MOBILE DNA IN OBLIGATE INTRACELLULAR BACTERIA

Seth R. Bordenstein and William S. Reznikoff*‡*

Abstract | The small genomes of obligate intracellular bacteria are often presumed to be impervious to mobile DNA and the fluid genetic processes that drive diversification in free-living bacteria. Categorized by reductive evolution and streamlining, the genomes of some obligate intracellular bacteria manifest striking degrees of stability and gene synteny. However, recent findings from complete genome sequences of obligate intracellular species and their mobile genetic associates favour the abandonment of these wholesale terms for a more complex and tantalizing picture.

> "... little if any effort has been made to detect transposition of mutators, modifiers, suppressors, or some types of inhibitors in other organisms, and the degree to which it may occur is not yet known. It would be surprising, indeed, if controlling elements were not found in other organisms ..."[1]
>
> *McClintock, 1956*

Barbara McClintock's seminal discovery of 'controlling' (that is, transposable) genetic elements in maize in the 1940s directly challenged the doctrine that genes are in fixed positions on a chromosome. Her detection of a dynamic genome that included mobile DNA was revolutionary, and her forecast on the ubiquity of mobile elements was an unconventional, but intuitive, vision[1]. Perhaps the single best measure of McClintock's forecast is that, at present, biologists are challenged not by determining the number of organisms that have mobile DNA elements, but instead, by discovering those that lack them.

The genome sciences are producing a wealth of new information on the abundance and distribution of mobile DNA in prokaryotes[2–4], examples of which include plasmids, bacteriophages and transposable elements (FIG. 1). Findings so far indicate that most bacterial genomes harbour prophages, some of which occupy up to 20% of the host genome[5]. In fact, mobile-related DNA, such as prophages, account for more than 50% of the strain-specific DNA in several important pathogens[6–8] and are the most common transporters of virulence genes in bacteria[9–11]. Plasmids and transposable elements have also had a considerable effect on bacterial genome architecture. Therefore, the importance of mobile DNA to our understanding of microbial genomes is profound.

In this review, we assess the data on mobile DNA in obligate intracellular bacteria, a group of organisms that has been characterized in the microbiology literature as having few if any mobile genes, owing to their intracellular confinement and accelerated rates of gene loss. It is this notion that makes the growing discovery of mobile genetic elements in these species surprising.

The obligate intracellular bacteria

The prokaryotes can be divided into three broad categories on the basis of their lifestyle: free-living, facultative intracellular and obligate intracellular bacteria. Free-living bacteria tend to have large population sizes and genomes (4–10 Mb) with a moderate composition of mobile DNA. Facultative intracellular species, which are not confined to intracellular replication, tend to be pathogenic with intermediate population sizes and a genome size that is similar to free-living species (2–7 Mb). Obligate intracellular bacteria, also known as obligate endosymbionts, replicate exclusively inside the cells of mostly eukaryotic organisms, and

**Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, The Marine Biological Laboratory, 7 MBL Street, Woods Hole, Massachusetts 02543, USA.*
‡Department of Biochemistry, University of Wisconsin, 433 Babcock Drive, Madison, Wisconsin 53706, USA.
Correspondence to S.B.
e-mail: sbordenstein@mbl.edu
doi:10.1038/nrmicro1233
Published online 10 August 2005

COSPECIATION PATTERNS
The speciation in parallel by closely associated species, such as a symbiont and host.

PARASITIC ASSOCIATION
A symbiotic relationship in which the bacteria benefits and the host is harmed.

MUTUALISTIC ASSOCIATION
A symbiotic relationship that benefits both the bacteria and its host.

COMMENSAL ASSOCIATION
A symbiotic relationship that benefits the bacteria but causes no important harm or benefit to the host.

REDUCTIVE EVOLUTION
The process by which genomes of obligate intracellular bacteria shrink and undergo extreme levels of gene degradation and loss.

DELETION BIASES
The mutational bias by which DNA deletions outnumber DNA insertions.

typically have no extracellular state. They tend to have small population sizes, and their genomes are usually small (0.5–2 Mb) and show marked AT nucleotide biases, accelerated sequence evolution and a loss of genes that are involved in recombination and repair pathways[12–14].

Obligate intracellular species can be further divided into species that show strict vertical transmission and species that show at least some horizontal transmission (BOX 1). The former includes the dietary endosymbionts that are required for the survival and reproduction of their insect hosts (for example, *Buchnera* spp. of aphids, *Wigglesworthia* spp. of tsetse flies, and *Blochmannia* spp. of ants). These genera of γ-proteobacteria manifest strict maternal inheritance, obligate mutualistic associations and precise COSPECIATION PATTERNS with their hosts[15–17]. By contrast, species that show at least some horizontal transmission include human and plant pathogens (for example, *Chlamydia* spp., *Rickettsia* spp. and *Phytoplasma* spp.) and the reproductive parasites of arthropods (for example, *Wolbachia* spp. and *Candidatus Cardinium hertigii*) that can distort sex ratios and sex determination[18–20] (BOX 2). Species that can switch from one host to another tend to form PARASITIC ASSOCIATIONS, but can also form MUTUALISTIC ASSOCIATIONS or COMMENSAL ASSOCIATIONS.

Comparative genomic studies of obligate intracellular species mostly reveal remarkable conservation in gene content, genome size and gene order[21,22]. The impact of REDUCTIVE EVOLUTION facilitated by small population sizes, DELETION BIASES and constrained access to novel gene pools in these species might promote genome streamlining[23] and a lack of horizontal gene transfer. Indeed, the published genomes of five endosymbiotic γ-proteobacteria of insects that are obligate mutualists, including the genomes of three *Buchnera* strains[21,24,25], one *Wigglesworthia* strain[26] and one *Blochmannia* strain[27], are devoid of mobile genetic elements. Furthermore, comparisons between the genomes of different species of *Buchnera* indicate that there has been no gene influx (duplications or horizontal-gene-transfer events) over the past 50 million years[21]. For these reasons, obligate intracellular species are commonly presumed to be impervious to mobile genetic elements and, for the long-term, vertically transmitted obligate mutualists, this presumption is probably correct. However, the study of species that can switch hosts will probably provide the greatest insights and will facilitate tractable hypotheses on the evolution of mobile DNA and genome instability in obligate intracellular bacteria.

Here, we summarize the evidence that amends the view that obligate intracellular bacteria are devoid of genetic parasites. Instead, we propose a more intricate picture — one in which differences in the modes of transmission of these bacteria partly predict distinct genomic outcomes for mobile DNA in obligate intracellular species.

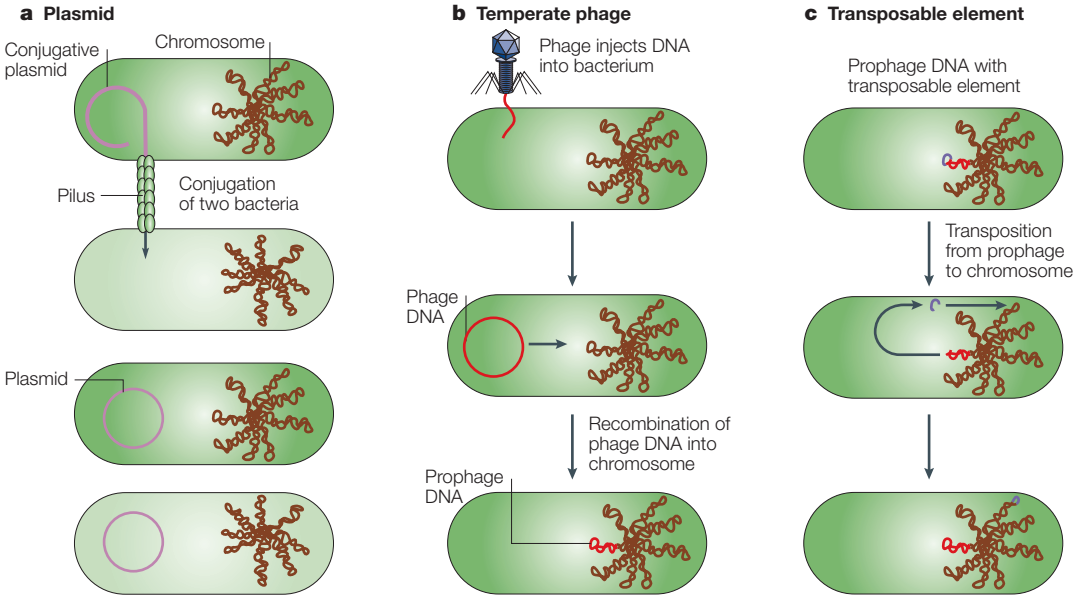

Figure 1 | **Examples of mobile genetic elements in prokaryotes.** Three main classes of mobile genetic elements occur in prokaryotes. **a** | Plasmids are small pieces of extrachromosomal DNA that are either linear or circular and that typically replicate independently of the host cell. Conjugative plasmids are laterally transferred from a donor bacterial cell to a recipient bacterial cell by direct physical contact between the cells. **b** | Phages are viruses of bacteria that use the host machinery to replicate. The DNA of a temperate phage enters the host cell and integrates into the bacterial genome as a prophage. Integrated prophage DNA is passively inherited until DNA excision and phage-induced lysis of the bacterial cell takes place. **c** | Transposable elements contain segments of DNA that are flanked by short inverted repeats that typically encode proteins which faciliate movement from one chromosomal location to another. In this case, a transposable element that is embedded within prophage DNA is shown excising and transferring to a new site in the bacterial chromosome.

Box 1 | **Transmission of obligate intracellular bacteria**

Symbiotic bacteria that exclusively inhabit animal or plant cells are called endosymbionts or obligate intracellular bacteria. They are widespread in nature and have diverse effects on their hosts. Obligate intracellular bacteria can be transmitted by many different mechanisms, ranging from completely vertical transmission to completely horizontal transmission.

