multicolor control would be a feasible development to broaden our ability to probe multicomponent systems, such as those recently discovered for nucleoli and stress granules. These cellular bodies appear to be composed of phase-separated structures formed from a static gel-like core surrounded by a dynamic liquid phase (Feric et al., 2016; Jain et al., 2016).

However, even the most sophisticated light-based control mechanisms will only be able to resolve parts of the mysteries around phase separation. It is intriguing that many liquid-liquid phase separating proteins contain intrinsically disordered domains (IDD), which lack stable structure in their native state, as well RNA binding domains (RBB). In fact, the underlying design principle of the fusion construct presented by Shin et al. (2017) was to swap the RBB domain with CRY2 and fuse this to the IDD domain of FUS, HNRNPA1, and DDX4. Future studies combining the optoDroplet method with systematic mutagenesis could enable a better understanding of the functional relevance of sequence composition and complexity of the IDD. However, the very design of the optoDroplet tool precludes asking at the same time what role the properties of RNA play. As RNA mixtures are frequently added to in vitro phase separation experiments and are abundant in many of the cellular bodies studied for phase separation, questions about whether it is an important but inert bystander or what specific aspects of RNA structure or chemistry are critical for functional phase separated droplets still remain to be investigated. Right now, our tools to control and study RNA function are underdeveloped compared to proteins.

With the innovative concept introduced here, the authors have ushered in a new generation of in vivo, quantitative, phase separation studies, which will hopefully shed light on the physiological and pathogenic aspects of such processes.

REFERENCES


Vesicles Spread Susceptibility to Phages

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Extracellular membrane vesicles from bacteria are now shown to transfer phage receptors from susceptible to resistant cells, thus making them transiently sensitive to phage infection (Tzipilevich et al.).

Viruses that infect bacteria, or phages, play key roles in microbial ecology and apply constant evolutionary pressure on bacterial communities. Phage infection begins with the attachment (adsorption) of the virus to its cognate receptor—an extracellular molecule on the bacterial cell envelope. This adsorption is followed by injection of the viral genetic material into the bacteria, expression of phage genes and phage DNA replication, and eventually, virion assembly and cell lysis. The presence of the phage receptor on the bacterial cell is essential for phage attachment, and without it, no infection can take place. Indeed, lab evolution experiments and metagenomic studies of natural populations have demonstrated that development of bacterial resistance to phage is very frequently achieved by a mutation in the phage receptor, preventing phage adsorption and prohibiting infection (Avrani et al., 2011; Mizuno et al., 2014). In this issue of Cell, Tzipilevich et al. (2017) report that bacteria lacking phage receptors can become transiently sensitive to phage and get infected. This is mediated by membrane vesicles, released from phage-sensitive cells, which carry the phage receptor and transfer it to the resistant cells.

The study of Tzipilevich et al. (2017) begins with the observation that, when
phage-resistant *Bacillus subtilis* cells—which completely lack the gene for the phage receptor—are co-cultured with phage-susceptible cells, a small proportion of the resistant cells are infected and lysed by phages. By directly visualizing fluorescently labeled adsorbed phages on fluorescently labeled resistant cells, the researchers verify that phages actively infect these cells. They further validate that the phage genome is injected and expressed in the resistant cells by fluorescently marking the phage DNA and phage proteins and observing them in the resistant cells. Strikingly, this phenomenon is not limited to co-culture of sensitive and resistant cells of the same species; for example, phages naturally infecting *Bacillus subtilis* also adsorb to *Bacillus cereus* cells when these cells are co-cultured with *Bacillus subtilis* cells during infection.

The authors suspected that the acquired phage sensitivity is due to transfer of the phage receptor between cells. Indeed, they directly visualize phage receptors on resistant cells, but only if these cells are first co-cultured with sensitive, receptor-expressing cells. Surprisingly, the acquired susceptibility to phage is transient. The authors show this by isolating resistant cells that have undergone conversion and demonstrate that their progeny are resistant to the phage and do not express the phage receptor. The rapid loss of phage susceptibility suggests that the basis to this phenomenon is not genetic, ruling out horizontal gene transfer as the underlying mechanism.

If not horizontal gene transfer, what may be the mode of phage receptor transfer? The authors first suspected nanotubes, which are physical bridges existing between *B. subtilis* and other cells (Dubey and Ben-Yehuda, 2011). However, experiments with nanotube-deficient mutants did not prevent the receptors from traveling to resistant cells. Instead, the mode of transfer of surface molecules is shown to occur via extracellular membrane vesicles. Such vesicles are membrane-enclosed small spheres that are known to be generated by both Gram negative and Gram positive bacteria (Brown et al., 2015; Kulp and Kuehn, 2010). The authors demonstrate that vesicles released by phage-sensitive cells carry the phage receptor YueB and that addition of purified extracellular vesicles from phage-sensitive cells is sufficient to induce phage susceptibility in resistant cells. As phage-induced cell lysis promotes the formation of membrane vesicles (Turnbull et al., 2016), the tendency of resistant cells to acquire susceptibility to phage is increased by lysis of phage-sensitive cells in their surroundings (Figure 1).

