LETTERS

Somatic stem cell niche tropism in *Wolbachia*

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Wolbachia **are intracellular bacteria found in the reproductive tissue of all major groups of arthropods[1,2]. They are transmitted vertically from the female hosts to their offspring, in a pattern analogous to mitochondria inheritance. But *Wolbachia* phylogeny does not parallel that of the host, indicating that horizontal infectious transmission must also occur[3–5]. Insect parasitoids are considered the most likely vectors, but the mechanism for horizontal transfer is largely unknown[4,6,7]. Here we show that newly introduced *Wolbachia* cross several tissues and infect the germline of the adult *Drosophila melanogaster* female. Through investigation of bacterial migration patterns during the course of infection, we found that *Wolbachia* reach the germline through the somatic stem cell niche in the *D. melanogaster* germarium. In addition, our data suggest that *Wolbachia* are highly abundant in the somatic stem cell niche of long-term infected hosts, implying that this location may also contribute to efficient vertical transmission. This is, to our knowledge, the first report of an intracellular parasite displaying tropism for a stem cell niche.**

The presence of *Wolbachia* in 20–80% of all insect species[1,2] is attributed to extensive horizontal transfer. However, propagation within a new population ultimately requires vertical transmission, which entails not only infection of the host soma, but also infection of its germline. Previous studies using a number of different insect and crustacean species have shown that inoculation of *Wolbachia* into the abdominal cavity of females results in stable vertically transmitted infection[8–10], suggesting that *Wolbachia* is able to reach

