenstein SR, Uy JJ, Werren JH. 2003. Host genotype determines cytoplasmic incompatibility type in the haplodiploid genus Nasonia. *Genetics* 164:223–33
12. Bordenstein SR, Wernegreen JJ. 2004. Bacteriophage flux in endosymbionts (*Wolbachia*): infection frequency, lateral transfer, and recombination rates. *Mol. Biol. Evol.* 21:1981–91
13. Bork P. 1993. Hundreds of ankyrin-like repeats in functionally diverse proteins: mobile modules that cross phyla horizontally? *Proteins* 17:363–74
14. Deleted in proof
15. Boyle L, O'Neill SL, Robertson HM, Karr TL. 1993. Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. *Science* 260:1796–99
16. Braig HR, Zhou W, Dobson SL, O'Neill SL. 1998. Cloning and characterization of a gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. *J. Bacteriol.* 180:2373–78
17. Breeuwer JA, Jacobs G. 1996. *Wolbachia*: intracellular manipulators of mite reproduction. *Exp. Appl. Acarol.* 20:421–34
18. Breeuwer JA, Werren JH. 1990. Microorganisms associated with chromosome destruction and reproductive isolation between two insect species. *Nature* 346:558–60
19. Brendza RP, Serbus LR, Duffy JB, Saxton WM. 2000. A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. *Science* 289:2120–22
20. Brendza RP, Serbus LR, Saxton WM, Duffy JB. 2002. Posterior localization of dynein and dorsal-ventral axis formation depend on kinesin in *Drosophila* oocytes. *Curr. Biol.* 12:1541–45
21. Bressac C, Rousset F. 1993. The reproductive incompatibility system in *Drosophila simulans*: DAPI-staining analysis of the *Wolbachia* symbionts in sperm cysts. *J. Invertebr. Pathol.* 61:226–30
22. Brumell JH, Scidmore MA. 2007. Manipulation of rab GTPase function by intracellular bacterial pathogens. *Microbiol. Mol. Biol. Rev.* 71:636–52
23. Byers JR, Wilkes A. 1970. A rickettsialike microorganism in *Dahlbominus fuscipennis* (Zett.) (Hymenoptera, Eulophidae): observations on its occurrence and ultrastructure. *Can. J. Zool.* 48:959–64
24. Callaini G, Dallai R, Riparbelli MG. 1997. *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(Pt 2):271–80
25. Callaini G, Riparbelli MG, Dallai R. 1994. The distribution of cytoplasmic bacteria in the early *Drosophila* embryo is mediated by astral microtubules. *J. Cell Sci.* 107(Pt 3):673–82

26. Carpenter T, Khalid S, Sansom MS. 2007. A multidomain outer membrane protein from *Pasteurella multocida*: modelling and simulation studies of PmOmpA. *Biochim. Biophys. Acta* 1768:2831–40
27. Deleted in proof
28. Casiraghi M, Bordenstein SR, Baldo L, Lo N, Beninati T, et al. 2005. Phylogeny of *Wolbachia pipientis* based on gltA, groEL and ftsZ gene sequences: clustering of arthropod and nematode symbionts in the F supergroup, and evidence for further diversity in the *Wolbachia* tree. *Microbiology* 151:4015–22
29. Cha BJ, Serbus LR, Koppetsch BS, Theurkauf WE. 2002. Kinesin I-dependent cortical exclusion restricts pole plasm to the oocyte posterior. *Nat. Cell Biol.* 4:592–98
30. Charlat S, Calmet C, Mercot H. 2001. On the mod resc model and the evolution of *Wolbachia* compatibility types. *Genetics* 159:1415–22
31. Chauvatcharin N, Ahantarig A, Baimai V, Kittayapong P. 2006. Bacteriophage WO-B and *Wolbachia* in natural mosquito hosts: infection incidence, transmission mode and relative density. *Mol. Ecol.* 15:2451–61
