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Endosymbiosis: The Remarkable Healing Powers of *Wolbachia*

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***Wolbachia*, a maternally transmitted bacterium globally present in arthropods, favors its own transmission by producing dramatic changes in host reproduction. Insight into the underlying molecular and cellular mechanisms comes from the identification of the *Wolbachia* effector protein TomO, which maintains host germline stem cells in an undifferentiated state.**

Wolbachia never ceases to amaze. First described in 1923, this intracellular bacterium resides in the germline and somatic cells of arthropods and filarial nematodes [1,2]. Present in over half the insect species on the planet, *Wolbachia*'s success is largely due its efficient vertical transmission and extraordinary ability to manipulate host reproduction. Because *Wolbachia* is exclusively transmitted through the female germline, it has evolved a number of varied and dramatic strategies to favor *Wolbachia*-infected females. In insects, this includes feminization of genetically male offspring, induction of parthenogenesis, cytoplasmic incompatibility — whereby infected females mated with infected or uninfected males produce viable embryos, while uninfected females mated with infected males produce inviable embryos — and even killing of infected males [3]. In addition to these dramatic effects on host reproduction, *Wolbachia* imparts more subtle but equally significant effects. This includes increasing the division rate of germline stem cells, such that infected females produce more eggs than uninfected females [4]. *Wolbachia* also induces a number of changes in host reproductive behaviors [5]. While the mechanisms are unknown, it is intriguing that *Wolbachia* localizes to regions of the brain that control these behaviors [6,7]. Recently, interest in *Wolbachia* has surged also in the wider public. This is because mosquitos and other insects infected with *Wolbachia* have a potent ability to suppress replication of the dengue, yellow fever, chikungunya and zika viruses in the same co-infected host [8]. This property has made it one of the most promising approaches to combat these devastating mosquito-borne diseases. A new study by Ote *et al.* [9], in this issue of *Current Biology*, provides a first glimpse into the mechanisms by which *Wolbachia* perturbs the cell biology of its host in such an extraordinary way.

Wolbachia naturally infects *Drosophila melanogaster*, enabling investigators to apply this organism's sophisticated arsenal of genetic tools to explore insect host factors that interact with *Wolbachia*. However, identifying *Wolbachia* proteins that mediate these host interactions has been difficult, largely because *Wolbachia* is an obligate intracellular bacterium that cannot multiply outside of its host cell. Consequently, standard microbiological methods of unbiased and targeted mutagenesis are not applicable. In addition, although many have tried, an efficient method of transforming *Wolbachia* has not been developed.

Wolbachia contains a type IV secretion complex and many genes with eukaryotic-like sequences, which strongly indicates that it secretes effector proteins [10]. *Wolbachia* effector proteins secreted into the host cell are of special interest because they might reveal how *Wolbachia* manipulates its insects hosts. Realizing that conventional approaches used in genetically tractable microbes cannot be applied, Ote *et al.* [9] successfully developed a novel approach. By transforming *Drosophila* S2 cells with expression clones from a comprehensive genomic *Wolbachia* library, they identified 9 clones that disrupted cell growth. Using the UAS/GAL4 system to ubiquitously express these clones in whole flies, one clone resulted in lethality. Restricting the expression of this clone to the female germline produced a dramatic reduction in fertility and egg hatch rates. Through a combination of sequence analysis and functional studies, the authors identified a single conserved *Wolbachia* gene, *TomO*, as responsible for these deleterious host effects. The TomO protein contains two conserved hydrophobic regions and in some instances ankyrin repeats, a common eukaryotic motif often mediating protein–protein interactions. Immunoflourescent studies in infected *Drosophila* provide compelling evidence that TomO is a bona fide *Wolbachia* effector protein: analyses of the female germline stem cells and pole cells of the early embryo reveal TomO expression is concentrated around *Wolbachia* and present in infected, but not uninfected, *Drosophila*. A set of clever antibody injection studies reveal TomO is indeed secreted into the cytoplasm.

One of the most remarkable features of *Wolbachia* is its ability to suppress host mutant phenotypes, which was originally discovered with the suppression of mutations in the *Sex-lethal* (*Sxl*) gene, a RNA splicing factor and master regulator of germline and somatic sex determination [11,12]. More recently, *Wolbachia* was also shown to suppress *bag-of-marbles (bam)* mutations, a gene required for differentiation of the daughter cell derived from female germline stem cell

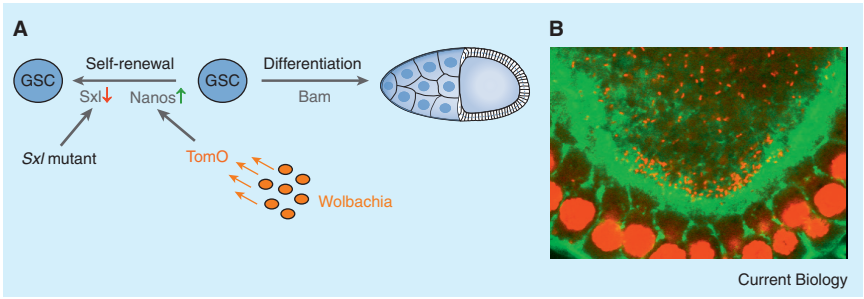

Figure 1. The Wolbachia effector TomO promotes germline stem cell self-renewal.
(A) Relief of translation suppression of *nanos* mRNA by the *Wolbachia* effector protein TomO results in elevated levels of Nanos. As Nanos promotes germline stem cell (GSC) self-renewal, this compensates mutants in Sxl, a protein also required for germline stem cell self-renewal. (B) *Wolbachia* (red puncta) closely associates with actin (green) and other germplasm components at the posterior pole (image by Serbus and Sullivan).

division [13]. At the cellular level, loss of the Sxl gene product results in a reduction in the number of female germline stem cells and inappropriate expression of *bam*. Thus, Sxl is required to maintain the germline stem cells in an undifferentiated state. Ote and colleagues found that *Wolbachia* infection suppressed both germline stem cell loss and bam up-regulation in *Sxl* mutants [9]. In a particularly striking result, the authors demonstrated that expressing TomO in the female germline prevented the ectopic Bam expression and loss of GSC through inappropriate differentiation. Thus, TomO appears to be a *Wolbachia* effector protein maintaining germline stem cells in an undifferentiated state, countering the effects of the *Sxl* mutants (Figure 1).

