4. Peterson, K.J., Lyons, J.B., Nowak, K.S., Takacs, C.M., Wargo, M.J., and McPeek, M.A. (2004). Estimating metazoan divergence times with a molecular clock. Proc. Natl. Acad. Sci. USA *101*, 6536–6541.

5. Philippe, H., Lartillot, N., and Brinkmann, H. (2005). Multigene analysis of Bilaterian animals corroborate the monophyly of Ecdysozoa, Lophotrochozoa, and Protostomia. Mol. Biol. Evol. *22*, 1246–1253.

6. Dunn, C., Hejnol, A., Matus, D., Pang, K., Browne, W., Smith, S., Seaver, E., Rouse, G., Obst, M., and Edgecombe, G. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. Nature *452*, 745–749.

7. Jenner, R.A., and Littlewood, D.T.J. (2008). Problematica old and new. Philos. Trans. R. Soc. B: Biol. Sci. *363*, 1503–1512.

8. Struck, T.H., Golombek, A., Weigert, A., Franke, F.A., Westheide, W., Purschke, G., Bleidorn, C., and Halanych, K.M. (2015). The evolution of annelids reveals two adaptive routes to the interstitial realm. Curr. Biol. *25*, 1993–1999.

9. Laumer, C.E., Bekkouche, N., Kerbl, A., Goetz, F., Neves, R.C., Sørensen, M.V., Kristensen, R.M., Hejnol, A., Dunn, C.W., Giribet, G., and Worsaae, K. (2015). Spiralian phylogeny informs the evolution of microscopic lineages. Curr. Biol. *25*, 2000–2006.

10. Worsaae, K., and Rouse, G.W. (2008). Is Diurodrilus an annelid? J. Morphol. *269*, 1426–1455.

11. Westheide, W. (1987). Progenesis as a principle in meiofauna evolution. J. Nat. Hist. *21*, 843–854.

12. Egger, B., Lapraz, F., Tomiczek, B., Müller, S., Dessimoz, C., Girstmair, J., Škunca, N., Rawlinson, K.A., Cameron, C.B., and Beli, E. (2015). A transcriptomic-phylogenomic analysis of the evolutionary relationships of flatworms. Curr. Biol. *25*, 1347–1353.

13. Strausfeld, N.J., and Hirth, F. (2013). Deep homology of arthropod central complex and vertebrate basal ganglia. Science *340*, 157–161.

14. De Robertis, E. (2008). The molecular ancestry of segmentation mechanisms. Proc. Natl. Acad. Sci. USA *105*, 16411–16412.

15. Budd, G.E., and Jensen, S. (2000). A critical reappraisal of the fossil record of the bilaterian phyla. Biol. Rev. *75*, 253–295.

16. Erwin, D.H., Laflamme, M., Tweedt, S.M., Sperling, E.A., Pisani, D., and Peterson, K.J. (2011). The Cambrian Conundrum: Early Divergence and Later Ecological Success in the Early History of Animals. Science *334*, 1091–1097.

17. Martin, M.W., Grazhdankin, D.V., Bowring, S.A., Evans, D.A.D., Fedonkin, M.A., and Kirschvink, J.L. (2000). Age of Neoproterozoic bilaterian body and trace fossils, White Sea, Russia: Implications for metazoan evolution. Science *288*, 841–845.

18. Sperling, E.A., Frieder, C.A., Raman, A.V., Girguis, P.R., Levin, L.A., and Knoll, A.H. (2013). Oxygen, ecology, and the Cambrian radiation of animals. Proc. Natl. Acad. Sci. USA *110*, 13446–13451.

19. Philippe, H., Brinkmann, H., Lavrov, D.V., Littlewood, D.T.J., Manuel, M., Wörheide, G., and Baurain, D. (2011). Resolving difficult phylogenetic questions: Why more sequences are not enough. PLoS Biol. *9*, e1000602.

Microbial Evolution: Towards Resolving the Plasmid Paradox

R. Craig MacLean* **and Alvaro San Millan**
Department of Zoology, University of Oxford, South Parks Road, Oxford OX1 3PS, UK
*Correspondence: craig.maclean@zoo.ox.ac.uk
http://dx.doi.org/10.1016/j.cub.2015.07.006

Plasmids play a key role in bacterial evolution by providing bacteria with new and important functions, such as antibiotic resistance. New research shows how bacterial regulatory evolution can stabilize bacteria–plasmid associations and catalyze evolutionary innovation.

