A Cellular Basis for *Wolbachia* Recruitment to the Host Germline

Laura R. Serbus, William Sullivan*

Molecular, Cell, and Developmental Biology, University of California Santa Cruz, Santa Cruz, California, United States of America

*To whom correspondence should be addressed. E-mail: sullivan@biology.ucsc.edu

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**Introduction**

*Wolbachia* are among the most widespread intracellular bacteria, carried by thousands of metazoan species. The success of *Wolbachia* is due to efficient vertical transmission by the host maternal germline. Some *Wolbachia* strains concentrate at the posterior of host oocytes, which promotes *Wolbachia* incorporation into posterior germ cells during embryogenesis. The molecular basis for this localization strategy is unknown. Here we report that the *wMel Wolbachia* strain relies upon a two-step mechanism for its posterior localization in oogenesis. The microtubule motor protein kinesin-1 transports *wMel* toward the oocyte posterior, then pole plasm mediates *wMel* anchorage to the posterior cortex. Trans-infection tests demonstrate that factors intrinsic to *Wolbachia* are responsible for directing posterior *Wolbachia* localization in oogenesis. These findings indicate that *Wolbachia* can direct the cellular machintery of host oocytes to promote germline-based bacterial transmission. This study also suggests parallels between *Wolbachia* localization mechanisms and those used by other intracellular pathogens.

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*Wolbachia* are among the most widespread intracellular bacteria, carried by an estimated 15%–76% of insect species as well as by some crustaceans, mites, and filarial nematodes [1,2]. *Wolbachia* are closely related to the *Rickettsia* family, a collection of tick-borne pathogens known for causing typhus and spotted fevers in humans. *Wolbachia* are also linked to human disease via a symbiotic relationship with pathogenic nematodes [3]. For example, the *Wolbachia*-bearing nematode *Onchocerca volvulus* is linked to the condition African river blindness in humans. Of the 18 million people infected by *O. volvulus*, nearly one million are visually impaired or already blind [4]. Recent work has implicated *Wolbachia* directly as the cause of ocular inflammation leading to river blindness [5].

The effect of *Wolbachia* infection on its host is as varied as the hosts are themselves. *Wolbachia* act as endosymbionts of some host organisms, such as the filarial nematode *O. volvulus* and the wasp *Asobara tabida*, which require *Wolbachia* in order to complete oogenesis properly [3,6]. *Wolbachia* appear to cause little phenotypic impact in certain hosts, such as in *Drosophila melanogaster*. In other cases, *Wolbachia* manipulate the host to their advantage. *Wolbachia* bias host reprodution to favor infected females by inducing phenotypes such as male-killing, feminization, sperm–egg cytoplasmic incompatibility, and parthenogenesis (virgin birth) [1,2]. This is thought to promote the spread of *Wolbachia* throughout host populations.

Infectious agents often spread to new hosts by becoming inhaled or ingested by that host. In the case of *Wolbachia*, however, bacterial transmission occurs within the host maternal germline [1,2]. Though *Wolbachia* appear in both male and female germlines, the bacteria are removed from sperm cysts at the end of spermatogenesis [7,8], creating a reliance upon maternal transmission. In arthropods, this maternal transmission is accomplished via incorporation of *Wolbachia* into germline precursor cells, also known as “pole cells” [9–11]. This ensures that infected females resulting from those embryos will carry bacteria in their germlines as well, thus perpetuating the *Wolbachia* transmission cycle. *Wolbachia* transmission rates have been reported at over 97% for wild-caught *D. melanogaster* flies, and at 100% for laboratory-reared *D. melanogaster* and *D. simulans* flies [12,13], suggesting that the pole cell–based transmission strategy is highly efficient.

