2. Algammal, A., Hetta, H.F., Mabrok, M., and Behzadi, P. (2023). Editorial: Emerging multidrug-resistant bacterial pathogens "superbugs": A rising public health threat. Front. Microbiol. *14*, 1135614.

3. Denissen, J., Reyneke, B., Waso-Reyneke, M., Havenga, B., Barnard, T., Khan, S., and Khan, W. (2022). Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health. Int. J. Hyg Environ. Health *244*, 114006.

4. Livermore, D.M. (2004). The need for new antibiotics. Clin. Microbiol. Infect. *10* (*Suppl 4*), 1–9.

5. Liu, G., Catacutan, D.B., Rathod, K., Swanson, K., Jin, W., Mohammed, J.C., Chiappino-Pepe, A., Syed, S.A., Fragis, M., Rachwalski, K., et al. (2023). Deep learning-guided discovery of an antibiotic targeting Acinetobacter baumannii. Nat. Chem. Biol. *19*, 1342–1350.

6. Wong, F., Zheng, E.J., Valeri, J.A., Donghia, N.M., Anahtar, M.N., Omori, S., Li, A., Cubillos-Ruiz, A., Krishnan, A., Jin, W., et al. (2024). Discovery of a structural class of antibiotics with explainable deep learning. Nature *626*, 177–185.

7. Stokes, J.M., Yang, K., Swanson, K., Jin, W., Cubillos-Ruiz, A., Donghia, N.M., MacNair, C.R., French, S., Carfrae, L.A., Bloom-Ackermann, Z., et al. (2020). A Deep Learning Approach to Antibiotic Discovery. Cell *180*, 688–702.e13.

8. Granato, E.T., Meiller-Legrand, T.A., and Foster, K.R. (2019). The Evolution and Ecology of Bacterial Warfare. Curr. Biol. *29*, R521–R537.

9. Santos-Júnior, C.D., Torres, M.D.T., Duan, Y., Rodríguez Del Río, Á., Schmidt, T.S.B., Chong, H., Fullam, A., Kuhn, M., Zhu, C., Houseman, A., et al. (2024). Discovery of antimicrobial peptides in the global microbiome with machine learning. Cell *187*, 3761–3778.e9. https://doi.org/10.1016/j.cell.2024.05.013.

Chaperones help TACkle phage infection

Shally R. Margolis[1] **and Alexander J. Meeske**[1,*]
[1]Department of Microbiology, University of Washington, Seattle, WA, USA
*Correspondence: meeske@uw.edu
https://doi.org/10.1016/j.chom.2024.06.009

Bacteria have evolved anti-viral defenses, but the mechanisms of sensing and stopping infection are still under investigation. In this issue of *Cell Host & Microbe*, Mets, Kurata, Ernits et al. describe how direct sensing of a phage protein by a bacterial toxin-antitoxin-associated chaperone unleashes toxin activity to prevent infection.

Bacteriophages (phage), the viruses that infect bacteria, are the most numerous biological entities on the planet. The threat of infection posed to bacteria has led to the evolution of a great many anti-phage immune defenses whose diverse mechanisms we are only beginning to understand. Toxin-antitoxin (TA) systems are two-gene operons that are widespread in bacterial genomes, and while their functions have been hotly debated over the years, many have been increasingly appreciated to serve anti-phage defense functions.[1] Type II TA systems are composed of a toxic protein and a labile neutralizing antitoxin protein, and their mechanisms of anti-phage immunity are incompletely understood. In general, it has been observed that infection-associated signals lead to the destabilization of antitoxins. This causes release of the toxin, which interferes with an essential cellular process, impeding the phage life cycle and usually leading to growth arrest or death of the infected cell. A subset of TA systems contain an additional gene encoding a SecB-like chaperone, which is required for stabilization of associated antitoxins that contain intrinsically disordered chaperone addiction (ChAD) domains.[2] These toxin-antitoxin-chaperone (TAC) systems were recently shown to protect their hosts from phage infection.[3] However, it remained unclear how TAC systems sense invading phage, and activate toxicity to neutralize infection.