Plasmids are autonomously replicating, mobile genetic elements that exist as small, circular DNA molecules within bacterial cells [1]. Plasmids are widely distributed across bacteria, and it is common for clones from many families of bacteria to carry multiple different plasmids within the same cell [2]. Plasmid genomes consist of 'core' genes that are purely involved in plasmid transmission and replication, and 'accessory' genes that can increase bacterial fitness under some ecological conditions [3]. For example, many plasmids carry antibiotic-resistance and heavy-metal-resistance genes that increase bacterial survival and competitive fitness in the presence of these toxins. Unlike bacterial chromosomal DNA, which is only vertically transmitted from mother cell to daughter cells, plasmid DNA can also be transmitted horizontally between even distantly related bacteria. Plasmids therefore provide bacteria with potential access to a vast reservoir of genes, and horizontal gene transfer mediated by plasmids acts as a very important source of evolutionary innovation in bacteria [4,5]. For example, antibiotic resistance in many pathogenic bacteria has evolved by the acquisition of plasmids carrying antibiotic-resistance genes derived from distantly related environmental bacteria, including many bacteria that actually produce antibiotics [6].

Although plasmids carry genes that can potentially benefit their bacterial hosts, it remains challenging to understand how plasmids can persist in bacterial populations over the long term — an evolutionary dilemma that has been called the 'plasmid paradox' [7]. First, plasmids impose a fitness cost on their bacterial hosts that generates selection against plasmid carriage under conditions in which plasmid genes do not provide any benefit to the host [8,9]. In this scenario, selection acting on the bacteria favours the loss of

plasmids simply because they are parasites that reduce bacterial fitness. The great irony of the plasmid paradox is that exposure to conditions that select for plasmid-carried genes can also ultimately lead to plasmid loss. Ecologically important genes on plasmids are often found in mobile genetic elements, such as transposons, that can move from plasmids onto chromosomes. The movement of beneficial genes from plasmids onto bacterial chromosomes therefore has the potential to render plasmids redundant and expose their fitness costs, resulting in selection against plasmid carriage. In this scenario, plasmids initially act as symbionts that increase bacterial fitness, but movement of beneficial genes onto the bacterial chromosome transforms plasmids from symbionts to parasites. Recurrent horizontal gene transfer of plasmids between bacteria could theoretically resolve the plasmid paradox and allow plasmids to stably persist in bacterial populations [10]. However, the general consensus is that rates of plasmid transfer are simply too low for horizontal transfer to maintain plasmids in the absence of selection for plasmid-carried genes [11]. For example, whole-genome sequencing has shown that only about 50% of plasmids are capable of transmitting themselves horizontally via conjugation [12]; the remaining 50% must rely on alternative mechanisms, such as phage-mediated transduction, and uptake of free plasmid DNA by naturally competent bacteria, both of which are thought to be inefficient mechanisms for plasmid transfer.

