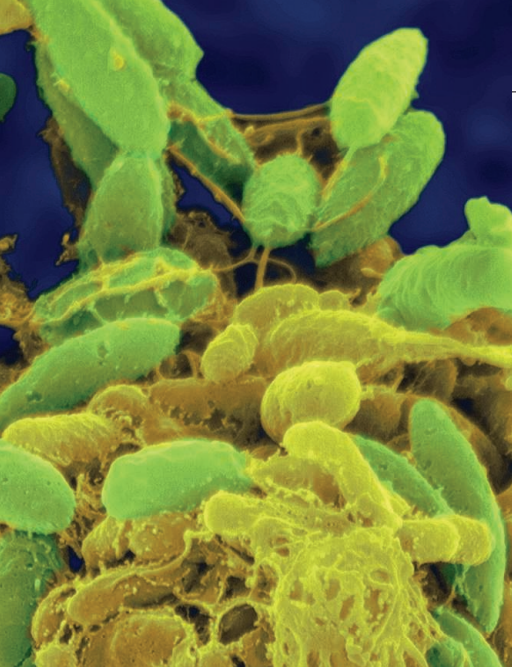


A scanning electron micrograph reveals a multiorganism biofilm formed on an implantable medical device. Life-threatening biofilm-associated infections resist antibiotic treatment and interfere with medical device function.

photobleaching than that seen with previous methods. This advance allowed the authors to carry out simultaneous imaging of 10,000 *V. cholerae* cells for the 16 hours it takes for the biofilm to develop, with 3-min intervals between subsequent images. This frequent imaging made it possible to track the trajectories of micrometer-sized cells, giving an unprecedented view into the behaviors of individual cells as the biofilm developed (see the figure).

The measurements revealed a qualitative transition in an individual cell's behavior, in which Brownian motion changes to ballistic motion as the biofilm develops. This transition corresponds to a new phase of collective growth, when the biofilm as a whole begins its vertical expansion away from the substrate. In this phase, cells displayed two types of trajectories. Some of the cells expanded ballistically outward, whereas others became trapped at the substrate. Overall, these trajectories gave rise to a collective fountain-like flow, which transported some cells to the biofilm front, while bypassing the cells trapped at the substrate. This fountain-like flow allowed for fast lateral expansion of the biofilm.

Cell tracking allowed Qin *et al.* to precisely quantify the dynamics of various cells, while also assessing how these dynamics differ for mutant cells that overproduce matrix components. To interpret the results, the authors built a mathematical model for the mechanics of biofilm expansion, balancing growth with substrate friction. By modeling different surface frictions and comparing the predicted cell motion with the observed cell motion, Qin *et al.* were able to explain the observed behavior as long as friction between the cells and surface was a dominant effect.

This study of *V. cholerae* offers an exciting insight into how collective behavior can arise from processes operating at the single-cell level. The mechanisms uncovered with a gram-negative bacterial species likely will be generalizable across other bacterial types. For example, the qualitative transitions in biofilm expansion observed in this study have analogs in other bacterial biofilms. With the gram-positive bacterium *Bacillus subtilis*, a qualitative change in colony expansion is triggered by a cellular bistable switch in which cells expressing flagella produce extracellular matrices (*12*, *13*). Osmolarity associated with matrix production drives colony expansion (*14*).

More broadly, this study demonstrates the great potential for advances in imaging technology and computer vision to help unravel how collective behavior arises from the activity of individual cells and their interactions. However, there is much more going on inside a biofilm that cannot yet be seen. More complete information would allow researchers to not only reconstruct the motion of cells but also uncover their phenotypic states. Previous work on *B. subtilis* with fluorescent labeling of genetic components shows detailed spatial arrangement of various cell types, with cells carrying out different biological functions in distinct parts of the biofilm (*3*, *15*).