These questions are beautifully tackled in an exciting new study by Mets, Kurata, Ernits et al.[4] The authors first identify two TAC systems, HigBAC and CmdTAC, within different *E. coli* prophages, and then show that they protect bacterial hosts from a diverse range of phages. They also confirm that these function as TAC systems; that is, expression of the toxin causes growth inhibition, and only the presence of both the antitoxin and the stabilizing chaperone can rescue this growth defect. The authors then sought to understand how the toxins in these systems interfere with the phage life cycle. HigBAC homologs have been studied previously,[2,5] and as expected, HigB, the predicted mRNase toxin, inhibited protein synthesis. The toxin from the CmdTAC system, CmdT, is a member of the ADP-ribosyltransferase (ART) family, and surprisingly also inhibited protein synthesis. ART family enzymes have previously been shown to ADP-ribosylate proteins and more recently DNA,[6] but for the first time here (and in complementary work by Vassallo et al.[7]), CmdT was shown to ADP-ribosylate mRNA in a sequence-specific manner, leading to the inhibition of translation. The discovery of a distinct molecular function for ART enzymes highlights the value of studying anti-phage immune proteins, and the importance of performing experiments to validate predicted functions.

Anti-phage toxin-antitoxin systems are often thought to act by killing the infected cell, eliminating the viral replicative niche and sparing the rest of the population. The toxins in this study both lead to the loss of new protein synthesis and growth inhibition when overexpressed. However, both CmdTAC and HigBAC allowed for host survival and growth during infection with some (but not all) phages, even when infected with viral particles far

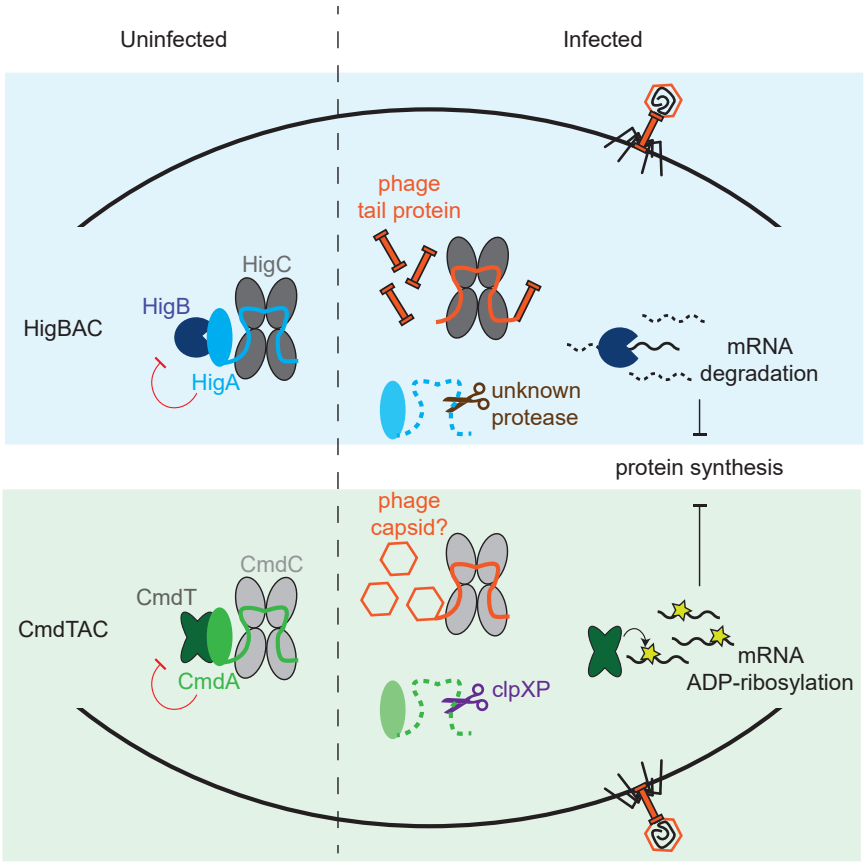

Figure 1. Model for the predicted mechanism of action of the two TAC systems in this study

exceeding the number of cells. This indicates that somehow the translation inhibition from RNA degradation by HigB or ADP-ribosylation by CmdT impacts the phage more than the host. Further studies will be needed to shed light on how these systems preferentially target viruses.

