Lab shows phage attacks in new light

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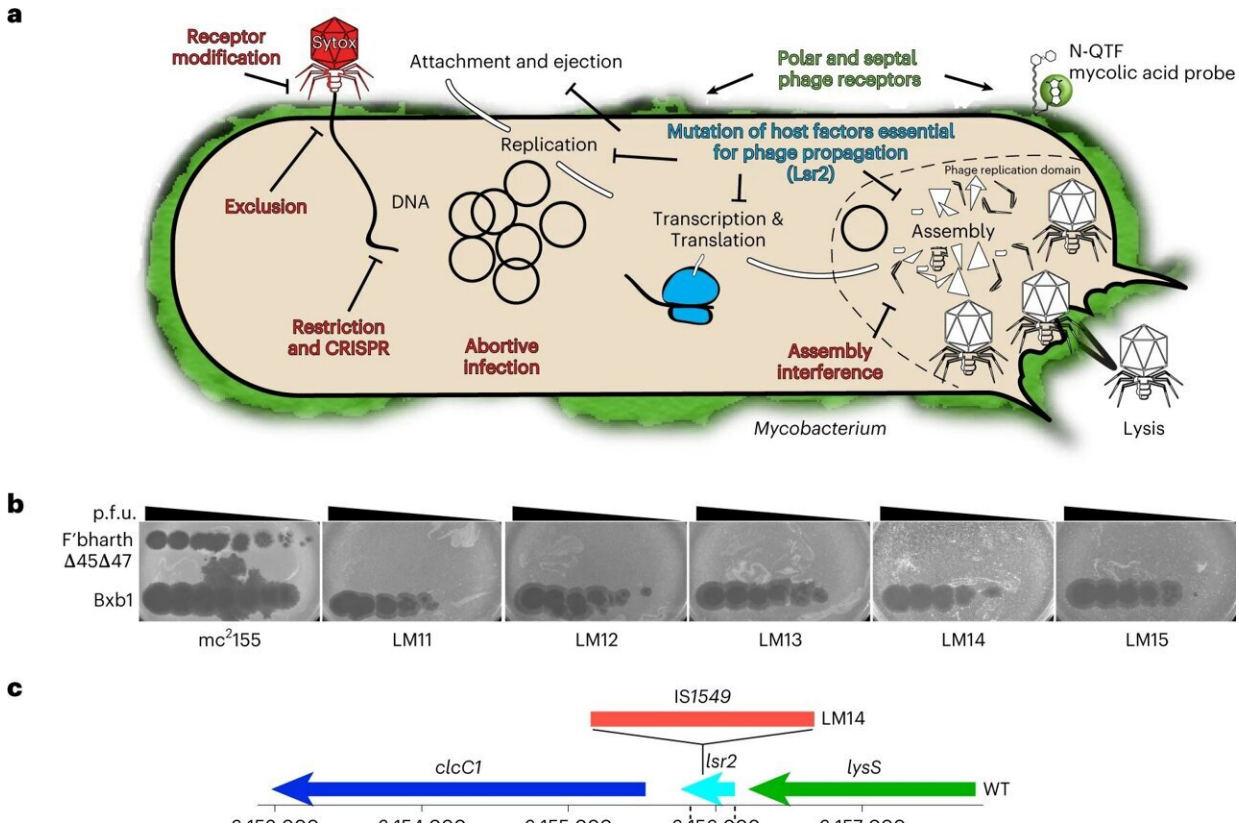

M. smegmatis Lsr2 is required for infection of diverse mycobacteriophages. **a**, Schematic of the mycobacteriophage lytic life cycle and resistance mechanisms. Infection begins with adsorption of phage particles to surface bacterial receptors and DNA injection into the host cell. Phage receptors are enriched at the actively growing poles and septa of mycobacterial cells. After DNA injection, the phage hijacks the host replication, transcription and translation machinery to produce and assemble progeny within the phage replication domain (dashed line). The phage expresses lytic enzymes that digest and lyse the host cell envelope, liberating the mature phage particles to initiate new infections. Bacteria resist phage infection via phage defense mechanisms (red text) such as CRISPR, and