One can only hypothesize about the diversity of cellular types and functions inside the beautiful fountain revealed in the present study. A deeper understanding of bacterial multicellular behavior will increase our ability to treat bacterial infections, control natural bacterial communities, and engineer synthetic ones for specific purposes. ■

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MOLECULAR BIOLOGY

Shutting down RNA-targeting CRISPR

The discovery of an anti-CRISPR reveals viral escape from CRISPR immunity

By **Rodolphe Barrangou**[1] and **Erik J. Sontheimer**[2]

Explorations of the evolutionary arms race between bacteria and bacteriophages (viruses that infect bacteria) have unearthed a variety of defense mechanisms that include CRISPR-Cas (CRISPR-associated nuclease) adaptive immune systems (*1*). Understanding the mechanisms of CRISPR-mediated immunity, involving DNA-encoded, RNA-guided, sequence-specific targeting of invasive nucleic acids (*2*, *3*), has spawned powerful genome engineering platforms based on diverse Cas effectors. Subsequent studies have also revealed anti-CRISPR proteins (*4*) that have proven valuable as control switches for Cas molecular machines. CRISPR-Cas immune systems encompass diverse families including DNA-targeting effectors such as Cascade-Cas3, Cas9, and Cas12 as well as the recently characterized RNA-targeting Cas13 ribonuclease (RNase) (*5*). The evolving immune arsenal in bacteria has been matched by diverse anti-CRISPRs that enable viruses to escape Cas nuclease targeting. On page 54 of this issue, Meeske *et al.* (*6*) report an anti-CRISPR mechanism that inhibits Cas13a RNase activity, with potential utility as a CRISPR effector control switch.

The Cas13 RNase is the signature protein for type VI CRISPR-Cas systems (*5*), which use their CRISPR RNA (crRNA) guides to target viral RNA sequences and trigger both the specific degradation of target phage RNA and the nonspecific destruction of host transcripts (*7*), leading to host dormancy and prevention of virus proliferation (*8*). Meeske *et al.* identified and

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characterized a type VI anti-CRISPR protein, AcrVIA1^{Lse}, encoded by the genome of the ϕLS46 listeriophage (see the figure). This anti-CRISPR binds to *Listeria seeligeri* Cas13a (LsCas13a) and prevents target RNA access and conformational activation of the RNase. AcrVIA1^{Lse} comprises 232 amino acids, which is notably larger than the typically hypervariable, <150–amino acid anti-CRISPRs previously characterized. The additional residues augment the RNase domain with a DNA-binding motif that contributes to anti-CRISPR transcriptional regulation. This AcrVIA1^{Lse} protein was able to completely thwart CRISPR-Cas13 immunity against otherwise susceptible listeriophage.

Meeske *et al*. investigated the interplay between AcrVIA1^{Lse} and the Cas13a:crRNA complex and showed dose-dependent inhibition of RNase activity through formation of an AcrVIA1^{Lse}:Cas13a:crRNA complex that prevents target RNA binding. Critically, both cis phage-targeting and trans host dormancy–inducing activities are abolished by AcrVIA1^{Lse}, enhancing not only the capacity of the listeriophage to replicate but also the ability of the host *L. seeligeri* to continue metabolically. This interaction was dissected structurally, and the authors used cryo–electron microscopy to establish that LsCas13a adopts a bilobed architecture encompassing a recognition domain and an RNase domain, directed by a 51-nucleotide crRNA with the target-complementary portion configured to pair with viral RNA. AcrVIA1^{Lse} interacts with both LsCas13a and the crRNA to prevent target RNA binding and RNase activation. Future studies will undoubtedly define AcrVIA1^{Lse} inhibitory specificity across Cas13 homologs, as well as the impact of type VI anti-CRISPR viral resistance on bacterial biology and host-virus dynamics.