32. Chen Y, Poon RY. 2008. The multiple checkpoint functions of CHK1 and CHK2 in maintenance of genome stability. *Front. Biosci.* 13:5016–29
33. Clark I, Giniger E, Ruohola-Baker H, Jan LY, Jan YN. 1994. Transient posterior localization of a kinesin fusion protein reflects anteroposterior polarity of the *Drosophila* oocyte. *Curr. Biol.* 4:289–300
34. Clark IE, Jan LY, Jan YN. 1997. Reciprocal localization of Nod and kinesin fusion proteins indicates microtubule polarity in the *Drosophila* oocyte, epithelium, neuron and muscle. *Development* 124:461–70
35. Clark ME, Veneti Z, Bourtzis K, Karr TL. 2002. The distribution and proliferation of the intracellular bacteria *Wolbachia* during spermatogenesis in *Drosophila*. *Mech. Dev.* 111:3–15
36. Clark ME, Veneti Z, Bourtzis K, Karr TL. 2003. *Wolbachia* distribution and cytoplasmic incompatibility during sperm development: the cyst as the basic cellular unit of CI expression. *Mech. Dev.* 120:185–98
37. de Crespigny FE, Pitt TD, Wedell N. 2006. Increased male mating rate in *Drosophila* is associated with *Wolbachia* infection. *J. Evol. Biol.* 19:1964–72
38. de Saint Phalle B, Sullivan W. 1998. Spindle assembly and mitosis without centrosomes in parthenogenetic Sciara embryos. *J. Cell Biol.* 141:1383–91
39. Dedeine F, Bouletreau M, Vavre F. 2005. *Wolbachia* requirement for oogenesis: occurrence within the genus Asobara (Hymenoptera, Braconidae) and evidence for intraspecific variation in *A. tabida*. *Heredity* 95:394–400
40. Dedeine F, Vavre F, Fleury F, Loppin B, Hochberg ME, Bouletreau M. 2001. Removing symbiotic *Wolbachia* bacteria specifically inhibits oogenesis in a parasitic wasp. *Proc. Natl. Acad. Sci. USA* 98:6247–52
41. Dedeine F, Vavre F, Shoemaker DD, Bouletreau M. 2004. Intra-individual coexistence of a *Wolbachia* strain required for host oogenesis with two strains inducing cytoplasmic incompatibility in the wasp *Asobara tabida*. *Evol. Int. J. Org. Evol.* 58:2167–74
41b. Dobson SL. 2003. *Wolbachia pipientis*: impotent by association. In *Insect Symbiosis*, ed. K Bourtzis, TA Miller, pp. 199–215. New York: CRC Press
42. Duron O, Bernard C, Unal S, Berthomieu A, Berticat C, Weill M. 2006. Tracking factors modulating cytoplasmic incompatibilities in the mosquito *Culex pipiens*. *Mol. Ecol.* 15:3061–71
43. Duron O, Boureux A, Echaubard P, Berthomieu A, Berticat C, et al. 2007. Variability and expression of ankyrin domain genes in *Wolbachia* infecting the mosquito *Culex pipiens*. *J. Bacteriol.* 189:4442–48
44. Duron O, Fort P, Weill M. 2006. Hypervariable prophage WO sequences describe an unexpected high number of *Wolbachia* variants in the mosquito *Culex pipiens*. *Proc. Biol. Sci.* 273:495–502
45. Duron O, Fort P, Weill M. 2007. Influence of aging on cytoplasmic incompatibility, sperm modification and *Wolbachia* density in *Culex pipiens* mosquitoes. *Heredity* 98:368–74
46. Dutton TJ, Sinkins SP. 2004. Strain-specific quantification of *Wolbachia* density in *Aedes albopictus* and effects of larval rearing conditions. *Insect. Mol. Biol.* 13:317–22
47. Ephrussi A, Dickinson LK, Lehmann R. 1991. Oskar organizes the germ plasm and directs localization of the posterior determinant nanos. *Cell* 66:37–50