How might *Wolbachia* ensure their incorporation into host pole cells? Many *Wolbachia* strains have been reported to concentrate at the posterior of mature oocytes [1,9–11,14–17]. Interestingly, the oocyte posterior pole corresponds to the location where pole cell formation takes place later in embryogenesis. For this reason, the posterior concentration of *Wolbachia* during oogenesis is thought to promote *Wolbachia* incorporation into the embryonic germline [9–11]. The cellular and molecular basis underlying this posterior *Wolbachia* localization in oogenesis is unknown to date, however.

A recent study indicated that *Wolbachia* can associate with host cell microtubules in *D. melanogaster* oocytes [18]. These oocytes contain an extensive network of microtubules that serves as a scaffold for cargo transport by motor proteins [19]. Up to stage 6 of oogenesis, microtubule minus ends are generally concentrated at the oocyte posterior with plus ends toward the anterior [20–22]. At stage 7, microtubules reorient such that minus ends are concentrated at the antero-lateral cortex of the oocyte, and plus ends are biased toward the posterior [23–27]. Work from *D. melanogaster* demonstrated...
This study focuses on *Wolbachia*, a genus of intracellular bacteria carried by insect and nematode host species. It was recently shown that *Wolbachia* carried into the human body by the host nematode *Onchocerca volvulus* trigger an immune response that leads to African river blindness. Findings like these raise fundamental questions of how *Wolbachia* interact with host cells to perpetuate *Wolbachia* infection. Distinct from many pathogenic bacteria, *Wolbachia* are transmitted throughout host populations primarily from females to their offspring, similar to mitochondrial inheritance. The molecular basis for this transmission strategy is unclear. Here we show that *Wolbachia* transmission is aided by a complex mechanism in egg development. Our study suggests that *Wolbachia* are transported inside the egg as cargo of molecular motors that walk along microtubule filaments. This directs *Wolbachia* to the posterior of maturing eggs, thus placing *Wolbachia* at the site where reproductive cells form during embryogenesis and ensuring *Wolbachia* integration into those cells. Furthermore, both factors intrinsic to *Wolbachia* and host molecules specifying reproductive cell fates are necessary to maximize posterior concentration of *Wolbachia* in the egg. This suggests that *Wolbachia* manipulate conserved cellular machinery in egg development to direct their transmission to the next host generation.

**Author Summary**

This study addresses how *Wolbachia* posterior localization is achieved by examining the roles of microtubules, motor proteins, pole plasm assembly, and *Wolbachia*. Our findings indicate that during mid- to late oogenesis, kinesin-1 transports *wMel Wolbachia* toward the posterior cortex where pole plasm components mediate posterior *wMel* anchorage. The functions of kinesin-1 and pole plasm contribute independently to posterior *Wolbachia* localization. Furthermore, *wMel* can direct its localization to the oocyte posterior pole, unlike the homogeneously distributed *wRi* *Wolbachia* strain carried by *D. simulans*. This distinction between posteriorly concentrating and evenly dispersed *Wolbachia* strains may be due to different abilities of those strains to interact with posterior pole plasm.

**Results**

**Wolbachia Concentrate at the Oocyte Posterior Pole in Mid- to Late Oogenesis**

To understand the basis for *wMel* incorporation into embryonic pole cells, ovaries were stained with propidium iodide. This showed *wMel* to be anteriorly concentrated in stage 3–6 oocytes (Figure 1A and 1B) and homogeneously distributed in stage 7–9 oocytes (Figure 1E, 1E', 1F, and 1F') [18]. From late stage 9 to stage 12, a subset of *wMel* bacteria concentrated at the oocyte posterior cortex (Figure 1I, 1I', 1J, and 1J'; Table 1) [10]. *wMel* posterior localization persisted through early embryogenesis, facilitating *wMel* incorporation into the pole cells (Figure S1) [9–11]. Thus, concentration of *wMel* at the posterior of late stage oocytes promotes germ-line-based transmission of *wMel*.

