Sensing danger

Luciano A. Marraffini[a,1]

CRISPR-Cas loci encode for an adaptive immune system in prokaryotes that provides defense against viruses (1) and plasmids (2) that infect these organisms. CRISPR loci contain a series of repetitive sequences intercalated with short sequences derived from invading viruses and plasmids (3–5). These short sequences, called spacers, are acquired from the invader upon infection during an "immunization" event (1). This is the first phase of CRISPR-Cas immunity. The information stored in spacers is then used to target the invader in the second phase of the CRISPR immune response (6). Each spacer sequence is transcribed and processed as a short RNA called CRISPR RNA (crRNA) that directs RNA-guided Cas nucleases to their matching target in the invader's genome to cleave it. Little is known about the regulation of the CRISPR-Cas immune response. The CRISPR-Cas locus present in *Escherichia coli* has been shown to be tightly repressed by HNS, a negative regulator of widespread effects in bacteria (7). Repression can be relieved by the LeuO activator (8), but environmental or physiological conditions that lead to the expression of the CRISPR-Cas machinery have not been identified.

In PNAS, Høyland-Kroghsbo et al. (9) describe the regulation of the CRISPR-Cas locus of the bacterial pathogen *Pseudomonas aeruginosa* through quorum sensing (QS) pathways. QS is a mechanism that bacteria use to communicate with each other and organize collective behaviors (10), mediated by genes that are responsible for the production and detection of extracellular chemicals known as autoinducers. Upon bacterial growth, the increase in the concentration of autoinducers triggers a signaling cascade that modifies the response of the bacterial population to different environmental cues (Fig. 1). Høyland-Kroghsbo et al. (9) first investigate whether the expression of the *cas* (CRISPR-associated) genes is affected by QS. *cas* genes flank the CRISPR array of repeats and spacers and are responsible for the execution of both phases of the CRISPR-Cas immune response (6). It was found that *cas* transcription is significantly increased at high cell density, and that this increase is dependent on both *lasI* and *rhlI*, the genes encoding for QS autoinducer synthases. Høyland-Kroghsbo et al.

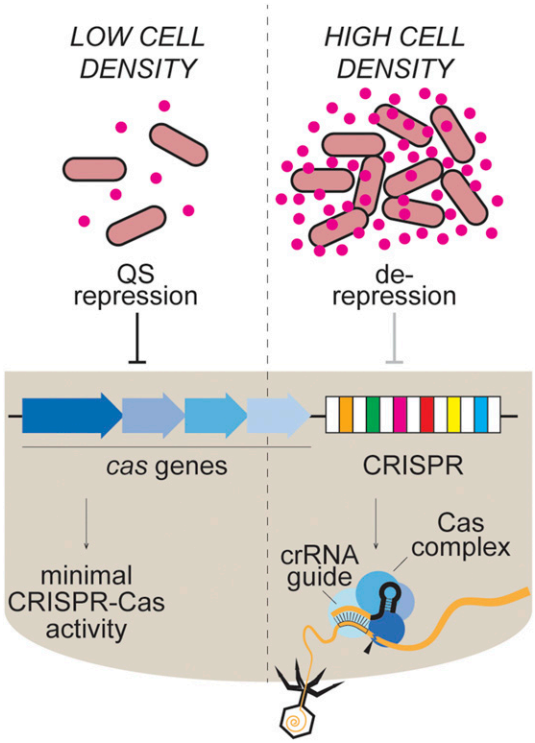

Fig. 1. Regulation of CRISPR-Cas activity by QS. At low cell density, low autoinducer (pink dot) concentrations result in the repression of CRISPR-*cas* loci through the QS pathway, keeping the CRISPR-Cas immune response at a minimum. In contrast, at high densities of bacteria (when they are most susceptible to phage attack) the increase in the concentration of autoinducer triggers the de-repression of CRISPR-*cas* loci. Expression of the Cas protein complex and crRNA guide prepares the cell for the cleavage (arrowhead) of incoming viruses.