48. Fenn K, Blaxter M. 2006. *Wolbachia* genomes: revealing the biology of parasitism and mutualism. *Trends Parasitol.* 22:60–65

49. Ferree PM, Frydman HM, Li JM, Cao J, Wieschaus E, Sullivan W. 2005. *Wolbachia* utilizes host microtubules and Dynein for anterior localization in the *Drosophila* oocyte. *PLoS Pathog.* 1:e14
50. Ferree PM, Sullivan W. 2006. A genetic test of the role of the maternal pronucleus in *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila melanogaster*. *Genetics* 173:839–47
51. Foster J, Ganatra M, Kamal I, Ware J, Makarova K, et al. 2005. The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. *PLoS Biol.* 3:e121
52. Francois S, El Benna J, Dang PM, Pedruzzi E, Gougerot-Pocidalo MA, Elbim C. 2005. Inhibition of neutrophil apoptosis by TLR agonists in whole blood: involvement of the phosphoinositide 3-kinase/Akt and NF-kappaB signaling pathways, leading to increased levels of Mcl-1, A1, and phosphorylated Bad. *J. Immunol.* 174:3633–42
53. French DM, Brown WC, Palmer GH. 1999. Emergence of *Anaplasma marginale* antigenic variants during persistent rickettsemia. *Infect. Immun.* 67:5834–40
54. Frydman HM, Li JM, Robson DN, Wieschaus E. 2006. Somatic stem cell niche tropism in *Wolbachia*. *Nature* 441:509–12
55. Fujii Y, Kubo T, Ishikawa H, Sasaki T. 2004. Isolation and characterization of the bacteriophage WO from *Wolbachia*, an arthropod endosymbiont. *Biochem. Biophys. Res. Commun.* 317:1183–88
56. Fuller MT. 1993. Spermatogenesis. In *The Development of Drosophila melanogaster*, ed. M Bate, AM Arias, pp. 71–147. New York: Cold Spring Harbor Lab. Press
57. Fuller MT. 1998. Genetic control of cell proliferation and differentiation in *Drosophila* spermatogenesis. *Semin. Cell Dev. Biol.* 9:433–44
58. Gavotte L, Henri H, Stouthamer R, Charif D, Charlat S, et al. 2007. A survey of the bacteriophage WO in the endosymbiotic bacteria *Wolbachia*. *Mol. Biol. Evol.* 24:427–35
59. Ghelelovitch S. 1952. Genetic determinism of sterility in the cross-breeding of various strains of *Culex autogenicus* Roubaud. *C. R. Hebd. Seances. Acad. Sci.* 234:2386–88
59b. Glover DM. 1992. The centrosome in cell division and development of Drosophila. In *The Centrosome*, ed. VI Kalnins, pp. 219–34. New York: Academic
60. Gonzalez-Reyes A, Elliott H, St. Johnston D. 1995. Polarization of both major body axes in *Drosophila* by gurken-torpedo signalling. *Nature* 375:654–58
61. Greenwood J, Gautier J. 2005. From oogenesis through gastrulation: developmental regulation of apoptosis. *Semin. Cell Dev. Biol.* 16:215–24
62. Hadfield SJ, Axton JM. 1999. Germ cells colonized by endosymbiotic bacteria. *Nature* 402:482
63. Harris HL, Braig HR. 2003. Sperm chromatin remodelling and *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila*. *Biochem. Cell Biol.* 81:229–40
64. Herranz H, Perez L, Martin FA, Milan M. 2008. A Wingless and Notch double-repression mechanism regulates G1-S transition in the *Drosophila* wing. *EMBO J.* 27:1633–45
65. Hilgenboecker K, Hammerstein P, Schlattmann P, Telschow A, Werren JH. 2008. How many species are infected with *Wolbachia*? A statistical analysis of current data. *FEMS Microbiol. Lett.* 281:215–20