**Directed Transport by Kinesin-1 Is Important for Posterior *Wolbachia* Localization**

The redistribution of *wMel* from the oocyte anterior to posterior suggests that an active localization mechanism is involved. To test a role for microtubule-based transport in posterior *wMel* localization, oocytes were treated with colcemid and colchicine. Some colcemid-treated oocytes exhibited *wMel* at both the lateral and posterior cortex (n = 7 of 15 cases; Figure 2A and 2A'), while others displayed a non-cortical, homogeneous distribution of *wMel* throughout the cytoplasm (n = 8 of 15 cases; Figure 2B and 2B'). Colchicine-treated oocytes displayed similar broad cortical or homogeneous *wMel* localization (n = 13 of 20 and n = 5 of 20 cases, respectively). This differed from control oocytes that mainly exhibited posterior *wMel* localization (19 of 22 cases; Figure 2C and 2C'). These data indicate that microtubules are required for focused posterior localization of *wMel*.

A role for microtubules in *wMel* localization implies that a posteriorly directed microtubule motor such as kinesin-1 is involved. To determine if kinesin-1 participates in *wMel* posterior localization, we created germline mutants for the *Kinesin heavy chain* (*Khc*) gene [23,27,33,34]. *Khc*27 oocytes, null for kinesin function, showed normal anterior *wMel* localization during early stages (Figure S2). However, stage 10A *Khc*27 oocytes exhibited abnormal *wMel* distribution, with *wMel* absent from the posterior cortex in 83% of oocytes (Figure 2D, 2D', 2F, and 2F'; Table 1). *wMel* was also strikingly depleted from the posterior half of *Khc*27 oocytes (Figure 2D and 2F). Thus, kinesin-1 is important to both localize *wMel* to...
the posterior cortex and redistribute wMel into the posterior region.

The role for kinesin-1 in wMel posterior localization may reflect a direct or indirect Wolbachia localization mechanism. One possibility is that kinesin-1 transports wMel to the posterior as a cargo. However, kinesin-1 also drives bulk cytoplasmic streaming during mid- to late oogenesis [27,35,36]. Perhaps streaming currents sweep wMel passively toward the posterior cortex. To test a requirement for streaming in wMel localization, we examined oocytes carrying the hypomorphic mutations Khc17 and Khc23. These alleles give rise to streaming-capable and streaming-deficient oocytes, respectively [27]. Posterior Wolbachia were exhibited by 70% of Khc17 mutant oocytes and 62% of Khc23 mutant oocytes.

### Table 1. Wolbachia Localization in Late Stage 9 and Stage 10A Oocytes

| Condition Tested          | Host Genotype Description | Posterior Wolbachia | Oocytes Scored |
|---------------------------|----------------------------|---------------------|----------------|
|                           |                            | None       | Weak   | Strong |                   |
| wRi in D. simulans        | Wild-type                  | 86%        | 14%    | —      | 22                |
| wMel in D. simulans       | Wild-type                  | —          | —      | 100%   | 18                |
| w; Sp/Cyo; St/TM6Hu       | Wild-type                  | —          | —      | 90%    | 21                |
| khc27 clone               | Null                       | 83%        | 17%    | —      | 23                |
| khc23 clone               | Strong hypomorph           | 38%        | 50%    | 12%    | 32                |
| khc27 clone               | Weak hypomorph             | 30%        | 50%    | 20%    | 20                |
| khc27/Cyo                 | Null/+                     | —          | 23%    | 77%    | 13                |
| ask+/+/Df(3R)XT103        | Null/deficiency            | 65%        | 35%    | —      | 26                |
| ask+/+/Tm6Hu              | Null/+                     | —          | 46%    | 54%    | 28                |
| Df(3R)iptp-XT103/Tm6Hu    | Deficiency/+               | —          | 29%    | 71%    | 21                |
| ask+/Df(3R)iptp-XT103     | Hypomorph/deficiency       | 36%        | 28%    | 36%    | 11                |
| ask+/TM6Hu                | Hypomorph/+                | —          | 33%    | 67%    | 9                 |
| stauR9/+/Df(2R)pC17B      | Null/deficiency            | 37%        | 47%    | 16%    | 19                |
| stauR9/Cyo                | Null/+                     | 12%        | 12%    | 76%    | 17                |
| stauD3/+/Df(2R)pC17B      | Null/deficiency            | 16%        | 49%    | 35%    | 37                |
| stauD3/Cyo                | Null/+                     | —          | 31%    | 69%    | 13                |
| stau1/+/Df(2R)pC17B       | Hypomorph/deficiency       | 17%        | 42%    | 42%    | 12                |
| stau1/Cyo                 | Hypomorph/+                | —          | —      | 100%   | 12                |

