Leto, D., and Saltiel, A.R. (2012). Regulation of glucose transport by insulin: traffic control of GLUT4. Nat. Rev. Mol. Cell Biol. *13*, 383–396.

Li, D.-D., Jawale, C.V., Zhou, C., Lin, L., Trevejo-Nunez, G.J., Rahman, S.A., Mullet, S.J., Das, J., Wendell, S.G., Delgoffe, G.M., et al. (2022). Fungal sensing leads to increased metabolic fitness of neutrophils by selectively regulating antifungal Glut1 activity. Cell Host Microbe *30*, 530–544.

Thorens, B., and Mueckler, M. (2010). Glucose transporters in the 21st Century. Am. J. Physiol. Endocrinol. Metab. *298*, E141–E145.

Wong, S.L., Demers, M., Martinod, K., Gallant, M., Wang, Y., Goldfine, A.B., Kahn, C.R., and Wagner, D.D. (2015). Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. Nat. Med. *21*, 815–819.

Xu, X., Xia, C., and Huang, Y. (2020). Different roles of intracellular and extracellular reactive oxygen species of neutrophils in type 2 diabetic mice with invasive aspergillosis. Immunobiology *225*, 151996.

A single bacterial enzyme i(NHI)bits phage DNA replication

Erin Huiting[1] **and Joseph Bondy-Denomy**[1,2,3,*]
[1]Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA 94158, USA
[2]Quantitative Biosciences Institute, University of California, San Francisco, San Francisco, CA 94158, USA
[3]Innovative Genomics Institute, Berkeley, CA 94720, USA
*Correspondence: Joseph.Bondy-Denomy@ucsf.edu
https://doi.org/10.1016/j.chom.2022.03.025

In this issue of *Cell Host & Microbe*, Nayeemul Bari et al. discover an anti-phage immune system in bacteria that uses a single enzyme to accomplish the challenging feat of detecting phage DNA and limiting its replication. Unlike CRISPR-Cas and restriction modification (R-M) systems, which use sequence motifs, nuclease-helicase immunity (Nhi) is proposed to target phage-specific replication intermediates.

Bacteria have evolved multiple lines of defense in order to protect themselves against highly abundant and diverse bacteriophage (phage) populations. In recent years, there has been a substantial increase in the discovery of new anti-phage immune systems (Doron et al., 2018; Gao et al., 2020), and this has re-established the niche of "bacterial immunology" as an essential field in the broader push for discovery-driven microbiology. The best-studied anti-phage immune systems are CRISPR-Cas and restriction modification (R-M) systems, which target phage nucleic acids and often require multiple components to curb phage replication (Koonin et al., 2017; Wilson and Murray, 1991). However, a study by Nayeemul Bari et al. (2022) reported in this issue of *Cell Host & Microbe* discovers a single enzyme that is capable of the challenging feat of recognizing *and* limiting phage DNA replication (nuclease-helicase immunity [Nhi]). The basis of this discovery defies the typical mold because it utilizes a native model organism, *Staphylococcus epidermidis*, and a diverse panel of staphylococcal phages. Given the overwhelming number of phages that exist in nature and their co-evolution with bacteria, phages serve as powerful molecular tools for the discovery and characterization of bacterial immunity.

The authors initiate their study with an *S. epidermidis* strain that harbors numerous genomic deletions which surround a known "defense island"—a genomic locus that is rich in known anti-phage immune systems—and they identify a strain that is uniquely sensitive to phage compared to its phage-resistant parent (Figure 1A). Through a series of complementation tests, a single gene was identified as necessary and sufficient for phage resistance. Follow-up experiments meticulously examine each stage of the phage replication cycle and demonstrate that Nhi does not inhibit phage adsorption nor does it induce abortive cell death (Figure 1B). Upon measuring phage DNA levels, the authors show that Nhi limits DNA replication. Analysis of the Nhi protein reveals that it contains domains that are derived from an HsdR Type I R-M endonuclease and an SF1 helicase family. The enzymatic activities are confirmed through the use of biochemical assays that demonstrate that the Nhi protein can cleave ssDNA 3'-5' and nick supercoiled DNA, and that display helicase activity on dsDNA, which requires overhangs (Figure 1C). Furthermore, the Nhi enzyme (formerly known as DUF2075) is widespread in Gram-positive and -negative bacteria, and it can be found in defense islands, but it is not very abundant; these are common signatures of immune genes. The anti-phage Nhi enzyme exhibits a distant relationship to the human proteins Schlafen5 and Schlafen13, which have endonuclease-based antiviral roles and which suggest conservation of immune function.