66. Hoffmann AA, Hercus M, Dagher H. 1998. Population dynamics of the *Wolbachia* infection causing cytoplasmic incompatibility in *Drosophila melanogaster*. *Genetics* 148:221–31
67. Hoffmann AA, Turelli M, Harshman LG. 1990. Factors affecting the distribution of cytoplasmic incompatibility in *Drosophila simulans*. *Genetics* 126:933–48
68. Huigens ME, de Almeida RP, Boons PA, Luck RF, Stouthamer R. 2004. Natural interspecific and intraspecific horizontal transfer of parthenogenesis-inducing *Wolbachia* in Trichogramma wasps. *Proc. Biol. Sci.* 271:509–15
69. Huigens ME, Luck RF, Klaassen RH, Maas MF, Timmermans MJ, Stouthamer R. 2000. Infectious parthenogenesis. *Nature* 405:178–79
70. Iturbe-Ormaetxe I, Burke GR, Riegler M, O'Neill SL. 2005. Distribution, expression, and motif variability of ankyrin domain genes in *Wolbachia pipientis*. *J. Bacteriol.* 187:5136–45
71. Jaenike J. 2007. Spontaneous emergence of a new *Wolbachia* phenotype. *Evolution Int. J. Org. Evolution* 61:2244–52
72. Jiggins FM, Hurst GD, Yang Z. 2002. Host-symbiont conflicts: positive selection on an outer membrane protein of parasitic but not mutualistic Rickettsiaceae. *Mol. Biol. Evol.* 19:1341–49

73. Kawamura N. 2001. Fertilization and the first cleavage mitosis in insects. *Dev. Growth Differ.* 43:343–49
74. Kim-Ha J, Smith JL, Macdonald PM. 1991. oskar mRNA is localized to the posterior pole of the *Drosophila* oocyte. *Cell* 66:23–35
75. King RC. 1970. *Ovarian Development in Drosophila melanogaster*. New York: Academic. 227 pp.
76. Kittayapong P, Mongkalangoon P, Baimai V, O'Neill SL. 2002. Host age effect and expression of cytoplasmic incompatibility in field populations of *Wolbachia*-superinfected *Aedes albopictus*. *Heredity* 88:270–74
77. Kitzmiller JB. 1959. Parthenogenesis in *Culex fatigans*. *Science* 129:837–38
78. Kondo N, Shimada M, Fukatsu T. 2005. Infection density of *Wolbachia* endosymbiont affected by coinfection and host genotype. *Biol. Lett.* 1:488–91
79. Kose H, Karr TL. 1995. Organization of *Wolbachia pipientis* in the *Drosophila* fertilized egg and embryo revealed by an anti*Wolbachia* monoclonal antibody. *Mech. Dev.* 51:275–88
80. Lassy CW, Karr TL. 1996. Cytological analysis of fertilization and early embryonic development in incompatible crosses of *Drosophila simulans*. *Mech. Dev.* 57:47–58
81. Li K, Kaufman TC. 1996. The homeotic target gene centrosomin encodes an essential centrosomal component. *Cell* 85:585–96
82. Li M, McGrail M, Serr M, Hays TS. 1994. *Drosophila* cytoplasmic dynein, a microtubule motor that is asymmetrically localized in the oocyte. *J. Cell Biol.* 126:1475–94
83. Lohr CV, Brayton KA, Shkap V, Molad T, Barbet AF, et al. 2002. Expression of *Anaplasma marginale* major surface protein 2 operon-associated proteins during mammalian and arthropod infection. *Infect. Immun.* 70:6005–12
84. Lohr CV, Rurangirwa FR, McElwain TF, Stiller D, Palmer GH. 2002. Specific expression of *Anaplasma marginale* major surface protein 2 salivary gland variants occurs in the midgut and is an early event during tick transmission. *Infect. Immun.* 70:114–20
85. Loppin B, Bonnefoy E, Anselme C, Laurencon A, Karr TL, Couble P. 2005. The histone H3.3 chaperone HIRA is essential for chromatin assembly in the male pronucleus. *Nature* 437:1386–90