Genotypes represent D. melanogaster unless otherwise indicated.

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oocytes (Figure 2E; Table 1). The similarity of posterior wMel localization in these Khc mutants suggests streaming is not needed for posterior Wolbachia localization. Rather, as both Khc27 and Khc23 oocytes retain some kinesin-1 function [27,37], these results indicate that wMel is transported toward the posterior as a cargo of kinesin-1.

Pole Plasm Mediates Posterior Concentration of Wolbachia

A dependency of wMel on kinesin-1 for its posterior localization in oogenesis suggests wMel may rely on the kinesin-1 cargoes osk mRNA and Stau as well. Perhaps wMel hitchhikes to the oocyte posterior as a passenger on osk/Stau messenger ribonucleoprotein particles (mRNPs). Alternatively, wMel may require osk-induced pole plasm for efficient anchorage to the oocyte posterior cortex. To test these possibilities, osk and stau were disrupted with maternal-effect mutations. The majority of these mutant oocytes exhibited depletion or absence of wMel from the posterior cortex compared to wild-type (Figure 2G–2I, 2G–2I'; Table 1), indicating that osk and stau gene products are important for efficient posterior wMel localization. Furthermore, osk and stau mutant oocytes lacking posteriorly concentrated wMel still exhibited a homogeneous bacterial distribution throughout the cytoplasm, differing sharply from the anterior wMel concentrations seen in Khc27 oocytes (compare Figure 2D to 2G). This suggests that kinesin-1 can transport wMel into the posterior half of the oocyte independently of osk/Stau mRNPs. However, kinesin-1 is insufficient to drive robust wMel concentration at the posterior cortex in oocytes with disrupted pole plasm (Figure 2G and 2G'; Table 1). This suggests that pole plasm is important for posterior wMel anchorage.

Kinesin-1 and Pole Plasm Contribute Independently to Posterior Wolbachia Enrichment

To test whether pole plasm is sufficient to drive wMel localization, we examined wMel in oocytes with anteriorly localized pole plasm. To this end, an osk-bicoid 3'UTR transgene was used to target osk mRNA to the oocyte anterior margin [38]. This ectopically localized osk is translated and assembles functional pole plasm at the antero-lateral cortex [38]. wMel co-localized with wild-type Osk protein at the oocyte posterior cortex (Figure 3A–3C, 3A’–3C’). However, wMel did not concentrate at the anterior margin with ectopically localized Osk in osk-bicoid 3'UTR oocytes (Figure 3D–3F, 3D’–3F’), suggesting that pole plasm alone is insufficient to recruit wMel from the cytoplasm. This result, taken together with those above, suggests that individual functions of kinesin-1 and pole plasm are both needed for robust posterior wMel localization in late stages 9 and 10A. This is consistent with a two-step mechanism for wMel localization: kinesin-1-mediated transport of wMel toward the oocyte posterior, followed by pole plasm-mediated anchorage of wMel to the posterior cortex (Figure 4).