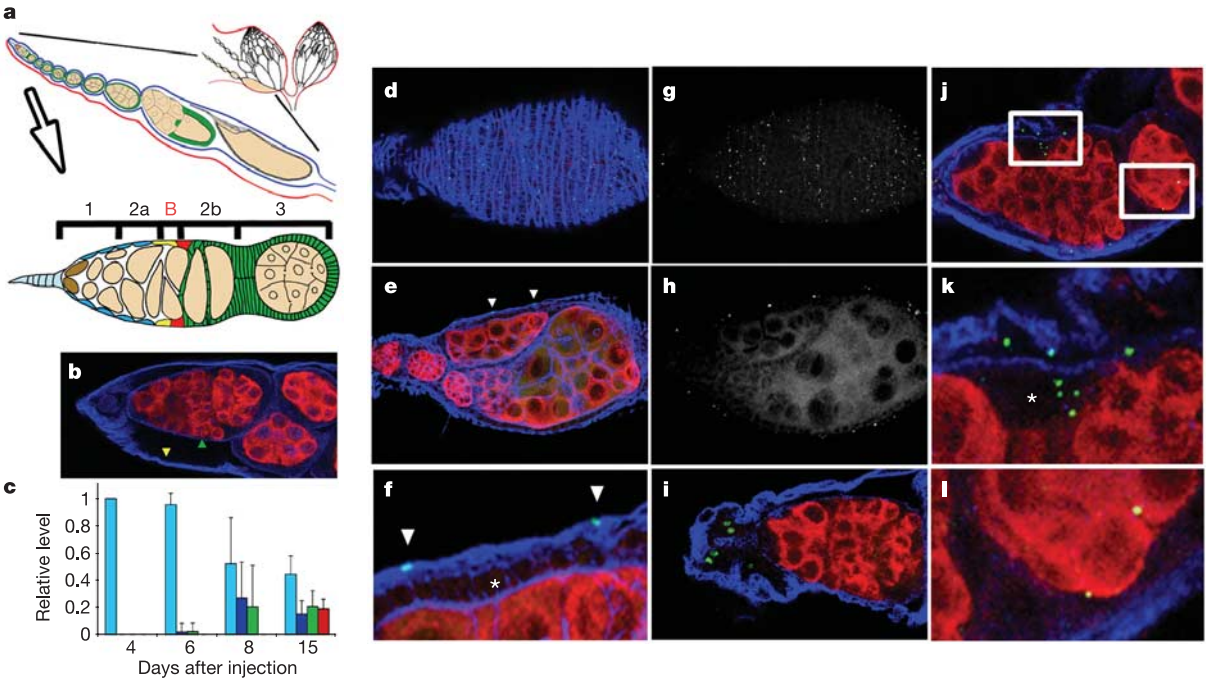

Figure 1 | *Wolbachia* introduced in the abdominal cavity reaches the germline. **a**, A drawing of the *Drosophila* ovary (top right) surrounded by a thin peritoneal sheath (red). Each ovariole (middle) is encased by a muscle epithelium (blue). Egg chambers are formed in the germarium (magnified at the bottom). Germarial regions are indicated. As the germline progresses from region 2a to 2b, it becomes enveloped by the somatic cells (green) derived from 2–3 somatic stem cells (SSC, red cells). For the purpose of this study, region 2 is divided in 2a, 'border' and 2b. The 'border' contains the SSC and its niche ('B', in red), formed by the anteriorly localized inner germarium sheath cell (IGS, yellow cells) and extracellular matrix. **b**, Control germarium injected with haemolymph from non-infected flies. Germline is in red (all figures). Laminin at muscle (yellow arrow) and germarium basement membrane (green arrow) are shown in blue. **c**, Relative levels of *Wolbachia* at muscle (light blue), muscle lumen (dark blue), follicle cells (green) and germline (red), at different days after *Wolbachia* injection ($n = 33$ ovarioles; 2,392 bacteria particles; error bars represent standard deviation). **d–l**, Ovaries of hosts injected with *Wolbachia* (green). Germline is shown in red. In **d–f**, muscle and follicle cell cortex actin are in blue. In **i–l**, laminin at muscle and germarium basement membrane are shown in blue. **d**, Ovary muscle 6 days after injection, showing muscle actin fibres with high levels of *Wolbachia*. **g**, *Wolbachia* channel. **e**, Same ovary as in **d**, germline plane. *Wolbachia* (arrowheads) at the muscle. **h**, *Wolbachia* channel of **e**. **f**, Higher magnification of **e**, *Wolbachia* not present at the follicular epithelium (asterisk). **i**, Germarium 8 days after injection. *Wolbachia* present at the muscle lumen around terminal filament. **j**, Germarium 15 days after injection. Boxes are regions depicted at higher magnification in **k** and **l**. *Wolbachia* present in the border region (asterisk in **k**) and germline (**l**).

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the germline from the infected soma. However, the mechanism underlying this mode of infection has not been characterized. To monitor the dynamics of germline colonization, we injected the *Wolbachia* wMel strain into the abdominal cavity of uninfected *D. melanogaster* females (see Methods) and imaged ovaries at different days (see below). Stocks established from injected flies still maintained *Wolbachia* in the germline 6, 20 and 29 generations after initial injection (Supplementary Fig. 1), indicating that our injection resulted in invasion of the germline, production of mature eggs containing *Wolbachia* and ultimately stable vertical transmission.

To reach the female host germline, injected bacteria need to cross three different tissues: a peritoneal sheath membrane surrounding the entire ovary, a muscle epithelium enclosing each individual ovariole and the somatic tissue enveloping the germline (Fig. 1a). We find that *Wolbachia* sequentially infect tissues of the host ovary in discrete stages (Fig. 1c) and ultimately colonize the germline 15 days following inoculation (Fig. 1l). Furthermore, the pattern of infection suggests that the route to reach the germ cells is through the germarium (Fig. 1). By days 4 and 6, high levels of *Wolbachia* were seen at the muscle surface (Fig. 1d–h), and between the muscle and the germarium basement membrane after 8 days (Fig. 1i). 65% (117 out of 220) of the *Wolbachia* associated with the germaria at this stage were found in the lumen between the terminal filament and the overlying muscle layer. However, *Wolbachia* entered the ovary preferentially at the border of the germarial regions 2a and 2b (hereafter referred as the 'border' region, Fig. 1j, k). This site is notable for harbouring two to three somatic stem cells (SSC)[11,12] (Fig. 1a).

The injection experiments suggest that specific somatic regions of the germaria may provide preferred sites for targeting during new infection. To determine whether the same regions would also be targeted in the case of long term maternally transmitted infections, we examined the *Wolbachia* levels of specific ovarian cell types in long term maternally infected flies. In such females, the germline cytoplasm contains higher levels of *Wolbachia* than the cytoplasm of follicle cells at all stages of oogenesis (Supplementary Fig. 2a). The only exception was in the border region, where we observe a high accumulation of *Wolbachia* (Fig. 2a–c) reminiscent of transient infections (Fig. 2i).