86. Louis C, Nigro L. 1989. Ultrastructural evidence of *Wolbachia* Rickettsiales in *Drosophila simulans* and their relationships with unidirectional cross-incompatibility. *J. Invertebr. Pathol.* 54:39–44
87. Mach JM, Lehmann R. 1997. An Egalitarian-BicaudalD complex is essential for oocyte specification and axis determination in *Drosophila*. *Genes Dev.* 11:423–35
88. Mahowald AP. 2001. Assembly of the *Drosophila* germ plasm. *Int. Rev. Cytol.* 203:187–213
89. Mahowald AP, Strassheim JM. 1970. Intercellular migration of centrioles in the germarium of *Drosophila melanogaster*. An electron microscopic study. *J. Cell Biol.* 45:306–20
90. Markussen FH, Michon AM, Breitwieser W, Ephrussi A. 1995. Translational control of oskar generates short OSK, the isoform that induces pole plasma assembly. *Development* 121:3723–32
91. Masui S, Kamoda S, Sasaki T, Ishikawa H. 2000. Distribution and evolution of bacteriophage WO in *Wolbachia*, the endosymbiont causing sexual alterations in arthropods. *J. Mol. Evol.* 51:491–97
92. Maxfield FR, McGraw TE. 2004. Endocytic recycling. *Nat. Rev. Mol. Cell Biol.* 5:121–32
93. McGraw EA, Merritt DJ, Droller JN, O'Neill SL. 2001. *Wolbachia*-mediated sperm modification is dependent on the host genotype in *Drosophila*. *Proc. Biol. Sci.* 268:2565–70
94. McGraw EA, Merritt DJ, Droller JN, O'Neill SL. 2002. *Wolbachia* density and virulence attenuation after transfer into a novel host. *Proc. Natl. Acad. Sci. USA* 99:2918–23
95. McGraw EA, O'Neill SL. 1999. Evolution of *Wolbachia pipientis* transmission dynamics in insects. *Trends Microbiol.* 7:297–302
96. Min KT, Benzer S. 1997. *Wolbachia*, normally a symbiont of *Drosophila*, can be virulent, causing degeneration and early death. *Proc. Natl. Acad. Sci. USA* 94:10792–96
97. Mohan Nair MK, Venkitanarayanan K. 2007. Role of bacterial OmpA and host cytoskeleton in the invasion of human intestinal epithelial cells by *Enterobacter sakazakii*. *Pediatr. Res.* 62:664–69
98. Mosavi LK, Cammett TJ, Desrosiers DC, Peng ZY. 2004. The ankyrin repeat as molecular architecture for protein recognition. *Protein Sci.* 13:1435–48
99. Mouton L, Henri H, Bouletreau M, Vavre F. 2006. Effect of temperature on *Wolbachia* density and impact on cytoplasmic incompatibility. *Parasitology* 132:49–56

100. Navarro C, Puthalakath H, Adams JM, Strasser A, Lehmann R. 2004. Egalitarian binds dynein light chain to establish oocyte polarity and maintain oocyte fate. *Nat. Cell Biol.* 6:427–35
101. Neuman-Silberberg FS, Schupbach T. 1993. The *Drosophila* dorsoventral patterning gene gurken produces a dorsally localized RNA and encodes a TGF alpha-like protein. *Cell* 75:165–74
102. Noh SM, Brayton KA, Knowles DP, Agnes JT, Dark MJ, et al. 2006. Differential expression and sequence conservation of the *Anaplasma marginale* msp2 gene superfamily outer membrane proteins. *Infect. Immun.* 74:3471–79
103. O'Neill SL, Pettigrew MM, Sinkins SP, Braig HR, Andreadis TG, Tesh RB. 1997. In vitro cultivation of *Wolbachia pipientis* in an *Aedes albopictus* cell line. *Insect. Mol. Biol.* 6:33–39
104. Pannebakker BA, Loppin B, Elemans CP, Humblot L, Vavre F. 2007. Parasitic inhibition of cell death facilitates symbiosis. *Proc. Natl. Acad. Sci. USA* 104:213–15
