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Title: A symbiont's guide to the germline

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Abstract:
Microbial symbioses exhibit astounding adaptations, yet all symbionts face the problem of how to reliably associate with host offspring every generation. A common strategy is vertical transmission, in which symbionts are directly transmitted from the female to her offspring. The diversity of symbionts and vertical transmission mechanisms is as expansive as the diversity of eukaryotic host taxa that house them. However, there are several common themes among these mechanisms based on the degree to which symbionts associate with the host germline during transmission. In this review, we detail three distinct vertical transmission strategies, starting with associations that are transmitted from host somatic cells to offspring somatic cells, either due to lacking a germline or avoiding it. A second strategy involves somatically-localized symbionts that migrate into the germline during host development. The third strategy we discuss is one in which the symbiont maintains continuous association with the germline throughout development. Unexpectedly, the vast majority of documented vertically inherited symbionts rely on the second strategy: soma-to-germline migration. Given that not all eukaryotes contain a sequestered germline and instead produce offspring from somatic stem cell lineages, this soma-to-germline migration is discussed in the context of multicellular evolution. Lastly, as recent genomics data have revealed an abundance of horizontal gene transfer events from symbiotic and non-symbiotic bacteria to host genomes, we discuss their impact on eukaryotic host evolution.

Keywords:
endosymbiosis, germline, vertical transmission, cell-to-cell transfer

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Background: symbiont transmission modes maintain symbiotic associations

Symbiotic associations between microbes and eukaryotes are ubiquitous in nature and provide functions that enable their members to adopt novel niches relative to their ancestors. These associations range from highly integrated intracellular associations where bacteria reside in the host cytoplasm, often encompassed by a host-derived membrane, to extracellular associations where symbionts are housed in special structures or epithelial surfaces [12,95,96]. Functionally, symbioses range from associations in which both host and symbiont benefit from the interaction, termed mutualism, to associations in which only one partner benefits, termed commensalism, to associations in which one member benefits at the expense of the other, termed parasitism. For this review, we will be focusing on interactions toward the mutualistic end of the spectrum (although, some are perhaps better described as addictive [131]), as these situations select for symbiont association with the host germline.

Across the diversity of symbiotic associations, many reliable mechanisms have evolved to facilitate symbiont transmission. They range from horizontal transmission strategies, in which symbionts pass through an environmental intermediate to reach new hosts, to vertical strategies, in which symbionts are inherited directly through parental host tissues (Figure 1). While paternal transmission via sperm occurs [142], it is rare, and vertically inherited symbionts are typically transmitted through the female germline or maternally-brooded embryos [10]. Vertical transmission is associated with highly dependent associations [33] and is thought to evolve through non-additive (epistatic) genetic interactions between host and symbiont that improve the fitness of both partners [25]. Thus, many associations have independently evolved elaborate routes and mechanisms for symbionts to reach host offspring [10].

Remarkably, many associations exhibit evidence of both vertical and horizontal transmission modes [10,28,114], indicating that the mechanisms or constraints required by each mode do not necessarily preclude the other mode. The evolutionary reasons for the existence of these “mixed modes” that incorporate horizontal and vertical transmission are not well understood, but may involve selection for some amount of symbiont gene flow via horizontal transmission to maintain symbiont genome function [114]. Mixed mode transmission certainly requires mechanisms for cell-to-cell transfer to be maintained for the horizontal component of transmission. As we discuss below, many vertical transmission strategies also utilize cell-to-cell transfer to enable symbiont migration from somatic host cells to the germline.

Regarding the evenness of symbiont distribution among host cells and tissues, there is an apparent tendency towards highly specific tissue tropism, or distribution, with increasing association obligacy/dependence. Mutualistic bacterial symbionts often reside in specialized host-derived cells termed bacteriocytes (or sometimes mycetocytes in insects). Bacteriocytes localize to specific host tissues or organs relevant to the symbiont function in the host, termed bacteriomes [45,51,96,105]. For example, chemosynthetic symbionts of bivalves reside in gill bacteriocytes where they have access to reduced chemicals for oxidation and carbon fixation [129], and the amino acid-synthesizing symbionts of aphids reside in bacteriocytes in paired bacteriome structures near the gut [66]. Interestingly, at least in insects, differentiation of these cells is controlled by host genes including the homeotic gene *ultrabithorax* [87]. More recently evolved or facultative associations exhibit less specific patterns of tissue tropism. For example, the more recently evolved *Rickettsia* symbionts of ticks exhibit nonspecific tissue tropism compared to their obligate *Coxiella* symbionts [73]. Similarly, the secondary, facultative symbiont *Serratia* of aphids exhibits a far more disorganized and less specific tissue distribution than the primary, obligate symbiont *Buchnera* [66]. Tissue distribution is an important consideration, as it often indicates how symbionts pass from one host generation to the next.

In this chapter, we examine vertical transmission strategies exhibited by endosymbionts associated with single-cell hosts to multicellular asexual and sexual hosts. By examining associations at these different levels of organismal complexity, we show how strategies scale across cellular and tissue complexity. As symbiotic associations exist across many eukaryotic and bacterial taxa, it is important to consider how strategies correlate with a particular taxonomic group or for some shared lifestyle, etc. For example, while intracellular symbioses are abundant in plants and invertebrate animals, excluding pathogens, they appear to be rare in vertebrates [99]. The only example found to date is in the spotted salamander, *Ambystoma maculatum*, which was thought to exclusively host its algal symbiont, *Oophila amblystomatis,* extracellularly in its egg capsules. However, more detailed investigation showed that some of the algae invade salamander cells and tissues during embryogenesis [62]. Many other examples of vertebrate symbioses exist, such as gut-associated microbiomes [125], but they are all extracellular associations permitted by both the innate and adaptive immune systems [14]. Thus, it appears that aspects of adaptive immunity prevent intracellular bacterial establishment in vertebrates [14,99], limiting symbiont access to the germline.

As we will show, distinct mechanisms of vertical transmission exist across associations, but these mechanisms are united by common strategies for navigating between the soma and/or germline. For clarity, we use germline to refer to the specific lineages in the male and female reproductive organs that lead to the formation of sperm and eggs, respectively. We use the term germ stem cell (GSC) to refer to the stem cells that produce a self-renewing daughter cell and a daughter cell that produces the lineage leading to gamete production [80]. Given that all known endosymbionts descended from free-living ancestors, the mechanisms for interacting with host somatic cells likely predated those for interacting with germ cells. Consequently, we find distinct differences in the continuity of symbiont association with the germline during vertical transmission. The following section describes the three major forms of vertical transmission: soma to soma, soma to germline, and germline to germline (Figure 1, y-axis). Where possible, we also describe the underlying molecular and cellular mechanisms driving vertical transmission processes.

Soma-to-soma strategies of vertical symbiont transmission

There are many examples of transmission strategies that accomplish vertical transmission without symbionts directly associating with the germline. Naturally, this is the only option in host species that do not sequester a protected germline, such as basal metazoans and plants [109]. However, strategies of soma-to-soma transmission also occur in host lineages with germlines, suggesting that germline association is either not necessary, not permitted, or has not yet evolved in these vertically transmitted associations.

An excellent example of vertical transmission exclusively through somatic lineages occurs in the obligate asexual catenulid flatworm, *Paracatenula galateia*, as illustrated in Figure 2A. These platyhelminthes contain chemosynthetic bacterial symbionts and very basic body plans consisting of a limited number of cell types [22]. Reproduction is by asexual fragmentation and relies on stem-cell-like neoblast cells. Neoblasts are also responsible for the worm's regenerative abilities, and produce new symbiont-containing bacteriocytes restricted to the posterior of the worm. In natural reproduction, fragmentation begins along the anterior-posterior axis of the worm, splitting the symbiont population in half [22]. Interestingly, although the neoblasts can become bacteriocytes, they themselves are not infected [22], and so symbionts must be acquired after differentiation by cell-to-cell transfer from the existing infected bacteriocytes [21].