To determine whether the accumulation of *Wolbachia* in the border region is mainly concentrated in the SSC themselves, we characterized the *Wolbachia* distribution in germaria in which the SSC were marked by labelling of clonal lineages. These experiments indicated that the highest bacterial levels are observed anterior to the stem cells, often distributed in two major clusters (Fig. 2d–f). In 8 out

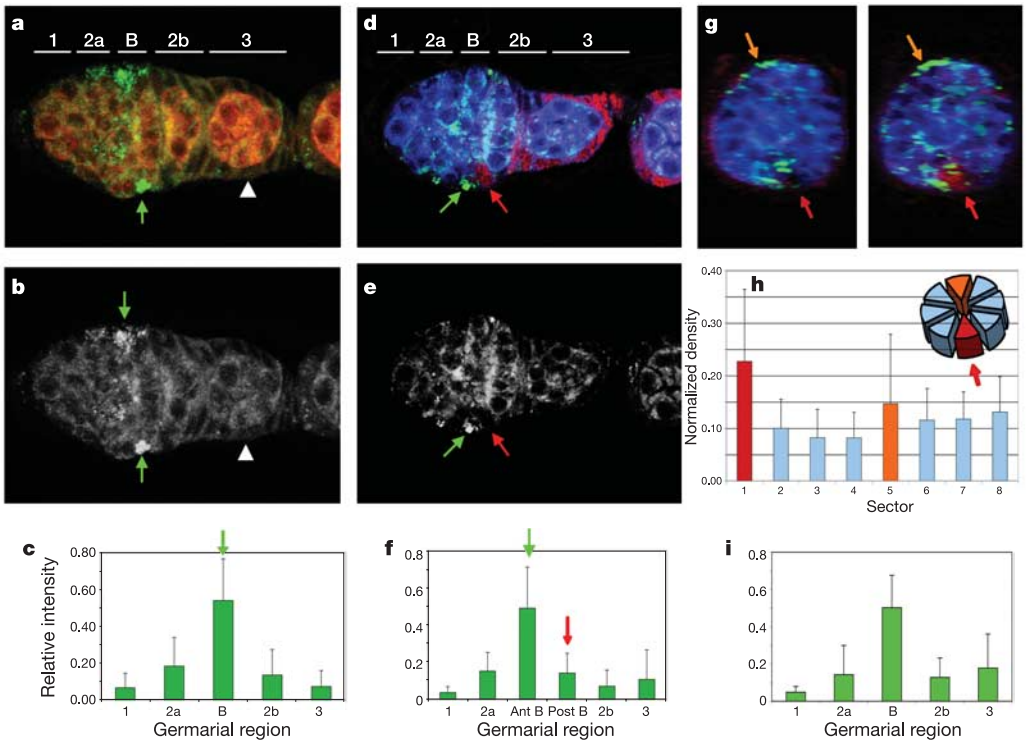

Figure 2 | *Wolbachia* preferentially infect the somatic stem cell niche (SSCN). **a**, Germarium of a maternally infected stock. High levels of *Wolbachia* (green) are seen in the somatic tissue at the 2a/2b border (regions indicated on top). Most of the remaining somatic tissue (arrowhead) has low levels of *Wolbachia* relative to the germline (red). **b**, *Wolbachia* channel, showing two major clusters of *Wolbachia*. **c**, Relative intensities of *Wolbachia* in the soma at the different germarial regions ($n = 26$). Green arrows in **a**, **b** and **c** point to *Wolbachia* accumulation at the border. **d**, SSC and its lineage are labelled in red, *Wolbachia* in green, germline in blue. SSC is the most anteriorly labelled cell (see Methods). *Wolbachia* accumulation (green arrow) is at the SSCN, anterior to the SSC (red arrow). **e**, *Wolbachia* channel from **d**. **f**, The border region is divided into the anterior portion containing the SSCN (green arrow) and the posterior portion containing the SSC (red arrow). Relative *Wolbachia* intensities are shown ($n = 14$). **g**, Overlays of reconstructed slice data at the SSC (red staining on right image) and immediately anterior to the SSC (left image). Reconstructions were performed to yield slice data perpendicular to the anterior-posterior axis. A radial axis consisting of eight sectors was defined on the SSC plane and oriented so that sector 1 contained the SSC (red arrow). This radial axis was then used to divide the slices of the 2a/2b border region that were anterior to the SSC into eight sectors (see drawing at **h**). Arrows point to *Wolbachia* staining at the sector defined by the SSC (red arrow) and at the sector radially opposed to it (orange arrow, see below). **h**, Normalized *Wolbachia* density by sector in the portion of the 2a/2b border region anterior to the SSC. Sector 1 (red bar at histogram and red arrow at drawing) contains the SSCN which is anterior to the labelled SSC. As the 2a/2b border region usually contains two radially opposed SSC as well as their associated SSCN, sector 5 (orange) may contain the SSCN of a second unmarked SSC ($n = 10$). **i**, Relative intensity of *Wolbachia* in ovarioles from uninfected flies inoculated with *Wolbachia*. Relative levels in the soma for germarial regions 15 days after injection are similar to maternally infected flies ($n = 8$). Error bars in **c**, **f**, **h**, **i** represent standard deviation.

of 10 cases, the highest levels of *Wolbachia* were contained within a sector directly anterior to the SSC ($P = 0.037$; Fig. 2g, h). This sector is predominantly composed of inner germarium sheath (IGS) cells anterior to the SSC that are constituents of the microenvironment harbouring the SSC, the so-called somatic stem cell niche (SSCN, Fig. 1a)[13–15].