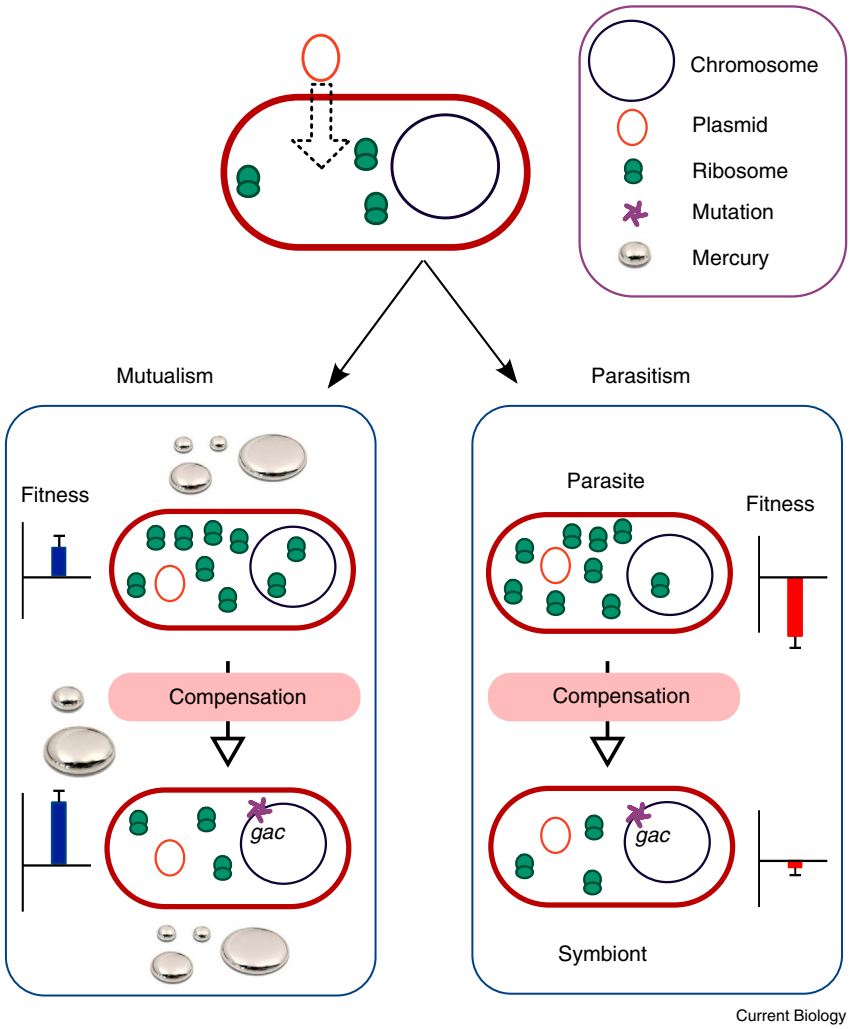

Figure 1. Compensatory evolution stabilizes a bacteria–plasmid association.
The acquisition of plasmid pQBR103 imposes a large biosynthetic burden on the bacterium *P. fluorescens*. Bacterial populations adapt to this burden via compensatory mutations in the GacA–GacS regulatory system that reduce plasmid gene expression. Compensatory adaptation therefore stabilizes the association between *P. fluorescens* and pQBR103, in both the presence and absence of mercury, by increasing the fitness of plasmid-bearing clones.

A key question in microbial evolution and ecology has therefore been to understand how plasmids persist in bacterial populations over evolutionary timescales. In a recent paper in *Current Biology*, Harrison and colleagues [13] address this problem by using experimental evolution to study interactions between the bacterium *Pseudomonas fluorescens SBW25* and pQBR103, a large plasmid that carries a mercury-resistance transposon. In experimental evolution, bacterial populations are serially passaged in laboratory culture media that impose distinct selective pressures. The large population size and short generation time of bacteria allows for rapid evolutionary responses to these manipulations, and the great advantage of this method is that it allows evolutionary outcomes to be observed in real time [14,15]. Previous studies that have examined evolution in bacteria–plasmid associations have tended to focus exclusively on studying evolution under conditions in which plasmids carry a cost. This study is novel in that it captures the dual role that plasmids play as both symbionts and parasites by studying evolution under high and low doses of mercury, respectively. The plasmid was maintained in the presence of mercury, where pQBR103 was initially a beneficial symbiont, as expected. Crucially, the plasmid was also maintained in populations that evolved in the absence of mercury, where pQBR103 initially acted as a costly parasite.

To understand the evolutionary mechanisms underpinning plasmid maintenance, the authors used whole-genome sequencing on a large number of clones that evolved under conditions in which the plasmid was initially either symbiotic or parasitic. The authors repeatedly observed

evolution in a relatively small number of 'target' genes across the bacterial chromosome, especially the GacA–GacS regulatory system. Such parallel molecular evolution is a tell-tale sign of positive selection for beneficial mutations in these target genes, implying that common evolutionary paths are followed to stabilize plasmids in bacterial populations across the parasite-to-symbiont continuum of bacteria–plasmid interactions. One simple explanation for parallel evolution in the GacA–GacS system is that mutations in these genes stabilized pQB103 by compensating for its costs. Consistent with this idea, the authors were able to show that knock-out mutations in *gacA* and *gacS* were sufficient to eliminate the cost of pQBR103 carriage.

To uncover the functional basis of compensatory evolution, the authors used microarrays to measure changes in gene expression during plasmid domestication. The acquisition of pQBR103 altered the expression of approximately 16% of bacterial chromosomal genes; in particular, the expression of genes involved in protein synthesis was massively upregulated. The expression of both plasmid genes and chromosomal biosynthetic genes was reduced in evolved clones, strongly suggesting that the cost of pQBR103 carriage stems from a huge additional biosynthetic burden associated with the expression of plasmid genes. Mutations in the GacA–GacS system clearly eliminate this burden under the conditions of this experiment. However, we speculate that this is likely to be a more complex problem in nature, because upper-level regulatory systems, like GacA–GacS, often have pleiotropic roles, and loss of these systems may carry costs in nature that are not evident in lab experiments.

