FORUM Microbiology

Manipulation of the manipulators

***Wolbachia* bacteria infect insects and can cause mating incompatibilities, an outcome that is used to fight insect-transmitted disease. The proposed genes responsible illuminate this process and the disease-control mechanisms.** SEE LETTER P.243

THE PAPERS IN BRIEF

- *Wolbachia* bacteria can infect the male and female germ line of insects.
- This bacterial infection can manipulate insect reproductive outcomes to benefit *Wolbachia* transmission through a phenomenon known as cytoplasmic incompatibility.
- *Wolbachia* has promise as a potent means of pest control and is being used to limit mosquito-transmitted human diseases such as dengue and Zika.
- Understanding the molecular basis of cytoplasmic incompatibility could aid efforts to improve disease-control strategies.
- On page 243 and in *Nature Microbiology*, respectively, LePage *et al.*[1] and Beckmann *et al.*[2] present evidence that they have identified bacterial genes responsible for cytoplasmic incompatibility.

Incompatibility pathway

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The ability of bacterial pathogens to usurp the core processes of host cells has long fascinated biologists. The bacterium *Wolbachia* can infect more than half[3] of all insect species and manipulates the reproduction of its host[4]. Although *Wolbachia* infects the germ line of both sexes, only females can transmit it to their offspring.

Cytoplasmic incompatibility (CI) refers to the sterility or strikingly low hatch rate that can arise when a *Wolbachia*-infected male mates with an uninfected female (Fig. 1). Matings between insects that are both infected with *Wolbachia* (known as rescue matings) and matings between *Wolbachia*-infected females and uninfected males all result in normal hatch rates. *Wolbachia*-infected females have a selective advantage over uninfected females because they have a normal brood size, regardless of the *Wolbachia*-infection status of the males they mate with. CI can therefore help to spread a *Wolbachia* infection rapidly through an insect population.

Although CI was discovered more than 45 years ago[5], its molecular basis has remained unknown until now. Previous work has provided descriptive observations of the cellular consequences of CI. When a *Wolbachia*-infected sperm nucleus enters the egg of an uninfected female, this nucleus encounters problems that include delays to DNA replication and cell-cycle progression[6]. These abnormalities result in embryonic lethality. The identification by LePage *et al.* and Beckmann *et al.* of genes in *Wolbachia* strains that seem to be required to induce CI should provide a major step forward in the efforts to understand this process.

Through a combination of genomic, bioinformatic and molecular approaches, LePage *et al.* identified potential CI-associated sequences in the *Wolbachia wMel* strain that contain the genes *cifA* and *cifB*. These sequences originate from viral DNA that has integrated into the *Wolbachia* genome. LePage and colleagues observed that *cifA* and *cifB* sequences are present in CI-inducing *Wolbachia* strains, but are absent, or present in a highly divergent form, in non-CI-inducing *Wolbachia* strains. The CI-effect strength, in terms of the level of embryonic lethality, correlates with the number of copies of *cifA* and *cifB* present in crosses between *Wolbachia*-infected males and uninfected females. This strongly suggests

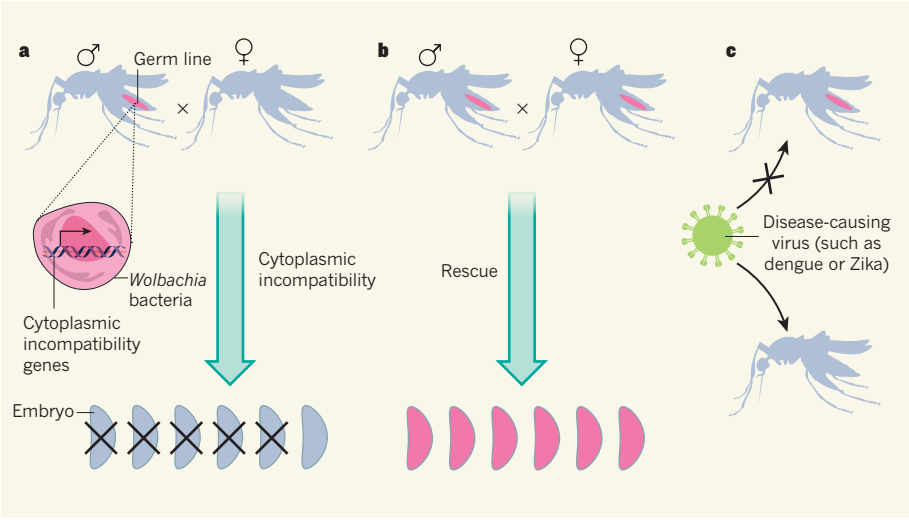

Figure 1 | Cytoplasmic incompatibility and *Wolbachia* in disease control. **a**, *Wolbachia* bacteria can infect the germ lines of insects, including mosquitoes (bacterial infection shown in pink), and only females can transmit the bacteria to their offspring. In the phenomenon known as cytoplasmic incompatibility (CI), matings between *Wolbachia*-infected males and uninfected females results in embryo lethality or low hatch rates. This phenomenon is being used to suppress insect populations in some pest-control approaches. LePage *et al.*[1] and Beckmann *et al.*[2] present evidence that they have identified bacterial genes responsible for CI. **b**, CI can be prevented in rescue matings between *Wolbachia*-infected males and *Wolbachia*-infected females. Because the effect of *Wolbachia* infection on insect reproduction favours the survival of *Wolbachia*-infected females over uninfected females, *Wolbachia* can rapidly spread through an insect population. **c**, *Wolbachia* infection can prevent insects from being infected by some viruses that cause human disease such as dengue or Zika, and the spread of *Wolbachia* through an insect population is being used to control insect-transmitted human diseases.

that this gene pair directly mediates CI.