(9) then check for the effects of the deletion of these genes in both stages of the CRISPR-Cas immune response: targeting and immunization. The expectation is that the down-regulation of *cas* genes that results from the absence of autoinducer production in the *lasI*,*rhlI* double mutant should negatively affect CRISPR-Cas immunity. Targeting is tested through transformation with a plasmid harboring a target sequence, which is considerably

[a]Laboratory of Bacteriology, The Rockefeller University, New York, NY 10065
Author contributions: L.A.M. wrote the paper.
The author declares no conflict of interest.
See companion article on page 131.
[1]Email: Luciano.Marraffini@rockefeller.edu.

reduced in the QS double mutant. Immunization is explored via PCR amplification of the CRISPR array, showing that wild-type, but not QS mutant, bacteria were able to expand the array with new spacers. Equivalent results were recently obtained in the antibiotic-producing bacterium *Serratia* (11), indicating that QS control of CRISPR-Cas expression may be a general regulatory mechanism of bacterial adaptive immunity.

These are important findings that open new avenues of research for some of the outstanding questions of CRISPR immunity. First, the findings show how bacteria can mount a coordinated antiphage response. It has been argued that at high cellular densities the danger of phage predation is markedly increased (12). Dense bacterial populations offer the possibility of extreme propagation to lytic phages, the phage progeny quickly finding a new, readily accessible host to infect next. In addition, rapidly growing bacteria tend to be clonal, reducing the chances that the next and nearby host is equipped with a phage-resistance genotype. In line with this theory, it has been already shown that QS can induce envelope resistance in *E. coli* (13) and *Vibrio anguillarum* (14) through the down-regulation of genes encoding for phage receptors. The involvement of QS in the CRISPR-Cas response adds another layer of organization to the bacterial antiphage resistance pathways. Second, the new findings provide a possible answer for a long-standing question about CRISPR immunity: how does this system prevent the acquisition of spacers from the chromosomal DNA? At the moment, experiments suggest that it is not possible for CRISPR-Cas systems to distinguish chromosomal vs. phage or plasmid DNA (they have the same chemistry after all). One study has demonstrated that the Cas machinery prefers to acquire new spacers from free DNA ends (15). Although this favors the acquisition of spacers from phages (at least during DNA injection they can expose a free DNA end) over the bacterial chromosome (which is a closed circle most of the time), many studies have shown that there still is extensive spacer acquisition from the bacterial genome (15–19). The low expression of the *cas* genes involved in spacer integration when the CRISPR immune response is less needed provides one route to decrease the "autoimmunity" cost associated with bacterial adaptive immunity.

Finally, the findings of Høyland-Kroghsbo et al. (9) provide new avenues for the development of phage therapies to treat *Pseudomonas* infections. *P. aeruginosa* causes chronic airway infections in cystic fibrosis patients and recently has become resistant to many antimicrobial drugs (20). The need for new antibiotics has led to the exploration of phage therapy for *Pseudomonas* infections (21); however, there is the worrying possibility that bacteria will use phage-resistance pathways, such as CRISPR, to become refractory to phage treatment. The use of QS inhibitors that maintain the CRISPR-Cas immune response at a minimum level could provide a strategy to confront phage resistance. As a big plus, *Pseudomonas* virulence genes are regulated by QS and therefore the inhibition of these pathways can also lead to a reduction of pathogenicity (22). This will represent an interesting addition to the list of CRISPR-Cas systems applications for biotechnology, which includes strain immunization for the dairy industry (23), the development of sequence-specific antimicrobials (24, 25) and, most notably, genome editing (26–28), an addition that requires the shut-down and not the exploitation of the CRISPR mechanisms. But the really exciting prospects lie in the future research that will elucidate the molecular mechanisms and implications behind QS regulation of the CRISPR-Cas immune response.

