pathogens by interacting with receptor kinases (*8*, *9*). It was previously shown that a mutation in the gene encoding RALF4 leads to misshapen pollen tubes, tube bursting, and cell wall composition changes (*10*). In addition, RALF4 forms a tetrameric complex with cell wall LEUCINE-RICH REPEAT EXTENSIN 8 (LRX8), which exposes a positively charged patch on RALF4 that is hypothesized to interact with negatively charged pectin (*11*). RALF4 also binds to members of the *Catharanthus roseus* RLK1-like family of receptor kinases and influences pollen tube function (*10*, *12*). On the basis of these multiple activities, Moussu *et al.* hypothesized that RALF4 triggered two parallel signaling pathways in the pollen tube: one inside the cell through receptor kinases and one outside the cell through interaction with leucine-rich extensins (*11*).

Using thermal shift assays, Moussu *et al.* showed that two RALF4 peptides form a complex with two LRX8 proteins and that the complex binds two deesterified HG molecules, one for each RALF4 subunit. Immunolabeling and high-resolution microscopy revealed that LRX8 and RALF4 are present both in the pollen tube tip, where they are exocytosed in vesicles into the tip, and also in the pollen tube shank, demonstrating that both are incorporated into the shank wall. This was unexpected, given that RALF4 is a signaling molecule. Super-resolution microscopy identified the heterotetrameric LRX8-RALF4 as punctate domains in a reticulated network in the wall. Deesterified pectin was present in the pollen tube wall as interconnected fibers (see the image) that colocalized with RALF4 in the reticulate network, reminiscent of the latticed and ring structures previously identified in pollen tubes (*3*).

To determine whether the association between LRX8-RALF4 and the pectin-labeled fibers occurred on preexisting fibers or as an active process in which LRX8-RALF4 influenced pectin fiber architecture, Moussu *et al.* analyzed the interaction of the LRX8-RALF4 complex with pectin. They showed that the ionic binding of RALF4 in the LRX8-RALF4 complex with pectin condensed demethylesterified pectin. Furthermore, compared with wild-type pollen tubes, pollen tubes with RALF4 that were mutated to remove the positively charged patch (to prevent the formation of the LRX8-RALF4 complex) displayed a relatively unstructured cell wall, grew at a greater velocity, and had premature tube bursting. This shows that the interaction of the LRX8-RALF4 complex with deesterified pectin is necessary for pollen tube patterning, growth, and integrity.

The study by Moussu *et al.* provides evidence that a protein-peptide-pectin interaction establishes and stabilizes a reticulate cell wall architecture in pollen tube walls that strengthens the pollen tube during growth. The results establish RALF4 as a multifunctional peptide with both signaling and structural roles in pollen tubes. A critical question for the future is to determine whether RALF4 or other RALF peptides generate cell wall architecture in non–tip growing cells, such as elongating epidermal or seedling stem hypocotyl cells. It will also be important to establish whether other RALF peptides have a dual signaling and structural role.

Another outstanding question regards the full polymeric structure of pectin in the lattice. There is little evidence that HG is an independent polysaccharide in plant cell walls. Rather, HG is covalently connected to the pectin rhamnogalacturonan I (RG-I) or to a pectic arabinogalactan protein proteoglycan and/or contains regions of the highly branched pectic RG-II (*5*, *13*, *14*). To understand how the LRX8-RALF4 stabilized lattice affects pollen tube shape, strength, and elongation, the structure of the pectic fibers in the lattice must be determined. Moussu *et al.* have identified RALF4 as a peptide that has a structural role in the cell wall and a signaling role in response to cell wall integrity fluctuations. This establishes RALF4 as a sentinel for rapid communication between the cell and the cell wall when rapid changes in cell wall architecture are required. ■