Factors Intrinsic to Wolbachia Are Needed for Posterior Wolbachia Localization

The extensive requirement of host components for posterior wMel concentration raises questions about whether wMel contributes to its localization. To investigate this, a trans-infection approach was employed using the host species, D. simulans, that normally carries the wRi Wolbachia strain [39]. In D. simulans oogenesis, wRi exhibited an anterior concentration during stages 3–6 and homogeneous distribution throughout the rest of oogenesis (Figure 1C, 1G, 1G’, 1K, and 1K’; Table 1) [18]. Is this lack of posterior concentration due to differences between host oogenesis machinery or between the wRi and wMel strains? To address this, we examined D. simulans oocytes ectopically transformed with wMel [40]. wMel-infected D. simulans oocytes exhibited anterior Wolbachia concentration during early stages, homogeneous distribution in middle stages, and a striking posterior localization in late stages (Figure 1D, 1H, 1H’, 1L, and 1L’; Table 1). This demonstrates that host components required for Wolbachia posterior localization are present in both D. melanogaster and D. simulans oocytes. Due to strain-specific differences, however, wMel engages those host components to
enhance its posterior concentration in late oogenesis, whereas wRi does not.

Which oocyte components are engaged by wMel but not by wRi? Comparing wMel in osk mutant oocytes to wRi localization in D. simulans reveals a similar homogeneous distribution (Figures 1K and 2G). A speculative interpretation of this similarity is that wMel and wRi are similarly transported into the posterior half of the oocyte by kinesin-1. A further possibility is that wRi is unable to interact with host pole plasm, unlike wMel, which requires pole plasm for efficient posterior localization (Figure 2G and 2G'; Table 1). Perhaps unlike wRi, factors intrinsic to wMel drive interactions with posterior pole plasm that facilitate posterior Wolbachia anchorage (Figure 4).

Discussion

Wolbachia Localization Shares Some Common Features with Other Pathogens

The involvement of kinesin (this study) and dynein [18] in Wolbachia localization during oogenesis is reminiscent of microtubule-based transport employed by a number of human pathogens. Viruses such as herpes simplex virus type 1 rely on dynein and dynactin for their transport to a perinuclear position referred to as their “replication site” [41]. Kinesin transports the viruses back to the cell periphery, enabling their exit from the cell. Bacteria such as Salmonella are transported toward the host cell nucleus in a dynactin/ dynein-dependent manner, which then facilitates bacterial replication [41]. Salmonella also actively recruits kinesin-1 to its surrounding membrane [42]. These observations suggest some parallels with wMel, which requires dynein and dynactin for anterior localization during early oogenesis [18] and kinesin-1 for posterior localization in late oogenesis. While the function of Wolbachia anterior localization is unclear, Wolbachia titer increases substantially at that location, suggesting that dynein-driven localization creates a replication site for Wolbachia within the oocyte [18]. Once replicated, kinesin-1-based transport enables Wolbachia to traverse the entire length of the growing oocyte, promoting Wolbachia incorporation into posterior pole cells. Wolbachia may therefore have sophisticated interactions with host motor proteins analogous to those used by other bacteria and viruses. The basis for a switch between dynein- and kinesin-1-dependent Wolbachia localization is currently unknown. In some systems the dynactin complex coordinates alternation of kinesin- and dynein-driven organelle motility [43]. Perhaps a regulatory agent like dynactin directs the changing Wolbachia localization pattern in oogenesis.

Posterior Wolbachia Anchorage May Be a Cooperative Process

Upon reaching the posterior pole, wMel becomes anchored in a pole plasm-mediated manner. How might this occur? The simplest interpretation is that wMel associates directly with pole plasm components. However, a minority of osk null oocytes exhibited weak posterior Wolbachia localization (Table 1), although pole plasm is absent in this mutant background [38]. This suggests that other factors in addition to pole plasm assist posterior Wolbachia anchorage. Perhaps wMel has a dual affinity for pole plasm and an as-yet-unidentified posterior anchor. In such a case, the combined presence of those substrates may be important for robust Wolbachia anchorage to the posterior cortex. Alternatively, pole plasm may indirectly promote Wolbachia localization by stabilizing Wolbachia anchorage sites. A recent report indicated that Osk regulates actin polymerization at the oocyte posterior cortex [44]. It may be that wMel has a high affinity for unknown factors that associate with the posterior actin cortex, creating an indirect dependency of wMel upon posterior Osk.