To understand the activation mechanism that follows phage infection, the authors take advantage of related phages that display disparate sensitivities to Nhi. In a serendipitous case of useful contamination, the authors isolate phages that appear to escape Nhi immunity, only to find they have recombined with a related phage to generate hybrids (Figure 1D).The authors isolate recombinant phages that pinpoint a single-stranded DNA binding protein (SSB) as the key phage-encoded factor that

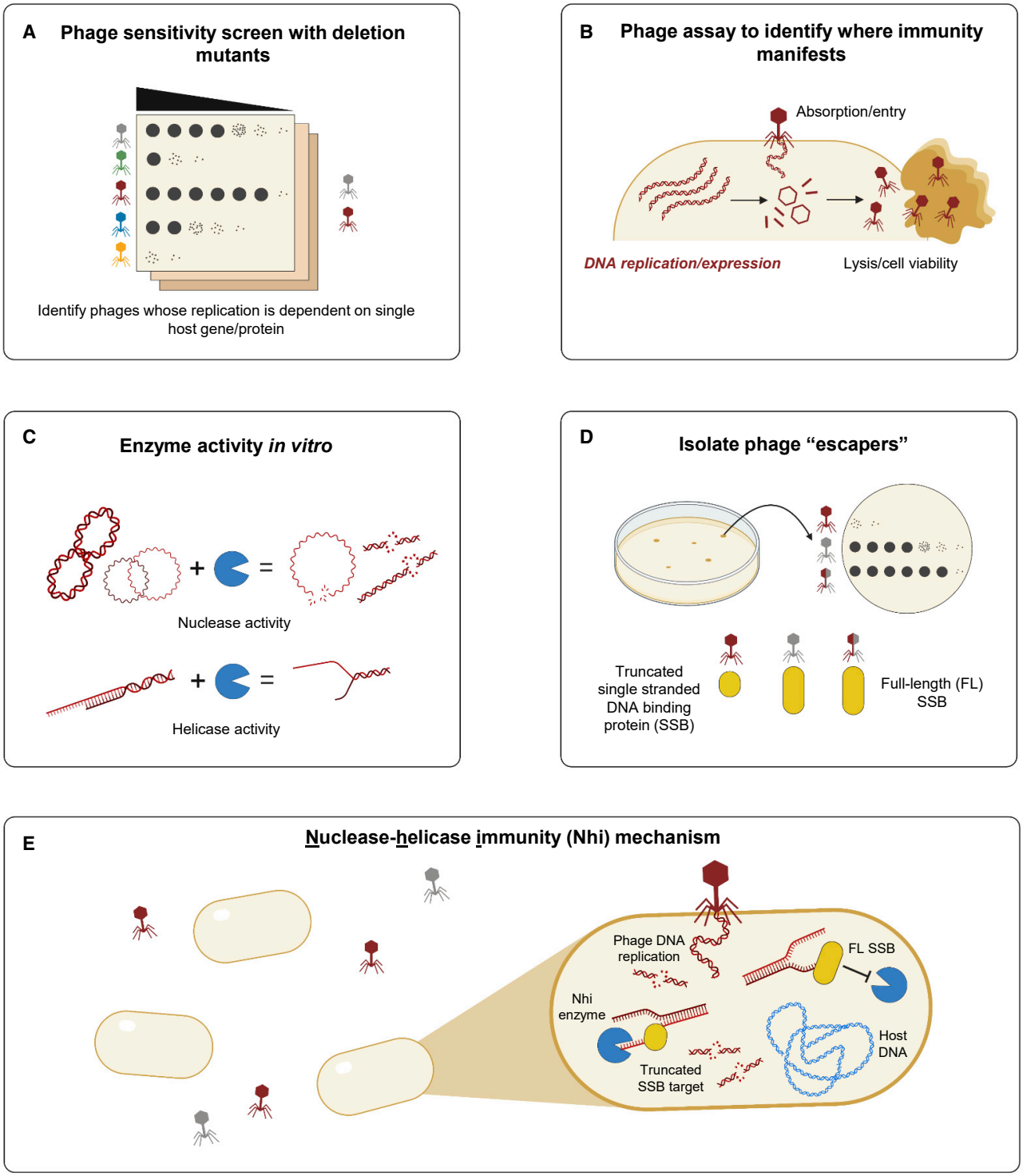

Figure 1. Summary of the work flow and mechanistic model for Nuclease-helicase anti-phage immunity (Nhi)
(A–D) A schematic summarizing the workflow that led to the discovery of Nhi (nuclease-helicase immunity).
(E) A final model for Nhi, which includes phage-encoded single-stranded DNA binding proteins (SSBs) that determine whether Nhi targets the phage genome.

determines sensitivity to Nhi. Specifically, phages that are sensitive to Nhi encode truncated SSB proteins, whereas the gain of Nhi resistance is associated with acquiring or possessing a full-length SSB. Follow-up experiments with staphylococcal phages from genetically distinct families converge on the unique N terminus of SSB likely serving as the main pathogen-associated molecular pattern (PAMP) of Nhi. In the context of the phage replication cycle, the authors ultimately

propose a model in which the phage-encoded SSB is sensed by Nhi and subsequently activates the Nhi enzyme to selectively degrade phage replication intermediates while leaving self-nucleic acids intact and the host protected from phage infection (Figure 1E).

