Virology

Phages hijack their host's defence

The discovery of a functional CRISPR/Cas system in bacteriophages is the first example of this defence mechanism in viruses, and beautifully illustrates the evolutionary tit-for-tat between viruses and the bacteria they infect. See Letter p.XXX

Manuela Villion & Sylvain Moineau

If you belong to the most abundant biological entities on the planet, you need a few tricks to stay on top. The global population of bacterial viruses (bacteriophages or phages) has been estimated to be 10^{31}, outnumbering their bacterial hosts by ten-fold (ref. 2). Bacteria have developed a formidable arsenal of sophisticated strategies to neutralize viruses[3], yet phages always seem to find a way to evolve, persist, and abound. Studies of the complex evolutionary dynamics between phages and bacteria led to the discovery of a widespread bacterial defence system called CRISPR/Cas[4]. In this issue, Seed *et al.*[1] report the remarkable finding that a group of phages infecting *Vibrio cholerae* have also acquired a functional CRISPR/Cas system in their own genome that allows them to neutralize an unrelated anti-virus system in their bacterial host.

CRISPR/Cas stands for clustered regularly interspaced short palindromic repeats (CRISPR), which are often flanked by *cas* (CRISPR-associated) genes[5]. These directly repeating nucleotide sequences are separated by short stretches of non-repetitive DNA called spacers. CRISPR/Cas regions have been found in 45% of bacterial species and 90% of archaea. These CRISPR/Cas-

harbouring microorganisms can acquire small pieces of DNA directly from the genomes of invading phages[4] or plasmids[6] (small non-chromosomal DNA molecules that can be transmitted between bacteria) and insert them as spacers within a CRISPR site. The spacers are then transcribed and processed, leading to the production of small RNA molecules called crRNAs. The crRNAs, coupled with Cas proteins, act as a surveillance system that is primed to quickly target, through base-pairing, similar nucleic acids from subsequent invaders, and then to cleave them[6]. Thus, the bacteria can acquire genetic information from invaders and use it to mount a defence. This process is reminiscent of our own immune system, and hence CRISPR/Cas has been called an adaptive microbial immune system.

A few stealthy phages have been discovered that can bypass this protection through mutation or deletion of the targeted region[7] or through acquisition of anti-CRISPR genes[8]. Metagenomic studies have also identified CRISPR/Cas systems in viral genomes[9], but no biological relevance was proposed for these. Enter Seed and colleagues, who elegantly demonstrate that phage genomes are no longer just ammunition and targets for CRISPR cassettes — certain *V. cholerae* phages have hijacked the entire system for their own defence and persistence.

V. cholerae is responsible for cholera, which affects hundred of thousands of people each year[10]. Phages are among the factors that may modulate the incidence of cholera in endemic regions, so understanding the interactions between the bacteria and their infecting phages is of interest. Seed *et al*. analysed the genomes of eleven phages isolated from stool samples of patients with cholera, and found that five of them contained a CRISPR/Cas system. When the authors examined the sequence of their spacers, they found them to match regions in the genome of the host bacteria.

Specifically, the spacer sequences matched an 18 kilobase 'genomic island' that is present in several other strains of *V. cholerae*.

This genomic element resembles phage-inducible chromosomal islands (PICIs), which are found, among others, in some *Staphylococcus aureus* strains. In *S. aureus*, these regions are known as SaPIs, and they represent pathogenicity islands that contain genes encoding virulence factors[11]. When a SaPI-containing cell is infected by certain phages, the SaPI sequence excises from the bacterial chromosome, circularizes and replicates, presumably to exit the infected bacterium. During this process, the bacterium also activates a largely uncharacterized defence system in an attempt to stop phage propagation and thereby ensure its own persistence and the persistence of the surrounding phage-susceptible bacterial population[12]. Seed *et al.* demonstrate that the 18-kb element in *V. cholerae* also circularizes following phage infection, and that it encodes an active anti-phage system. Consequently, the authors refer to it as a PICI-like element, or PLE.

Further studies of one of the isolated phages, phage ICP1, which carries a CRISPR/Cas system with two PLE-targeting spacers, showed that it can replicate and kill a PLE-harbouring *V. cholerae* strain isolated from the same stool sample. However, the authors show that a mutant phage ICP1 that lacks the matching spacer cannot replicate in this PLE+ strain, but that it can replicate in a mutated *V. cholerae* strain lacking the PLE, further supporting the targeted action of the CRISPR/Cas system.

The authors also performed an elaborate set of experiments to confirm the hallmarks of an active CRISPR/Cas system. For example, they show that crRNAs are transcribed and processed from the phages, and that they could isolate derivative phages that had acquired new CRISPR spacers

targeting PLE. Overall, these results demonstrate that phages can hijack a functional adaptive immune-evasion system to benefit their own multiplication. Furthermore, because bacterial cell death and DNA damage is inherent to virulent phage infection, CRISPR-mediated DNA cleavage of the targeted bacterial genome does not negatively impact phage proliferation .

This study illustrates another extraordinary turn of events in the evolution of phages and bacteria, in which the phages outright defeat the bacteria by using one of its own weapons against it. It remains to be seen how frequently such an event occurs and whether a phage that contains a CRISPR/Cas system remains stable. Nevertheless, these findings will certainly fuel selected applications. For example, the discovery of other phages with a CRISPR/Cas system targeting host genes, or phages with anti-CRISPR genes[8], may provide additional leverage to design an efficient cocktail of natural or engineered phages to prevent or treat bacterial contamination or infection. On the other hand, this finding suggests biotechnological industries relying solely on CRISPR/Cas systems to protect key bacterial strains from phage infection should be ready to go back to the drawing board. Because, as always, phages will find a way. They may already have.

Manuela Villion & Sylvain Moineau are in the Département de biochimie, microbiologie et bio-informatique, Faculté des sciences et de génie, Université Laval, Québec, Canada, G1V 0A6. S. M. holds the Canada Research Chair in Bacteriophages and is also in the Groupe de recherche en écologie buccale, Faculté de médecine dentaire, and the Félix d'Hérelle Reference Center for Bacterial Viruses, Université Laval
e-mail : Sylvain.Moineau@bcm.ulaval.ca

1. Seed, K. D., Lazinski, D. W., Calderwood, S. B. & Camilli, A. http://dx.doi.org/10.1038/xxx (2013).

2. Suttle, C. A. *Nature* **437,** 356–361 (2005).

3. Labrie, S. J., Samson, J. E., & Moineau, S. *Nature Rev. Microbiol.* **8,** 317–327 (2010).

4. Barrangou, R. *et al*. *Science* **315,** 1709–1712 (2007).

5. Wiedenheft, B., Sternberg, S. H., & Doudna, J. A. *Nature* **482,** 331–338 (2012).

6. Garneau, J. E. *et al. Nature* **468,** 67–71 (2010).

7. Deveau, H. *et al*. *J. Bacteriol*. **190,** 1390–1400 (2008).

8. Bondy-Denomy, J., Pawluk, A., Maxwell, K. L., & Davidson, A. R. *Nature* **493**, 429–32 (2013).

9. Minot, S. *et al. Genome Res.* **21,** 1616–1625 (2011).

10. (http://www.who.int/topics/cholera/en/): Cholera , 2011, *Weekly Epidemiological Record* **87**, 289–-304 (2012).

11. Novick, R. P., Christie, G. E., & Penadés, J. R. *Nature Rev. Microbiol.* **8**, 541–51 (2010).

12. Ram G. R., *et al. Proc. Natl Acad. Sci. USA* **109**, 6300–16305 (2012).