One apparent conflict with these selective anchorage hypotheses is the finding that some colcemid- and colchicine-treated oocytes exhibit Wolbachia in association with the lateral cortex of the oocyte (Figure 2A). One interpretation of this result is that Wolbachia may have a general affinity for cortical actin independent of pole plasm. In such a scenario, one would predict that kinesin must normally drive wMel away from the lateral cortex and restrict it to the oocyte posterior where wMel is permitted to bind actin. This type of model has previously been proposed in the context of osk mRNA localization to the posterior pole [24,27]. If this prediction is accurate for wMel also, then oocytes lacking kinesin function should exhibit wMel localization to the antero-lateral cortex. However, wMel did not concentrate on the cortex of Khc null oocytes (Figure 2D). This suggests wMel does not have a general affinity for the actin cortex analogous to osk mRNA. An alternative interpretation of cortical wMel localization in colchicine- and colcemid-treated oocytes is that the drug treatments permitted microtubule remnants to
The study presented here is one of the few to examine host–pathogen interactions in a developmental context. What emerges from this analysis is that the *Wolbachia* localization pattern is unique and does not follow specific morphogens or organelles during oogenesis. The *Wolbachia* localization pattern is distinct from mitochondria, which are concentrated on the posterior side of the oocyte nucleus during early stages, homogeneously distributed during mid-oogenesis, and posteriorly concentrated in stages 9 and 10 [45]. The anterior localization of *Wolbachia* precedes that of the determinant *bicoid* mRNA, which concentrates anteriorly from stages 6 to 14 of oogenesis [46]. *Wolbachia* posterior localization also appears later than *osk* mRNA, which concentrates posteriorly from stages 3 to 6, anteriorly in stage 8, and posteriorly again from stages 8 to 10 of oogenesis. Furthermore, our study indicates that *Wolbachia* do not localize to the posterior cortex in association with *osk* Stau mRNPs. Taken together, these observations suggest that the demands of replication and localization are unique to *Wolbachia* and may preclude these bacteria from hitchhiking on morphogens or organelles.

**Posterior Localization as an Adaptive Strategy for *Wolbachia***

The posterior localization strategy described in our report is exhibited by *Wolbachia* strains carried within multiple *Drosophila* and *Hymenoptera* species [1,9–11,14–17]. This recurrent localization pattern may reflect bacterial adaptations to the host environmental conditions. *D. simulans* allows *wRi* to persist at a high titer during embryogenesis, which is sufficient to promote *wRi* incorporation into posterior pole cells [10]. This environment may provide little incentive for *wRi* to evolve or retain a posterior localization strategy. The *wMel* strain, by contrast, is maintained at lower concentrations in *D. melanogaster* embryos [10]. This may pressure *wMel* to evolve and/or retain mechanisms that drive its posterior localization in oogenesis, thus enhancing its incorporation into embryonic pole cells. Taking advantage of kinesin-1 and pole plasm assembly at the oocyte posterior, as demonstrated by this study, provides an excellent means by which *Wolbachia* can accomplish this goal.

**Materials and Methods**

**Fly strains.** *wMel Wolbachia* were crossed into wild-type *D. melanogaster* flies carrying the markers and balancers *w*; *Sp/Cyo, Sh/ Tm6Hu*. This infected stock was used to cross *wMel* into all the *D. melanogaster* mutants used for this study, ensuring that all carried *wMel* strains of a comparable genetic background.

**Immunolabeling.** Ovaries were dissected and fixed using standard methods [23], then stained and imaged as previously [18]. Rabbit anti-Osk antibodies were used at 1:5000 [49]. Embryos were dechorionated with 50% bleach, fixed 20 min in a 1:1 mixture of 3.7% formaldehyde and heptane, and devitellinized by vigorous agitation in methanol. Embryos were stained with rabbit anti-Vasa at 1:2000 [50] and mouse anti-Hsp60 (Sigma) at 1:100 [18] in PBS/0.1% Triton, followed by 1:500 dilutions of Alexa-488- and Alexa-594-conjugated secondary antibodies (Molecular Probes).