REFERENCES AND NOTES

1. P. K. Hepler, C. M. Rounds, L. J. Winship, *Mol. Plant* **6**, 998 (2013).
2. P. A. Bedinger, K. J. Hardeman, C. A. Loukides, *Trends Cell Biol.* **4**, 132 (1994).
3. J. Derksen *et al.*, *Plant J.* **68**, 495 (2011).
4. S. Moussu *et al.*, *Science* **382**, 719 (2023).
5. M. A. Atmodjo, Z. Hao, D. Mohnen, *Annu. Rev. Plant Biol.* **64**, 747 (2013).
6. C. Cameron, A. Geitmann, *Curr. Opin. Genet. Dev.* **51**, 11 (2018).
7. T. E. Proseus, J. S. Boyer, *Plant Signal. Behav.* **7**, 1490 (2012).
8. G. Pearce, D. S. Moura, J. Stratmann, C. A. Ryan Jr., *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12843 (2001).
9. M. Haruta, G. Sabat, K. Stecker, B. B. Minkoff, M. R. Sussman, *Science* **343**, 408 (2014).
10. M. A. Mecchia *et al.*, *Science* **358**, 1600 (2017).
11. S. Moussu *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **117**, 7494 (2020).
12. Z. Ge *et al.*, *Science* **358**, 1596 (2017).
13. L. Tan *et al.*, *Carbohydr. Polym.* **301**, 120340 (2023).
14. F. Dardelle *et al.*, *Plant Physiol.* **153**, 1563 (2010).

ACKNOWLEDGMENTS

The work of D.M. is partially funded by The Center for Bioenergy Innovation, a US Department of Energy (DOE) Research Center supported by the Office of Biological and Environmental Research in the DOE Office of Science and the DOE, Office of Science, Basic Energy Sciences, Chemical Sciences, Geosciences and Biosciences Division, under award DE-SC0015662.

10.1126/science.adl1198

INFECTIOUS DISEASE

Preparing for the next pandemic

New lead drugs to treat COVID-19 are beginning to emerge

By **Brian K. Shoichet** *and* **Charles S. Craik**

Early in the COVID-19 pandemic, it was suggested that drugs to meaningfully treat the disease would come from either the current antiviral armamentarium or from drug repurposing (*1*). Genuinely new therapeutics, although likely to be the most effective, would take too long to develop. As it happened, drug repurposing paid few lasting dividends (*2*, *3*), and the effective drugs that did emerge, such as remdesivir and nirmatrelvir [the active ingredient in Paxlovid that targets the main protease (Mpro) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)], were drawn from preexisting antiviral pipelines. On page 663 of this issue, Boby *et al.* (*4*) report an open science effort to discover new Mpro drugs to treat COVID-19. Such work could help develop treatments for emerging drug-resistant variants of SARS-CoV-2 (*5*) and other coronaviruses that are likely to emerge in the future.

The effort described by Boby *et al.* began early in the pandemic and combined innovations in the techniques and organization of drug discovery. The scientific innovations included high-throughput crystallographic fragment screening: The structures of more than 70 fragments—which are about a third to a half of the size of a druglike inhibitor—bound to the SARS-CoV-2 enzyme Mpro were determined (*6*) as were the structures of more than 500 more-advanced compounds. A second area of innovation was computational free-energy calculations and machine learning that helped guide medicinal chemistry; both techniques remain at the cutting edge of drug development.

Organizationally, the study of Boby *et al.* resulted from the integration of the expertise of 212 scientists in 47 organizations across 15 countries, mostly in academia but also including researchers from pharmaceuti-

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cal companies. All donated their time in an open access model, capturing some of the strengths of a vertically integrated pharmaceutical company in a collaborative academic environment. They report several lead compounds with cellular antiviral potencies that are comparable to those of nirmatrelvir with in vivo pharmacokinetics that may support advancement toward clinical trials.