Also lacking a sequestered germline, plants in the families Rubiaceae, Primulaceae, and Dioscorea host endophytic extracellular *Burkholderia*-related bacterial symbionts that induce the formation of the leaf nodules they are housed in [105]. The function of these symbionts is less well-understood than that for rhizobia root symbionts, but might involve cofactor metabolism and/or protection from reactive oxygen species. In these associations, leaf symbionts become associated with embryos when they are trapped after the axillary shoot meristems (*i.e.,* stem cells) differentiate into inflorescences (see Figure 2B). These leaf symbionts exhibit reduced genome sizes relative to their free-living relatives (2-6Mb vs 8Mb) and have more non-coding elements [105], as expected for vertically transmitted symbionts [135]. Thus, while they are extracellular, these bacterial symbionts appear to have high fidelity mechanisms for localizing to specific plant tissues during embryogenesis. Transmission of leaf endophytes in *Dioscorea sansibarensis* occurs through the colonization of asexual reproductive tissues, tubers and bulbils [105], likely enabling far more symbionts to associate than is possible in small seeds. Interestingly, horizontally transmitted rhizobia bacteria localize to cells that respond to bacterially-induced mitotic signals, as opposed to existing dividing cells [39], which may partially explain why none of the rhizobia symbionts are found to be vertically transmitted [10,57].

In species with viviparous development, vertically transmitted symbionts have the option of colonizing offspring during development or during birth. In tsetse flies [111], and bat flies [52], the gammaproteobacterial symbionts *Candidatus* Wigglesworthia glossinidia and *Candidatus* Aschnera chinzeii, respectively, migrate from the soma to colonize the milk gland. During embryogenesis, symbionts colonize offspring through milk secretions fed to the developing offspring [111], as shown in Figure 2C. In the marine ascidian *Lissoclinum punctatum*, intracellular cyanobacterial symbionts are housed within tunic cells (a mesenchyme-like tissue that overlies the epidermis [47]). Instead of colonizing embryos during embryogenesis, symbionts transfer to offspring by direct tissue contact when larvae swim out of the mother's tunic [67]. Similarly, genetic data on the host-associated epithelial microbiome communities in vertebrates indicates that many of these associations are transmitted from mother to offspring by contact [38].

Soma-to-soma vertical transmission strategies need not be contained within host tissues. This is best exemplified by the wide diversity of stink bug species that transfer their symbionts externally, in extracellular host-derived secretions. There are two main strategies for external transmission reported to date among stink bugs and relatives (Insecta: Heteroptera: Pentatomomorpha): egg-smearing with symbiont-containing secretions or deposition of symbiont-containing capsules. Species in the families Acanthosomatidae, Cydnidae, Pentatomidae, Pyrrhocoridae, and Scutelleridae apply symbiont-containing secretions to egg surfaces during oviposition [49,58,59,63,107,108]. After hatching, nymphs probe the surface of the remaining egg mass and acquire symbionts [49]. In an amazing behavioral modification of egg-smearing, females of the subsocial stink bug *Parastrachia japonensis* wait until five minutes before egg hatching to apply symbiont-containing mucus to the eggs, conferring their infection [50]. A modification of egg-smearing is seen in stink bugs in the family Urostylididae, which produce a symbiont-infected jelly that coats eggs upon oviposition [60]. Production and deposition of symbiont-containing capsules can be seen in the Japanese common plataspid stink bug, *Megacopta punctatissima*. In this species, gammaproteobacterial symbionts are deposited in specialized capsules alongside egg masses. Upon hatching, nymphs consume these capsules, acquiring the symbionts needed for normal growth and development [37].

Vertical transmission through the germline Part 1: migration from the soma to oocytes/embryos

The vast majority of vertically transmitted symbioses exhibit transmission strategies involving the transfer of symbionts from somatic tissue directly to mature gametes or offspring (vertical transmission: soma-germline diagram in Figure 1 and Supplemental Table 1). It is perhaps not surprising that the majority of endosymbionts exhibit very specific tissue distributions and only reside within bacteriocytes in adults, but it does suggest that many vertically transmitted symbionts cannot or have not evolved mechanisms to remain continuously associated with the germline. Below, we describe three general categories of this pattern based upon how and when symbionts become associated with host gametes or offspring, and the complexity of host tissues.

Transmission without tissue types: sponge symbiont transmission

Basal animal taxa such as sponges present an excellent system to investigate basic regulation of symbiont localization and transmission, as they lack true tissues, and simply consist of multiple cell types [11]. As depicted in Figure 3A, sponge bodies consist of choanocyte (feeding cell) chambers suspended in mesohyl, lined with a layer of pinacocytes to serve as an external barrier. The mesohyl makes up most of the sponge by volume, and is an extracellular matrix consisting of connective tissue, sponge cells, and bacterial symbionts [44]. Oocytes are located in the mesohyl [139], and in cases of internal fertilization and viviparity, embryos are also brooded in the mesohyl, sometimes in specialized chambers [61,120,126,140].

During sexual reproduction in sponges, four main strategies for vertical symbiont transmission have been observed [30]: 1) symbionts are phagocytosed into oocytes from their extracellular locations in the adult mesohyl [85], 2) bacteriocytes transfer symbionts to brooded embryos [85,119], 3) symbionts are transferred extracellularly via mucus [61], and 4) symbionts are sequestered in the extracellular space between the egg and the follicle cells during oogenesis, with the symbionts entering the embryo during cleavage [29]. Even in the simplest of multicellular body plans, there are a wide diversity of mechanisms and strategies for accomplishing proper localization patterns in the soma and germline. Sponges are also capable of asexual reproduction by fragmentation [43], further increasing the strategies available to them for symbiont transmission.

Extracellular storage of vertically transmitted intracellular symbionts

A distinct transmission strategy involves not only the extracellular transfer of endosymbionts during the vertical transmission process, but also their storage. For example, in the whiteflies *Aleurochiton aceris* and *Bemisia tabaci*, symbionts are positioned under the vitelline envelope, outside the oocyte plasma membrane. In these host species, the primary and secondary bacterial symbionts travel together in host bacteriocytes from the body cavity to the ovary, beginning at the last larval instar stage. There, they pass between the posterior follicle cells to sit next to the oocyte plasma membrane, becoming enclosed between it and the vitelline envelope produced by the follicle cells [18,134] (Figure 3B). Between five and seven bacteriocytes associate with each oocyte, which contain dense aggregations of actin. The bacteriocytes appear to maintain an extracellular position in both *A. aceris* [134] and *B. tabaci* [18] through the end of oogenesis. In a similar strategy, the gammaproteobacterial symbionts of *Greenisca brachypodii* scale insects, *Candidatus* Kotejella greeniscae and *Arsenophonus*, migrate between and through follicle cells to cluster at the connection between the oocyte and supporting cells. Once there, they cluster at the anterior of the developing oocyte in a deep, but extracellular depression of the oocyte plasma membrane, and are enclosed by the egg envelope [89] (Figure 3C).

While extracellular routes of oocyte-mediated symbiont transmission are relatively rare in insects, they may be common in marine molluscs, as the vesicomyid and solemyid species examined to date exhibit evidence consistent with this trend. In the deep-sea chemosynthetic clam *Calyptogena okutanii*, the

chemosynthetic gammaproteobacterial symbionts were shown to be located under the vitelline envelope at the vegetal/posterior pole of mature oocytes [54] (Figure 3D). Similarly, in *Solemya velum*, symbionts may be associated with the oocyte perimeter [116]. It is not clear why this strategy is used in these species, as both broadcast spawn their gametes directly into the oceanic water column, exposing them to environmental dangers. Perhaps these related chemosynthetic symbionts, which are more closely related to free-living colorless sulfur-oxidizing bacteria than other endosymbionts, do not possess the mechanisms needed to colonize the oocyte directly, or colonization is prohibitive to host development.

Intracellular symbiont transmission from the soma to the germline involves cell-to-cell transfer

Obligate endosymbiosis is at a very high frequency among hemipteran insect taxa, such as aphids, mealybugs, whiteflies, and planthoppers, due to an exclusive diet of nutrient-poor plant fluids [42]. In these associations, symbionts provide metabolites such as amino acids to supplement the host's nitrogen-poor diet [24]. The majority of these taxa host one primary symbiont, which is always present and co-speciates with the host, and one or more secondary symbionts, which are more facultative and have their own evolutionary histories independent of the host. Both primary and secondary symbionts are vertically transmitted in these associations [24], but they may have different cellular routes of inheritance [66]. In many of these associations, intracellular symbionts pass from adult bacteriocyte cells to gametes or embryos through cell-to-cell transfer mechanisms. Below, we describe what is known about the best studied of these associations.