Vertical transmission of bacterial endosymbionts occurs when the bacteria are inherited directly from mother to offspring. These bacteria typically infect cells of the female reproductive tissues (ovaries) of the host and get passed directly into the developing eggs to infect the next generation. Bacteria with strict vertical transmission typically establish obligate and irreversible associations with their hosts, so that the bacteria-free hosts grow poorly and produce few offspring, whereas the bacteria themselves cannot survive outside of the host cell. Beneficial associations ensue in which the intracellular bacteria typically provide essential nutrients to the host that it cannot generate for itself. Examples include *Buchnera* of the plant-sap-feeding aphids and *Wigglesworthia* of the blood-feeding tsetse flies.

Horizontal transmission occurs when a bacteria is transmitted from one individual or species to another. These bacteria might replicate intracellularly, but have the ability to switch hosts. They tend to form parasitic associations with their animal or plant hosts. For example, the plant pathogen *Phytoplasma* is transmitted to plants by leafhoppers (insects) and is not usually transmitted vertically within the leafhopper itself. By contrast, the reproductive parasite *Wolbachia* shows a mixed pattern of transmission among arthropods, with primarily vertical inheritance within host species and horizontal transmission between species. Horizontal transmission can be promoted by various mechanisms, which include parasite–host interactions, TROPHALLAXIS and blood feeding.

The composition of mobile DNA

FIGURE 2 compares the number of genes that have mobile-DNA functions in obligate versus facultative intracellular bacteria. The bacteria represented in FIG. 2 comprise most species that have well defined intracellular lifestyles and genomes that have been completely annotated in the Comprehensive Microbial Resource v15.2 of The Institute for Genomic Research (TIGR)[28]. To generate this database, TIGR carries out an automated annotation of completed microbial genomes and classifies genes into 19 functional-role categories, which include a mobile-DNA category that specifies prophage, transposable element and plasmid genes. Other types of horizontally acquired or mobile DNA such as pathogenicity islands[29], integrons[30] and conjugative transposons[31] are not catalogued in this analysis. We caution that little to no experimental work has confirmed the presence of mobile elements or their remnants for most of the species described in this review. As variations in genome annotation methods can produce different estimates of mobile DNA composition[2], we confined our analysis to the TIGR data. Furthermore, as open reading frames (ORFs) can be assigned several different functions during annotation, the number of genes in a role category can be overestimated. Therefore, we analysed genes predicted to solely have mobile-DNA functions. We highlight three findings from this synopsis.

Box 2 | ***Wolbachia* — a parasite and mutualist**

Wolbachia infect the reproductive tissues of many species of arthropods and filarial nematodes, and are primarily transmitted through females by transovarial transmission. Phylogenetic and experimental evidence also indicate a low frequency of horizontal transmission in arthropods. Maternally inherited bacteria can invade a host population as long as the number of infected female progeny per infected mother is greater than the number of uninfected female progeny per uninfected mother. This imbalance can be accomplished by increasing the fitness of infected females (known as mutualism) or by a strategy known as reproductive parasitism, in which the host sex ratio or fitness is manipulated in favour of the transmitting sex.

Mutualism is typically expressed in the filarial nematode hosts in which the *Wolbachia* bacteria infect the lateral chords of the hypodermis and the ovaries. Antibiotic-curing experiments have determined that the bacteria are required for embryogenesis and larval molting. *Wolbachia* bacteria are an increasingly promising chemotherapy target for control of the symptoms and agents of human filarial diseases, including lymphatic filariasis and onchocerciasis.

Reproductive parasitism in *Wolbachia*-infected arthropods is accomplished by several mechanisms — feminization, male-killing, parthenogenesis and a post-fertilization failure called cytoplasmic incompatibility. Feminization, which takes place in infected crustaceans, is the conversion of a genetic male into a functional female by the suppression of hormones that are required for male development. Male-killing, which occurs in various insects, results in the death of infected male embryos so that infected female progeny can survive and reproduce in a resource-limited environment. Parthenogenesis (virgin birth) occurs in hymenopteran wasps and exploits their haplodiploid sex-determination system — usually, diploid fertilized eggs develop into females, and unfertilized eggs become males. In *Wolbachia*-infected wasps, however, all eggs undergo endoreplication to become diploid and develop into females without fertilization. Cytoplasmic incompatibility is the most common reproductive alteration induced by *Wolbachia* and primarily results in early embryonic inviability of uninfected progeny produced from a cross between an infected male and an uninfected female. Infected females do not suffer this same crossing incompatibility.

TROPHALLAXIS
The regurgitation of food from one adult or larvae to another that is most common in social insects such as termites, bees and wasps.

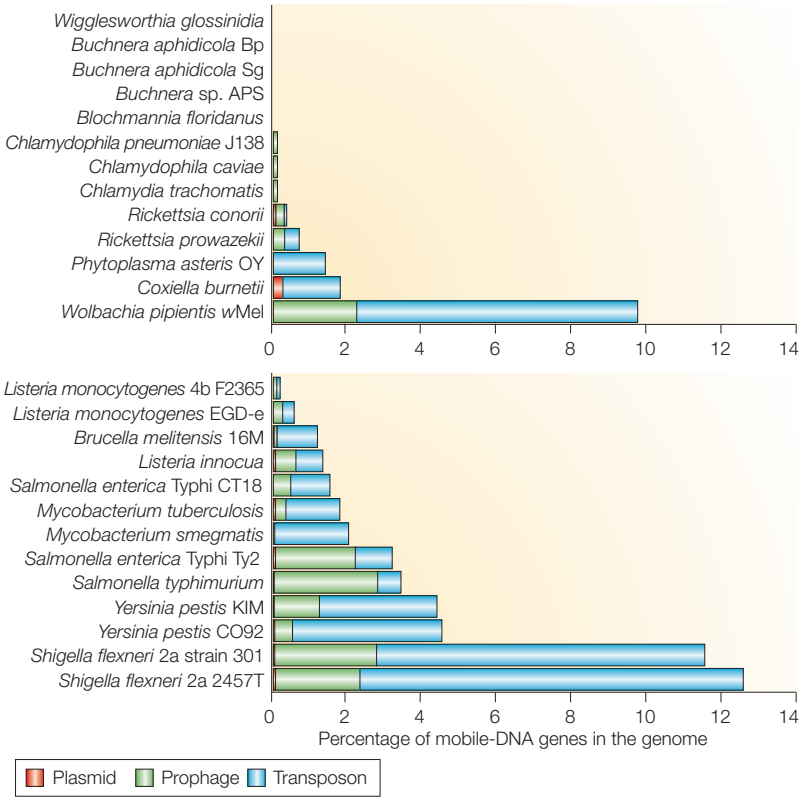

Figure 2 | **Mobile-DNA composition in the genomes of intracellular bacteria.** Genes that encode mobile-DNA functions vary in their abundance and diversity across obligate (top) and facultative (bottom) intracellular bacteria. This stacked bar graph depicts the number of mobile-DNA genes per species, normalized to the total number of genes in the genome for 26 completed prokaryotic genomes. Three categories of mobile DNA are shown: plasmids, prophages and transposable elements. In general, transposable-element genes comprise the largest fraction of mobile DNA per genome. Among obligate intracellular species, the parasitic genera that host-switch (*Wolbachia*, *Coxiella*, *Phytoplasma*, *Rickettsia*, *Chlamydia* and *Chlamydophila*) tend to harbour mobile DNA in their genomes, whereas the stable mutualistic genera (*Buchnera*, *Blochmannia* and *Wigglesworthia*) do not.

First, the amount of mobile DNA in the genomes of obligate intracellular species spans almost the same range as the genomes of facultative intracellular species. However, facultative intracellular bacteria contain an average of four-fold more mobile DNA than obligate intracellular bacteria ($p=0.0015$, Mann–Whitney U-test). This finding is consistent with predictions that facultative intracellular bacteria have mobile-DNA compositions that are more similar to free-living than to obligate species. It also dispels the assumption that obligate intracellular bacteria lack mobile genetic elements or their remnants — the genomes of at least half of the obligate species analysed contained some mobile DNA. However, with the exception of *Wolbachia pipientis* *w*Mel, in obligate intracellular species, mobile DNA comprises less than 2% of the total genome — a level that is similar to the lower end of the range found in facultative intracellular species.

The second finding answers a basic question that has emerged from genomic analyses — what type of mobile genetic element is most common in intracellular bacteria? As is evident in FIG. 2, transposable elements constitute the largest portion of mobile DNA. The proportion of plasmid genes per genome is consistently small, and the proportion of prophage genes per genome is intermediate to that of plasmid and transposable-element genes. Transposable elements might predominate in bacterial genomes because they often do not require site specificity for insertion and can integrate into a genome that already has a copy of the same transposable element. By contrast, genome insertion by phages generally shows site specificity and confers immunity to multiple infections. It should also be noted that phages serve as vectors that shuttle other mobile elements, such as transposable elements, into a host genome. However, the difference between the amount of transposable-element and prophage-related genes found in intracellular bacteria is nonetheless striking, as a transposable element typically carries a single gene (encoding a transposase or reverse transcriptase/maturase), whereas a prophage genome consists of tens of genes.

Third, this analysis provides a first glimpse of the impact of lifestyle differences on the mobile-DNA composition of obligate intracellular species. The genera that have mobile-DNA genes are strictly pathogenic (*Coxiella*, *Chlamydia*, *Chlamydophila*, *Phytoplasma* and *Rickettsia*) or parasitic (*Wolbachia*) genera that undergo at least some horizontal transmission. By contrast, those obligate species that lack mobile genetic regions include all of the dietary mutualists of insects that are vertically transmitted (*Wigglesworthia*, *Blochmannia* and three strains of *Buchnera*). This indicates that transmission differences among obligate intracellular species might shape genome plasticity. In particular, the evolutionary processes that operate in the small population sizes of these strictly maternally inherited species (for example, GENETIC DRIFT) might accelerate the loss of mobile DNA. By contrast, the more permissive lifestyles of host-switching pathogens and parasites might augment their contact with novel gene pools and the uptake of foreign DNA. One exception could be the unpublished report of a high mobile-DNA content in the *Sitophilus oryzae* primary endosymbiont (SOPE)[4], which is a recently derived, γ-proteobacterial mutualist of *Sitophilus* grain weevils. Maternally transmitted bacteria that have recently adopted an intracellular lifestyle might have mobile-DNA contents that are more similar to their free-living or facultative intracellular relatives, because not enough time has elapsed to streamline the genome by deletion of mobile DNA.