In summary, this study shows that plasmids can be stably maintained in bacterial populations in both the presence and absence of selection for plasmid-carried genes (Figure 1). This is an important finding because it suggests that the conditions for the maintenance of plasmids in bacterial populations may be less restrictive than the plasmid paradox would suggest. This study also raises some interesting observations that question some of the logic underlying the plasmid paradox. For example, the authors repeatedly observed that the mercury transposon moved to the bacterial chromosome in populations that evolved in the presence of mercury, and this was accompanied by plasmid loss. However, clones carrying chromosomal mercury-resistance genes were not able to successfully invade into bacterial populations. One possible explanation for this result is that movement of the transposon onto the chromosome was a rare event and, by chance, the transposon tended to move into chromosomal backgrounds with low fitness. Alternatively, it is possible that this evolutionary path was not followed because chromosomal mercury-resistance transposons tend to carry a greater fitness cost than plasmid-borne copies. If this is the case, then it is possible that plasmids are so ubiquitous because it is advantageous to have copies of some genes on plasmids. An important goal for future work will be to understand the constraints on chromosomal gene acquisition from plasmids and the advantages associated with the plasmid location of genes.

Fitness costs associated with plasmid carriage are the most important obstacle to plasmid maintenance in bacterial populations. Although a large number of studies have measured the cost of plasmid acquisition [7–9], the molecular mechanisms underpinning this cost have remained largely obscure. This study provides clear evidence that the cost of plasmid maintenance is ameliorated by the reduction of plasmid gene expression, which is in good agreement with a second recently published study [16]. This is important, because it suggests that the cost of plasmid carriage is produced by the burden associated with plasmid gene expression and/or by the negative interactions between plasmid-encoded proteins and the bacterial host [8]. Understanding the chromosomal mechanisms that contribute to modulating plasmid gene expression is therefore a key area for future work aimed at understanding the molecular biology of bacteria–plasmid associations.

Plasmids play a key role in the evolution and ecology of bacteria, but our understanding of the fundamental processes that allow plasmids to persist over the long term remains incomplete. Recent empirical work has made progress towards resolving this problem by simultaneously studying the ecology and molecular biology of bacteria–plasmid associations in the presence and absence of selection for plasmid-encoded traits [13,16,17]. Hopefully future studies will translate the conceptual advances being made by these studies to understanding bacteria–plasmid associations in environmental and clinical settings.

REFERENCES

1. Funnell, B.E., and Phillips, G.J., eds. (2004). Plasmid Biology (Washington, D.C: American Society for Microbiology).
2. San Millan, A., Heilbron, K., and MacLean, R.C. (2014). Positive epistasis between co-infecting plasmids promotes plasmid survival in bacterial populations. ISME J. *8*, 601–612.
3. Gogarten, J.P., and Townsend, J.P. (2005). Horizontal gene transfer, genome innovation and evolution. Nat. Rev. Microbiol. *3*, 679–687.
4. Wiedenbeck, J., and Cohan, F.M. (2011). Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol. Rev. *35*, 957–976.
5. Treangen, T.J., and Rocha, E.P.C. (2011). Horizontal transfer, not duplication, drives the expansion of protein families in prokaryotes. PLoS Genet. *7*, e1001284.
6. Martínez, J.L. (2008). Antibiotics and antibiotic resistance genes in natural environments. Science *321*, 365–367.
7. Harrison, E., and Brockhurst, M.A. (2012). Plasmid-mediated horizontal gene transfer is a coevolutionary process. Trends Microbiol. *20*, 262–267.
8. Baltrus, D.A. (2013). Exploring the costs of horizontal gene transfer. Trends Ecol. Evol. *28*, 489–495.
9. Vogwill, T., and MacLean, R.C. (2015). The genetic basis of the fitness costs of antimicrobial resistance: a meta-analysis approach. Evol. Appl. *8*, 284–295.
10. Stewart, F.M., and Levin, B.R. (1977). The population biology of bacterial plasmids: a priori conditions for the existence of conjugationally transmitted factors. Genetics *87*, 209–228.
11. Bergstrom, C.T., Lipsitch, M., and Levin, B.R. (2000). Natural selection, infectious transfer and the existence conditions for bacterial plasmids. Genetics *155*, 1505–1519.

12. Smillie, C., Garcillán-Barcia, M.P., Francia, M.V., Rocha, E.P., and de la Cruz, F. (2010). Mobility of plasmids. Microbiol. Mol. Biol. Rev. *74*, 434–452.

13. Harrison, E., Guymer, D., Spiers, A.J., Paterson, S., and Brockhurst, M.A. (2015). Parallel compensatory evolution stabilizes plasmids across the parasitism–mutualism continuum. Curr. Biol. *25*, 2034–2039.