In pea aphids, *Acyrthosiphon pisum*, females reproduce by either sexual or asexual reproduction depending on the season. Over the summer months, many asexual generations are produced via telescoping viviparous parthenogenesis (in which adult aphids contain embryonic aphids, containing embryonic aphids), and sexual eggs are produced for overwintering. In aphid sexual reproduction, the gammaproteobacterial endosymbiont *Candidatus* Buchnera aphidicola is delivered directly to the posterior pole of the oocyte in the final stages of oogenesis via cell-to-cell transfer from the follicle cells [94] (Figure 4). During parthenogenetic embryogenesis, *Buchnera* are transported in host bacteriocytes to the posterior pole of the blastula [94]. There, *Buchnera* are exocytosed from the bacteriocytes and endocytosed by the blastula membrane, and incorporated into the syncytial cytoplasm [66]. These bacteria localize near host nuclei in the mesodermal syncytium and become enclosed in individual cells during cellularization, producing a new generation of bacteriocytes (Figure 4). Interestingly, the secondary symbiont *Serratia* is taken up by the blastula from the hemolymph, and sorts separately from *Buchnera* during cellularization [66]. During later stages of embryogenesis, bacteriocytes cluster together, forming a paired bacteriome organ that remains in close proximity to the germ cells throughout development, maintaining this position in the adult [66,94]. As a limited number of *Buchnera* are transferred in either reproduction mode, the vertical transmission process imposes a fairly harsh bottleneck on within-host symbiont population sizes [91].

Appropriation of symbionts to the germline or embryo from somatic tissues late in development is a common strategy for vertical transmission, and routes between tissues can be complex. For example, the *Arsenophonus*-like bacterial endosymbiont of human lice, *Candidatus* Riesia pediculicola [3], completes a complex pattern of migration across host cells and tissues during development, crossing extracellularly from germline in embryogenesis to the soma during the nymphal stages, and back to the ovary in the adult. There, symbionts pass into fully developed eggs through hydropyle structures in the shell [103]. Similarly, during embryogenesis the fat-body bacteroidetes symbionts of cockroaches and the related basal termites (genus *Mastotermes*) migrate extracellularly from the embryonic bacteriome to pre-bacteriocyte cells in the fat body [5,74], and then migrate from there to the ovary in the second nymphal instar [118]. During the third and fourth instars, symbionts exit bacteriocytes and migrate

extracellularly across the ovariole sheath, between the follicle cells, and to the plasma membrane of the oocyte. There they are surrounded by microvilli until after vitellogenesis when these bacteria are taken up by oocytes via pseudopod-like extensions [118]. In *Camponotus floridanus* carpenter ants, endosymbionts also have a dynamic pattern of migration during development, ending up in the midgut prior to metamorphosis. They are thought to migrate from this tissue to the ovary [130], colonizing oocytes shortly after division from the stem cell [72]. Lastly, in one of the more bizarre localization patterns reported, the gammaproteobacterial symbionts of mealybugs reside within a second betaproteobacterial endosymbiont, and are transported to oocytes in this configuration. The nested symbiont cells are transported within bacteriocytes from the symbiont-housing organ (bacteriome) in the abdomen to the ovary. There, they are released from host cells, cluster around the connection between the oocyte and supporting cells (similar to scale insects [89]), and are taken into the germline at this point [23].

Interestingly, several associations demonstrate the phenomenon of the "symbiont ball", where symbionts cluster together in a clumped, ball-shaped structure during transmission and embryogenesis. While some symbiont balls are bound by the oocyte membrane [89], the examples below are not. In brown planthoppers, yeast-like symbionts appear to migrate from the adult fat body to the ovary, passing between follicle cells to enter the posterior oocyte cytoplasm in late oogenesis. There, they form a ball of symbionts that migrates, and ultimately colonizes the fat body in the embryonic abdomen [98]. Similarly, the *Carsonella* and *Profftella* symbionts of the Asian citrus psyllid, *Diaphorina citri*, migrate extracellularly from the abdomen to the oocyte where they pass between the follicle cells and are incorporated in the oocyte as a ball [19]. In the stink bugs *Nysius ericae, Nysius plebius*, and *Nithecus jacobaeae*, bacteriocytes containing gammaproteobacterial symbionts exist within membranes adjoining those of previtellogenic oocytes, and transfer symbionts across the membranes. As oocytes mature, symbionts form a ball at the oocyte anterior [86,133]. Although it is not its normal distribution, a symbiont ball can also be seen in wMel-infected *Drosophila melanogaster* when symbiont transport via host microtubules is increased [115].

Passage of symbionts between follicle cells for direct uptake by vitellogenic host oocytes is another common theme in ovarial symbiont transmission strategies, as described above for scale insects, termites/cockroaches, planthoppers, and psyllids, and has been shown for the adelgid aphid *Adelges viridis* and its betaproteobacterial symbionts [88]. Fortunately, this is also how the *Spiroplasma* symbionts of the model organism *Drosophila melanogaster* are transmitted, enabling experiments to determine the underlying molecular and cellular mechanisms. Work by Herren *et al.* 2013 has shown that in infected *D. melanogaster*, *Spiroplasma* colonize the oocyte following extracellular transport from the hemolymph. After passing between the follicle cells of vitellogenic oocytes (stages 8-10), symbionts are endocytosed with yolk granules and use the Yolkless receptor involved in normal yolk uptake from follicle cells [46]. Given the diversity of symbionts that enter the oocyte through the peri-follicular space [19,94,98], the high yolk content of insect embryos [56], and that intracellular pathogens have also been found to co-opt the yolk machinery for ovarial transmission [46], this is a potential mechanism for other endosymbionts.

Infection of oocytes in pre-vitellogenic stages of oogenesis is observed in other associations in addition to those in stink bugs. In the bulrush bug, *Chilacis typhae*, symbionts are housed in bacteriocyte-like cells in the midgut epithelium as well as in the ovary germaria. Symbionts enter oocytes from the surrounding cells near the posterior of the germarium [71]. Other examples of transfer to pre-vitellogenic oocytes may exist, however, resolving the position of symbionts in the germarium's dense tissue structure may limit the detection of this transmission route.

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The full and precise details about how symbionts colonize host tissues during host development is not known for many associations, but much can be inferred from their localization patterns in adults. For example, *Rhipicephalus* spp. ticks host *Coxiella* sp. symbionts, which are present in the malpighian tubules of both males and females, as well as in the female gonad. While it is unclear when in development the *Coxiella* symbionts migrate to the ovary, Lalzar *et al.* 2014 showed that they are at high concentration in the oviduct and interstitial ovary cells, and associate with host oocytes beginning in mid-oogenesis. Interestingly, *Coxiella* concentrate at opposite poles during mid-oogenesis (stage 3) and become restricted to one pole by late oogenesis (stages 4-5) [73]. Based on studies in *Drosophila,* this suggests that these symbionts may rely on the host actin and microtubule cytoskeleton and microtubule motor proteins [32,115,124].

Vertical transmission through the germline Part 2: migration from the soma to germ stem cells (GSCs)

While the examples above demonstrate that many symbionts colonize the germline late in gametogenesis, or even after fertilization during embryonic development, some symbionts have evolved strategies for colonizing the primordial germ stem cells. There are a number of reasons that a symbiont might be selected to colonize the germ stem cell. These include: 1) sequestration with the GSC to prevent the symbiont genome from accumulating mutations during cell division and to protect the symbionts from host cellular defenses [109,113], 2) manipulation of host reproduction [31,35], and 3) insurance that the symbiont is present in all host offspring.

Transfer from somatic cells to the germ stem cell is well documented in *Wolbachia*, a widespread bacterial endosymbiont of insects and filarial nematodes [16,144]. During early embryogenesis in filarial nematodes, *Wolbachia* preferentially concentrate in the blastomere leading to lineages that produce the germline and hypodermal chords, a somatic tissue that runs the length of the body. However, when this blastomere divides, the bacteria are excluded from the daughter blastomere destined to form the germline, and instead concentrate in the daughter blastomere destined to form the hypodermis [75]. *Wolbachia* remains there until the fourth larval instar stage when a portion of the population migrates extracellularly from the hypodermis to the neighboring distal somatic sheath cells of the gonad, and from there colonize the germline stem cells. This process occurs in the female germline, but not the male. This migration is associated with a disruption in cortical actin, consistent with a *Wolbachia*-induced endocytic event. The bacteria then concentrate in the ovary syncytium, possibly using the host's actin-rich rachis structure for motility, or replication [35,75]. Interestingly, *Wolbachia's* requirement for host fertility may be partially explained by its localization to the germline stem cell. In *B. malayi*, the wBm strain of *Wolbachia* confers mitotic quiescence in the stem cells, but then activates transit-amplifying mitotic replication when cells divide away from the niche. In uninfected hosts, this process is incorrectly regulated, resulting in apoptosis and sterile oocytes [35,75].