GENETIC DRIFT
An evolutionary process that is characterized by random variation in gene frequencies over time owing to random sampling in finite populations. Genetic drift is often seen in small populations owing to the increased effect of random occurrences in these communities.

Correlation of mobile DNA with genome size

The relationship between the total number of genes and the proportion of mobile DNA in a genome reflects the balance between the inflow and outflow of mobile DNA. Whereas gene-gain events depend on rates of horizontal gene transfer and gene duplication, gene-loss events depend on rates of gene inactivation and deletion. If mobile-DNA loss is

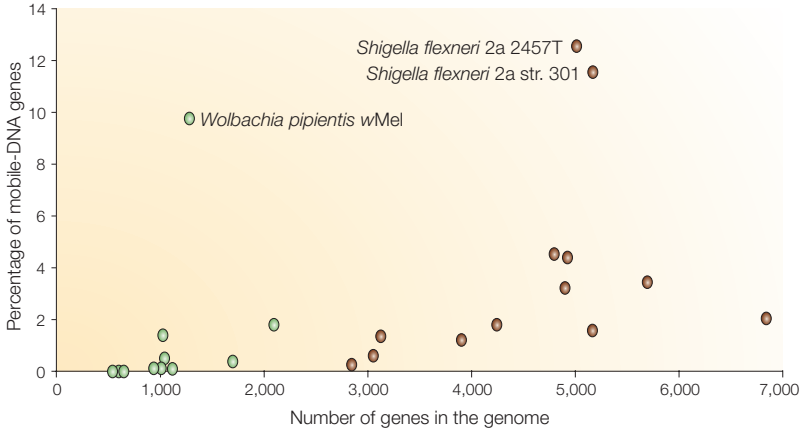

Figure 3 | **Correlation of genome size and mobile-DNA composition.** Fractions of mobile DNA per genome significantly increase with genome size among intracellular bacteria. Obligate intracellular bacteria are denoted by green circles and facultative intracellular bacteria are denoted by red circles. There is a significant, positive correlation (p=0.013) between genome size and the percentage of genomic mobile-DNA genes in these bacteria, and a simple linear regression analysis indicates that genome size can partially predict mobile-DNA content (R^2 =23.2%). The analysis was repeated after removing the three extreme values with 9% or more mobile DNA genes (*Wolbachia pipientis* *w*Mel, *Shigella flexneri* 2a strain 301, and *Shigella flexneri* 2a 2457T), and it yielded a higher R^2 value of 62.4% (p<0.001).

enhanced in the genomes of long-term obligate intracellular species or if mobile-DNA gain is enhanced in the genomes of facultative intracellular species, the fraction of mobile-DNA genes in the obligate species will be reduced compared with that of facultative or free-living species. Alternatively, if the loss and gain of mobile DNA is random, the proportion of mobile-DNA genes should remain constant across species. Genome analysis shows that the relative mobile-DNA composition of intracellular bacteria increases with total gene number (FIG. 3). Therefore, although we now know that obligate intracellular species do have mobile genetic elements, the smaller genomes of obligate intracellular species show preferential deletion of these mobile elements.

The conventional explanation for the reduction of mobile DNA in obligate intracellular bacteria is that these bacteria have a general mutational bias for deletions and, therefore, the rate of gene loss in such species exceeds the rate of new gene acquisitions by horizontal gene transfer or gene duplications[32–34]. This bias is thought to result in the relatively small genomes of prokaryotes and the close packing of genes in bacterial genomes. Indeed, a comparative analysis of pseudogene evolution across a wide range of prokaryotes shows that deletions are far more frequent than insertions[35]. Elevated deletion rates in bacteria might even be due to deterministic evolutionary forces such as selection against the continuous influx of dangerous genetic parasites[36]. Therefore, an inherent mutational bias for deletions in prokaryotes, coupled with no or relatively limited inflow of new mobile elements in endosymbionts, could accelerate mobile-DNA reduction in obligate intracellular species.

Plasmids

Plasmids are extrachromosomal elements that move horizontally between bacterial cells in at least four ways: self-directed transmission, in which the plasmid encodes the conjugative machinery for host-cell fusion; mobilizable methods, in which one plasmid parasitizes the self-directed transmission of another plasmid; transduction, in which a plasmid gets packaged in a phage particle; and transformation, in which cell lysis releases plasmid elements from the host bacterium. Plasmids also move vertically by transmission through dividing host cells. Similar to phages, plasmids might have an important role in microbial evolution by acting as natural gene vectors[37].

Among intracellular bacteria, instances of plasmid genes inserted into the host chromosome are uncommon. Only two of the obligate intracellular bacterial genomes represented in FIG. 2 have genes with plasmid functions. Of these species, the maximum number of plasmid genes per genome is five (*Coxiella burnetti*), and the average number of plasmid genes in obligate and facultative intracellular bacteria is approximately one (TABLE 1). Plasmid genes therefore do not significantly affect the genetic architecture of intracellular bacterial genomes. However, this does not preclude their importance as natural gene vectors of foreign DNA that might be advantageous[38] or deleterious to the recipient host genome.

Among obligate intracellular prokaryotes, at least six different genera harbour extrachromosomal plasmids, including the insect mutualists *Buchnera*, *Wigglesworthia* and *Sodalis* and the obligate intracellular parasites *Chlamydia*, *Chlamydophila* and *Phytoplasma*. The two plasmids in *Buchnera* are vertically transmitted[39] and carry amino-acid biosynthesis genes that aid the main role of *Buchnera* symbionts in aphids — the provision of amino acids that are deficient in the insect phloem-sap diet[40]. However, not all *Buchnera* strains carry plasmids, and the *leuABCD* plasmid might experience only rare horizontal transmission[41] and genetic exchange with the *Buchnera* host chromosome[38]. Much less is known about the plasmids that have been isolated from *Wigglesworthia glossinidia*[26] and *Sodalis glossinidius*[42]. The former has a single 5.3-kb plasmid called pWig1, and the latter has a 134-kb plasmid and possibly a 10-kb plasmid. Also, members of the diverse genus of *Phytoplasma* have at least 12 plasmids of unknown function[43]. All are less than 11 kb in size, share varying amounts of homology and structure[44], and frequent rearrangements might affect their size and structure. Plasmid pOYM notably encodes a unique replication protein that has domains that are related to both prokaryotic plasmids and eukaryotic viruses[45].

Bacteriophages

Bacteriophages are viruses of prokaryotes and are among the most abundant biological entities in the biosphere[46]. They are one of the most effective vectors that convey foreign DNA into recipient bacteria and can cause significant amounts of genome diversification[47,48]. Virulent

Table 1 | **Examples of mobile-DNA content in intracellular bacteria**

Species	Total genes	Mobile-DNA genes	Plasmid genes	Prophage genes	Transposase genes	RT genes	Disrupted mobile-DNA genes (%)
Obligate intracellular bacteria							
Wigglesworthia glossinidia	653	0	0	0	0	0	-
Buchnera aphidicola Bp	539	0	0	0	0	0	-
Buchnera aphidicola Sg	661	0	0	0	0	0	-
Buchnera sp. APS	649	0	0	0	0	0	-
Blochmannia floridanus	602	0	0	0	0	0	-
Chlamydia trachomatis serovar D	935	1	0	1	0	0	0.0%
Chlamydophila caviae	1,013	1	0	1	0	0	100.0%
Chlamydophila pneumoniae J138	1,120	1	0	1	0	0	0.0%
Rickettsia conorii	1,705	6	1	4	1	0	0.0%
Rickettsia prowazekii	1,041	5	0	1	4	0	0.0%
Phytoplasma asteris OY	1,021	14	0	0	14	0	0.0%
Coxiella burnetii RSA 493	2,096	37	5	0	32	0	10.8%
Wolbachia pipientis wMel	1,271	123	0	28	81	14	43.9%
Facultative intracellular bacteria							
Listeria monocytogenes 4b F2365	2,847	7	0	3	4	0	14.3%
Listeria monocytogenes EGD-e	3,058	18	0	9	9	0	0.0%
Brucella melitensis 16M	3,901	47	1	4	42	0	0.0%
Listeria innocua	3,121	42	2	18	22	0	0.0%
Salmonella enterica Typhi CT18	5,165	80	0	25	54	1	0.0%
Mycobacterium tuberculosis CDC1551	4,245	76	2	14	60	0	9.2%
Mycobacterium smegmatis	6,844	140	0	4	136	0	10.0%
Salmonella enterica Typhi Ty2	4,895	156	2	107	46	1	0.0%
Salmonella enterica serovar Typhimurium	5,695	195	1	159	33	2	0.0%
Yersinia pestis KIM	4,924	216	1	61	154	0	0.0%
Yersinia pestis CO92	4,796	216	2	24	189	1	0.0%
Shigella flexneri 2a strain 301	5,155	593	2	141	449	1	0.0%
Shigella flexneri 2a 2457T	5,005	628	4	113	509	2	0.0%

Data are derived from the Comprehensive Microbial Resource of The Institute for Genomic Research (TIGR). Annotation of mobile-DNA genes is based on TIGR's automated annotation of completed microbial genomes and might in some cases differ slightly from the primary annotation of the published bacterial genomes. To curb overestimation of mobile-DNA genes, we enumerate genes that solely have mobile-DNA functions. RT, reverse transcriptase.

phages always lyse their hosts after invasion, whereas temperate phages can integrate their DNA into the bacterial host chromosome as a prophage, so that the DNA is passively inherited (FIG. 1). On prophage integration, associated DNA elements, such as pathogenicity islands or other mobile genetic elements, are also transferred into the host chromosome. Prophages consequently account for large fractions of horizontally acquired DNA in bacterial genomes[5–8]. Intact, complete phages have been purified, isolated and sequenced from two groups of obligate intracellular bacteria, *Wolbachia* and *Chlamydiaceae* (TABLE 2). A third case of phage infection occurs in the secondary endosymbionts (γ-proteobacteria) of aphids, which have not been firmly characterized as obligate or facultative intracellular bacteria[49–52].