105. Pare C, Suter B. 2000. Subcellular localization of Bic-D::GFP is linked to an asymmetric oocyte nucleus. *J. Cell Sci.* 113(Part 12):2119–27
106. Poinsot D, Bourtzis K, Markakis G, Savakis C, Mercot H. 1998. *Wolbachia* transfer from *Drosophila melanogaster* into *D. simulans*: host effect and cytoplasmic incompatibility relationships. *Genetics* 150:227–37
107. Poinsot D, Charlat S, Mercot H. 2003. On the mechanism of *Wolbachia*-induced cytoplasmic incompatibility: confronting the models with the facts. *BioEssays* 25:259–65
108. Pokrywka NJ, Stephenson EC. 1995. Microtubules are a general component of mRNA localization systems in *Drosophila* oocytes. *Dev. Biol.* 167:363–70
109. Popov VL, Chen SM, Feng HM, Walker DH. 1995. Ultrastructural variation of cultured *Ehrlichia chaffeensis*. *J. Med. Microbiol.* 43:411–21
110. Power CP, Wang JH, Manning B, Kell MR, Aherne NJ, et al. 2004. Bacterial lipoprotein delays apoptosis in human neutrophils through inhibition of caspase-3 activity: regulatory roles for CD14 and TLR-2. *J. Immunol.* 173:5229–37
111. Presgraves DC. 2000. A genetic test of the mechanism of *Wolbachia*-induced cytoplasmic incompatibility in *Drosophila*. *Genetics* 154:771–76
112. Reed KM, Werren JH. 1995. 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.* 40:408–18
113. Renault AD, Axton JM. 2003. Identification of plu genes and *cis*-acting elements of PCNA in the *Drosophila* genus using conservation of gene order. *Gene* 307:77–86
114. Renault AD, Zhang XH, Alphey LS, Frenz LM, Glover DM, et al. 2003. giant nuclei is essential in the cell cycle transition from meiosis to mitosis. *Development* 130:2997–3005
115. Reynolds KT, Thomson LJ, Hoffmann AA. 2003. The effects of host age, host nuclear background and temperature on phenotypic effects of the virulent *Wolbachia* strain popcorn in *Drosophila melanogaster*. *Genetics* 164:1027–34
116. Riegler M, Sidhu M, Miller WJ, O'Neill SL. 2005. Evidence for a global *Wolbachia* replacement in *Drosophila melanogaster*. *Curr. Biol.* 15:1428–33
117. Riparbelli MG, Giordano R, Callaini G. 2007. Effects of *Wolbachia* on sperm maturation and architecture in *Drosophila simulans* Riverside. *Mech. Dev.* 124:699–714
118. Robinson JT, Wojcik EJ, Sanders MA, McGrail M, Hays TS. 1999. Cytoplasmic dynein is required for the nuclear attachment and migration of centrosomes during mitosis in *Drosophila*. *J. Cell Biol.* 146:597–608
119. Rousset F, Bouchon D, Pintureau B, Juchault P, Solignac M. 1992. *Wolbachia* endosymbionts responsible for various alterations of sexuality in arthropods. *Proc. Biol. Sci.* 250:91–98
120. Ryan SL, Saul GB 2nd. 1968. Post-fertilization effect of incompatibility factors in Mormoniella. *Mol. Gen. Genet.* 103:29–36
121. Salcedo SP, Holden DW. 2005. Bacterial interactions with the eukaryotic secretory pathway. *Curr. Opin. Microbiol.* 8:92–98
122. Deleted in proof

123. Sanogo YO, Eitam A, Dobson SL. 2005. No evidence for bacteriophage WO orf7 correlation with *Wolbachia*-induced cytoplasmic incompatibility in the *Culex pipiens* complex (Culicidae: Diptera). *J. Med. Entomol.* 42:789–94
124. Sasaki T, Braig HR, O'Neill SL. 1998. Analysis of *Wolbachia* protein synthesis in *Drosophila* in vivo. *Insect. Mol. Biol.* 7:101–5