Several studies have deciphered how anti-CRISPRs can inhibit Cas effectors by blocking target nucleic acid loading and preventing Cas nuclease activity (*4*), but the primary focus had been on DNA-targeting Cascade-Cas3, Cas9, and Cas12. These studies revealed that physiological anti-CRISPR activity often only partially inhibits CRISPR-Cas, providing limited immune escape (*9*, *10*). This appears to reflect a race between anti-CRISPR expression in phages and host immune defense by the CRISPR effector complexes. Viral infections can fail because anti-CRISPR expression does not reach inhibitory thresholds in time to overcome CRISPR immunity, but repeated failed infections can yield gradually increasing anti-CRISPR amounts that immunosuppress the host and allow later infections to succeed. In the case of type VI CRISPR immunity, however, the DNA genome of the invasive phage is not specifically cleaved; instead, viral and host transcripts are the targets for Cas13-mediated degradation, and virus propagation is suppressed primarily by the induction of bacterial host cell dormancy (*8*). This fundamental distinction reduces the time pressure on anti-CRISPR–mediated inhibition of Cas13 because the phage genome is not specifically cleared and dormancy can often be reversed.

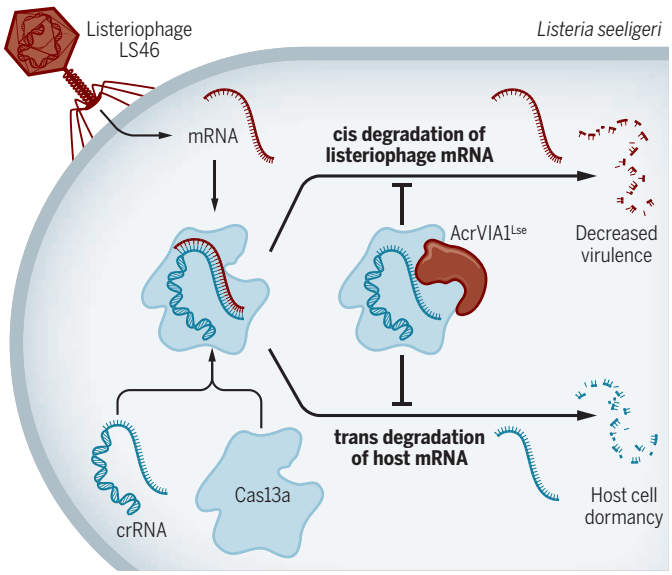
A single dose of AcrVIA1^{Lse} could completely abrogate LsCas13 activity, even in unfavorable conditions such as very low viral multiplicity of infection, targeting by multiple crRNAs, or immunity activation by early phage transcripts. Thus, mechanistically establishing how these different types of CRISPR systems function is a critical step toward understanding and repurposing these molecular machines and ultimately manipulating, engineering, and controlling Cas13-based tools.

Besides the continued rise of DNA-targeting CRISPR effectors such as Cas9 and Cas12 for genome editing, several recent studies have illustrated how valuable RNA-targeting Cas13 effectors can be, including for detection of RNA viruses such as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19) (*11*). There is also much potential for Cas13 as an RNA-targeting and RNA-editing platform (for example, to correct disease-causing mutations in cellular RNAs) (*12*, *13*), but this depends on whether its activity can be controlled. The study of Meeske *et al*. provides a means to reduce or eliminate Cas13 activity, opening new avenues for the use of CRISPR to control and shape the transcriptomes of organisms and viruses. Indeed, type VI anti-CRISPRs can readily control Cas13a RNA targeting and dCas13a RNA editing in human cells (*14*). However, more studies are needed to harness this tool and establish how broadly exploitable such Cas13-based technologies (and their off switches) could be. ■

"...opening new avenues for the use of CRISPR to control and shape the transcriptomes of organisms and viruses."

Inhibiting CRISPR-associated ribonuclease activity

Listeriophage LS46 encodes the anti-CRISPR protein AcrVIA1^{Lse}. The host, *Listeria seeligeri*, encodes a type VI CRISPR-Cas13a (CRISPR-associated 13a) immune system. Cas13a is a ribonuclease that, through complementarity between CRISPR RNA (crRNA) and target sequences in listeriophage messenger RNA (mRNA), cleaves and thus inactivates phage mRNA in cis and host mRNA in trans. AcrVIA1^{Lse} can bind and inhibit the Cas13a:crRNA complex.



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