Wolbachia *of arthropods.* *Wolbachia* are a genus of cytoplasmically transmitted α-proteobacteria that infect millions of arthropod species and many filarial nematodes[53,54], and which are phylogenetically related to emerging human pathogens in the Anaplasmataceae[55]. Their roles in inflammatory-mediated FILARIAL DISEASE[56,57] and their unusually high levels of genomic and phenotypic plasticity[18,19,58] have attracted recent interest. *Wolbachia* are most well known for inducing several selfish forms of reproductive parasitism in arthropods (BOX 2), which might affect key evolutionary processes, including speciation[59,60], sex determination[61] and sexual selection[62]. Despite early microscopy studies that reported phage particles in *Wolbachia* of the

FILARIAL DISEASE
Diseases such as human river blindness and elephantiasis that are caused by filarial nematodes and their *Wolbachia* endosymbionts.

Table 2 | **Examples of phage-related elements in obligate intracellular bacteria**

Phage name	Prophage/bacteriophage	Host bacteria	Genome size	Refs
WO-A	Prophage	*Wolbachia*	44.5 kb	79
WO-B	Bacteriophage	*Wolbachia*	20.5 kb	80
WO-B	Prophage	*Wolbachia*	33–75.9 kb	64,79
Py	Prophage-like	*Wolbachia*	5.5 kb	79
Chp1	Bacteriophage	*Chlamydophila psittaci*, avian strain	4.9 kb	62
Chp2	Bacteriophage	*Chlamydophila abortus*	4.6 kb	74
Chp3	Bacteriophage	*Chlamydophila pecorum*	4.6 kb	66
ϕCPAR39	Bacteriophage	*Chlamydophila pneumoniae*	4.5 kb	75
ϕCPG1	Bacteriophage	*Chlamydophila psittaci*, pig strain	4.5 kb	73

mosquito *Culex pipiens*[63], insect endosymbionts in general were previously presumed to be refractory to mobile genetic parasites. However, after the isolation and sequencing of prophage WO (for *Wolbachia*) in 2000 (REF. 64), the analysis of molecular markers and phage-filtration techniques established the connection between the virus particles of *Wolbachia* and prophage WO[65]. The genome sequence of *Wolbachia* strain *w*Mel from *Drosophila melanogaster* reveals two divergent prophage WO families, WO-A and WO-B (TABLE 2). Molecular evolution analyses indicate that family WO-B groups into three CLADES[66–68], and distribution surveys indicate that WO-B homologues occur in at least 89% of two main lineages of *Wolbachia* that infect arthropods[66]. Notably, WO-B is the only bacteriophage found in insect endosymbionts that is known to elicit gene expression, carry transposable elements, recombine at fast rates and laterally transfer between bacteria[64,66,69]. Owing to its high prevalence in *Wolbachia*, WO-B might be one of the most abundant viruses in all obligate intracellular bacteria, and it could be an important source of genomic flux in the evolutionarily important *Wolbachia*.

The *Wolbachia w*Mel genome sequence also contains a small pyocin-like element (prophage Py) that contains nine genes. Pyocin elements encode bacterial products that morphologically resemble bacteriophage tails and that often have bactericidal activities towards bacterial strains and species that are closely related to the producer[70]. All of the *Wolbachia w*Mel phage elements have a low GC content, similar to the host chromosome, which is indicative of a long association with *Wolbachia* and/or other intracellular bacteria. Because these elements do not exist in *Rickettsia* relatives, we infer that they entered the *Wolbachia* system from an intracellular species that is not closely related to the Anaplasmataceae family of α-proteobacteria. However, ongoing lateral exchange of bacteriophage WO-B between different strains of *Wolbachia* on co-infection of cells does seem to take place[64,66], and these cases represent the first clear findings of recent lateral transfer in obligate intracellular bacteria.

Chlamydiaceae. The family Chlamydiaceae is an abundant group of obligate intracellular bacteria composed of two separate genera, *Chlamydia* and *Chlamydophila*, which contain three and six species, respectively[71]. *Chlamydia trachomatis* and *Chlamydophila pneumoniae* are human pathogens. All Chlamydiaceae exist in two alternating developmental forms, which include a metabolically inert extracellular form, termed the elementary body, and an intracellular replicating cell, termed the reticulated body. Virus particles that infect the reticulated body were first observed in the avian strain *Chlamydophila psittaci*[72], and at least five single-stranded DNA (ssDNA) Microviridae bacteriophages are now known to infect the *Chlamydophila* genus[73–76] (TABLE 2).

All five phages constitute a divergent subfamily of the well known coliphage ϕX174. In total, four of the phages, ϕCPG1, ϕCPAR39, Chp2, and Chp3, are closely related, with genome identities greater than 90% (REF. 75), and these are divergent from the fifth phage, Chp1. The genomes of all five bacteriophages encode at least three viral structural proteins and a few additional genes. Phage infection typically reduces bacterial viability in the laboratory, but further work remains to determine how phage infection generally impacts chlamydial disease, evolution and genetics. Recent horizontal acquisition of phage ϕCPAR39 is inferred, based on its sporadic distribution across nearly identical *C. pneumoniae* host strains. The five ssDNA phages of the *Chlamydophila* genus are unrelated to the double-stranded-DNA phage WO-B, and therefore represent a completely separate event of phage evolution in obligate intracellular bacteria.

Transposable elements

Transposable elements are mobile genetic elements that can move (transpose) from one site in the genome to a second site, or from one DNA molecule (that is, an infecting phage genome or a plasmid) to a second DNA molecule (the bacterial chromosome). They are the most abundant type of mobile genetic element in the genomes of intracellular bacteria. The number and distribution of TRANSPOSASE GENES in various intracellular bacteria analysed from the TIGR

CLADE
A group of genetically related organisms that includes an ancestor and all of its descendants.

TRANSPOSASE GENE
A gene that encodes an enzyme that mediates movement of one segment of genomic DNA into another location.