Vertical transmission through the germline Part 3: continuous germ cell association throughout development

Continuous association with the germline presents the most basic form of linkage with host reproduction for symbionts and appears quite important for bacterial taxa such as *Wolbachia*. While it is likely that other manipulative bacteria such as *Rickettsia* may exhibit similar patterns, the data are limited, so we present what is known currently about continuous germline association strategies, starting with the most basic form in unicellular hosts.

Segregation with host cell division: the first form of vertical transmission

The most rudimentary form of vertical transmission is symbiont segregation to host daughter cells during mitosis in unicellular hosts. Conceptually, this can be accomplished two ways: 1) via active coordination with the host cell machinery or 2) by having a high concentration of symbionts passively distributed around the dividing cell. Mitochondria, which arose via an ancient endosymbiotic event in the single-celled eukaryotic ancestor, appear to use both strategies in different organisms and cell types. Active mechanisms that shuttle mitochondria along microtubules to daughter cells are used during asymmetric cell division in budding yeast and during *Drosophila* oogenesis [92]. Passive mechanisms involve mitosis-induced fission of multiple, fused mitochondria, which then are subdivided into daughter cells by cytokinesis [69].

In more recently evolved unicellular endosymbioses, both active and passive inheritance strategies are observed. For example, in *Hartmannella* species of amoeba, the alphaproteobacterial symbiont *Nucleicultrix* localizes to the host nucleus and segregates with the daughter nuclei during binary fission (*i.e.,* cell division) [121]. In contrast, the methanotrophic archaeal symbionts of *Nyctotherus* spp. ciliates are randomly distributed among daughter cells following host cell division [48], suggesting they likely utilize a passive strategy.

These mechanisms for vertical transmission in single-celled hosts form the basis for intracellular interactions that not only enable transmission to the next generation, but facilitate the proper colonization of the somatic cells in multicellular hosts. For example, as discussed above, the wBm strain of *Wolbachia* segregates with particular host cell lineages during early embryogenesis in filarial nematodes [75]. If mechanisms exist to precisely control patterns of symbiont segregation to host daughter cells during cell division, these can be used to ensure proper tissue tropism during development. An example of this was discussed earlier for the pea aphid, *Acyrthosiphon pisum,* in which *Buchnera* and *Serratia* symbionts exhibit independent, tightly coordinated movements with host syncytial nuclei and cells during embryogenesis [66] (Figure 4). Similarly, in *Drosophila, Wolbachia* segregate asymmetrically during the larval neuroblast stem cell divisions such that they fate map to specific regions of the adult brain [2]. In this way, vertically transmitted symbionts can achieve specific tissue localization patterns via faithful segregation with particular cell lineages during development.

Symbiont segregation with the primordial germline

Given their affinity for the germline, it not surprising that many *Wolbachia* strains that infect insect species appear to localize to the germline continuously throughout development. Patterns in continuous germline localization are variable from strain to strain, but commonalities exist among strategies in a somewhat modular fashion, indicating that different strains share different combinations of mechanisms. An overview of what is known about *Wolbachia* localization to the germ line during development is presented below (and see Figure 5).

In adult *Drosophila* flies, the wMel, wWil, and wAu strains of *Wolbachia* are found within the germline stem cells at the end of the ovariole terminal filaments [32,90]. The wMel strain has been shown to segregate with both the regenerating germline stem cell and the differentiating daughter cell in *Drosophila melanogaster*, ensuring high vertical transmission fidelity [2]. Interestingly, while wMel segregate equally between germline stem cells and their daughters, they segregate unequally between neuroblast stem cells and daughter cells [2], suggesting that *Wolbachia* are able to manipulate these processes depending on the cell type.

As oogenesis progresses, wMel replicate and are transported on host microtubules to colonize the nurse cells and developing *D. melanogaster* oocyte. In early oogenesis (stages 3-6), *Wolbachia* concentrate at the anterior end of the oocyte [32,124]. This localization pattern is dependent on the host minus-end directed microtubule motor protein dynein, as both the depolymerization of microtubules and knockdown of dynein heavy chain or its dynactin linker protein disrupt anterior localization [32]. Following microtubule reorganization at stage 7 of oogenesis, *Wolbachia* become evenly distributed throughout the oocyte cytoplasm [124]. Starting at stage 9, the germ plasm begins to form in the cytoplasm at the posterior pole of the oocyte, which will form the germline in embryogenesis [84,127]. Slightly after germ plasm assembly begins, wMel use the host plus-end directed microtubule-dependent motor protein kinesin heavy chain to move to the posterior pole and colocalize with the germ plasm, as evidenced by inhibition of this process via microtubule depolymerization and kinesin heavy chain knockdown [124]. Importantly, wMel achieve kinesin-mediated transport without interfering in host development by being a poor competitor for this motor protein (see Box 1). Following stage 10a of oogenesis, the remaining *Wolbachia* contained in the nurse cells are dumped into the oocyte during nurse cell dumping [115,124], which also appears to occur in the *Westeberhardia* gammaproteobacterial symbionts of *Cardiocondyla obscurior* ants [65].

Germ plasm colocalization during oogenesis is also reported for wMel in *Drosophila simulans,* wWil in *Drosophila willistoni*, wSty in *Drosophila teissieri, Drosophila yakuba, and Drosophila santomea,* wCle in *Cimex lectularius* bed bugs, wAtab in *Asobara tabida,* the strain in *Aphytis,* the strain in *Trichogramma, and* the strain in *Nasonia* wasps [9,20,51,90,106,141,148]. Interestingly, other strains exhibit alternative localization patterns that may mimic other patterning factors. *Wolbachia* strains wMo, wKa, and wKi in *Drosophila simulans* exhibit anterior localization, similar to the *bicoid* mRNA gradient during embryogenesis. In a range of *Drosophila* species, the wRi strain achieves an even distribution across late oocytes and embryos [2]. Compared to wMel, wRi colonizes *D. simulans* and *D. melanogaster* oocytes at higher titers, ensuring that at least some *Wolbachia* are included in the posterior-located germ plasm [124,141].

In the embryo, wMel within the germ plasm become cellularized with the pole cells during cycle 11 of embryogenesis [124,141], and from within these primordial germ cells, undergo migration to the future gonad as in normal fly development [4]. While strains such as wAu and wMel colonize the somatic embryo in addition to the pole cells [2,90,124], wWil from *D. willistoni* exhibits strict tropism for the pole cells and the resulting germline stem cells [90]. Thus, several strains of *Wolbachia* are able to target the germline continuously throughout development, and some do so to the exclusion of the somatic tissue. See Boxes 2 and 3 for further discussions about how *Wolbachia* interacts with the germline to influence host reproduction.

Over the evolutionary history of *Wolbachia*, many strains have switched hosts [110,137] through horizontal transmission events [1,78,82]. Host switching requires mechanisms of cell-to-cell transfer and germline targeting to restart the vertical transmission process. Processes for both the release from and uptake into host cells happen readily for wMel in cell culture [145]. *Wolbachia's* route from the soma to the germline is observed in experiments on whole female flies injected with wMel-infected hemolymph [36]. After injection, *Wolbachia* migrate through the hemolymph to the ovary. There, they enter the oocytes through the germarium's somatic stem cell niche, as opposed to localizing to the germ stem cells or their niche directly. The normal pattern of germ stem cell localization is, however, resumed in the next vertically transmitted generation [36]. Given that the somatic stem cell niche is a route to the oocytes in other associations (discussed above for stink bugs and bulrush bugs), it may be the ancestral route for *Wolbachia,* used before these bacteria gained access to the germ stem cell.