125. Sasaki T, Massaki N, Kubo T. 2005. *Wolbachia* variant that induces two distinct reproductive phenotypes in different hosts. *Heredity* 95:389–93
126. Schupbach T. 1987. Germ line and soma cooperate during oogenesis to establish the dorsoventral pattern of egg shell and embryo in *Drosophila melanogaster*. *Cell* 49:699–707
127. Serbus LR, Cha BJ, Theurkauf WE, Saxton WM. 2005. Dynein and the actin cytoskeleton control kinesin-driven cytoplasmic streaming in *Drosophila* oocytes. *Development* 132:3743–52
128. Serbus LR, Sullivan W. 2007. A cellular basis for *Wolbachia* recruitment to the host germline. *PLoS Pathog.* 3:e190
129. Sinkins SP, Walker T, Lynd AR, Steven AR, Makepeace BL, et al. 2005. *Wolbachia* variability and host effects on crossing type in Culex mosquitoes. *Nature* 436:257–60
130. Sironi M, Bandi C, Sacchi L, Di Sacco B, Damiani G, Genchi C. 1995. Molecular evidence for a close relative of the arthropod endosymbiont *Wolbachia* in a filarial worm. *Mol. Biochem. Parasitol.* 74:223–27
131. Sluder G, Thompson EA, Rieder CL, Miller FJ. 1995. Nuclear envelope breakdown is under nuclear not cytoplasmic control in sea urchin zygotes. *J. Cell Biol.* 129:1447–58
132. Snook RR, Cleland SY, Wolfner MF, Karr TL. 2000. Offsetting effects of *Wolbachia* infection and heat shock on sperm production in *Drosophila simulans*: analyses of fecundity, fertility and accessory gland proteins. *Genetics* 155:167–78
133. Sokolova MI, Zinkevich NS, Zakharov IA. 2002. Bacteria in ovarioles of females from maleless families of ladybird beetles *Adalia bipunctata* L. (Coleoptera: Coccinellidae) naturally infected with Rickettsia, *Wolbachia*, and Spiroplasma. *J. Invertebr. Pathol.* 79:72–79
133b. Sonnenblick BP. 1950. The early embryology of *Drosophila melanogaster*. In *Biology of Drosophila*, ed. M Demerec, pp. 62–167. New York: Wiley
134. Starr DJ, Cline TW. 2002. A host parasite interaction rescues *Drosophila* oogenesis defects. *Nature* 418:76–79
135. Stouthamer R, Breeuwer JA, Hurst GD. 1999. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53:71–102
136. Stouthamer R, Breeuwert JA, Luck RF, Werren JH. 1993. Molecular identification of microorganisms associated with parthenogenesis. *Nature* 361:66–68
137. Szollosi A, Debec A. 1980. Presence of Rickettsias in haploid *Drosophila melanogaster* cell lines. *Extr. Biol. Cell.* 38:129–34
138. Takada S, Kelkar A, Theurkauf WE. 2003. *Drosophila* checkpoint kinase 2 couples centrosome function and spindle assembly to genomic integrity. *Cell* 113:87–99
139. Theurkauf WE, Smiley S, Wong ML, Alberts BM. 1992. Reorganization of the cytoskeleton during *Drosophila* oogenesis: implications for axis specification and intercellular transport. *Development* 115:923–36
140. Tram U, Ferree PM, Sullivan W. 2003. Identification of *Wolbachia*–host interacting factors through cytological analysis. *Microbes Infect.* 5:999–1011
141. Tram U, Fredrick K, Werren JH, Sullivan W. 2006. Paternal chromosome segregation during the first mitotic division determines *Wolbachia*-induced cytoplasmic incompatibility phenotype. *J. Cell Sci.* 119:3655–63
142. Tram U, Sullivan W. 2002. Role of delayed nuclear envelope breakdown and mitosis in *Wolbachia*-induced cytoplasmic incompatibility. *Science* 296:1124–26