The discovery that phages can successfully infect resistant cells following transient acquisition of receptor molecules has several intriguing implications. First, it provides an avenue for phage-mediated horizontal gene transfer (also called transduction) into species not serving as natural hosts for these phages. As many phages have a narrow host range, this new mechanism can explain how phages can serve as vectors to transfer genes into bacteria that are genetically resistant to them. Although most transduction-mediated horizontal gene transfer is thought to occur between closely related bacteria (Popa et al., 2016), the observation that receptors can travel between *Bacillus subtilis, Bacillus amyloliquefaciens*, and *Bacillus cereus* suggests that the mechanism discovered by Tzipilevich et al. (2017) can broaden the effective host range for phage-dependent gene transfer. This possibility necessitates more research, though, as Tzipilevich et al. (2017) only demonstrate adsorption of phages, and the question of whether DNA injection takes place following the adsorption remains open.

It is important to note that receptor transfer to resistant cells is a rare event, probably affecting less than 1% of the resistant cells, with limited effect on the population dynamics of these cells during infection. Tzipilevich et al. (2017) observe and document this rare event through high-resolution microscopic examination of the co-culture infection process at single-cell resolution, combined with phage transduction assays and other methods. This demonstrates how, despite many decades of research on phage-bacteria interactions, application of new methodologies can yield surprising discoveries in the field.

In a broader context, these results provide another example for the increasingly appreciated roles of membrane vesicles in the sophisticated molecular exchange between bacteria. Such extracellular membrane vesicles have been shown to carry genetic material, as well as proteins and lipids, and are known to be involved in pathogenicity, communication, and antagonistic interactions between bacteria (Kulp and Kuehn, 2010; MacDonald...
and Kuehn, 2012). Previously, extracellular vesicles were shown to provide protection against phages by serving as decoys to phage adsorption, thus lowering the effective phage concentrations (Manning and Kuehn, 2011). Now, their capabilities to promote phage infection shed a new light on their molecular roles and suggest that bacteria using membrane vesicles as decoys against phage may inflict collateral damage on bystander bacteria that are naturally resistant to the phage.

Transfer of phage receptors might be just the first example of surface components sharing between bacteria through membrane vesicles. As surface molecules are involved in multiple aspects of bacterial life in addition to phage susceptibility, this new interaction mechanism may lead to surprising discoveries in the future.

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Staying in Touch while on the Go

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While chemical forms of cell-to-cell communication are well recognized to coordinate bacterial populations, electrical signaling has been relatively ignored. Humphries et al. show that Bacillus subtilis biofilms utilize potassium production to attract far away, motile cells of even phylogenetically distant species by altering their membrane potential.

All living cells maintain an electrical potential across the cytoplasmic membrane by establishing differences in ionic concentrations inside versus outside using ion channels and pumps. Classic work by Hodgkin and Huxley established how electrical signaling between ion channels in nerve fibers creates action potentials (Hodgkin and Huxley, 1952), forming the basis of information transfer in the nervous system. Until recently, the vast majority of studies into cell-cell communication in bacteria have focused on chemical signaling, whereby cells secrete and detect the concentration of autoinducer molecules to synchronize cell density-dependent behaviors (Waters and Bassler, 2005). In this issue of Cell, Humphries et al. (2017) show that biofilms of the Gram-positive bacterium Bacillus subtilis can use electrical signaling mediated by potassium ion channels to affect the motility of cells far from the biofilm. This work elucidates a general mechanism for coordinating isolated cells with communities and has the potential to rejuvenate the investigation of the role of membrane potential in myriad biological processes.

Previous work by the same group showed that cells within B. subtilis biofilms actively produce extracellular potassium in a manner dependent on their metabolic state, leading to the propagation of electrical waves through the biofilm (Prindle et al., 2015). This electrical activity drives metabolic co-dependence that resolves the conflict for nutrients within the community, thereby increasing the fitness of the biofilm (Liu et al., 2015). Humphries et al. (2017) now report that potassium directs the motility of distant cells toward a biofilm by altering their membrane potential, leading to the periodic accumulation of motile cells at the biofilm edge with a frequency matching the oscillations in biofilm membrane potential, as well as an increase in the probability of motile cells becoming embedded in the biofilm (Figure 1). A mutation that interferes with the activity of the ion channel responsible for generating the extracellular potassium signal from the biofilm abolished motile cell attraction, and a mutant lacking the major potassium pump had a more negative resting membrane potential and failed to accumulate at the biofilm edge (Figure 1) (Humphries et al., 2017). Thus, potassium production by the biofilm and sensing by motile cells are necessary for electrical signaling.

Given that ions are common currency for all cells, electrical signaling may attract...