One other association which may prioritize appropriating symbionts to the germline over the functional somatic tissue is the leafhopper *Scaphoideus titanus*. In *S. titanus*, *Cardinium* symbionts (Bacteroidetes) are transmitted to oocytes from adjacent bacteriocyte cells in the ovary. Interestingly, during nymphal development, symbionts are continuously associated only with the ovary, as the fat body and salivary gland are not colonized until adulthood [117], suggesting that the bacteria associated with the germline form a discrete population from the bacteria in the soma, with little need for exchange between the two.

Continuous germ line association is not the norm among strictly vertically transmitted symbionts

While continuous association with the germline may conceptually be the most intimate/integrated form of vertical transmission, it clearly cannot be in reality because few endosymbionts exhibit this pattern of inheritance. According to patterns of genome evolution, the obligate endosymbionts of plant-feeding insects are some of the most extreme examples of evolutionarily derived symbionts (relative to their free-living relatives) known. Compared to free-living taxa with genome sizes of 3-7Mb, vertically transmitted insect symbionts possess genomes down to 0.2-0.6Mb, often only containing genes for their symbiotic functions and even lacking genes for cellular maintenance [135]. However, few of these associations remain associated with the germline continuously, and instead exhibit one of the various forms of soma-to-germline routes of vertical transmission.

In contrast, the genomes of many *Wolbachia* endosymbionts exhibit evidence that they experience environments other than the host cell, suggesting that they are less integrated with their hosts than some of the endosymbionts that have to migrate to the ovary from the soma. The genomes of the various *Wolbachia* strains are around 1.3Mb and contain abundant mobile elements [64,83,147]. As the cut and paste activities of mobile elements across the genome are generally deleterious, the elements become inactivated and lost over time. Thus, finding active copies in the genome indicates that these bacteria experience regular exposure to the environment, during which time they acquire new, functional mobile elements [135]. Interestingly, the strains in supergroups C and D do exhibit signs of genome degradation and stasis, consistent with their obligate nature and strict vertical transmission strategy [15]. So, while highly integrated endosymbionts may continuously associate with the germline, they do not seem to require it.

Parallels between symbiont transmission and the origin of the germline

Similar to the evolution of multicellularity and the germline itself, a range of solutions have evolved for handling and distributing inherited endosymbionts among cells and tissues in organisms of differing cellular and structural complexity. Multicellularity has independently evolved at least 25 times in organisms ranging from bacteria to single-celled eukaryotes [112]. While all of these make some form of reproductive cell type, not all have a dedicated and sequestered tissue type for it, i.e., a germline. For example, no discrete germline evolved in plants and basal animal taxa, such as cnidaria, and platyhelminthes. Instead, gametes are generated in the adult from pluripotent stem cells in the soma [109]. Thus, a germline is clearly not necessary for sexual reproduction and multicellular development. Similarly, despite vertically-transmitted endosymbionts' ties to host reproduction for their own reproduction, continuous localization within the germline or pluripotent stem cells is not generally observed (see sections II and III above, and Supplemental Table 1).

It is interesting to consider how the population of host tissues and sequestration of a germline affects mitochondria, the intracellular relict of the endosymbiont that enabled oxidative phosphorylation in the

ancestral eukaryote. Oogamy and uniparental mitochondrial inheritance evolved long before germline sequestration, and cannot be implicated in its evolution via older hypotheses such as to avoid competition among mitochondrial genotypes [17,27] or to enable tissue complexity [55]. Instead, it has been proposed that mitochondrial loss of function mutations drove the sequestration of the germline in animal early development. When per-generation mitochondrial mutation rates are high, either due to a high per-division mutation rate (*e.g.*, due to metabolically-induced damage [40]) or a high number of genome/cellular replications, or both, sequestration of the germline serves to limit the number of mutations acquired by the mitochondrial genome. Over-replication of mitochondria during oogamy further enables effective selection for the best phenotype/genotype, restoring the functions served by selecting mitochondria from a large somatic population [109].

During multicellular development endosymbionts are not distributed to every cell type in the body like mitochondria organelles, but are restricted to particular tissues like differentiated cell types [13,22,143]. Assuming the same population-level principles apply to endosymbionts as mitochondria, the Radzvilavicius *et al.* 2016 theory suggests that mutation accumulation is not an issue for most endosymbionts. This could potentially be due to low symbiont mutation rates and/or the inability to ramp up symbiont copy number during oogamy to enable within-individual selection for fit genotypes [109]. In these cases, the large variance in mutational loads of symbionts across host cells may help ensure a functional symbiont is available for transmission. Many endosymbiont populations number in the millions to billions of cells in somatic tissue [26,68,93,122,146] and are typically at much lower abundances in the germline [32,54,91,116]. Thus, mechanisms may be selected that enable the choice of symbionts from the much larger somatic pool of genotypes to occupy the germline. Perhaps relevant to this topic, population sizes of the human head lice endosymbiont [103] and the carpenter ant endosymbionts [130] change radically throughout development.

Another popular theory for the evolution of multicellularity and germline sequestration proposes that cellular processes required for animal life cause mutational damage, thus somatic cells have been relegated to the job, protecting germ cells from mutations that could be passed to offspring [40]. While this is unlikely to be the driver for endosymbionts, which are generally thought to have evolved associations with their hosts for their functions in somatic tissues [95], it may offer insights into influential processes. For example, if symbionts are unable to regulate their metabolic functions on a tissue-level basis, then they could cause more harm than benefit if they were to continuously associate with the germline. By restricting symbionts to very specific cell types (*i.e.*, bacteriocytes) in host tissues, often in large numbers, the mutational damage can be kept isolated, and a large symbiont pool is left available to be selected from to occupy the germline. See Box 4 for a further discussion of the evolutionary pressures facing somatic and germline-associated symbiont populations.

Conclusions

Our review of the literature reveals that the majority of microbial symbionts across a diversity of host and symbiont taxa are transmitted to the host germline from somatic cells or tissues, making association with the host germline rare for endosymbionts. It is not clear why this pattern prevails, but it could be due to either a lack of need for continuous association or a constraint involving its evolution. For example, it is conceivable that the presence of symbionts at particular stages of primordial germline formation could disrupt the process.

Bacterial access to the germline has impacted host genome evolution and has prepared hosts for interacting with microbial symbionts. It has been repeatedly observed that insect genomes contain

horizontally transferred genes from bacterial genomes. As any horizontally transferred gene must make its way to the germline to be inherited with the rest of the genome, transfers from germline-associated symbionts have high likelihoods of inheritance. While some of these transfers are from recent endosymbionts, such as the new sex chromosome in the isopod *Armadillidium vulgare* [79], others are from unassociated, or not currently associated bacteria. For example, mealybug genomes contain genes from diverse bacterial lineages that likely supplement functions lost from their endosymbionts' reduced genomes [53]. Psyllid genomes contain metabolic genes from many different bacteria that complement symbiont pathways [128], and aphid genomes contain non-*Buchnera* genes whose products are transferred to the *Buchnera* symbionts [97]. Furthermore, in a recent preprint, Blondel *et al.* propose that the *oskar* gene, which is necessary and sufficient to induce the formation of the germline in holometabolous insects, is the product of a horizontal gene transfer event combined with a fusion event between a bacterial gene and a eukaryotic gene [7]. Thus, germline-associated endosymbionts may even be implicated in the formation of the germline itself.

While these studies have provided significant insights regarding the patterns and mechanisms of vertical endosymbiont transmission, much remains unknown. Key outstanding questions include: Why is continuous association with the germline rare in vertically inherited endosymbionts? Are there events during germline development that are particularly susceptible to interference by the presence of an endosymbiont? What are the molecular and cellular mechanisms by which different endosymbionts target and associate with the host germline? What are the host and endosymbiont factors that determine endosymbiont abundance in the germline? How genetically diverse are within-host symbiont populations, and how does that diversity impact the inclusive fitness of symbionts relegated to the soma (and thus destined never to reproduce)? And similarly, how do vertical transmission strategies impact the size and genetic diversity (*i.e.*, genetic bottleneck) of inherited symbiont populations? As more knowledge is gained about host and symbiont genomics, and we learn how symbiont and host genes function, answers to these questions will be forthcoming.