1 Barrangou R, et al. (2007) CRISPR provides acquired resistance against viruses in prokaryotes. *Science* 315(5819):1709–1712.
2 Marraffini LA, Sontheimer EJ (2008) CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. *Science* 322(5909):1843–1845.
3 Bolotin A, Quinquis B, Sorokin A, Ehrlich SD (2005) Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology* 151(Pt 8):2551–2561.
4 Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E (2005) Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol* 60(2):174–182.
5 Pourcel C, Salvignol G, Vergnaud G (2005) CRISPR elements in *Yersinia pestis* acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology* 151(Pt 3):653–663.
6 Marraffini LA (2015) CRISPR-Cas immunity in prokaryotes. *Nature* 526(7571):55–61.
7 Pul U, et al. (2010) Identification and characterization of *E. coli* CRISPR-cas promoters and their silencing by H-NS. *Mol Microbiol* 75(6):1495–1512.
8 Westra ER, et al. (2010) H-NS-mediated repression of CRISPR-based immunity in *Escherichia coli* K12 can be relieved by the transcription activator LeuO. *Mol Microbiol* 77(6):1380–1393.
9 Høyland-Kroghsbo NM, et al. (2017) Quorum sensing controls the *Pseudomonas aeruginosa* CRISPR-Cas adaptive immune system. *Proc Natl Acad Sci USA* 114:131–135.
10 Papenfort K, Bassler BL (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat Rev Microbiol* 14(9):576–588.
11 Patterson AG, et al. (November 17, 2016) Quorum sensing controls adaptive immunity through the regulation of multiple CRISPR-Cas systems. *Mol Cell*, 10.1016/j.molcel.2016.11.012.
12 Abedon ST (2012) Spatial vulnerability: Bacterial arrangements, microcolonies, and biofilms as responses to low rather than high phage densities. *Viruses* 4(5):663–687.
13 Høyland-Kroghsbo NM, Maerkedahl RB, Svenningsen SL (2013) A quorum-sensing-induced bacteriophage defense mechanism. *MBio* 4(1):e00362-12.
14 Tan D, Svenningsen SL, Middelboe M (2015) Quorum sensing determines the choice of antiphage defense strategy in *Vibrio anguillarum*. *MBio* 6(3):e00627.
15 Levy A, et al. (2015) CRISPR adaptation biases explain preference for acquisition of foreign DNA. *Nature* 520(7548):505–510.
16 Heler R, et al. (2015) Cas9 specifies functional viral targets during CRISPR-Cas adaptation. *Nature* 519(7542):199–202.
17 Paez-Espino D, et al. (2013) Strong bias in the bacterial CRISPR elements that confer immunity to phage. *Nat Commun* 4:1430.
18 Wei Y, Terns RM, Terns MP (2015) Cas9 function and host genome sampling in Type II-A CRISPR-Cas adaptation. *Genes Dev* 29(4):356–361.
19 Yosef I, Goren MG, Qimron U (2012) Proteins and DNA elements essential for the CRISPR adaptation process in *Escherichia coli*. *Nucleic Acids Res* 40(12):5569–5576.
20 Folkesson A, et al. (2012) Adaptation of *Pseudomonas aeruginosa* to the cystic fibrosis airway: An evolutionary perspective. *Nat Rev Microbiol* 10(12):841–851.
21 Krylov VN (2014) Bacteriophages of *Pseudomonas aeruginosa*: Long-term prospects for use in phage therapy. *Adv Virus Res* 88:227–278.
22 O'Loughlin CT, et al. (2013) A quorum-sensing inhibitor blocks *Pseudomonas aeruginosa* virulence and biofilm formation. *Proc Natl Acad Sci USA* 110(44):17981–17986.
23 Barrangou R, Horvath P (2012) CRISPR: New horizons in phage resistance and strain identification. *Annu Rev Food Sci Technol* 3:143–162.
24 Bikard D, et al. (2014) Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nat Biotechnol* 32(11):1146–1150.
25 Citorik RJ, Mimee M, Lu TK (2014) Sequence-specific antimicrobials using efficiently delivered RNA-guided nucleases. *Nat Biotechnol* 32(11):1141–1145.
26 Cong L, et al. (2013) Multiplex genome engineering using CRISPR/Cas systems. *Science* 339(6121):819–823.
27 Jiang W, Bikard D, Cox D, Zhang F, Marraffini LA (2013) RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nat Biotechnol* 31(3):233–239.
28 Mali P, et al. (2013) RNA-guided human genome engineering via Cas9. *Science* 339(6121):823–826.