Taken together, this study identifies a single host enzyme that is necessary and sufficient for anti-phage immunity, characterizes the dual helicase-nuclease function of the enzyme, and presents a unique model for targeting phage-specific replication intermediates. An emerging theme in the discovery of new anti-phage systems has involved numerous examples of >2 component systems, such as CBASS, retrons, and Pycsar, that first recognize a phage infection event and then are proposed to act on the host to curb the infection (Cohen et al., 2019; Millman et al., 2020; Tal et al., 2021). Nhi goes against the grain with an ''old school'' mechanism that appears to directly antagonize the phage. The mechanistic challenge of both recognizing phage-specific molecules and acting on them in an inhibitory fashion in a way that does not appear to be sequence-specific is a significant and exceptional finding.

The ''start-to-finish'' narrative of this paper can serve as a model for future discoveries in bacterial immunity because it systemically and cleverly combines phage/bacterial genetics, bioinformatics, and reductionist biochemistry. Furthermore, by establishing a native model system of Nhi within *S. epidermidis* bacteria and its cognate phages, the authors are able to isolate phages that escape immunity and provide important insights into the conservation and mechanism of Nhi. Many open questions remain, of course. Although the current Nhi model suggests that phage-specific replication intermediates is the target and, by extension, is sensed by the Nhi enzymes, substrates that mirror these intermediates—complete with SSB proteins that do or do not license Nhi targeting—are not examined *in vitro* in this study. This will certainly be important to study in future work in order to solidify or alter the model. In addition, the authors fortuitously discover that the N-terminal fragment of the Nhi protein possesses nuclease activity, but this activity is auto-inhibited with the full-length protein. Whether the enzyme is constitutively in an auto-inhibited state, which is then activated by phage infection, remains to be seen.