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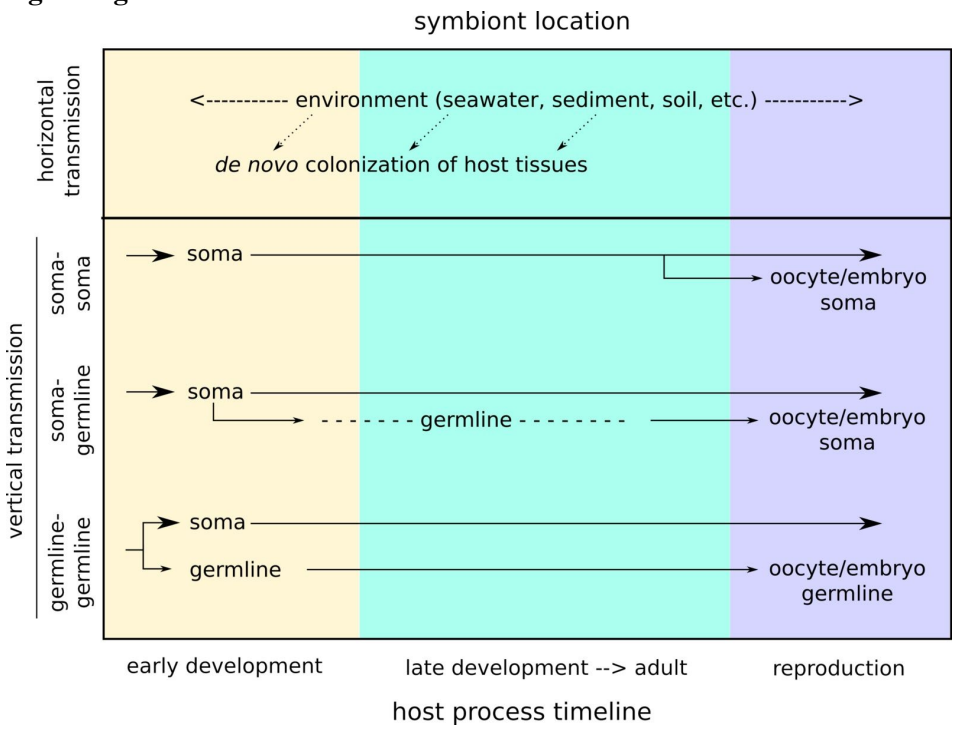

Figure 1. General location patterns of symbionts during host development and reproduction in horizontally transmitted associations and vertically transmitted associations with three different strategies based upon when and how symbionts colonize the soma, germline, gametes, or offspring.

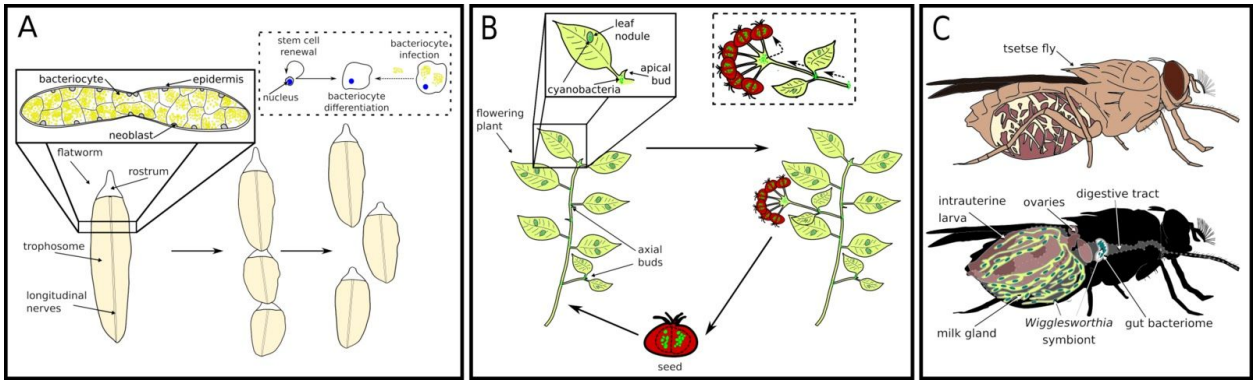

Figure 2. Examples of soma-to-soma vertical transmission strategies. A) Transmission of chemosynthetic alphaproteobacterial symbionts during asexual reproduction by fragmentation in *Paracatenula galateia* requires cell-to-cell transfer to bacteriocytes after they divide from neoblasts and differentiate [22]. B) Cyanobacterial plant leaf nodule symbionts are transmitted vertically by colonizing the apical and axillary bud tissue after germination, from which they colonize either vegetative shoots or reproductive shoots [105]. C) The obligate intracellular gammaproteobacterial symbiont of tsetse flies, *Wigglesworthia glossinidia*, is housed in paired bacteriomes off the midgut. Symbionts are thought to colonize the milk gland through the digestive tract, where they are transmitted extracellularly via milk gland secretions to intrauterine larvae [111].

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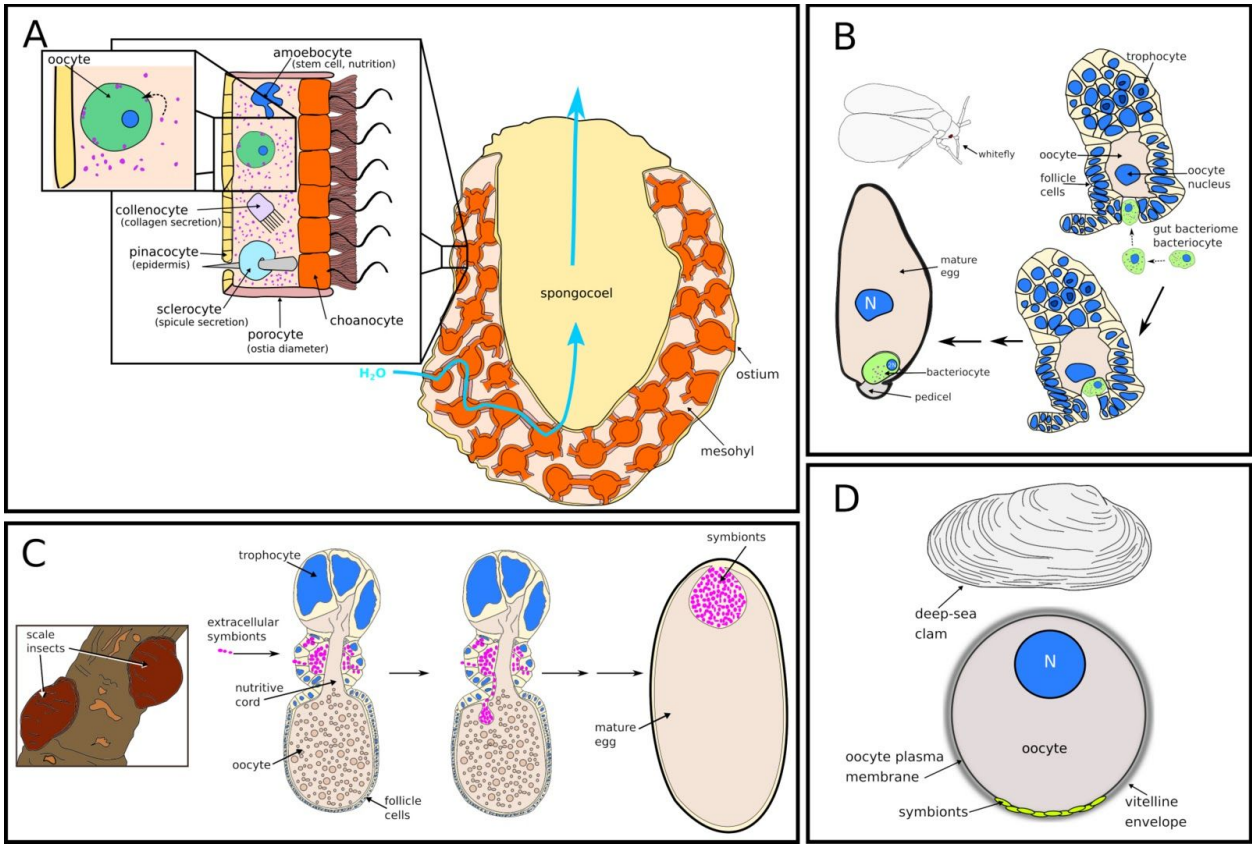

Figure 3. Examples of soma-to-germline vertical transmission strategies that use extracellular routes. (A) Illustration of a demosponge body plan and route of symbiont transfer to oocytes/embryos from extracellular populations in the mesohyl. (B) In whiteflies, bacteriocytes containing bacterial symbionts migrate from the gut bacteriome to the ovary and become associated with the perivitelline space between the oocyte plasma membrane and the follicle cells prior to vitelline envelope formation [134]. This results in the bacteriocyte being located under the shell, but outside the oocyte, at the posterior of mature eggs [18], as illustrated. (C) The two gammaproteobacterial symbionts of scale insects migrate extracellularly from dissociated bacteriocytes in the gut bacteriome to the ovary, where they cross between or through follicle cells to become associated with the perivitelline space between oocyte and follicle cell plasma membranes. As illustrated, this collection of extracellular symbionts becomes enclosed in the perivitelline space in the mature oocyte [89]. (D) Similarly, the vertically transmitted symbionts of deep-sea chemosynthetic clams localize to the perivitelline space of broadcast spawned eggs [54], as illustrated.