14. Buckling, A., Maclean, R.C., Brockhurst, M.A., and Colegrave, N. (2009). The Beagle in a bottle. Nature *457*, 824–829.

15. Barrick, J.E., and Lenski, R.E. (2013). Genome dynamics during experimental evolution. Nat. Rev. Genet. *14*, 827–839.

16. San Millan, A., Toll-Riera, M., Qi, Q., and MacLean, R.C. (2015). Interactions between horizontally acquired genes create a fitness cost in Pseudomonas aeruginosa. Nat. Commun. *6*, 6845.

17. San Millan, A., Peña-Miller, R., Toll-Riera, M., Halbert, Z.V., McLean, A.R., Cooper, B.S., and MacLean, R.C. (2014). Positive selection and compensatory adaptation interact to stabilize non-transmissible plasmids. Nat. Commun. *5*, 5208.

Signal Transduction: A Different Kind of Toll Is in the BAG

Dennis H. Kim
Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
Correspondence: dhkim@mit.edu
http://dx.doi.org/10.1016/j.cub.2015.06.057

A recent study identifies a role for a Toll pathway with likely non-canonical features in the developmental specification of BAG neurons in *Caenorhabditis elegans*. These neurons function to sense carbon dioxide, which is shown to facilitate avoidance of pathogenic bacteria.

The celebrated Toll pathway was initially discovered from a genetic screen for mutants defective in embryonic dorsal–ventral patterning in *Drosophila* [1]. Subsequently, another role for Toll was identified in the response of *Drosophila* to pathogenic fungi, where activation of the Toll pathway was found to induce expression of antimicrobial peptides [2]. The hypothesis that innate immunity is an ancient, evolutionarily conserved system of defense [3] was strikingly validated by the discoveries of mammalian Toll homologs in innate immune signaling. A human Toll-like receptor (TLR) was shown to activate the transcription factor NF-κB, homologous to *Drosophila* Dorsal, which controls the expression of cytokines and serves as a pivotal bridge to the activation of the adaptive immune response [4]. In parallel, positional cloning of a locus responsible for conferring to mice sensitivity to the effects of bacterial lipopolysaccharide also converged on TLR4 [5]. The molecular genetic elucidation of TLR signaling pathways in mammals established TLR-mediated pathogen recognition and signaling as a paradigm for understanding innate immunity in mammals [6].

The identification of TLR signaling components in basal cnidarian species underscored the ancient origins of Toll, but also suggested loss of Toll pathway components in some species, including *Caenorhabditis elegans* [7], where the function of the Toll pathway has remained elusive. Although the *C. elegans* genome encodes a single TLR homolog, TOL-1, no homologs of the canonical TLR signaling pathway components NF-κB and MyD88 have been identified, and only distant homology observed in other TLR signaling components (Figure 1). Moreover, a *tol-1* mutant did not have enhanced susceptibility to killing by pathogenic *Pseudomonas aeruginosa*, casting doubt on a role for canonical Toll signaling in *C. elegans* innate immunity [8]. The only tantalizing hint of what Toll might be doing in *C. elegans* was the observation that wild-type animals avoided the pathogenic bacterium *Serratia marcescens*, but *tol-1* mutant animals did not [8].

Now, Brandt and Ringstad [9], as reported in this issue of *Current Biology*, reveal that Toll signaling in *C. elegans* is required for the development of a pair of neurons, termed the BAG neurons, which function in the sensation of carbon dioxide [10] and molecular oxygen [11]. The authors started with a forward genetic approach to isolate mutants defective in the expression of genes normally expressed in the BAG neurons and identified a requirement for a mitogen-activated protein kinase (MAPK) module that includes *C. elegans* homologs of mammalian TAK1 MAPKKK and p38 MAPK in BAG neuron specification. The authors show that the PMK-3 p38 MAPK pathway functions during development and not in the direct sensation of carbon dioxide. Because the TAK1 MAPKKK lies at the nexus of multiple major signal transduction pathways, including not only MAPK signaling but also TLR signaling, the authors also examined the possibility that TOL-1 might also function in the BAG neurons. Strikingly, the genetic analysis of Brandt and Ringstad demonstrates that animals carrying mutations in *tol-1*, or mutations in genes encoding homologs of the TLR signaling components IRAK and TRAF, all exhibit defects in carbon dioxide sensing due to aberrant BAG neuron function. Mutations in a *C. elegans* homolog of mammalian IκB, which is negatively regulated by canonical TLR signaling, can suppress the defective BAG neuron phenotypes of *tol-1* and *pmk-3* mutants. The authors also demonstrate using genetically encoded

