Reproduction

Germ cells colonized by endosymbiotic bacteria

Wolbachia (Rickettsiaceae) is a genus of maternally inherited endosymbiotic bacteria commonly found in the reproductive tissues of arthropods. These bacteria manipulate host reproduction to increase the number of infected individuals within the population, erecting intraspecific fertility barriers, causing parthenogenesis or resulting in the feminization of genetic males[1]. They are usually transmitted vertically, so we predicted that they should have evolved a mechanism to target the host's germ cells during development. Here we show that *Wolbachia* become concentrated in the germ plasm of the *Drosophila* egg. Mutations in developmental patterning genes[2] demonstrate that this localization is dependent on the assembly of germ plasm.

The endosymbiotic bacteria (fluorescent staining in Fig. 1) are maternally inherited and sensitive to the antibiotic tetracycline. Analysis of their DNA after amplification by polymerase chain reaction (PCR) revealed an *ftsZ* sequence[3] that was identical to that of *Wolbachia* strain A. This sequence was the same in both the Canton S wild-type *Drosophila* stock and the laboratory stock[4] that we used as a source of endosymbionts for all the other stocks.

These bacteria are found throughout the syncytial embryos of infected strains of *Drosophila*, concentrating in the region of the cortical actin cytoskeleton and accumulating with the mitochondrial motor protein KLP67A on the microtubules of mitotic asters[4–6]. These mechanisms might ensure transmission of the bacteria, as would colonization of the posterior pole, the site of germ-cell formation. This has been reported for *Nasonia* wasp embryos, which also show cortical localization[7,8], but not for *Drosophila*.

To identify the host genes required for posterior localization of *Wolbachia* in the absence of asters, we counted the bacteria in four regions of unfertilized eggs (Table 1) from wild-type females and from females bearing mutations in *gurken* or *oskar*, two maternally acting genes that determine the site of germ-cell formation. The posterior pole region P1 had a mean bacterial density

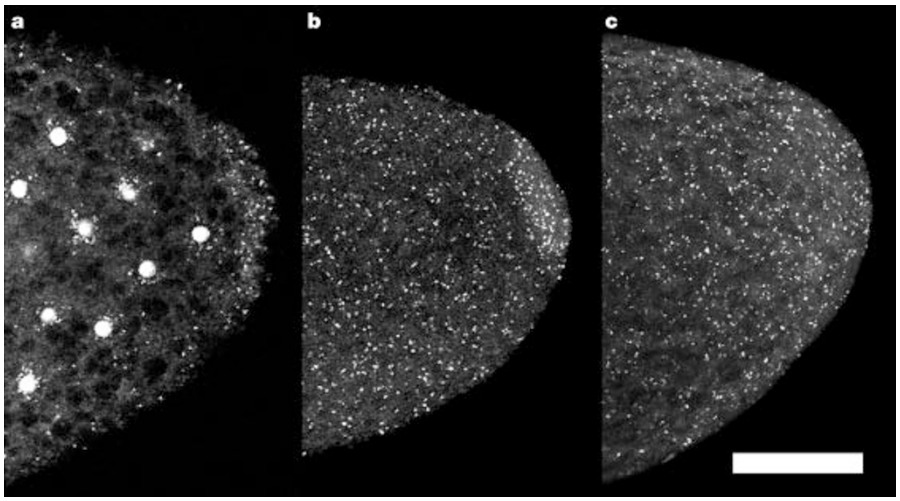

Figure 1 Colonization of *Drosophila* germ cells by *Wolbachia*. **a,** *Wolbachia* clustered on asters around proliferating nuclei and concentrated in the germ plasm on the posterior dorsal side of a wild-type *Drosophila* embryo (single optical section). **b,** Germ-plasm colonization in an unfertilized egg from a heterozygous *oskar*^{5}/+ mother. **c,** Unfertilized egg lacking germ-cell determinants from an *oskar*^{5}/*oskar*^{5} mother. Scale bar, 50 μm. Serial 2-μm optical sections of *Drosophila* eggs were fixed in methanol and stained for DNA with H33258, collected on a Bio-Rad MRC 1024ES confocal microscope with multi-photon excitation, using a 40× 0.85NA (air) lens set at a zoom of 1.5 and a pixel size of 0.388 μm, and then converted to a single two-dimensional image by maximum brightness projection.

significantly different from those of areas A1, A2 and P2 in eggs from wild-type and heterozygous mothers. The bacteria were not localized in eggs from homozygous mutant mothers. The posterior localization of *Wolbachia* therefore requires not only the functional anteroposterior axis of polarized microtubules specified by Gurken signalling[9], but also the germ plasm determined by Oskar[2].

The eggs of our Canton S strain had a density of $3.04 \pm 1.04 \times 10^6$ bacteria per mm^3. We used published data[7] to calculate the density in the *Nasonia vitripennis* egg as $0.51 \pm 0.13 \times 10^6$ bacteria per mm^3. In contrast to the tight posterior localization of *Wolbachia* in the *Nasonia* embryo, most bacteria in *Drosophila* colonize somatic cells. The fate of these bacteria is unknown, but they may infect the germ line later in development.

Alternatively, the symbiosis may be less well established in *Drosophila*. We considered that transferring *Wolbachia* from *Nasonia* to *Drosophila* would show whether the germ-cell signals of the two species are recognized by a conserved bacterial mechanism. We found that early germline association seems to be symbiotic: *Wolbachia* infection was not a significant variable influencing the number of germ cells or the fidelity of their migration to the gonad in the Canton S strain (results not shown).

Posterior localization of *Wolbachia* was also abolished in eggs of *Drosophila* that are mutant for the *vasa* or *tudor* genes, which encode germplasm components localized by Oskar (data not quantified). Functional germ-cell determinants are localized in the mature oocyte (stage 14)[10], but we could find no oocytes at stages 1–14 that had posterior localization of *Wolbachia*, in contrast to 80% of unfertilized eggs 0–3 hours after egg deposition (results not shown).

If germ plasm is merely a rich food source that stimulates local proliferation, why are *Wolbachia* not seen there earlier in oogenesis? Although we have not excluded the possibility of rapid local proliferation after egg deposition, we think it is more likely that the bacteria seen in ovarian nurse cells and oocytes relocate in response to a component of pole plasm that is translated only after egg activation[11].

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Table 1 Quantification of *Wolbachia* in regions of the *Drosophila* egg

Genotype	*Bacterial density (mean ± s.d. × 10^6 per mm^3)*			
Canton S	P2(2.57 ± 1.47)	A2(2.79 ± 0.98)	A1(3.29 ± 1.10)	P1(4.65 ± 2.10)
gurken^{3}/+	A2(2.04 ± 0.94)	A1(2.19 ± 0.76)	P2(2.34 ± 1.17)	P1(3.99 ± 1.65)
oskar^{5}/+	P2(2.22 ± 1.27)	A2(2.94 ± 1.67)	A1(2.96 ± 1.57)	P1(4.26 ± 1.80)
gurken^{3}/*gurken*^{3}	A2(2.10 ± 1.32)	P2(2.24 ± 1.00)	A1(2.29 ± 0.89)	P1(2.45 ± 1.36)
oskar^{5}/*oskar*^{5}	P2(1.88 ± 0.66)	P1(1.95 ± 0.66)	A2(2.06 ± 0.56)	A1(2.11 ± 0.76)

Bacterial density in 10 unfertilized eggs of each genotype, measured in a 95 × 29 μm ellipse placed at the anterior pole (A1), two sites (A2 and P2) at

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