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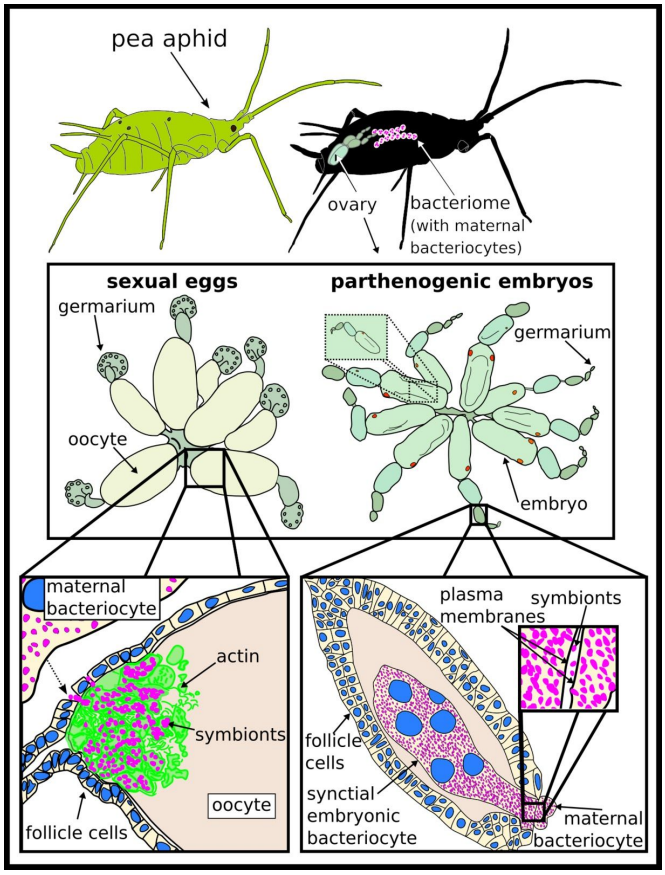

Figure 4. Intracellular soma-to-germline vertical transmission strategies illustrated for sexual and parthenogenic pea aphids infected with the primary gammaproteobacterial symbiont, *Buchnera aphidicola*. In both modes of host reproduction symbionts are transmitted from maternal bacteriocytes, that originate in the gut bacteriome, to the posterior of developing oocytes or embryos through cell-to-cell transfer. During development, the bacteriome remains in close contact with the germband, ultimately residing near both the gut and the ovary [8,66,94].

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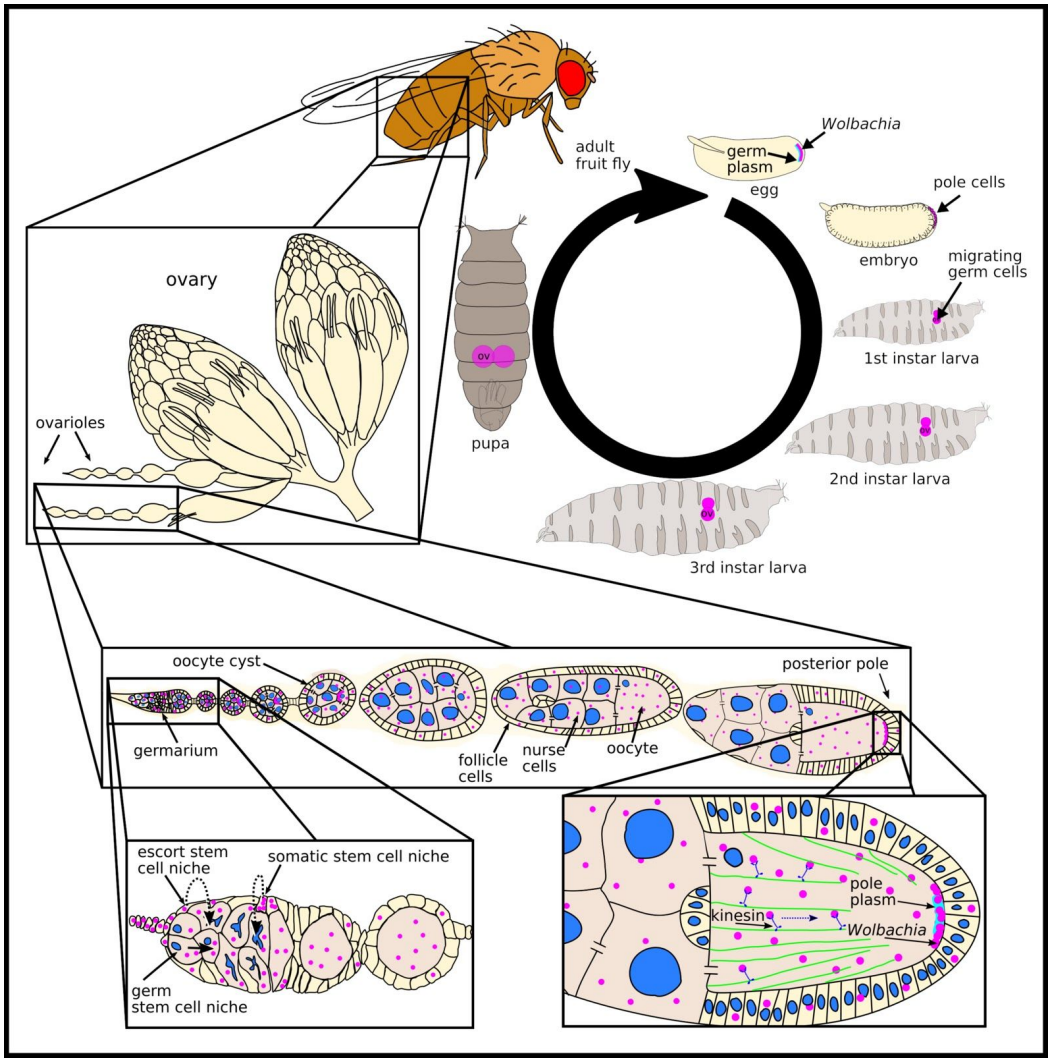

Figure 5. Continuous germline association, as exemplified for *Drosophila* fruit flies infected with strains of *Wolbachia* bacteria. Some strains of *Wolbachia,* such as wWil in *D. willistoni*, localize to the germline throughout development by first localizing to the germline stem cell and somatic stem cell niches in the adult ovary. During oogenesis, *Wolbachia* localize to the germ plasm at the posterior pole of the oocyte. The localization patterns established during oogenesis persist in the embryo, and presence at the posterior pole of the oocyte/embryo enables the bacteria to be enclosed in host pole cells during cellularization. These cells then migrate during embryogenesis to form the primordial germline and future gonad [124,136,141]**.**

Inset Boxes:

Box 1. Wolbachia use but do not abuse the host transport system

Wolbachia's migration through the developing oocyte to the posterior pole plasm is coincident with recruitment of factors required for germline formation as well as for anterior/posterior and dorsal/ventral axis formation [124]. Thus, *Wolbachia* must navigate and use the host transport system without disrupting transport of vital host components. In oocytes in which *Wolbachia* titer is too high, dorsal/ventral axis determination is disrupted [123]. Insight into the mechanisms by which *Wolbachia* ensures its localization

to the posterior pole without disrupting germline establishment comes from a recent study by Russell *et al.* 2018 demonstrating that *Wolbachia* is a weak competitor for the plus-end directed motor protein kinesin heavy chain. Knocking down a key kinesin linker protein, kinesin light chain (KLC) that associates with a number of host components required for pole plasm formation, surprisingly results in a dramatic increase of *Wolbachia* at the posterior pole. One interpretation of this result is that knocking down KLC results in a greater concentration of kinesin for *Wolbachia's* poleward transport. That is, kinesin is limiting for *Wolbachia* but not host components. Experimental support for this idea comes from finding that overexpressing kinesin in the oocyte results in a dramatic increase of *Wolbachia* at the posterior pole, similar to KLC knockdown. Thus, *Wolbachia* may have evolved to weakly compete with host cargo for association with motor proteins, ensuring that germline formation is not disrupted. A key next aim is to identify the kinesin linker protein used by *Wolbachia*. Whether this is a host protein or a protein encoded by *Wolbachia* is unknown.

