that are distinct from the vesicles produced by free-living cells. Interestingly, the composition of vesicles produced by biofilm cells correlated with components of the extracellular matrix; for example, gas chromatography analysis revealed that the main polysaccharides in vesicles are mannan and glucan, which are two major matrix components. This suggests that vesicles may have a role in delivering extracellular matrix components.

To further corroborate this finding, the author generated mutants that are defective in components of the endosomal sorting complexes required for transport (ESCRT) complex, which is required for the production of exosomes in eukaryotes. Some of the mutants that were deficient in exosome production also exhibited hypersusceptibility to the antifungal fluconazole during biofilm growth in vitro and in an in vivo biofilm infection model, but this effect was not observed in planktonic mutant cells. Drug hypersusceptibility was reversed in complement strains. Moreover, ESCRT mutants with vesicle and drug-susceptibility defects also displayed defects in biofilm architecture and the extracellular matrix, including decreased quantities of the matrix components mannan and glucan. These effects could also be restored by the addition of exogenous wild-type vesicles. Similarly, vesicle cargo mutants exhibited increased drug resistance, and those mutants regained their ability to grow in the presence of fluconazole after the addition of exogenous wild-type vesicles, suggesting that cargo proteins in extracellular vesicles confer drug resistance, possibly through their role in matrix biogenesis and modification.

In summary, these findings suggest that extracellular vesicles produced by *C. albicans* biofilm cells have a crucial role in the production of the extracellular matrix and biofilm drug resistance, and that extracellular vesicles could present a potential therapeutic target to treat fungal infections.

Andrea Du Toit

ORIGINAL ARTICLE Zarnowski, R. et al. *Candida albicans* biofilm-induced vesicles confer drug resistance through matrix biogenesis. *PLOS Biol.* https://doi.org/10.1371/journal.pbio.2006872 (2018)

Credit: Loerie/Stockimo/Alamy Stock Photo

Different contemporary EV-D68 clades have been linked with AFM and, therefore, the authors tested the ability of all commercially available contemporary EV-D68 strains to infect SH-SY5Y cells. All of these strains replicated in the neuronal cells and reduced cell viability. By contrast, rhinoviruses, which also are non-polio enteroviruses but are not known to cause paralysis, did not infect the neuronal cells.

To further elucidate the mechanisms underlying neurotropism, the authors transfected SH-SY5Y cells with the full-length RNA genomes of the different isolates. Transfection led to the production of all isolates, which suggests that neurotropism is likely determined by permissive binding to and/or entry into neuronal cells. Indeed, the neurotropic EV-D68 strains showed higher binding to neuronal cells than the older, non-neurotropic strains. There was no difference in binding to epithelial cells.

The authors conclude that these results, taken together, support a causal link between EV-D68 infection of neurons and AFM, and the models used in this study now can be applied to further study the neuropathogenic potential of EV-D68.

Ursula Hofer

ORIGINAL ARTICLE Brown, D. M. et al. Contemporary circulating enterovirus D68 strains have acquired the capacity for viral entry and replication in human neuronal cells. *mBio* **9**, e01954-18 (2018)

FURTHER READING Baggen, J. et al. The life cycle of non-polio enteroviruses and how to target it. *Nat. Rev. Microbiol.* **16**, 368–381 (2018)

IN BRIEF

MICROBIOME

Plant probiotic supresses bacterial wilt

Plants differ in their resistance to soil-borne pathogens. Tomato variety Hawaii 7996 is resistant to *Ralstonia solanacearum* — a xylem-colonizing bacterium that induces wilt — whereas the Moneymaker variety is not. Now, Kwak et al. demonstrate that the rhizosphere microbiota enables resistance to wilt. Transplantation of the rhizosphere microbiota from resistant plants supressed symptoms of wilt in susceptible plants. By comparing the rhizosphere microbiomes of susceptible and resistant plants, the authors found marked differences in microbiome structures and a more abundant flavobacterial metagenome in the resistant plant rhizosphere microbiome. The authors cultured this bacterium (*Flavobacteriaceae* sp. TRM1) and found that it supressed bacterial wilt in susceptible plants. This study highlights that the native microbiota could be harnessed to protect plants from bacterial pathogens through the development of plant probiotics.

ORIGINAL ARTICLE Kwak, M.-J. et al. Rhizosphere microbiome structure alters to enable wilt resistance in tomato. *Nat. Biotechnol.* https://doi.org/10.1038/nbt.4232 (2018)

BACTERIAL PATHOGENESIS

A useful by-product

The intracellular pathogen *Shigella flexneri* replicates to high cell densities in colon epithelial cells and then spreads to neighbouring cells. This study found that formate (a metabolite of *S. flexneri* glycolysis and mixed acid fermentation within host cells) accumulation enhances virulence gene expression and intercellular spread. A mutant of pyruvate formate lyase (PFL; Δ*pflB*), which cannot convert pyruvate to acetyl-CoA and formate, produced smaller plaques in epithelial cell monolayers. The growth rate of this mutant was not affected, but its ability to spread cell to cell was. This phenotype was rescued by adding exogenous formate or by deleting the *S. flexneri* formate dehydrogenase gene *fdnG*, which increased formate accumulation. Formate was found to increase the expression of *S. flexneri* virulence genes *icsA* and *ipaJ*, which promote intercellular spread and inhibit the trafficking of the host innate immune effector STING, respectively, suggesting that formate is an intracellular signalling molecule that regulates virulence in response to *S. flexneri* metabolism and density.

ORIGINAL ARTICLE Koestler, B. J., Fisher, C. R. & Payne, S. M. Formate promotes *Shigella* intercellular spread and virulence gene expression. *mBio* **9**, e01777–18 (2018)

FUNGAL GENOMICS

An expanding fungal tree of life

Our knowledge of the fungal tree of life is biased towards model systems, fungi in biotechnology applications and pathogens, thus limiting our understanding of fungal evolution and their biosynthetic diversity. Now, Ahrendt et al. use single-cell genomics to expand the fungal tree of life. The authors sequenced the genomes of eight uncultured fungi from across the fungal tree of life, focusing primarily on early-diverging fungi from the Cryptomycota, Chytridiomycota and Zoopagomycota, which represent understudied fungal taxa. Each single amplified genome of the early-diverging lineages was placed within the tree of life, and the observed high number of heterozygous genomes at the base of the fungal tree led the authors to infer a diploid (or higher aneuploidy) ancestor of fungi. They also observed metabolic deficiencies and expansions that correlate with their parasitic nature and unculturability.

ORIGINAL ARTICLE Ahrendt, S. R. et al. Leveraging single-cell genomics to expand the fungal tree of life. *Nat. Microbiol.* https://doi.org/10.1038/s41564-018-0261-0 (2018)