CELLULAR MICROBIOLOGY

Bacterial networking

Exchange of cellular contents by tubular conduits between neighbouring cells is common in multicellular eukaryotic organisms. For example, plant cells can be connected by cytoplasmic tubes known as plasmodesmata, whereas mammalian cells use gap junctions and synapses to connect to adjacent cells and nanotubes for longer-distance communication. Now, Dubey and Ben-Yehuda report that intercellular nanotubes can be formed between bacterial cells of the same and of different species to facilitate the exchange of cytoplasmic content.

To test the hypothesis that bacteria could exchange cytoplasmic content in ways analogous to those previously described for plant and mammalian cells, the authors

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Cells of *Bacillus subtilis* grown on solid medium and visualized by high-resolution scanning electron microscopy. Nanotubes connecting bacterial cells are visible. Image courtesy of G. P. Dubey and S. Ben-Yehuda, The Hebrew University of Jerusalem, Israel.

cultured two strains of *Bacillus subtilis* side by side on solid medium, one strain of which (GFP⁺) had been engineered to express GFP from a chromosomal locus. Using fluorescence microscopy, they observed that the wild-type cells lying close to GFP⁺ cells progressively acquired a weak fluorescent signal. Conversely, the fluorescence of the GFP⁺ cells decreased over time, suggesting that their cytoplasmic GFP molecules (or GFP transcripts) were being distributed among adjacent cells. Remarkably, tubular protrusions (nanotubes) connecting neighbouring cells grown on solid media were observed by high-resolution scanning electron microscopy, but they were not detected between cells grown in liquid media. Nanotubes were up to 1 μm long and 30–130 nm wide, which could allow the passage of molecules larger than GFP, and they seemed to contain cell wall material, membrane and cytoplasmic content, as indicated by transmission electron microscopy. These characteristics make nanotubes clearly different from conjugative pili. In addition, clusters of smaller tubes connected nearby cells. The presence of GFP within nanotubes connecting *B. subtilis* GFP⁺ and wild-type cells was confirmed by immunoelectron microscopy using GFP-specific antibodies. Furthermore, the transfer of a non-conjugative plasmid was also detected between two *B. subtilis* strains when grown on solid medium.

To test whether nanotubes could mediate the exchange of enzymes conferring antibiotic resistance, the authors used two *B. subtilis* strains that expressed chromosomally

encoded resistance to either chloramphenicol or lincomycin. When cultured separately, each strain was able to grow in the presence of one of the antibiotics but not the other; however, mixtures of the two strains survived on agar plates containing both antibiotics. Survivor cells from these mixed cultures, when cultured again separately, exhibited resistance to one of the antibiotics but not both, indicating that they had not acquired an additional resistance gene from the other strain. The antibiotic resistance assay was used to screen a set of *B. subtilis* mutants for genes involved in nanotube formation, but none of the tested mutations reduced the antibiotic resistance of the mixed populations. Remarkably, GFP from *B. subtilis* was also transferred to *Staphylococcus aureus* and *Escherichia coli*, and nanotubes connecting cells of the same and of different species were observed in mixed populations of the three bacterial species.

These results suggest that the exchange of small molecules, proteins and nucleic acids between neighbouring cells through nanotubes may be widespread among bacteria growing in biofilms. The authors propose that nanotubes may represent a key form of intercellular bacterial communication in nature, allowing the emergence of new phenotypes in multispecies communities.

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ORIGINAL RESEARCH PAPER Dubey, G. P. & Ben-Yehuda, S. Intercellular nanotubes mediate bacterial communication. *Cell* **144**, 590–600 (2011)
FURTHER READING Keller, L. & Surette, M. G. Communication in bacteria: an ecological and evolutionary perspective. *Nature Rev. Microbiol.* **4**, 249–258 (2006)