By focusing on Mpro, the authors drew on a legacy of successful drug discovery against viral proteases. Beginning in the 1980s and 1990s, investigators began to move away from the nucleoside analogs that, until then, had dominated antiviral chemotherapy and toward viral protease inhibitors. This approach promised to disrupt a key step in the viral life cycle—the cleavage of the viral polypeptide chain to produce multiple functional proteins—with potentially less toxicity than conferred by the nucleoside antivirals that inhibit viral genomic replication. Since the introduction of the first HIV protease inhibitors in the early 1990s, proteases have been well-accepted targets for antivirals, and HIV protease inhibitors continue to be part of anti-AIDS cocktails to this day.

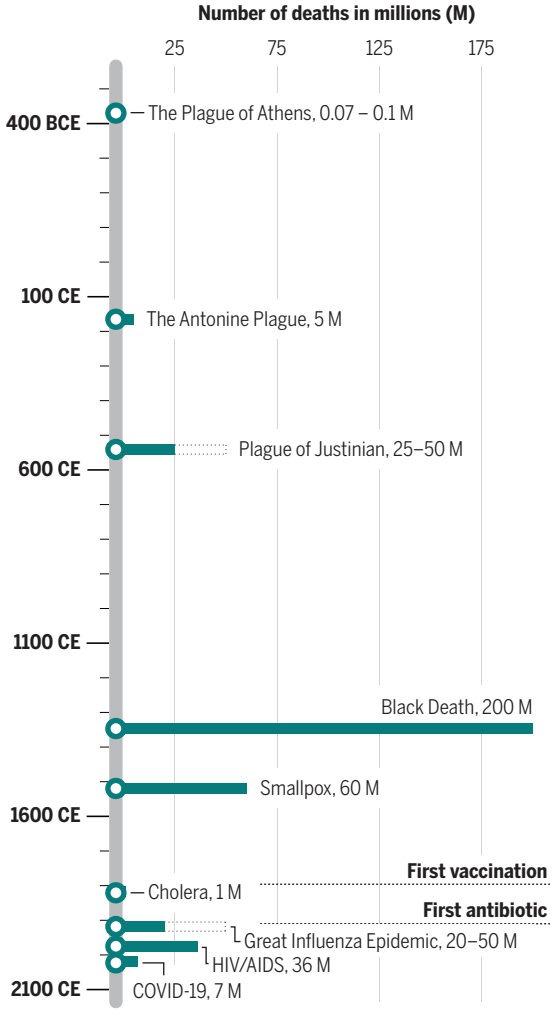
The success of the HIV protease inhibitors inspired the targeting of proteases in hepatitis C virus (HCV), the dengue protease, and the SARS-CoV-2 Mpro and papain-like protease (Plpro). Each has its own story; for example, the intensive combined efforts of academic and industrial scientists to identify a treatment for AIDS targeted HIV protease (PR) by using its three-dimensional structure to computationally screen chemical compounds to rapidly identify an inhibitor (*7*). This is one of the earliest examples of this technique that is now widespread in drug discovery. Notably, and as with Paxlovid, the initial protease inhibitors for HIV PR capitalized on the ~20 years of previous research on renin inhibitors, a human homolog of the viral aspartyl protease. There are now nine US Food and Drug Administration (FDA)–approved drugs against HIV PR. One of them, ritonavir, is used to boost the pharmacokinetic properties of the others, as it does for nirmatrelvir in Paxlovid—another example of borrowing from the past.

The lead therapeutics described by Boby *et al.* may not be ready in time to affect the current pandemic, considering the timelines and challenges of drug approval. Nevertheless, the compounds, and the techniques used to identify them, may well affect human health in the future. The leads themselves represent new departures for Mpro inhibitors, and the methodology used to find them, not to mention the organization of the project, will inspire others. More broadly, the COVID-19 pandemic led to the investigation of several other SARS-CoV-2 enzymes that, like HIV PR in the mid-1980s, have few precedents as antiviral targets but are known to drug discovery more broadly. These enzymes, including those that combat the cellular innate immune system, fool the cell into treating viral RNA as human RNA, and unwind viral RNA to support replication, are crucial for the life cycle of SARS-CoV-2 and are likely good targets for many other viruses as well. Against each of these classes of enzymes, new inhibitors have been discovered that show promise as drug leads (*8*–*11*).