Box 2. Manipulation of host reproduction by *Wolbachia*-induced cytoplasmic incompatibility

In addition to navigating the germline, *Wolbachia* interacts and dramatically influences a diversity of germline functions including transcription, translation, and the cell cycle [16]. Because *Wolbachia* is exclusively transmitted through the female germline, manipulations that serve to promote infected female fecundity and provide a selective advantage to infected females serve to increase *Wolbachia* infection frequencies in the population. These include *Wolbachia*-induced male killing, feminization, parthenogenesis and, most famously, cytoplasmic incompatibility (aka. CI).

Wolbachia-induced CI is a conditional form of male sterility. Matings between *Wolbachia*-infected males and uninfected females produce dramatically reduced hatch rates. However, if the females are infected, normal hatch rates occur whether she mates with infected or uninfected males. Thus, in an infected population, infected females are at a great selective advantage over uninfected females. Both in the laboratory and field settings, CI results in rapid sweeps of *Wolbachia* through insect populations [70,138]. Cellular analysis of embryos derived from the CI-cross exhibit a failure of the paternal chromosome complement to condense and properly align on the metaphase plate. Consequently, there is either a partial or complete failure of paternal chromosome segregation during the first zygotic division. Subsequent analysis revealed the proximal cause of these defects in paternal chromosome dynamics is a delay in the protamine-histone exchange that occurs as the sperm transitions into a pronucleus. Likely as a consequence of these delays, DNA replication, nuclear envelope breakdown, CDK1 activation, and entry of the paternal chromosome set into anaphase are delayed. Interestingly, in crosses between infected females and infected males, condensation and segregation of the paternal chromosome set is normal. Furthermore, neither CDK1 activation nor mitosis is delayed [76], as would be expected if the infected female simply had matching modifications on its chromosomes. Instead, some *Wolbachia*-generated product in the female germline may reverse the male modification.

Recent insight into the molecular basis of CI may help resolve these two models. Proteomic studies of sperm derived from infected and uninfected male mosquitoes identified a wPip *Wolbachia* protein only present in the former. This protein, currently named CidA, is encoded in an operon containing a second gene, *cidB*, which encodes a deubiquitylating enzyme. Transgenic male *Drosophila* expressing CidA and CidB produce paternal chromosome segregation defects strikingly similar to those observed in CI crosses [6]. Biochemical studies showed that CidA binds and inhibits CidB, inhibiting its deubiquitylating activity. These results support a toxin-antitoxin model for how sperm and oocyte modifications induced by *Wolbachia* function in cytoplasmic incompatibility. A parallel study by LePage *et al.* 2017 using wMel *Wolbachia* relied on bioinformatics approaches to identify bacterial genes that correlate with CI induction, returning CifA and CifB, homologs of the *cidAB* genes [81]. These genes are encoded by a

prophage integrated into the wMel genome. A number of lines of evidence strongly suggest CifA and CifB induce CI. First, these genes are present in CI-inducing strains but absent in non-CI inducing strains. Second, as with the Beckman *et al.* 2017 study, expression of *cifA* and *cifB* in the germline of males expressing CI resulted in chromosome segregation defects equivalent to those observed in CI crosses and reduced hatch rates. Furthermore, normal hatch rates were recovered when these transgenic males were mated to *Wolbachia*-infected females.

These exciting findings raise a number of questions, most significantly what are the targets of the CidB deubiquitylating enzyme? What is the relationship between CidAB and the cell cycle and chromosome defects observed in CI crosses? How is rescue achieved by infected female oocytes? It is an exciting era for *Wolbachia* functional and genomic research.

Box 3. *Wolbachia* control of germline differentiation

Accumulating evidence from different strains of *Wolbachia* suggests that these intracellular symbionts have the ability to modulate host cell differentiation by controlling host gene expression. In *Drosophila melanogaster,* the wMel strain is able to rescue loss of the *sex-lethal* (*sxl*) and *bag-of-marbles* (*bam*) genes, both of which are involved in controlling germ stem cell maintenance and daughter cell differentiation in early oogenesis [34,132]. Recently, Ote *et al.* (2016) found that expression of the *Wolbachia* protein Toxic manipulator of oogenesis (TomO) is able to rescue the germline stem cell maintenance function of Sxl. However, another *Wolbachia*-encoded factor must be involved in full *Wolbachia*-based *sxl* rescue, as TomO does not restore female fertility. The mechanism by which TomO rescues Sxl function appears to be through the disruption of mRNA complexes, which causes increased Nos expression [100]. TomO has also been found to bind and disrupt other mRNA complexes at later stages of oogenesis, such as those bound to orb mRNA [101], suggesting that this bacterial protein may broadly regulate host translation.

In support of the idea that *Wolbachia* is a general regulator of host cellular differentiation, *Wolbachia* control over this process has been shown in other strains and host taxa. Recently, it has been shown that the presence of wBm in *Brugia malayi* filarial nematode ovaries controls germ stem cell quiescence. If wBm are removed by antibiotic treatment, then the germ stem cells differentiate and are lost. Further along in the ovary, this inappropriate loss of quiescence manifests as oocyte apoptosis and polarity defects, culminating in a loss of fertility [35,77]. *Wolbachia* have also been reported to be required for normal oogenesis in *Asobara tabida* wasps because they inhibit apoptosis [102]. However, the results from filarial nematodes suggest that a mechanism also involving control of host cell differentiation may be involved upstream of the apoptotic effects.

Box 4. Endosymbionts: case-studies in kin selection

As a whole, endosymbionts with somatic populations that function to better host fitness and transmit a subset of their population to host offspring present a case study in kin selection [41]. Next to nothing is known about how or when symbionts are selected for transmission or relegation to a lifetime in the soma, with no individually-gained fitness. Maintaining low intrahost genetic diversity likely helps, as there is nothing on which to select [113]. Alternatively, selecting symbionts from the somatic population, as many associations do, may help keep the population honest and prevent “cheaters”, i.e., symbionts receiving benefit from, but not providing services to the host. Thus, as long as they are genetically related, symbionts in the soma increase their fitness by enabling symbionts in the germline to reproduce and be transmitted to host offspring. This is analogous to how sibling reproduction increases the inclusive fitness of sexual eukaryotes [41].

Endosymbionts with more general tissue tropisms and less well-resolved contributions to host fitness, such as *Wolbachia*, present an interesting thought experiment on how these situations evolve. Somatic support for germline symbionts can either evolve as primary factors, when symbionts start out as tissue infections, or secondary factors, when germline-associated bacteria colonize the germline to improve host fitness. As there are few reasons besides selfish ones for a symbiont to exclusively colonize the germline, the second situation likely represents an initially pathogenic/parasitic one. *Wolbachia's* fairly unspecific somatic tissue distribution, and its lack of a need for specific bacteriocyte cells [104] suggests that *Wolbachia* began as a general somatic infection, like some *Rickettsia* species [73], and has evolved highly specific germline-association mechanisms from there. However, intriguingly, in bed bugs, *Cimex lectularius, Wolbachia* occupies bacteriocytes and produces vitamin B12 for the host [51]. The alternative is also possible, that it was originally exclusively germline associated, which implies that a pathogenic relationship turned commensal and then beneficial, or at least addictive [131], sometime in the history of its relationship with insects. In either case, once symbiont and host reproductive interests have become linked, symbiont populations should be selected to be well mixed and have minimal diversity so that symbiont reproduction in the germline fulfills the fitness interests of the symbionts in the soma.

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