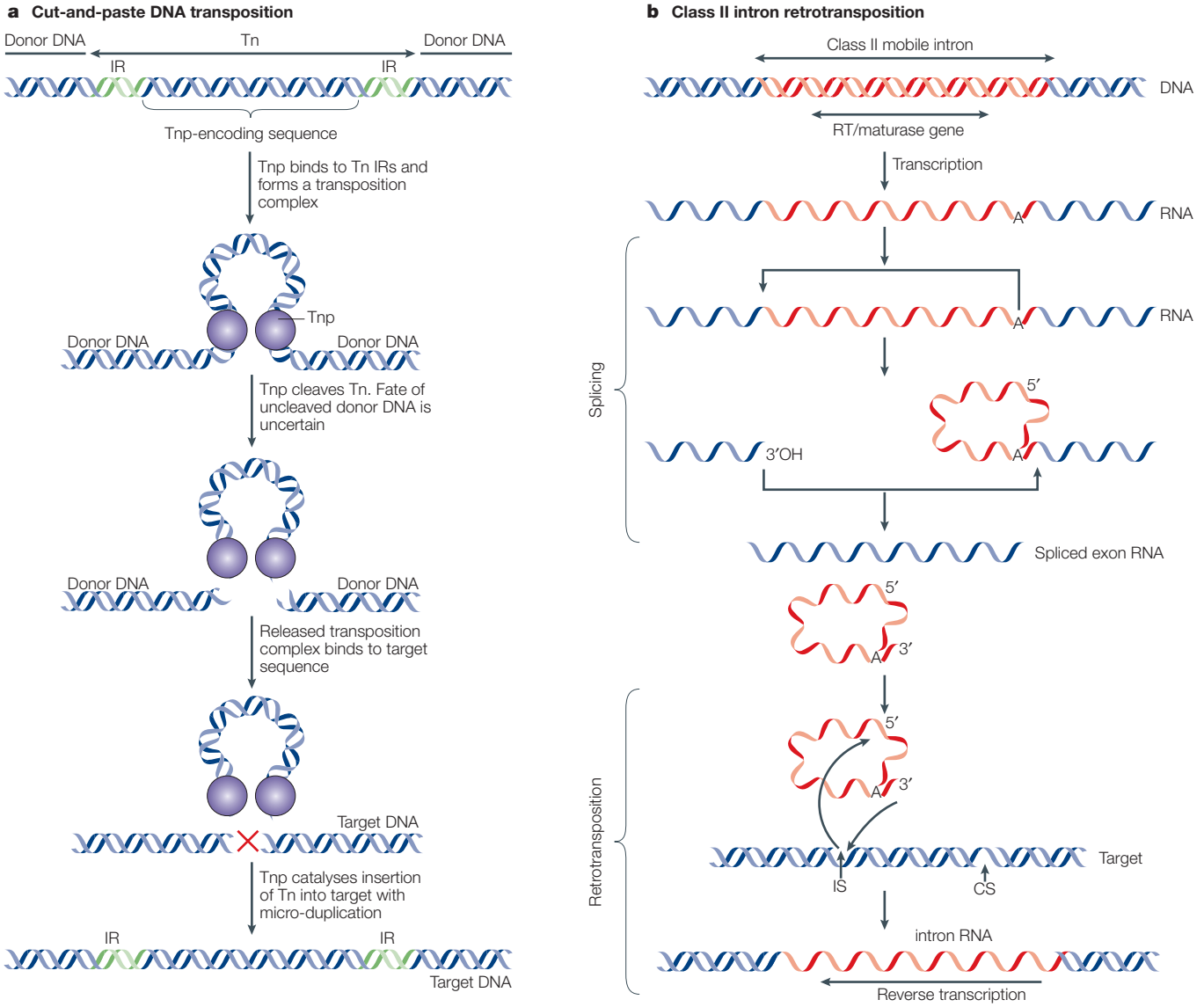

Figure 4 | **Transposable genetic elements. a** | DNA transposition. DNA transposition occurs by various mechanisms[99]. The figure shows the general pathway for the IS*4* transposable element (Tn) family that moves by a conservative cut-and-paste process. Other *Wolbachia w*Mel transposable elements probably use other conservative transposition mechanisms. DNA transposable elements have little or no target specificity. In eubacteria, a simple transposable element that encodes a single protein, the transposase (Tnp), is called an insertion sequence (IS). **b** | Class II mobile-intron retrotransposition. Intron removal from an mRNA involves two steps: the 2′OH group of a specific internal A attacks the 5′ end of the intron, forming a lariat structure. Then the 3′ end of the 5′ exon attacks the splice juncture site at the 3′ end of the intron, thereby releasing the intron lariat. The 3′ end of the lariat then attacks the target site (IS) for insertion, and the 3′ end of the nicked target attacks the 2′–5′ lariat bond, generating a DNA–RNA–DNA hybrid strand. The nuclease that is associated with the reverse transcriptase (RT) protein attacks the opposite strand of the target site (CS). The inserted intron then acts as a template used by RT to synthesize the intron cDNA. The second strand of intron DNA is synthesized. The donor DNA is not altered by retrotransposition. The target specificity for retrotransposition is determined by intron sequence and RT selectivity. This figure is similar to that presented in REF. 100. IR, inverted repeat.

database is shown in TABLE 1. Five out of thirteen obligate intracellular species and all thirteen of the facultative intracellular species harbour transposable elements. Among these species, the average number of transposable elements varies, with obligate intracellular bacteria and facultative intracellular bacteria harbouring an average of 29.2 and 131.9 transposase genes, respectively. There is also a report of an INSERTION SEQUENCE (IS) element in the primary endosymbiont SOPE of the *Sitophilus zeamais* grain weevil[77], the complete genome of which has not been sequenced.

Two classes of transposable elements are found in the genomes of obligate intracellular bacteria. The most 'genetically destructive' class is the DNA transposable element (FIG. 4a). These elements are identified by the presence of transposase-encoding genes that are

INSERTION SEQUENCE
A small segment of mobile DNA flanked by inverted repeat sequences that encodes a protein that catalyses transposition.

members of transposase families that have previously been found in free-living bacterial species[2]. For instance, *Wolbachia w*Mel contains transposase genes from the widely dispersed IS*3*, IS*4*, IS*5*, IS*110* and IS*256* families. DNA transposable elements from these families transpose by conservative mechanisms described in FIG. 4a. The impact of these transposition events includes possible inefficient repair of donor DNA molecules[78] and the disruptive effects of the transposable element insertions. Preliminary data also indicate the presence of previously undetected IS elements (IS*21*, IS*3* and IS*481*) in the genomes of *Buchnera* sp. APS, *Chlamydia muridarum* and *C. trachomatis*[2].

The second group of transposable elements comprises the retrotransposable elements that move through an RNA intermediate (FIG. 4b). The presence of these elements is indicated by the discovery of genes that encode reverse transcriptases (RT) in *Wolbachia w*Mel. There are fourteen RT-like genes, many of which are identical or nearly identical (TABLE 1). Of particular interest are three intact RT genes that contain MATURASE DOMAINS (WD0693, WD0995 and WD1138)[79]. A maturase domain in the RT genes is a strong genetic signature of a MOBILE GROUP II INTRON. Mobile introns should have no impact on donor DNA sequences and little impact on the integrity of target genes if they are inserted in the correct orientation, as splicing will remove the intron sequences from the target-gene mRNAs. Why there is a surplus and diversity of RT ORFs in *Wolbachia* remains a question for future study, but their presence in *Wolbachia* and absence from related *Rickettsia* genomes implies that they originated through horizontal-RT-gene transfer events.

Disrupted mobile-DNA genes

Eight of the thirteen obligate intracellular and all of the thirteen facultative intracellular species analysed in this review have genes that are annotated with mobile-DNA functions (FIG. 2, TABLE 1). Three patterns are evident with respect to the proportion of these mobile-DNA genes that are inactivated owing to mutations. First, the total proportion of disrupted mobile-DNA genes in the obligate (31.4%, 59/188) and facultative (0.9%, 22/2,414) intracellular species indicates an asymmetry in the coding capacity of mobile DNA between these two classes of bacteria. This asymmetry is consistent with the preferential degenerative evolution of mobile DNA in obligate intracellular species. Furthermore, analysis of the species listed in TABLE 1 also reveals that a greater fraction of obligate intracellular species that contain mobile DNA have inactivated mobile-DNA genes (three species out of eight) in comparison to that of the facultative intracellular species (three species out of thirteen). A closer look at the type of inactivated genes indicates a tendency to inactivate transposable-element genes across both classes of bacteria. In the three obligate intracellular species with inactivated mobile DNA, including *Wolbachia w*Mel, *C. burnetti* and *Chlamydophila caviae*, almost all of the disrupted genes encode presumed transposases or RTs. Similarly, in the three facultative intracellular species, including *Mycobacterium smegmatis*, *Mycobacterium tuberculosis* and *Listeria monocytogenes* 4b, almost all of the disrupted genes encode transposases.

Therefore, whereas obligate intracellular species are more likely to have disrupted mobile-DNA genes, perhaps owing to accelerated rates of mutation and gene inactivation, there seems to be a common selective force in bacteria that specifically drives inactivation of DNA transposable elements. A high fraction of non-site-specific DNA-transposition events would generate lethal mutations, ultimately posing a greater selective pressure against the presence of active transposable elements in a genome. Furthermore, excision of a DNA transposable element requires double-strand-break repair of the transposable-element donor chromosome, and this seems to be inefficient[78]. However, this does not explain the apparent high levels of RT-gene inactivation, as retrotransposition should have less damaging consequences. Instead, it is possible that the error-prone nature of RT activity results in an elevated mutation frequency for RT genes.

By contrast, phages often show site specificity in invading a host genome and might pose less of a fitness cost to their host bacterium if these sites are non-essential. Moreover, excision of prophages typically is efficient and self-repairing. Therefore, DNA-transposable-element inactivation might reflect deterministic forces to specifically eliminate harmful transposable elements from the genome.

Mobile DNA and the 'intracellular arena'

The presence of mobile DNA in obligate intracellular bacteria raises two crucial questions: first, what is its origin, and second, how does it spread in a host population of obligate intracellular bacteria? The first question can be answered using phylogenomic studies. Assuming that the mobile genetic elements in intracellular bacteria were originally derived from orthologous elements in free-living species and that enough microbial genomes will be sequenced to trace the historical pedigree of mobile-DNA donors and recipients, then phylogenetic analysis should piece together the evolutionary history of mobile DNA in obligate intracellular bacteria.

The amount of genome-sequence information that is currently available paints a cloudy picture of the origins of mobile DNA in obligate intracellular species. Although putative orthologues of transposable elements and bacteriophages can be identified among free-living and intracellular bacteria, we are unable to discern between the original and most recent donor species of mobile DNA. Two interesting cases of transposable-element acquisitions in the *Wolbachia w*Mel genome indicate that the bacteriophage WO-B might be a vector for introducing transposable elements into *Wolbachia*[69]. An IS*110*-like transposase sequence from bacteriophage WO-B from *Wolbachia w*CauB[80] is present in multiple (and often degenerate) copies throughout

MATURASE DOMAIN
A region of the reverse-transcriptase gene that ensures proper RNA folding for intron excision from the RNA.

MOBILE GROUP II INTRON
A catalytic RNA molecule that acts as a mobile genetic element as it encodes a reverse transcriptase and can insert site-specifically into target DNA.

the *w*Mel genome (both within and distant from prophage regions) and is sometimes flanked by additional transposase sequences. There is also an intact IS*5*0-like sequence in the WO-B prophage genome of *Wolbachia w*Tai[64]. IS*5*0-transposase homologues are rare, but occur in widely divergent bacteria, including *Wolbachia w*Mel[69]. The most recent donors of the bacteriophages themselves are probably other intracellular bacteria, as the shared AT nucleotide biases of intracellular bacteria with mobile-element sequences indicate a long association in species with an intracellular lifestyle.

There are several possible answers to the second question — how mobile DNA spreads among the obligate intracellular bacteria. Mobile DNA might confer a fitness benefit to the host and spread rapidly, but, so far, there is no clear evidence that transposable elements or bacteriophages carry genes that are adaptive for their obligate intracellular hosts. It is more probable that the mobile elements are neutral[4] or even deleterious to the bacterial host.

The 'intracellular arena' hypothesis posits that genetic material can move in and out of communities of obligate intracellular bacteria that co-infect the same intracellular host environment[66]. Therefore, one could view the eukaryotic host cell as a consortium of co-infecting intracellular bacteria that span different genotypes, species, genera and major orders of bacteria. Such an arena of interacting microorganisms could provide an escape from 'genetic confinement' for these specialized microorganisms and a window of opportunity for recombination and horizontal exchange of genomic elements.