143. Turelli M, Hoffmann AA. 1995. Cytoplasmic incompatibility in *Drosophila simulans*: dynamics and parameter estimates from natural populations. *Genetics* 140:1319–38
144. Veneti Z, Clark ME, Karr TL, Savakis C, Bourtzis K. 2004. Heads or tails: host-parasite interactions in the *Drosophila-Wolbachia* system. *Appl. Environ. Microbiol.* 70:5366–72
145. Voronin DA, Dudkina NV, Kiseleva EV. 2004. A new form of symbiotic bacteria *Wolbachia* found in the endoplasmic reticulum of early embryos of *Drosophila melanogaster*. *Dokl. Biol. Sci.* 396:227–29

146. Wasserman WJ, Smith LD. 1978. The cyclic behavior of a cytoplasmic factor controlling nuclear membrane breakdown. *J. Cell Biol.* 78:R15–22
147. Weeks AR, Turelli M, Harcombe WR, Reynolds KT, Hoffmann AA. 2007. From parasite to mutualist: rapid evolution of *Wolbachia* in natural populations of *Drosophila*. *PLoS Biol.* 5:e114
148. Werren JH. 1997. Biology of *Wolbachia*. *Annu. Rev. Entomol.* 42:587–609
149. Werren JH, Zhang W, Guo LR. 1995. Evolution and phylogeny of *Wolbachia*: reproductive parasites of arthropods. *Proc. Biol. Sci.* 261:55–63
150. Williams EH, Fields S, Saul GB 2nd. 1993. Transfer of incompatibility factors between stocks of *Nasonia* (=Mormoniella) *vitripennis*. *J. Invertebr. Pathol.* 61:206–10
151. Wiwatanaratanabutr S, Kittayapong P. 2006. Effects of temephos and temperature on *Wolbachia* load and life history traits of *Aedes albopictus*. *Med. Vet. Entomol.* 20:300–7
152. Wright JD, Barr AR. 1980. The ultrastructure and symbiotic relationships of *Wolbachia* of mosquitoes of the Aedes scutellaris group. *J. Ultrastruct. Res.* 72:52–64
153. Wright JD, Barr AR. 1981. *Wolbachia* and the normal and incompatible eggs of *Aedes polynesiensis* (Diptera: Culicidae). *J. Invertebr. Pathol.* 38:409–18
154. Wright JD, Sjostrand FS, Portaro JK, Barr AR. 1978. The ultrastructure of the rickettsia-like microorganism *Wolbachia pipientis* and associated virus-like bodies in the mosquito *Culex pipiens*. *J. Ultrastruct. Res.* 63:79–85
155. Wu M, Sun LV, Vamathevan J, Riegler M, Deboy R, et al. 2004. Phylogenomics of the reproductive parasite *Wolbachia pipientis wMel*: a streamlined genome overrun by mobile genetic elements. *PLoS Biol.* 2:E69
156. Xi Z, Dean JL, Khoo C, Dobson SL. 2005. Generation of a novel *Wolbachia* infection in *Aedes albopictus* (Asian tiger mosquito) via embryonic microinjection. *Insect Biochem. Mol. Biol.* 35:903–10
157. Yen JH, Barr AR. 1974. Incompatibility in *Culex pipiens*. In *The Use of Genetics in Insect Control*, ed. R Pal, MJ Whitten, pp. 97–118. Amsterdam: Elsevier
158. Yu KR, Saint RB, Sullivan W. 2000. The Grapes checkpoint coordinates nuclear envelope breakdown and chromosome condensation. *Nat. Cell Biol.* 2:609–15
159. Zabalou S, Apostolaki A, Pattas S, Veneti Z, Paraskevopoulos C, et al. 2008. Multiple rescue factors within a *Wolbachia* strain. *Genetics* 178:2145–60
160. Zchori-Fein E, Roush RT, Rosen D. 1998. Distribution of parthenogenesis-inducing symbionts in ovaries and eggs of Aphytis (Hymentoptera: Aphelinidae). *Curr. Microbiol.* 36:1–8

Annual Review of Genetics

Volume 42, 2008

Contents

Errata

An online log of corrections to *Annual Review of Genetics* articles may be found at http://genet.annualreviews.org/errata.shtml