**Microtubule inhibitor treatment.** Flies were starved 18 h, then fed 24–48 h with yeast paste containing 50 μM colcemid, 50 μM colchicine in DMSO, or comparable dilutions of DMSO alone. Mispositioning of the oocyte nucleus served as an internal control to verify that microtubule disruption had occurred [21,51].

**Microscopy and image analysis.** Images were acquired on a Leica DM IRB confocal microscope using a 63x oil objective and zoom factor of 1.5. Each oocyte was imaged as a z-series stack of 7–14 images spaced at 1.5-μm intervals. Optical sections deeper than 4.5 μm into the oocyte were examined for the presence of posterior *Wolbachia*. Oocytes were categorized in Table 1 as showing strong posterior localization if they exhibited striking *Wolbachia* staining, which consisted of either an intense linear array of *Wolbachia* puncta or a crescent-shaped area saturated with *Wolbachia* staining along the posterior cortex for four out of five consecutive z-sections. Oocytes were designated as showing weak posterior localization if they exhibited a) at least one z-section with striking posterior localization, or b) at least two z-sections with a higher *Wolbachia* density along the posterior cortex than in the cytoplasm of the cell. Oocytes were categorized as showing no posterior localization if they did not meet the above conditions.

**Supporting Information**

**Figure S1.** *wMel* and Pole Plasm Localization in Early Embryos

Embryos are shown (A–C) prior to meiosis, (D–F) in cycle 11, (G–I) in...
cycle 14, and (J–L) during gastrulation. Posterior is facing down.

3. Hise AG, Gillette-Ferguson I, Pearlman E (2004) The role of endosymbiotic Wolbachia bacteria in filarial disease. Cell Microbiol 6: 97–104.

4. Clark ME, Veneti Z, Karr TL (2002) Heads or tails: symbiont infection causing cytoplasmic incompatibility in Drosophila. Curr Opin Microbiol 7: 67–70.

5. Theurkauf WE (1994) Premature microtubule-dependent cytoplasmic exclusion restricts pole plasm to the oocyte posterior. Nat Cell Biol 4: 592–598.

6. Ly KT, Casanova JE (2007) Mechanisms of Salmonella entry into host cells. Cell Microbiol 9: 2103–2111.

7. Brainard AW, Mercot H (2005) The role of Wolbachia in Drosophila germ plasm. Curr Biol 15: 225–229.

8. Poinsot D, Bourtzis K, Markakis G, Savakis C, Mercot H (1998) Kinesin heavy chain (P17210), Staufen (P25159), and Vasa (P09052).

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mitochondrial inheritance during Drosophila oogenesis. Development 130: 1579–1590.

46. St Johnston D, Driever W, Berleth T, Richstein S, Nusslein-Volhard C (1989) Multiple steps in the localization of bicoid RNA to the anterior pole of the Drosophila oocyte. Development 107 Suppl.: 13–19.

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. Clegg NJ, Frost DM, Larkin MK, Subrahmanyan L, Bryant Z, et al. (1997) maelstrom is required for an early step in the establishment of Drosophila oocyte polarity: posterior localization of grk mRNA. Development 124: 4661–4671.

49. 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: 3723–3732.

50. Lasko PF, Ashburner M (1990) Posterior localization of vasa protein correlates with, but is not sufficient for, pole cell development. Genes Dev 4: 905–921.

51. Koch EA, Spitzer RH (1983) Multiple effects of colchicine on oogenesis in Drosophila: induced sterility and switch of potential oocyte to nurse-cell developmental pathway. Cell Tissue Res 228: 21–32.

52. Taylor MJ, Hoerauf A (1999) Wolbachia bacteria of filarial nematodes. Parasitol Today 15: 437–442.