Insight into potential host targets of TomO came from observations that overexpressed TomO is associated with germ granules, specialized membrane-less ribonucleoprotein particles in the germ cell lineage that prevent differentiation and maintain totipotency [14]. A candidate screen of germ granule components revealed that TomO associates with *nanos* mRNA, a key component of the germ granules. Nanos protein is essential for maintenance of the germline stem cells in an undifferentiated state [15]. Further, the authors demonstrate that TomO disrupts the association between *nanos* mRNA and the germ granule component Cup, an inhibitor of translation initiation [16]. This results in elevated levels of Nanos protein. Previous studies demonstrated Nanos is required for preventing germline stem cell differentiation [14]. Taken together, these experiments lead to a satisfying explanation of the mechanism of *Wolbachia*-mediated suppression of the *Sxl* mutant phenotype: *Wolbachia* secretion of TomO disrupts the Cup–*nanos* mRNA interaction, resulting in increased levels of Nanos maintaining germline stem cells in an undifferentiated state (Figure 1).

As the *Drosophila* germ plasm contains multiple distinct ribonucleoprotein complexes, secretion of TomO by *Wolbachia* may have additional functional consequences. Support for this idea comes from the finding that *Wolbachia* closely associates with the maternal Gurken complex and that oocytes with exceptionally high *Wolbachia* titers produce distinct dorsal/ventral patterning defects that phenocopy *gurken* mutants [17]. Significantly, the Gurken complex also contains Cup [16]. Perhaps *Wolbachia*-induced dorsal/ventral patterning defects are a direct result of TomO disrupting Cup function in the Gurken ribonucleoprotein complex.

The identification of TomO and its role in maintaining germ cells in an undifferentiated state by promoting *nanos* mRNA translation provides an explanation for the previous finding that *Wolbachia* increases germline stem cell division rates [4]. In other systems, the regulation of both stem cell self-renewal and division rate depends on translational control [18]. Thus, TomO may be promoting translation of mRNAs that promote mitosis in the germline stem cells. As described above, *Wolbachia* has a dramatic influence on host somatic cells as well as its germline. *Wolbachia*-induced male-killing, feminization, behavior modification and viral protection are all likely due to somatic effects. Given regulation of translational rates of specific host mRNAs could mediate each of these *Wolbachia*-induced host modifications, it will be of great interest to determine whether TomO is responsible.

Finally, the technical take-home message from this study is that employing this expression cloning technique is an efficient means of identifying *Wolbachia* effector proteins and it's certain that many more will be forthcoming using this technique [19]. Now if we could just induce some old-fashion mutants in *Wolbachia*!

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Neurodevelopment: Three's a Crowd, Four Is a Receptor Complex

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Sensory neurons detect environmental signals with dendritic processes near the skin, but the molecules that direct dendritic outgrowth to this location are largely unknown. A new study identifies a diffusible cue that mediates interactions with the epidermis to guide dendritic branching.

Neurons are specialized cells designed to send and receive signals. This dual function is defined by a highly asymmetric architecture. Elongated processes known as dendrites detect chemical information, and separate projections called axons relay signals to other cells. These wire-like structures arise during development and may grow out over substantial distances to produce functional networks of interconnected cells. Numerous environmental cues for steering axons to their destinations have been discovered, but the external signals that guide dendrites are largely unknown. This disparity likely derives from striking differences in structural complexity that have hindered efforts to understand dendrite morphogenesis: a single neuron gives rise to only one axon but typically multiple dendrites, each of which may be highly branched [1]. One strategy to overcome this challenge is to study a model neuron with a readily observed dendritic architecture and to exploit genetic tools available in simple organisms such as *Drosophila* or *Caenorhabditis elegans* to reveal the molecular pathways that control dendrite morphogenesis [2,3]. In this issue of *Current Biology*, Diaz-Balzac *et al.* [4] adopt this powerful approach to discover that the growth-promoting factor LECT2/chondromodulin II acts as a secreted ligand for a specific receptor complex to define the stereotypical dendritic arbor of PVD sensory neurons in the nematode *C. elegans*.

Sensory neurons in the skin respond to a wide array of external stimuli, including mechanical force, chemical agents and changes in temperature. Nociceptors, the sensory neurons that evoke painful sensations, may adopt highly branched dendrites that occupy discrete topical domains. Nociceptors are described as 'polymodal' because they typically respond to more than one noxious signal [5]. These fundamental features of sensory neurons are evolutionarily conserved and thus can be studied in simple organisms. In *C. elegans*, the PVD neurons, one on each side of the body, envelop the animal with a pair of highly branched dendritic arbors that respond to harsh touch, extreme temperature and hyperosmolarity [2,6,7]. Because the skin is transparent, this sensory network can be readily observed as it emerges during larval development by using fluorescent marker proteins (e.g., GFP) to illuminate PVD architecture. Initially, a single axon exits the cell body to innervate motor circuit targets in the ventral nerve cord. Next, 1° dendrites