Both CmdTAC and HigBAC protect host bacteria from viruses through a toxin that stops translation, but how is the toxin specifically activated during phage infection? The authors tackle this question with HigBAC and phage λ_{vir}, making use of structural predictions by AlphaFold that are well supported by genetic and biochemical experiments. In the absence of phage, the disordered ChAD element of the HigA antitoxin wraps around the HigC chaperone, with 4 conserved aromatic residues playing key roles in the interaction. This interaction stabilizes HigA, enabling neutralization of the HigB toxin. During infection with λ_{vir}, the phage major tail protein directly binds to HigC through analogous aromatic residues and displaces HigA. The free antitoxin is then degraded by a host protease: clpXP in the case of CmdA, and an unknown factor in the case of HigA (unlike the previously studied HigA, which is also degraded by clpXP[8]). With the antitoxin gone, the toxin is freed to inhibit translation and halt phage infection (Figure 1). Notably, Vassallo et al. also recently identified the T4 capsid protein as the activator of a distinct CmdTAC system.[7] In both cases, evidence suggests that the chaperone acts as the sensor of phage infection. Both capsid and tail are structural proteins produced late during infection, indicating it may be common for TACs to directly sense these types of pathogen-associated molecular patterns. Coupling sensing of proteins that are not expressed until the end of an infection cycle with induction of a response that is toxic to the host makes sense as a last resort and appears to be a common mechanism of abortive infection systems.

Finally, the authors sought to test whether the chaperone sensors are interchangeable and dictate phage specificity, as CmdTAC and HigBAC were found to protect against nonidentical sets of phages. Unexpectedly, a chimeric TAC composed of CmdTA and HigC was found to protect against all phages targeted by CmdTAC and HigBAC, and even some that were not targeted by either parent system. These findings show that the genes in these TAC systems could be swapped during evolution to provide protection against new threats. There are multiple plausible explanations for the expanded range of phages neutralized by the chimeric TAC system. Though the genetic and biochemical experiments in this paper thoroughly establish the chaperone as a central player in sensing infection, it is possible that the toxin and/or antitoxin also influence sensing. We also propose that some of the phages tested here may encode inhibitors of HigBA that account for their resistance to HigBAC but susceptibility to CmdTA/HigC. These possibilities may be distinguished in the future by investigating the stability of the antitoxin during infection with each phage.

Interestingly, the two systems studied here are often found on prophages, that is, phages that have integrated into the host genome. This raises the intriguing possibility that TAC systems play a role in inter-phage conflicts by protecting both the prophage and its host from infection by a competing phage. Until reactivation, prophages do not produce structural proteins such as the phage tail detected by HigBAC and could have ways to control the detection of these proteins during reactivation. Further studies on TAC systems in their native prophage context may provide interesting insights into conflicts between viruses and how hosts balance the risks and benefits of having a prophage.

An interesting outstanding question is why the SecB-like chaperones have evolved to perform this phage sensing function. TA systems alone can function in anti-phage defense, with the antitoxins usually acting as the sensors. TAC systems can even be converted to TA systems with the removal of the ChAD from the antitoxin (these no longer sense phage). Phages are known to use chaperones for proper protein folding,[9] so we wonder whether the SecB-like chaperones in TAC systems may serve as decoys to sense viral proteins that

rely on chaperones. This may limit the ability of phages to evolve away from detection by TAC systems; mutations in phage proteins that cause them to no longer be sensed by TACs may also inhibit their ability to interact with the necessary chaperones to form stable proteins.

In sum, this study from Mets, Kurata, Ernits et al. provides important mechanistic insights into how bacterial immune systems can both sense and inhibit phage infection and further confirms the biological function of TAC systems.

DECLARATION OF INTERESTS

A.J.M. is a co-founder of Profluent Bio.

REFERENCES

1. Kelly, A., Arrowsmith, T.J., Went, S.C., and Blower, T.R. (2023). Toxin-antitoxin systems as mediators of phage defence and the implications for abortive infection. Curr. Opin. Microbiol. *73*, 102293. https://doi.org/10.1016/j.mib.2023.102293.

2. Bordes, P., Sala, A.J., Ayala, S., Texier, P., Slama, N., Cirinesi, A.M., Guillet, V., Mourey, L., and Genevaux, P. (2016). Chaperone addiction of toxin-antitoxin systems. Nat. Commun. *7*, 13339. https://doi.org/10.1038/ncomms13339.