Could the infected SSCN itself be a source of *Wolbachia* into the germline? Germline cells contact the SSCN as they pass through this region, and afterwards remain in contact with precursors of the follicle cell epithelium arising from the SSC. Thus, an infected niche can potentially transmit *Wolbachia* to the germline by two routes, either directly or by infection of the SSC and its derived follicle cell population. We have evidence suggesting both of these possibilities. In occasional germaria with only one infected niche, we can compare levels of *Wolbachia* in the half containing the infected SSCN to those in the other half. In 16 such germaria (Supplementary Fig. 2b–d and Supplementary Movie), somatic and germline regions anterior to the SSCN show no bias in *Wolbachia* levels in the two halves of the germarium (Supplementary Table). However, the somatic regions posterior to the SSCN show increased *Wolbachia* levels in the half containing the infected SSCN in 14 out of 16 cases, with an average increase of 62 ± 21%, suggesting that a SSC residing in an infected SSCN also becomes infected and its follicular progeny consistently have a greater degree of *Wolbachia* infection ($P = 1.6 \times 10^{-5}$). In the border region, we also see an increase of 52 ± 22% in germline *Wolbachia* levels in the half of the germarium containing the infected niche ($P = 1.9 \times 10^{-3}$). However, that bias does not seem to be maintained as the interconnected cyst of germ cells continues its growth.

Although *Wolbachia* are found primarily in the reproductive tissues, they can also be detected throughout the soma of many insects[16–19]. We wondered whether *Wolbachia* present in the abdominal cavity of long term maternally infected hosts could serve as an additional source of bacteria into the germline, possibly via infection and accumulation in the SSCN. To test this hypothesis, we transplanted germaria into the abdominal cavity of infected *D. melanogaster* hosts (see Methods). If the vertically transmitted *Wolbachia* present in the abdominal cavity are competent to infect the germline and the SSCN, we should expect to see infection in the transplanted germarium. Two to three weeks after germarium transplantation, the SSCN had been colonized in 22 of 29 cases (Fig. 3a–e). *Wolbachia* levels were higher than in neighbouring somatic tissue in 18 out of the 22 cases. Five of the seven transplanted germaria with no *Wolbachia* in the niche had no *Wolbachia* in the germline, whereas 22 out of 22 germaria with infected niches had *Wolbachia* in the germline. These results demonstrate that endogenous levels of free *Wolbachia* present in the abdomen of maternally infected flies are sufficient to cause infection of germ cells that were previously free of the bacteria. We conclude that the likely source of bacteria into the germline is the infection of the SSCN.

The above experiment has shown that tropism for the stem cell niche and ultimate transfer to germ cells is not merely a property of newly injected bacteria, but may represent a mechanism that contributes to germline infection in long term maternal transmission. Re-infection of the germline through the SSCN might provide a route for *Wolbachia* to maintain infection in species like *Drosophila mauritiana* where, unlike *D. melanogaster*, no special accumulation is observed in germline precursors during early embryonic development[20,21]. We examined *Wolbachia* distributions in females from a maternally infected *D. mauritiana* line to determine whether if such a pathway is feasible. In the *D. mauritiana* ovary, we detect limited *Wolbachia* levels in most cell types but, consistent with the tropism presented above in *D. melanogaster*, we see high levels in the border region. Levels in the germline are lower, consistent with the possibility that enrichment in the SSCN might provide a stable reservoir for infecting the adjacent germ cells (Fig. 3f). The same enrichment at the border was also observed in *Wolbachia* wRi infecting *Drosophila simulans* and *Wolbachia* wPop infecting *D. melanogaster* (Supplementary Fig. 2e, f).