The new classes of antiviral drug leads, of which those reported by Boby *et al.* are among the most advanced, are being developed in both pharmaceutical companies and in universities, including in nine antiviral centers supported by the US government. These efforts seek to restock the antiviral armamentarium for the next pandemic. Such waves of infectious disease have swept through the human population not only in the past century (i.e., influenza, polio, AIDS, and COVID-19) but regularly throughout recorded history. From the plague of Athens (430 BCE), to that of the Antoinines (160 to 189 CE), to that of Justinian (beginning in 541 CE), to the Black Death (14th through 16th centuries), to the smallpox and cholera epidemics of the 17th through the 19th centuries, viral and bacterial plagues have devastated human populations (see the figure). There is every reason to expect these plague cycles to continue (*12*). Until the 1930s, there were no effective drugs to treat microbial pandemics, and, even in recent years, previously discovered drugs were initially relied on to target new threats. The drugs in which investments are made today will pay dividends in the inevitable pandemics of the future. ■

Pandemics from 500 BCE to the present

Pandemics have affected human lives throughout history. Up to 100,000 lives were claimed by the Plague of Athens (*13*) followed by 5 million deaths in the Antonine Plague and ~20 to 50 million deaths in the Plague of Justinian (*14*). The Black Death (*14*) and smallpox (*15*) caused considerable death tolls, and the cholera crisis took 1 million lives (*14*). The advent of the smallpox vaccine in 1796 and the first antibiotic, Salvarsan, in 1910 mark the transition into the modern era of therapeutics against infectious diseases. Vaccines and antibiotics supported the resistance to the Great Influenza Epidemic (*14*), the HIV/AIDS pandemic (*14*), and most recently, COVID-19 (*16*).



GRAPHIC: V. HERSHER/*SCIENCE*

REFERENCES AND NOTES

1. D. Lowe, "Covid-19 Small Molecule Therapies Reviewed," *In The Pipeline* (AAAS, 2020); www.science.org/content/blog-post/covid-19-small-molecule-therapies-reviewed.
2. A. Edwards, *J. Chem. Inf. Model.* **60**, 5727 (2020).
3. T. A. Tummino *et al.*, *Science* **373**, 541 (2021).
4. M. L. Boby *et al.*, *Science* **382**, eabo7201 (2023).
5. Y. Duan *et al.*, *Nature* **622**, 376 (2023).
6. A. Douangamath *et al.*, *Nat. Commun.* **11**, 5047 (2020).
7. R. L. DesJarlais *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6644 (1990).
8. T. Otava *et al.*, *ACS Infect. Dis.* **7**, 2214 (2021).
9. C.-H. Zhang *et al.*, *ACS Med. Chem. Lett.* **12**, 1325 (2021).
10. A. Luttens *et al.*, *J. Am. Chem. Soc.* **144**, 2905 (2022).
11. S. Gahbauer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2212931120 (2023).
12. C. Kenny, *The Plague Cycle: The Unending War Between Humanity and Infectious Disease* (Scribner, 2021).
13. R. J. Littman, *Mt. Sinai J. Med.* **76**, 456 (2009).
14. N. Srivastava *et al.*, in *Coronavirus Disease 2019 (COVID-19): Epidemiology, Pathogenesis, Diagnosis, and Therapeutics*, S. K. Saxena, Ed. (Springer, 2020), pp. 9–21.
15. A. Chaikhouni, *Heart Views* **21**, 235 (2020).
16. P. Jha *et al.*, *Lancet* **399**, 1937 (2022).

ACKNOWLEDGMENTS

The authors acknowledge A. L. Lourenço for his artwork. B.K.S. and C.S.C. are partly supported by National Institutes of Health grant U19AI171110.

10.1126/science.adk5868