This new hypothesis is motivated by reports of diverse bacteria that can co-infect the same host and molecular evolution studies that have examined chromosomal recombination and lateral exchange of phage DNA[66]. The α-proteobacteria *Wolbachia* often co-infect hosts with many other intracellular bacteria, including other *Wolbachia* strains[54], Anaplasmataceae relatives[81], γ-proteobacteria[82,83] and the Bacteroidetes parasite, *Candidatus Cardinium hertigii*[84,85]. The secondary endosymbionts of pea aphids horizontally transfer and co-infect aphids with primary endosymbionts and other strains of secondary endosymbionts at high frequencies[52,86]. Furthermore, the arthropod-borne pathogens *Rickettsia* and *Anaplasma* co-infect the same tick hosts[81], and different strains of the plant-pathogen *Phytoplasma* are also known to establish mixed infections in their plant hosts[87]. Also, *C. pneumoniae* can establish mixed infections in human hosts with *Mycoplasma pneumoniae*[88] and *Streptococcus pneumoniae*[89]. If there is transfer of mobile or chromosomal DNA among these microbial communities, then the view that obligate intracellular bacteria are devoid of genetic exchange might be overly simplistic.

So far, there is evidence in *Wolbachia* that supports the intracellular arena hypothesis. In three cases of insect species infected with two divergent strains of *Wolbachia*, recent lateral transfer of phage WO-B between the co-infecting strains was inferred based on comparative sequence analyses of a capsid-protein gene[64,66]. Results indicate that this evolutionarily recent exchange of phage DNA either occurred through recombination between prophage DNA sequences, horizontal transfer of complete or partial phage genomes or recombination between DNA from prophage and phage particles, as has been recently proposed in certain systems[46]. Determining the extent of horizontal DNA transfer associated with phage elements will be an interesting area of future research. Comparative analyses across sets of *Wolbachia* gene sequences specify that recombination frequently occurs among closely related strains[90–92] and possibly between divergent groups[93] in the gene encoding the *Wolbachia* surface protein Wsp. The extent to which genetic exchange in the intracellular arena influences the intracellular microbial community and the spread of mobile genetic elements in bacterial communities should be a topic of future study.

Interestingly, the recently sequenced genome of a *Wolbachia* strain that infects the pathogenic nematode *Brugia malayi* was found to have far fewer mobile-DNA genes than the genome of *Wolbachia w*Mel[94]. This streamlined version of the *Wolbachia* genome is not surprising when one considers its strict vertical transmission[95] and obligate mutualistic lifestyle[96–98]. All filarial nematodes are only infected by single strains of *Wolbachia* that are typically reduced in genome size compared with the *Wolbachia* that infect arthropods. The disparity in mobile-DNA content across a monophyletic clade of bacteria with varied transmission routes and host ranges clearly highlights the effects of lifestyle differences on mobile-DNA acquisition and invasion.

Future perspectives

Obligate intracellular bacteria are most often viewed as excellent model systems to understand the stable, symbiotic interactions that occur between prokaryotes and their eukaryotic hosts. The genomes of two strains of the aphid symbiont *Buchnera aphidicola* that are estimated to have diverged 50 to 70 million years ago show no indication of horizontal gene transfers[21], and many other obligate species have lost genes that encode DNA-repair and recombinase functions[12]. Therefore, just as the presence of horizontal gene transfer is a hallmark of genome evolution in bacteria with free-living lifestyles, its absence has become a hallmark of genome evolution in bacteria with obligate intracellular lifestyles.

Are these specialized bacteria sealed to a fate of perpetual gene loss with no gene inflow by horizontal gene transfer? We suggest that the answer depends, in part, on the lifestyle of the obligate species. Rates of horizontal gene transfer are affected by the rate of exposure to novel gene pools and, therefore, variations in transmission routes and host range will have a direct effect on rates of contact with foreign DNA. In support of this hypothesis, the obligate intracellular species with mobile DNA are those that host-switch (for example, *Wolbachia*, *Coxiella* and *Phytoplasma* species). Even the Chlamydiaceae family,

which has low genomic mobile-DNA contents, has extrachromosomal bacteriophages that infect the *Chlamydophila* genus. These data indicate that mobile genetic elements and horizontal gene transfer will not always be revealed by whole genome sequences, but might require the direct isolation of extrachromosomal elements.

The presence of mobile DNA alone does not specify whether a lateral transfer event has occurred in the recent or ancient evolutionary past. Instead, comparative sequence analyses of the mobile-DNA genes are the biologist's tool kit to reconstruct these evolutionary events. In many cases, the mobile DNA genes are disrupted by truncations and stop codons that reflect the ongoing degenerative processes in the genomes of intracellular bacteria. However, in other cases, it is clear that recent horizontal transfer of mobile DNA has occurred in obligate intracellular bacteria, particularly in cases associated with bacteriophages[64,66]. The recent discovery of mobile-DNA genes and horizontally acquired mobile DNA in host-switching obligate intracellular species argues for a new set of systems-biology methods that should complement the studies of single bacteria but also take into account the genetic interplay of multiple microorganisms that co-infect the same intracellular niche. Horizontal gene transfer could constitute both a serious threat to the stability of a highly integrated eukaryotic–prokaryotic association or a central source of evolutionary innovation to bacterial genomes that are otherwise experiencing extensive genome degeneration.

Note added in proof

Prior to publication, the discovery of the first putative conjugative plasmid in an obligate intracellular parasite (*Rickettsia felis*) was reported[101].