Both the somatic and germline stem cells require high mitotic rates in order to sustain the high rate of egg production. Under conditions of low or fluctuating *Wolbachia* levels, these proliferation rates may lead to occasional daughter cells with *Wolbachia* levels insufficient for efficient transmission. By contrast, the stromal neighbours that form the niche for these stem cells are more stable and undergo few if any divisions. We propose that the *Wolbachia* infection we observe in such stable niches contributes to the efficient spread of *Wolbachia* within a new host population by providing a point for stable accumulation before subsequent vertical transfer into stem cell derivatives. After introduction of *Wolbachia* into a new host organism, such as following a wasp or parasite mediated infection[4,6,7], the limited number of bacteria may be under selective pressure to target the germ cells. Under such low density conditions, direct entry into the germline would result in a reduced number of infected eggs. However, initial targeting of the niche offers the opportunity of population self-renewal, amplification and spreading into the germline, in a strategy similar to that of the SSC themselves. In the case of strict vertical transmission, if the levels of *Wolbachia* in the germline stem cells are not enough to supply every derived germline cyst with *Wolbachia*, the constant and renewed infection of the niche would provide an additional reservoir of *Wolbachia* for entry into the germline. The pathway we describe for infecting the SSCN is therefore a very efficient strategy for both horizontal and vertical transmission.

The mechanism for *Wolbachia* entry and accumulation at the niche is unknown. It has recently been reported that IGS cells originate near the tip of the ovary, where they encase the developing

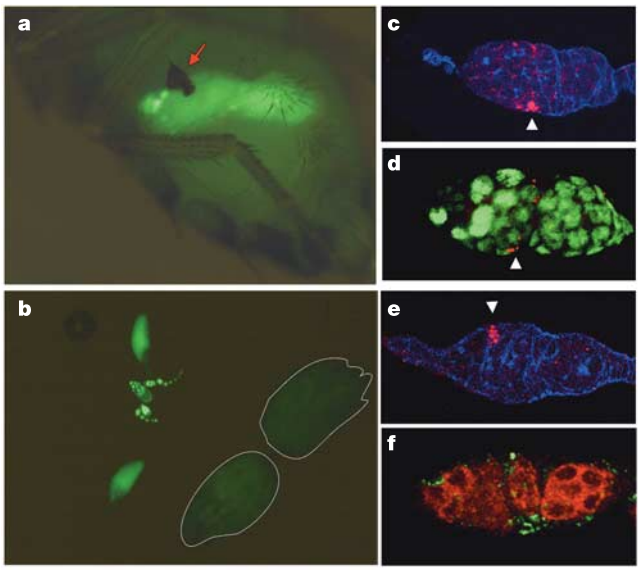

Figure 3 | *Wolbachia* at the abdominal cavity of maternally infected flies infect newly introduced germarium. **a**, Two germaria from a histone-GFP tetracycline treated stock (*Wolbachia*-free) transplanted into the abdominal cavity of a maternally infected fly (see Methods). The dark spot (arrow) is a melanotic scar from transplantation surgery. **b**, Fly from **a** dissected. Both transplanted germaria developed into GFP labelled ovarioles. The host germarium is outlined. **c**, Host germarium from **b** has high levels of *Wolbachia* (red) in the border region (arrowhead). Cortical actin is shown in blue. **d**, *Wolbachia* (red) infects the SSCN of transplanted histone-GFP (green) germarium. Frequently *Wolbachia* levels are considerably lower in the transplanted germarium than in host germaria (arrowhead). **e**, Transplanted germarium from **b**, with moderate levels of *Wolbachia* (red) in one of the SSCN (arrowhead). Actin is shown in blue. **f**, Germarium from *D. mauritiana*. High levels of *Wolbachia* wMau (green) are present in the border region. The germline is shown in red.

cyst and move towards the border, turning over at this region[22]. The dynamic behaviour of IGS cells may contribute to the accumulation of *Wolbachia* at the niche. We anticipate that future elucidation of the cellular basis for horizontal transfer and the mechanism responsible for *Wolbachia* tropism towards the SSCN will deepen our understanding of *Wolbachia* transmission and of the niche maintaining SSC.

METHODS

Immunocytochemistry. Ovarioles were stained as previously described[23,24]. The following antisera were used at the indicated dilutions: anti-hsp60[24,25] (Sigma) (1:50), rabbit polyclonal anti-Laminin A (a gift from H. Gutzeit; 1:3,000), rabbit polyclonal anti-β-galactosidase antibody (Cappel) (1:3,000), rat anti-vasa (a gift from P. Lasko; 1:500). Actin was stained with Alexa568 (Molecular Probes) conjugated to phalloidin (1:400).

Injections and transplantations. *Wolbachia* were isolated from adult flies as described previously[9], with modifications. To ensure the same initial inoculation, all flies analysed in Fig. 1 were injected with 0.2 μl of the same preparation. Germarium transplantations were performed as described[26] using uninfected 1–3-day-old histone-GFP flies[27] as germarium donors into newly eclosed (1–6 h) infected flies as hosts.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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