In summary, Nayeemul Bari et al. provide a comprehensive example of how investigating the co-evolution of phages with their native host uncovers a bacterial immune system that defies the status quo and utilizes a single-component enzyme for anti-phage activity. In the broader scope of the field, this scientific strategy to ''follow the phage'' will enable further discovery and address critical questions in fundamental phage and bacterial biology. Of equal importance, our understanding of bacterial immune systems, such as Nhi, can also provide essential insight into the driving pressures behind some immune processes found in humans and the success or failure of phages in microbial therapeutics. Altogether, this body of work, alongside future work, will shape our understanding of bacterial immunology and generate powerful tools in biotechnology and antimicrobial applications.

ACKNOWLEDGMENTS

Anti-phage immunity research in the Bondy-Denomy lab is supported by the National Institutes of Health (grant number R21AI168811), the Vallee Foundation, and the Searle Scholarship. E.H. is supported by the National Science Foundation Graduate Research Fellowship Program (grant number 2038436). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. Schematics for the figure were created on BioRender.com.

DECLARATION OF INTERESTS

J.B.-D. is a scientific advisory board member of SNIPR Biome, Excision Biotherapeutics, and LeapFrog Bio, and he is a scientific advisory board member and co-founder of Acrigen Biosciences. The Bondy-Denomy lab receives research support from Felix Biotechnology.

REFERENCES

Cohen, D., Melamed, S., Millman, A., Shulman, G., Oppenheimer-Shaanan, Y., Kacen, A., Doron, S., Amitai, G., and Sorek, R. (2019). Cyclic GMP-AMP signalling protects bacteria against viral infection. Nature *574*, 691–695. https://doi.org/10.1038/s41586-019-1605-5.

Doron, S., Melamed, S., Ofir, G., Leavitt, A., Lopatina, A., Keren, M., Amitai, G., and Sorek, R. (2018). Systematic discovery of antiphage defense systems in the microbial pangenome. Science *359*, eaar4120. https://doi.org/10.1126/science.aar4120.

Gao, L., Altae-Tran, H., Böhning, F., Makarova, K.S., Segel, M., Schmid-Burgk, J.L., Koob, J., Wolf, Y.I., Koonin, E.V., and Zhang, F. (2020). Diverse enzymatic activities mediate antiviral immunity in prokaryotes. Science *369*, 1077–1084. https://doi.org/10.1126/science.aba0372.

Koonin, E.V., Makarova, K.S., and Zhang, F. (2017). Diversity, classification and evolution of CRISPR-Cas systems. Curr. Opin. Microbiol. *37*, 67–78. https://doi.org/10.1016/j.mib.2017.05.008.

Millman, A., Bernheim, A., Stokar-Avihail, A., Fedorenko, T., Voichek, M., Leavitt, A., Oppenheimer-Shaanan, Y., and Sorek, R. (2020). Bacterial Retrons Function In Anti-Phage Defense. Cell *183*, 1551–1561.e12. https://doi.org/10.1016/j.cell.2020.09.065.

Nayeemul Bari, S.M., Chou-Zheng, L., Howell, O., Hossain, M., Hill, C.M., Boyle, T.A., Cater, K., Dandu, V.S., Thomas, A., Aslan, B., and Hatoum-Aslan, A. (2022). A unique mode of nucleic acid immunity performed by a single multifunctional enzyme. Cell Host Microbe *30*, 570–582.

Tal, N., Morehouse, B.R., Millman, A., Stokar-Avihail, A., Avraham, C., Fedorenko, T., Yirmiya, E., Herbst, E., Brandis, A., Mehlman, T., et al. (2021). Cyclic CMP and cyclic UMP mediate bacterial immunity against phages. Cell *184*, 5728–5739.e16. https://doi.org/10.1016/j.cell.2021.09.031.

Wilson, G.G., and Murray, N.E. (1991). Restriction and modification systems. Annu. Rev. Genet. *25*, 585–627. https://doi.org/10.1146/annurev.ge.25.120191.003101.