1. McClintock, B. Controlling elements and the gene. *Cold Spring Harb. Symp. Quant. Biol.* **21**, 197–216 (1956). **One of Barbara McClintock's seminal articles on transposable elements.**
2. Chandler, M. & Mahillon, J. in *Mobile DNA II* (eds Craig, N. L., Craigie, R., Gellert, M. & Lambowitz, A. M.) 305–366 (ASM Press, Washinton DC, 2002).
3. Dai, L., Toor, N., Olson, R., Keeping, A. & Zimmerly, S. Database for mobile group II introns. *Nucleic Acids Res.* **31**, 424–426 (2003).
4. Moran, N. A. & Plague, G. R. Genomic changes following host restriction in bacteria. *Curr. Opin. Genet. Dev.* **14**, 627–633 (2004).
5. Casjens, S. Prophages and bacterial genomics: what have we learned so far? *Mol. Microbiol.* **49**, 277–300 (2003). **Reviews the prevalence of prophage sequences in completed bacterial genomes and highlights the importance of prophages in prokaryotic genome evolution.**
6. Ohnishi, M., Kurokawa, K. & Hayashi, T. Diversification of *Escherichia coli* genomes: are bacteriophages the major contributors? *Trends Microbiol.* **9**, 481–485 (2001).
7. Banks, D. J., Beres, S. B. & Musser, J. M. The fundamental contribution of phages to GAS evolution, genome diversification and strain emergence. *Trends Microbiol.* **10**, 515–521 (2002).
8. Van Sluys, M. A. *et al.* Comparative analyses of the complete genome sequences of Pierce's disease and citrus variegated chlorosis strains of *Xylella fastidiosa*. *J. Bacteriol.* **185**, 1018–1026 (2003).
9. Miao, E. A. & Miller, S. I. Bacteriophages in the evolution of pathogen–host interactions. *Proc. Natl Acad. Sci. USA* **96**, 9452–9454 (1999).
10. Boyd, E. F., Davis, B. M. & Hochhut, B. Bacteriophage–bacteriophage interactions in the evolution of pathogenic bacteria. *Trends Microbiol.* **9**, 137–144 (2001).
11. Boyd, E. F. & Brussow, H. Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved. *Trends Microbiol.* **10**, 521–529 (2002).
12. Moran, N. A. & Wernegreen, J. J. Lifestyle evolution in symbiotic bacteria: insights from genomics. *Trends Ecol. Evol.* **15**, 321–326 (2000). **An excellent review on the origins and evolution of pathogenesis and mutualism in intracellular bacteria.**
13. Moran, N. A. Microbial minimalism: genome reduction in bacterial pathogens. *Cell* **108**, 583–586 (2002).
14. Klasson, L. & Andersson, S. G. Evolution of minimal-gene-sets in host-dependent bacteria. *Trends Microbiol.* **12**, 37–43 (2004).
15. Moran, N. A. & Baumann, P. Bacterial endosymbionts in animals. *Curr. Opin. Microbiol.* **3**, 270–275 (2000).
16. Buchner, P. Endosymbiosis of Animals with Plant Microorganisms (Interscience Publishers, John Wiley and Sons, New York, 1965).
17. Douglas, A. E. Mycetocyte symbiosis in insects. *Biol. Rev. Camb. Philos. Soc.* **64**, 409–434 (1989).
18. Werren, J. H. Biology of *Wolbachia*. *Annu. Rev. Entomol.* **42**, 587–609 (1997).
19. Stouthamer, R., Breeuwer, J. A. & Hurst, G. D. *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* **53**, 71–102 (1999).
20. Hunter, M. S., Perlman, S. J. & Kelly, S. E. A bacterial symbiont in the Bacteroidetes induces cytoplasmic incompatibility in the parasitoid wasp *Encarsia pergandiella*. *Proc. Biol. Sci.* **270**, 2185–2190 (2003).
21. Tamas, I. *et al.* 50 million years of genomic stasis in endosymbiotic bacteria. *Science* **296**, 2376–9237 (2002). **Compares the genomes of two ancient strains of the aphid endosymbiont *Buchnera* and reveals extreme genome stability and gene synteny. The genomes are devoid of mobile genetic elements.**
22. Silva, F. J., Latorre, A. & Moya, A. Why are the genomes of endosymbiotic bacteria so stable? *Trends Genet.* **19**, 176–180 (2003).
23. Andersson, S. G. *et al.* Comparative genomics of microbial pathogens and symbionts. *Bioinformatics* **18** (Suppl. 2), S17 (2002).
24. Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y. & Ishikawa, H. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* **407**, 81–86 (2000).
25. van Ham, R. C. H. J. *et al.* Reductive genome evolution in *Buchnera aphidicola*. *Proc. Natl Acad. Sci. USA* **100**, 581–586 (2003).
26. Akman, L. *et al.* Genome sequence of the endocellular obligate symbiont of tsetse flies, *Wigglesworthia glossinidia*. *Nature Genet.* **32**, 402–407 (2002).
27. Gil, R. *et al.* The genome sequence of *Blochmannia floridanus*: comparative analysis of reduced genomes. *Proc. Natl Acad. Sci. USA* **100**, 9388–9393 (2003).
28. Peterson, J. D., Umayam, L. A., Dickinson, T., Hickey, E. K. & White, O. The comprehensive microbial resource. *Nucleic Acids Res.* **29**, 123–125 (2001).
29. Lee, C. A. Pathogenicity islands and the evolution of bacterial pathogens. *Infect. Agents Dis.* **5**, 1–7 (1996).
30. Recchia, G. D. & Hall, R. M. Gene cassettes: a new class of mobile element. *Microbiology* **141**, 3015–3027 (1995).
31. Scott, J. R. & Churchward, G. G. Conjugative transposition. *Annu. Rev. Microbiol.* **49**, 367–397 (1995).
32. Andersson, J. O. & Andersson, S. G. Insights into the evolutionary process of genome degradation. *Curr. Opin. Genet. Dev.* **9**, 664–671 (1999). **Introduces the concept of deletion biases and determines that deletion mutations in *Rickettsia* pseudogenes are more frequent than insertion mutations.**
33. Andersson, J. O. & Andersson, S. G. Genome degradation is an ongoing process in *Rickettsia*. *Mol. Biol. Evol.* **16**, 1178–1191 (1999).
34. Andersson, J. O. & Andersson, S. G. Pseudogenes, junk DNA, and the dynamics of *Rickettsia* genomes. *Mol. Biol. Evol.* **18**, 829–839 (2001).
35. Mira, A., Ochman, H. & Moran, N. A. Deletional bias and the evolution of bacterial genomes. *Trends Genet.* **17**, 589–596 (2001).
36. Lawrence, J. G., Hendrix, R. W. & Casjens, S. Where are the pseudogenes in bacterial genomes? *Trends Microbiol.* **9**, 535–540 (2001).
37. Davison, J. Genetic exchange between bacteria in the environment. *Plasmid* **42**, 73–91 (1999).
38. Sabater-Munoz, B., van Ham, R. C., Moya, A., Silva, F. J. & Latorre, A. Evolution of the leucine gene cluster in *Buchnera aphidicola*: insights from chromosomal versions of the cluster. *J. Bacteriol.* **186**, 2646–2654 (2004).
39. Wernegreen, J. J. & Moran, N. A. Vertical transmission of biosynthetic plasmids in aphid endosymbionts (*Buchnera*). *J. Bacteriol.* **183**, 785–790 (2001).
40. Moran, N. A., Plague, G. R., Sandstrom, J. P. & Wilcox, J. L. A genomic perspective on nutrient provisioning by bacterial symbionts of insects. *Proc. Natl Acad. Sci. USA* **100** (Suppl. 2), 14543–14548 (2003).
41. Van Ham, R. C. *et al.* Postsymbiotic plasmid acquisition and evolution of the repA1-replicon in *Buchnera aphidicola*. *Proc. Natl Acad. Sci. USA* **97**, 10855–10860 (2000).
42. Akman, L., Rio, R. V., Beard, C. B. & Aksoy, S. Genome size determination and coding capacity of *Sodalis glossinidius*, an enteric symbiont of tsetse flies, as revealed by hybridization to *Escherichia coli* gene arrays. *J. Bacteriol.* **183**, 4517–4525 (2001).
43. Liefting, L. W., Shaw, M. E. & Kirkpatrick, B. C. Sequence analysis of two plasmids from the phytoplasma beet leafhopper-transmitted virescence agent. *Microbiology* **150**, 1809–1817 (2004). **A recent article that briefly summarizes the many plasmids in *Phytoplasma* and describes the discovery of two others.**
44. Nishigawa, H. *et al.* Evidence of intermolecular recombination between extrachromosomal DNAs in phytoplasma: a trigger for the biological diversity of *Phytoplasma*? *Microbiology* **148**, 1389–1396 (2002).
45. Oshima, K. *et al.* A plasmid of *Phytoplasma* encodes a unique replication protein having both plasmid- and virus-like domains: clue to viral ancestry or result of virus/plasmid recombination? *Virology* **285**, 270–277 (2001).
46. Hendrix, R. W. Bacteriophage genomics. *Curr. Opin. Microbiol.* **6**, 506–511 (2003).
47. Canchaya, C., Fournous, G., Chibani-Chennoufi, S., Dillmann, M. L. & Brussow, H. Phage as agents of lateral gene transfer. *Curr. Opin. Microbiol.* **6**, 417–424 (2003).
48. Krylov, V. N. [Role of horizontal gene transfer by bacteriophages in the origin of pathogenic bacteria]. *Genetika* **39**, 595–620 (2003) (in Russian).
49. Fukatsu, T. Secondary intracellular symbiotic bacteria in aphids of the genus *Yamatocallis* (Homoptera: Aphididae: Drepanosiphinae). *Appl. Environ. Microbiol.* **67**, 5315–5320 (2001).
50. Fukatsu, T., Nikoh, N., Kawai, R. & Koga, R. The secondary endosymbiotic bacterium of the pea aphid *Acyrthosiphon pisum* (Insecta: Homoptera). *Appl. Environ. Microbiol.* **66**, 2748–2758 (2000).
51. van der Wilk, F., Dullemans, A. M., Verbeek, M. & van den Heuvel, J. F. Isolation and characterization of APSE-1, a bacteriophage infecting the secondary endosymbiont of *Acyrthosiphon pisum*. *Virology* **262**, 104–113 (1999). **Reports the first genome sequence of a bacteriophage from an insect endosymbiont.**