phage resistance can arise de novo by mutating host bacterial genes (such as *lsr*2) that are essential for phage propagation. **b**, *M. smegmatis* strains LM11–LM15 were isolated as resistant to infection by mycobacteriophage Fionnbharth, using a lytic derivative of the parent temperate phage. Tenfold serial dilutions of phages Fionnbharth*Δ45Δ47* (in which both repressor and integrase genes are deleted) and Bxb1 were spotted onto lawns of *M. smegmatis* mc^2155 and LM11–LM15. **c**, Schematic representation of the *M. smegmatis lsr2* locus showing the position of the IS*1549* transposon insertion in the *lsr2* gene (MSMEG_6092) in *M. smegmatis* LM14 and the unmarked *lsr2* deletion mutant GWB142. The bottom shows the domain organization of Lsr2 with amino acid coordinates indicated, together with the location of a G100A substitution in the AT-hook-like DNA binding domain. **d**, Tenfold serial dilutions of a set of genetically diverse mycobacteriophages were spotted onto strains of *M. smegmatis* LM14 and *M. smegmatis* Δ*lsr2* together with their derivatives carrying integrative plasmid vector (pTTP1b), a plasmid with *lsr2* but no promoter (pCG52), a plasmid expressing *lsr2* from a phage BPs promoter (pCG54) or a plasmid derivative of pCG54 carrying a G100A Lsr2 substitution (pCG67); the control strain *M. smegmatis* mc^2155 on which the phages were propagated is also shown. Phage names are shown at the left and their cluster/subcluster/singleton (sin) designations shown at the right. Credit: *Nature Microbiology* (2023). DOI: 10.1038/s41564-023-01333-x

As antibacterial resistance continues to render obsolete the use of some antibiotics, some have turned to bacteria-killing viruses to treat acute infections as well as some chronic illnesses.

Graham Hatfull, the Eberly Family Professor of Biotechnology in the Kenneth P. Dietrich School of Arts and Sciences at Pitt, has pioneered the use of these viruses—bacteriophages, phages for short—to treat infections in chronic diseases such as cystic fibrosis. Although the importance of resistance may have eluded the early discovers of antibiotics, Hatfull is intent on understanding how bacteria become resistant to phages.

His lab has just discovered how a specific mutation in a bacterium results in phage resistance. The results were published Feb. 23, in the journal *Nature Microbiology*.

The new methodology and tools his team developed also gave them the opportunity to watch in unprecedented detail as a phage attacks a bacterium. As the use of phage therapy expands, these tools can help others better understand how different mutations protect bacteria against invasion by their phages.

For this study, the team started with Mycobacterium smegmatis, a harmless relative of the bacteria responsible for tuberculosis, leprosy and other hard-to-treat, chronic diseases. They then isolated a mutant form of the bacterium that is resistant to infection by a phage called Fionnbharth.

To understand how the specific mutation in the lsr2 gene helps these resistant bacteria fight off a phage, the team first needed to understand how phages killed a bacteria without the relevant mutation.

Carlos Guerrero-Bustamante, a fourth-year graduate student in Hatfull's lab, genetically engineered two special kinds of phages for this study. Some produced red fluorescence when they entered a bacterial cell. Others had segments of DNA that would stick to fluorescent molecules so phage DNA would light up in an infected cell.

Following the fluorescent beacons, "We could see where the phage DNA entered the cell," Guerrero-Bustamante said. The imaging methods they used were designed by Charles Dulberger, a collaborator and co-first author of the paper who was then at Harvard T.H. Chan School of Public Health.

"We saw for the first time how the phages take that first step of binding

to cells and injecting their DNA into the bacteria," said Hatfull, who is also a Howard Hughes Medical Institute Professor. "Then we applied those insights to ask, 'So, how's it different if we get rid of the Lsr2 protein?'"

The link between Lsr2 and phage resistance has not been previously known, but with their new methods and tools, the team clearly saw the critical role it played.

Typically, Lsr2 helps bacteria replicate its own DNA. When a phage attacks, however, the virus co-opts the protein, using it to replicate phage DNA and overwhelm the bacteria. When the lsr2 gene is missing or defective—as in the phage-resistant Mycobacterium smegmatis—the bacteria doesn't make the protein and phages don't replicate enough to take over the bacterial cell.

This was a surprise.

"We didn't know Lsr2 had anything to do with bacteriophages," Hatfull said.

These new tools can be used to uncover all manner of surprises written in the genes of phage-resistant bacteria. It may also help today's researchers and tomorrow's clinicians to better understand and take advantage of phages' abilities while avoiding the missteps that led to antibiotic resistance.

"This paper focuses on just one bacterial protein," and its resistance to just one phage, Hatfull said, but its implications are wide. "There are lots of different phages and lots of other proteins."

More information: Charles L. Dulberger et al, Mycobacterial nucleoid-associated protein Lsr2 is required for productive mycobacteriophage

infection, *Nature Microbiology* (2023). DOI: 10.1038/s41564-023-01333-x