than swarmers alone. This effect not only occurred in *E. coli* but also in *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Salmonella enterica* and *Serratia marcescens*, indicating that such 'necrosignalling' is common.

Interestingly, when cells were killed by heat, necrosignalling was abolished, indicating that the signal is heat-labile. Furthermore, treatment of supernatant from dead cells with protease, but not with RNAse or DNAse, also abolished necrosignalling, suggesting that a protein released by the dead cells is the causative factor for increased resistance. Mass spectrometry identified five candidate proteins, and further experiments with deletion and overexpression mutants confirmed that one of these proteins, AcrA, was responsible for necrosignalling.

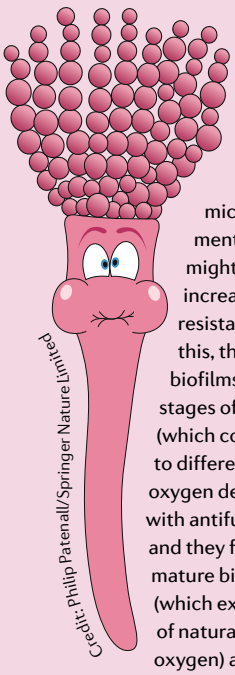
AcrA is part of the RND efflux pumps AcrAB-TolC and AcrAD-TolC. Deletion of *tolC* in the dead cells had no effect on their ability to increase resistance when added to the swarm, whereas *tolC* deletion in the recipient swarming cells prevented a response to dead cells, indicating that binding of dead cell-released AcrA to TolC on the surface of swarmers underlies the effect. The authors confirmed this assumption through imaging and experiments with strains carrying point mutations in residues important for binding.

Furthermore, AcrA induced upregulation of genes related to drug efflux and catabolism of reactive oxygen species in swarmers. Colorimetric assays confirmed the increased efflux in swarmers that received the necrosignal, as did measurements of intracellular antibiotic concentrations.

In summary, the data show that swarming cells can function as a 'canary in the coal mine' by releasing AcrA, which signals to otherwise susceptible cells to upregulate drug efflux.

Ursula Hofer

ORIGINAL ARTICLE Bhattacharyya, S., Walker, D. M. & Harshey, R. M. Dead cells release a 'necrosignal' that activates antibiotic survival pathways in bacterial swarms. *Nat. Commun.* **11**, 4157 (2020)



Credit: Philip Patenall/Springer Nature Limited

active fungal cells, led the authors to hypothesize that the induced hypoxic microenvironments in biofilms might lead to increase drug resistance. To test this, they treated biofilms at different stages of growth (which correspond to different levels of oxygen depletion) with antifungal drugs, and they found that mature biofilms (which exhibit zones of naturally depleted oxygen) are less sensitive to the drugs than younger biofilms, which is in agreement with the notion that hypoxia contributes to antifungal drug resistance and increased cell survival. In addition, growing biofilms on oxygen-permeable plates, which permits increased oxygen perfusion in the biofilms, resulted in decreased drug resistance of mature biofilms, further linking hypoxic microenvironments to drug resistance. Finally, the authors report that after treatment removal, the hypoxic zones reoxygenate, and quiescent hyphae in those areas resume growth.

In sum, the authors propose a model whereby self-generated hypoxic zones within mature biofilms induce a hypoxic response in fungal cells at the basal layer of the biofilm, which leads to reduced metabolic activity. These metabolically inactive cells are able to survive drug treatment and, upon drug removal, might function as a reservoir for regrowth. Further studies are now needed to understand the mechanisms of oxygen-mediated antifungal drug resistance in fungal biofilms.

Andrea Du Toit

ORIGINAL ARTICLE Kowalski, C. H. et al. Fungal biofilm architecture produces hypoxic microenvironments that drive antifungal resistance. *Proc. Natl Acad. Sci. USA* https://doi.org/10.1073/pnas.2003700117 (2020)

IN BRIEF

VIRAL INFECTION

Controlling the nucleus from a distance

Human cytomegalovirus (HCMV) induces nuclear rotation during infection, but precisely how and why nuclei rotate was unknown. Procter et al. used convolutional neural network-based automated cell classification and analyses to examine HCMV-mediated control of intranuclear polarity through cytoplasmic virus-assembled microtubule-organizing centres (MTOCs). The authors found that acetylation of tubulin in microtubules originating from the viral MTOC facilitates nuclear rotation by enabling microtubules to engage linker of nucleoskeleton and cytoskeleton complexes through the outer nuclear envelope protein nesprin-2G, which causes polarization of the inner nuclear membrane protein SUN1. This results in intranuclear polarization of emerin, which regulates nuclear actin filaments that segregate viral DNA from heterochromatin, thus promoting viral replication.

ORIGINAL ARTICLE Procter, D. J. et al. Cytoplasmic control of intranuclear polarity by human cytomegalovirus. *Nature* https://doi.org/10.1038/s41586-020-2714-x (2020)

VIRAL PATHOGENESIS

Zika virus enhances dengue risk

Sequential dengue virus (DENV) infections can result in antibody-dependent disease enhancement. As the Zika virus (ZIKV) and dengue virus envelope proteins are ~40% homologous, Katzelnick et al. explored whether immune interactions among different dengue virus serotypes extends to ZIKV. The authors followed a patient cohort in Nicaragua who have been sequentially exposed to DENV and ZIKV and found that the risk of symptomatic DENV serotype 2 (DENV-2) infection and severe disease was increased by one prior DENV or ZIKV infection. By contrast, multiple DENV infections reduced disease risk. High levels of pre-exisiting anti-DENV antibodies protected against DENV-1, DENV-3 and ZIKV infection, but intermediate levels of DENV or ZIKV antibodies increased the risk of severe disease in DENV-2 and DENV-3 infection, posing a challenge to DENV and ZIKV vaccine development.

ORIGINAL ARTICLE Katzelnick, L. C. et al. Zika virus infection enhances future risk of severe dengue disease. *Science* **369**, 1123–1128 (2020)

SYMBIOSIS

A perfect match

Like animals and plants, fungi establish symbiotic relationships with bacteria that are both antagonistic and mutualistic; however, the interactions that occur between fungi and bacteria in these different types of symbioses are not well-understood. Lastovetsky et al. analysed both fungal and bacterial transcriptional responses before and after physical contact between *Rhizopus microsporus* and *Mycetohabitans* sp. endobacteria, as well as the responses between the same bacterium and an isolate of *R. microsporus* that is naturally bacterium-free and interacts antagonistically with endobacteria. Endobacteria expressed a similar gene repertoire, regardless of host partner, whereas fungal transcriptional responses were markedly different in antagonistic *R. microsporus* compared with the mutualistic counterpart. In particular, antagonistic fungi increased reactive oxygen species (ROS) in response to endobacteria, whereas mutualistic fungi quenched ROS production, suggesting that ROS metabolism has a role in bacterial–fungal symbioses.

ORIGINAL ARTICLE Lastovetsky, O. A. et al. Molecular dialogues between early divergent fungi and bacteria in an antagonism versus a mutualism. *mBio* **11**, e02088-20 (2020).