52. Sandstrom, J. P., Russell, J. A., White, J. P. & Moran, N. A. Independent origins and horizontal transfer of bacterial symbionts of aphids. *Mol. Ecol.* **10**, 217–228 (2001).
53. Jeyaprakash, A. & Hoy, M. A. Long PCR improves *Wolbachia* DNA amplification: *wsp* sequences found in 76% of sixty-three arthropod species. *Insect Mol. Biol.* **9**, 393–405 (2000).
54. Werren, J. H. & Windsor, D. M. *Wolbachia* infection frequencies in insects: evidence of a global equilibrium? *Proc. Biol. Sci.* **267**, 1277–1285 (2000).
55. Dumler, J. S. *et al.* Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as subjective synonyms of *Ehrlichia phagocytophila*. *Int. J. Syst. Evol. Microbiol.* **51**, 2145–2165 (2001).
56. Saint Andre, A. *et al.* The role of endosymbiotic *Wolbachia* bacteria in the pathogenesis of river blindness. *Science* **295**, 1892–1895 (2002).
57. Taylor, M. J. *Wolbachia* endosymbiotic bacteria of filarial nematodes. A new insight into disease pathogenesis and control. *Arch. Med. Res.* **33**, 422–424 (2002).
58. Bandi, C., Slatko, B. & O'Neill, S. L. *Wolbachia* genomes and the many faces of symbiosis. *Parasitol. Today* **15**, 428–429 (1999).
59. Bordenstein, S. R., O'Hara, F. P. & Werren, J. H. *Wolbachia*-induced incompatibility precedes other hybrid incompatibilities in *Nasonia*. *Nature* **409**, 707–710 (2001).
60. Bordenstein, S. R. in *Insect Symbiosis* (eds Bourtzis, K. & Miller, T.) 283–304 (CRC Press, New York, 2003).
A recent review on the evolutionary consequences of *Wolbachia* infection, with an emphasis on the mechanisms of *Wolbachia*-assisted speciation.
61. Kageyama, D. & Traut, W. Opposite sex-specific effects of *Wolbachia* and interference with the sex determination of its host *Ostrinia scapulalis*. *Proc. Biol. Sci.* **271**, 251–258 (2004).
62. Jiggins, F. M., Hurst, G. D. & Majerus, M. E. Sex-ratio-distorting *Wolbachia* causes sex-role reversal in its butterfly host. *Proc. Biol. Sci.* **267**, 69–73 (2000).
63. Wright, J. D., Sjostrand, F. S., Portaro, J. K. & Barr, A. R. The ultrastructure of the rickettsia-like microorganism *Wolbachia pipientis* and associated virus-like bodies in the mosquito *Culex pipiens*. *J. Ultrastruct. Res.* **63**, 79–85 (1978).
64. Masui, S., Kamoda, S., Sasaki, T. & Ishikawa, H. Distribution and evolution of bacteriophage WO in *Wolbachia*, the endosymbiont causing sexual alterations in arthropods. *J. Mol. Evol.* **51**, 491–497 (2000).
Characterizes the first genome sequence of prophage WO-B and infers lateral transfer between different *Wolbachia* supergroups.
65. Masui, S. *et al.* Bacteriophage WO and virus-like particles in *Wolbachia*, an endosymbiont of arthropods. *Biochem. Biophys. Res. Commun.* **283**, 1099–1104 (2001).
66. Bordenstein, S. R. & Wernegreen, J. J. Bacteriophage flux in endosymbionts (*Wolbachia*): infection frequency, lateral transfer, and recombination rates. *Mol. Biol. Evol.* **21**, 1981–1991 (2004).
67. Gavotte, L. *et al.* Diversity, distribution and specificity of WO phage infection in *Wolbachia* of four insect species. *Insect Mol. Biol.* **13**, 147–153 (2004).
68. Sanogo, Y. O. & Dobson, S. L. Molecular discrimination of *Wolbachia* in the *Culex pipiens* complex: evidence for variable bacteriophage hyperparasitism. *Insect Mol. Biol.* **13**, 365–369 (2004).
69. Reznikoff, W. S., Bordenstein, S. R. & Apodaca, J. Comparative sequence analysis of IS50/Tn5 transposase. *J. Bacteriol.* **186**, 8240–8247 (2004).
70. Michel-Briand, Y. & Baysse, C. The pyocins of *Pseudomonas aeruginosa*. *Biochimie* **84**, 499–510 (2002).
71. Everett, K. D., Bush, R. M. & Andersen, A. A. Emended description of the order *Chlamydiales*, proposal of *Parachlamydiaceae* fam. nov. and *Simkaniaceae* fam. nov., each containing one monotypic genus, revised taxonomy of the family *Chlamydiaceae*, including a new genus and five new species, and standards for the identification of organisms. *Int. J. Syst. Bacteriol.* **49**, 415–440 (1999).
72. Richmond, S. J., Stirling, P. & Ashley, C. R. Virus infecting the reticulate bodies of an avian strain of *Chlamydia psittaci*. *FEMS Microbiol. Lett.* **14**, 31–36 (1982).
73. Hsia, R. C., Ting, L. M. & Bavoil, P. M. Microvirus of *Chlamydia psittaci* strain guinea pig inclusion conjunctivitis: isolation and molecular characterization. *Microbiology* **146**, 1651–1660 (2000).
74. Liu, B. L. *et al.* Molecular characterization of a bacteriophage (Chp2) from *Chlamydia psittaci*. *J. Virol.* **74**, 3464–3469 (2000).
75. Read, T. D., Fraser, C. M., Hsia, R. C. & Bavoil, P. M. Comparative analysis of *Chlamydia* bacteriophages reveals variation localized to a putative receptor binding domain. *Microb. Comp. Genomics* **5**, 223–231 (2000).
76. Garner, S. A., Everson, J. S., Lambden, P. R., Fane, B. A. & Clarke, I. N. Isolation, molecular characterisation and genome sequence of a bacteriophage (Chp3) from *Chlamydophila pecorum*. *Virus Genes* **28**, 207–214 (2004).
77. Dale, C., Wang, B., Moran, N. & Ochman, H. Loss of DNA recombinational repair enzymes in the initial stages of genome degeneration. *Mol. Biol. Evol.* **20**, 1188–1194 (2003).
78. Bender, J., Kuo, J. & Kleckner, N. Genetic evidence against intramolecular rejoining of the donor DNA molecule following IS*10* transposition. *Genetics* **128**, 687–694 (1991).
79. Wu, M. *et al.* Phylogenomics of the reproductive parasite *Wolbachia pipientis* wMel: a streamlined genome overrun by mobile genetic elements. *PLoS Biol.* **2**, E69 (2004).
Reports the first genome sequence of *Wolbachia* from *Drosophila melanogaster* and finds that the genome is unusually littered with repetitive and mobile DNA sequences.
80. Fujii, Y., Kubo, T., Ishikawa, H. & Sasaki, T. Isolation and characterization of the bacteriophage WO from *Wolbachia*, an arthropod endosymbiont. *Biochem. Biophys. Res. Commun.* **317**, 1183–1188 (2004).
81. Hartelt, K. *et al.* Pathogens and symbionts in ticks: prevalence of *Anaplasma phagocytophilum* (*Ehrlichia* sp.), *Wolbachia* sp., *Rickettsia* sp., and *Babesia* sp. in Southern Germany. *Int. J. Med. Microbiol.* **293** (Suppl. 37), 86–92 (2004).
82. Heddi, A., Grenier, A. M., Khatchadourian, C., Charles, H. & Nardon, P. Four intracellular genomes direct weevil biology: nuclear, mitochondrial, principal endosymbiont, and *Wolbachia*. *Proc. Natl Acad. Sci. USA* **96**, 6814–6819 (1999).
83. Gomez-Valero, L. *et al.* Coexistence of *Wolbachia* with *Buchnera aphidicola* and a secondary symbiont in the aphid *Cinara cedri*. *J. Bacteriol.* **186**, 6626–6633 (2004).
84. Weeks, A. R., Velten, R. & Stouthamer, R. Incidence of a new sex-ratio-distorting endosymbiotic bacterium among arthropods. *Proc. Biol. Sci.* **270**, 1857–1865 (2003).
85. Zchori-Fein, E. & Perlman, S. J. Distribution of the bacterial symbiont *Cardinium* in arthropods. *Mol. Ecol.* **13**, 2009–2016 (2004).
86. Russell, J. A., Latorre, A., Sabater-Munoz, B., Moya, A. & Moran, N. A. Side-stepping secondary symbionts: widespread horizontal transfer across and beyond the Aphidoidea. *Mol. Ecol.* **12**, 1061–1075 (2003).
87. Leyva-Lopez, N. E., Ochoa-Sanchez, J. C., Leal-Klevezas, D. S. & Martinez-Soriano, J. P. Multiple *Phytoplasmas* associated with potato diseases in Mexico. *Can. J. Microbiol.* **48**, 1062–1068 (2002).
88. Fernandez, M., Zagolin, M., Ruiz, M., Martinez, M. A. & Diaz, J. C. [Community acquired pneumonia in a hospitalized community: etiological study]. *Rev. Med. Chil.* **131**, 498–504 (2003) (in Spanish).
89. Fukano, H. *et al.* [Comparison of clinical presentation of mixed pneumonia with *Chlamydia pneumoniae* and *Streptococcus pneumoniae* and *S. pneumoniae pneumonia*]. *Kansenshogaku Zasshi* **78**, 108–113 (2004) (in Japanese).
90. Jiggins, F. M., von Der Schulenburg, J. H., Hurst, G. D. & Majerus, M. E. Recombination confounds interpretations of *Wolbachia* evolution. *Proc. Biol. Sci.* **268**, 1423–1427 (2001).
91. Reuter, M. & Keller, L. High levels of multiple *Wolbachia* infection and recombination in the ant *Formica exsecta*. *Mol. Biol. Evol.* **20**, 748–753 (2003).
92. Werren, J. H. & Bartos, J. D. Recombination in *Wolbachia*. *Curr. Biol.* **11**, 431–435 (2001).
93. Baldo, L., Lo, N. & Werren, J. H. Mosaic nature of the *Wolbachia* surface protein. *J. Bacteriol.* (in the press).
94. Foster, J. *et al.* The *Wolbachia* genome of *Brugia malayi*: endosymbiont evolution within a human pathogenic nematode. *PLoS Biol.* **3**, E121 (2005).
95. Casiraghi, M., Anderson, T. J., Bandi, C., Bazzocchi, C. & Genchi, C. A phylogenetic analysis of filarial nematodes: comparison with the phylogeny of *Wolbachia* endosymbionts. *Parasitology* **122**, 93–103 (2001).
96. Hoerauf, A. *et al.* Doxycycline in the treatment of human onchocerciasis: kinetics of *Wolbachia* endobacteria reduction and of inhibition of embryogenesis in female *Onchocerca* worms. *Microbes Infect.* **5**, 261–273 (2003).
97. Kramer, L. H., Passeri, B., Corona, S., Simoncini, L. & Casiraghi, M. Immunohistochemical/immunogold detection and distribution of the endosymbiont *Wolbachia* of *Dirofilaria immitis* and *Brugia pahangi* using a polyclonal antiserum raised against WSP (*Wolbachia* surface protein). *Parasitol. Res.* **89**, 381–386 (2003).
98. Casiraghi, M. *et al.* Tetracycline treatment and sex-ratio distortion: a role for *Wolbachia* in the moulting of filarial nematodes? *Int. J. Parasitol.* **32**, 1457–1468 (2002).
99. Mizuuchi, K. & Backer, T. A. in *Mobile DNA II* (eds Craig, N. L., Craigie, R., Gellert, M. & Lambowitz, A. M.) 12–23 (ASM Press, Washington DC, 2002).
100. Belfort, M., Derbyshire, V., Parker, M. M., Cousineau, B. & Lambowitz, A. M. in *Mobile DNA II* (eds Craig, N. L., Craigie, R., Gellert, M. & Lambowitz, A. M.) 761–783 (ASM Press, Washington DC, 2002).
Provides an excellent review of the distribution, stucture and splicing mechanisms of group I and group II mobile introns.
101 Ogata, H. *et al*. The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. *PLoS Biol*. **3**, e248 (2005).

Acknowledgements

Work for this article was supported by a NASA Astrobiology Institute grant and the Evelyn Mercer Professorship of Biochemistry and Molecular Biology, University of Wisconsin, USA. We thank M. Belfort, S. Biber, A. Lazarus, S. Roland and J. Wernegreen for their insightful comments.

Competing interests statement

The authors declare no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:
Entrez: http://www.ncbi.nlm.nih.gov/Entrez
ϕCPAR39 | ϕX174 | *Buchnera aphidicola* | *Buchnera* sp. APS | *Chlamydia muridarum* | *Chlamydia trachomatis* | *Chlamydophila caviae* | *Chlamydophila pneumoniae* | Chp1 | Chp2 | *Coxiella burnetti* | *Listeria monocytogenes* 4b | *Mycobacterium smegmatis* | *Mycobacterium tuberculosis* | *Mycoplasma pneumoniae* | *Streptococcus pneumoniae* | *Wigglesworthia glossinidia* | *Wolbachia pipientis*

FURTHER INFORMATION

Seth Bordenstein's laboratory:
http://jbpc.mbl.edu/bordenstein
William Reznikoff's homepage:
http://www.biochem.wisc.edu/reznikoff/index.html
The Comprehensive Microbial Resource: http://www.tigr.org/tigr-scripts/CMR2/CMRHomePage.spl
The Institute for Genomic Research: http://www.tigr.org
International Symbiosis Society: http://people.bu.edu/iss
***Wolbachia* FIBR Project:** http://research.amnh.org/FIBR
***Wolbachia*:**
http://www.Wolbachia.sols.uq.edu.au/index.html
Access to this interactive links box is free online.