3. Vassallo, C.N., Doering, C.R., Littlehale, M.L., Teodoro, G.I.C., and Laub, M.T. (2022). A functional selection reveals previously undetected anti-phage defence systems in the E. coli pangenome. Nat. Microbiol. *7*, 1568–1579. https://doi.org/10.1038/s41564-022-01219-4.

4. Mets, T., Kurata, T., Ernits, K., Johansson, M.J.O., Craig, S.Z., Evora, G.M., Buttress, J.A., Odai, R., Wallant, K.C., Nakamoto, J.A., et al. (2024). Mechanism of phage sensing and restriction by toxin-antitoxin-chaperone systems. Cell Host Microbe *32*, 1059–1073.e8. https://doi.org/10.1016/j.chom.2024.05.003.

5. Christensen-Dalsgaard, M., and Gerdes, K. (2006). Two higBA loci in the Vibrio cholerae superintegron encode mRNA cleaving enzymes and can stabilize plasmids. Mol. Microbiol. *62*, 397–411. https://doi.org/10.1111/j.1365-2958.2006.05385.x.

6. Jankevicius, G., Ariza, A., Ahel, M., and Ahel, I. (2016). The Toxin-Antitoxin System DarTG Catalyzes Reversible ADP-Ribosylation of DNA. Mol. Cell *64*, 1109–1116. https://doi.org/10.1016/j.molcel.2016.11.014.

7. Vassallo, C.N., Doering, C.R., and Laub, M.T. (2024). Anti-viral defense by an ADP-ribosyltransferase that targets mRNA to block translation. Preprint at bioRxiv. https://doi.org/10.1101/2024.02.24.581662.

8. Texier, P., Bordes, P., Nagpal, J., Sala, A.J., Mansour, M., Cirinesi, A.M., Xu, X., Dougan, D.A., and Genevaux, P. (2021). ClpXP-mediated Degradation of the TAC Antitoxin is Neutralized by the SecB-like Chaperone in Mycobacterium tuberculosis. J. Mol. Biol. *433*, 166815. https://doi.org/10.1016/j.jmb.2021.166815.

9. Zeilstra-Ryalls, J., Fayet, O., and Georgopoulos, C. (1991). The universally conserved GroE (Hsp60) chaperonins. Annu. Rev. Microbiol. *45*, 301–325. https://doi.org/10.1146/annurev.mi.45.100191.001505.

Breathe and bloom: Gut hypoxia limits *C. albicans* growth

Animesh A. Mishra[1] **and Andrew Y. Koh**[1,2,3,*]
[1]Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[2]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
[3]Harold C. Simmons Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
*Correspondence: andrew.koh@utsouthwestern.edu
https://doi.org/10.1016/j.chom.2024.06.006

Multiple host and microbial factors dictate whether *Candida albicans* can colonize the mammalian gastrointestinal tract. In this issue of *Cell Host & Microbe*, Savage et al. demonstrate that restoration of intestinal epithelial hypoxia is sufficient to restore *Candida albicans* colonization resistance, even when other *Candida* inhibitory effectors remain depleted.

Candida albicans, the most common human fungal pathogen, is a dominant member of the human mycobiota, particularly in individuals residing in western countries.[1] The colonization fitness of *Candida* is dictated by the interplay of host immune effectors (antimicrobial peptides, cellular immunity), fungal determinants (morphology, anti-microbial resistance) and gut bacterial microbiota.[2] *Candida albicans (Ca)* gastrointestinal colonization levels in a healthy host is limited in the presence of a healthy microbiome replete with *Clostridia* and *Bacteroidota* species. *Ca* rapidly expands after antibiotic-induced disruption of the gut microbiota, most pronounced with antibiotics effective in depleting *Clostridia* and *Bacteroidota* species.[3] Mechanistically, these gut microbiota induce intestinal epithelial antimicrobial peptides, which are effective in killing *Ca*.[3]

In the current study, Savage et al. demonstrate that gut microbiota-dependent modulation of intestinal epithelial oxygen levels is a critical determinant of *Ca* gut colonization.[4] This study builds on previous work showing that antibiotic-induced gut dysbiosis results in depleted levels of the short-chain fatty acid butyrate and a concomitant increase in the availability of intestinal epithelial oxygen allowing facultative anaerobic bacterial species (e.g., *Salmonella)* to utilize aerobic respiration pathways and expand in the gut.[5,6]

Here, the authors utilized an unbiased metabolomics screen coupled with