LePage and colleagues expressed *cifA* and *cifB* sequences in *Drosophila melanogaster* fruit flies. When these genes were expressed in the germ line of males mated to females that did not express *cifA* and *cifB*, the offspring had reduced hatch rates and embryonic cell-division defects that were strikingly similar to those observed in CI. The hatch rate was restored when males flies expressing *cifA* and *cifB* were mated to *Wolbachia*-infected females.

The findings by LePage *et al.* are consistent with the work of Beckmann and colleagues. Previous studies[7] by Beckmann *et al.* identified a protein called WPA0282 that is present in sperm from males infected with the *Wolbachia* strain *w*Pip. In the operon sequence that encodes the WPA0282 protein (renamed CidA by Beckmann *et al.*), the authors identified a gene they called *cidB*. This gene encodes a deubiquitylating enzyme, an enzyme that can remove ubiquitin proteins attached to other proteins. Such bound ubiquitins often act as a tag that targets a protein for destruction. When *D. melanogaster* males expressing CidA and CidB proteins in their germ line were mated with female flies that were not infected with *Wolbachia*, the offspring had the type of nuclear abnormalities during early embryonic divisions that are characteristic of CI.

It seems likely that *cifA* and *cifB* identified by LePage and colleagues are the same as (or related to) *cidA* and *cidB* identified by Beckmann and colleagues. The relationship between these genes, which were identified in different *Wolbachia* strains, will require further investigation.

If a deubiquitylating enzyme is responsible for CI induction, where does it localize, and what are its targets? What pathway connects the action of the identified genes to the observed embryonic defects in CI, and how does rescue of CI occur? Researchers are now positioned to rapidly address many of these important questions.

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Bacteria that fight disease

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There has been an upsurge in interest in the use of *Wolbachia* bacteria to control mosquito-transmitted viruses such as dengue and Zika — devastating diseases that threaten about half of the world's population[8]. Most of the current control measures for these diseases are proving to be ineffective.

Two main *Wolbachia*-based disease-control methods are being tested in the field. The first approach targets virus replication in mosquitoes. *Wolbachia* prevents a range of human viruses and parasites, such as dengue, chikungunya and Zika virus, from replicating in the *Aedes aegypti* mosquito. If *Wolbachia* can be successfully introduced into natural populations of these mosquitoes, it should greatly reduce the disease-transmission potential of the insect populations (Fig. 1). It is thought that the *Wolbachia*-mediated pathogen-interference mechanism involves a combination of upregulated mosquito immune pathways and competition for key lipid molecules such as cholesterol[9]. The second approach uses *Wolbachia* to directly suppress the abundance of mosquito populations and thereby reduce viral transmission. Both approaches rely on the CI phenomenon for their success.

For disease-control approaches that introduce *Wolbachia* into mosquito populations, such as the work being carried out by the Eliminate Dengue Program[10], CI is the central mechanism that allows *Wolbachia* to become established and maintain itself sustainably in the mosquito population. In the alternative suppression approach, the embryo mortality that CI induces is being used to reduce the mosquito population over time. In this approach, male mosquitoes are released that are infected with a *Wolbachia* strain that will induce CI in matings with wild females.

A deeper understanding of the CI mechanism from the work by LePage *et al.* and Beckmann *et al.* opens the door for manipulating the system and potentially tuning *Wolbachia*-induced CI to enhance the ability of different *Wolbachia* strains to invade insect populations. Perhaps the CI mechanism can be separated from the pathogen-blocking effects of different *Wolbachia* strains so that better strains for insect treatment can be generated. It might be possible in future to genetically engineer *Wolbachia* strains to replace strains that have been previously used in control programmes as a way of managing resistance issues, if *Wolbachia* were to lose effectiveness over time. Understanding CI might enable this phenomenon to be used in other insect species that *Wolbachia* does not naturally infect, or that are resistant to *Wolbachia* infection.

Many of these potential approaches would require the release of genetically modified insects into the environment. Recent controversy in Florida about the release of genetically modified mosquitoes for control of mosquito-transmitted viruses indicates that technology alone is insufficient for programmes to be successfully implemented in the field, and public understanding and acceptance are required for such technologies to be effective. ■

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NON-CODING RNA

More uses for genomic junk

It emerges that nascent non–coding RNAs transcribed from regulatory DNA sequences called enhancers bind to the enzyme CBP to promote its activity locally. In turn, the activities of CBP stimulate further enhancer transcription.

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In addition to protein-coding messenger RNAs, our cells produce a plethora of diverse non-coding RNA molecules. Many of these are generated from sequences that are distant from genes, and include regulatory DNA sequences called enhancers[1]. Transcription factors bound at enhancers are thought to regulate gene expression by looping towards genes in 3D space. The potential functions of non-coding enhancer RNAs (eRNAs) in this process have been avidly debated, but there has been a tendency to write them off as accidentally transcribed by-products of enhancer–gene interactions. After all, how