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Bacterial nanotubes: a conduit for intercellular molecular trade

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Bacteria use elaborate molecular machines for intercellular contact-dependent interactions. We discuss a relatively less explored type of intercellular connections mediated by tubular membranous bridges, termed nanotubes. Increasing evidence suggests that nanotube structures mediate cytoplasmic molecular trade among neighboring cells of the same and different species. Further, nanotubes were found to facilitate both antagonistic and cooperative interspecies interactions, thereby allowing the emergence of new non-heritable phenotypes in multicellular bacterial communities. We propose that nanotube-mediated cytoplasmic sharing represents a widespread form of bacterial interactions in nature, providing an enormous potential for the emergence of new features. Here we review the current knowledge on bacterial nanotubes, and highlight the gaps in our current understanding of their operation.

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Introduction

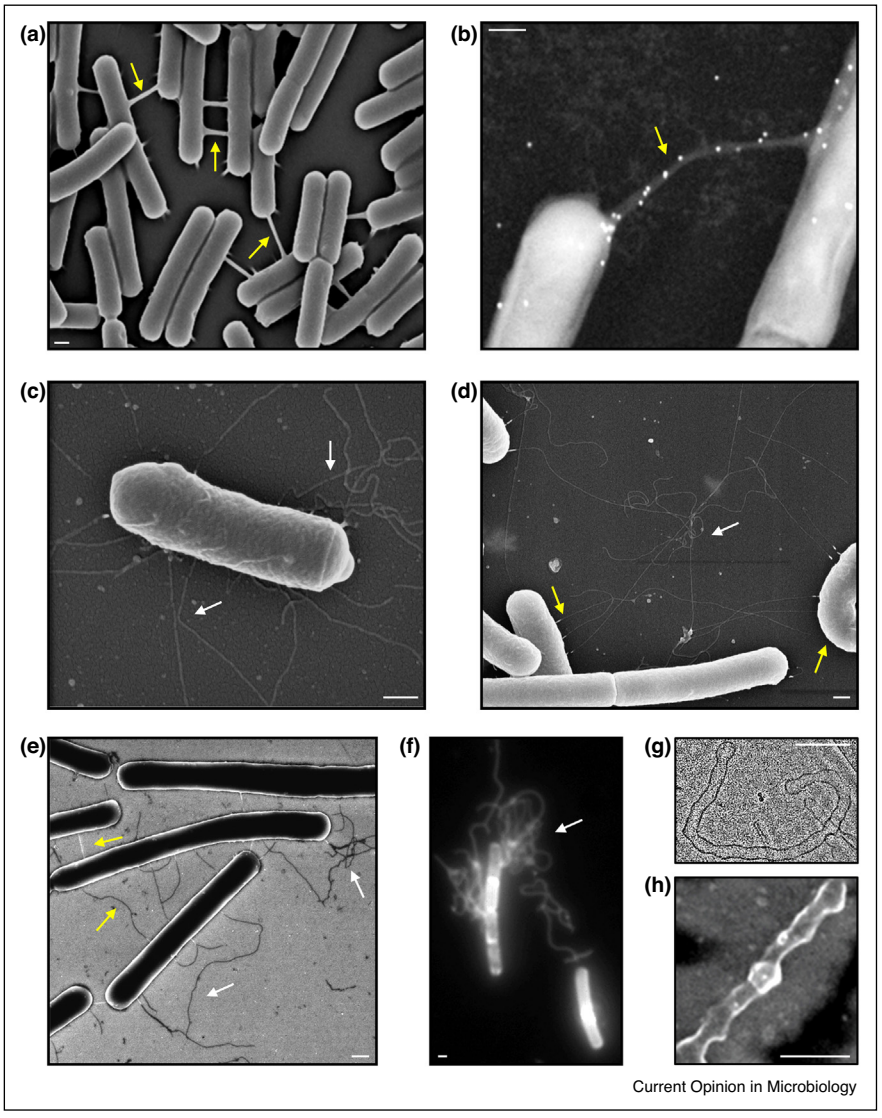
Intercellular molecular exchange among adjacent cells is abundant in multicellular eukaryotic organisms. In plants, neighboring cells are connected by cytoplasmic tubes termed plasmodesmata, which provide a route for intercellular transport of nutrients, signaling molecules, proteins and transcripts [1,2]. Mammalian cells utilize gap junctions for short distance molecular trade, and a network of intercellular membrane nanotubes for remote communication. These nanotubes can channel cytoplasmic molecules and even organelles and viruses in an intercellular manner [3–6]. We have previously reported the identification of analogous nanotubular conduits formed among bacterial cells grown in proximity [7••]. Such structures were shown to connect cells of the same and diverse bacterial species [7••,8••,9••]; yet, their prevalence in nature and their impact on shaping bacterial communities remain to be explored. Here we review the current knowledge on bacterial nanotubes, enlighten the potential effect of such intimate intercellular junctions, and address the future challenges in understanding nanotube biogenesis, structure and function.

Intercellular cytoplasmic molecular exchange

The initial investigation of bacterial nanotubes was triggered by the observation that cytoplasmic GFP molecules could be exchanged among neighboring cells of the Gram positive soil bacterium *Bacillus subtilis* [7••]. We noted that wild-type cells, neighboring GFP-producing cells, gradually acquired a weak fluorescent signal, suggesting that cytoplasmic GFP molecules were being distributed among adjacent cells. In addition, the spread of calcein, a small cytoplasmically caged fluorescent molecule, from one cell to another, occurred rapidly in a spatial and temporal manner. These results raised the possibility of the existence of intercellular connections that facilitate this molecular flow. Indeed, nanotubular protrusions bridging neighboring cells were revealed by electron microscopy (EM), along with GFP molecules that were localized to these protrusions, implicating the role of nanotubes in molecular trafficking (Figure 1a) [7••].

Contact-dependent molecular trade among bacteria can be beneficial when neighboring strains possess complementing features. Such an additive interaction was exemplified by co-culturing two *B. subtilis* strains, harboring different antibiotic resistance genes. Growth in a mixture enabled the cells to transiently acquire non-hereditary resistance to both antibiotics [7••]. Additionally, non-conjugative plasmids could be transferred from one cell to another, independently of the competence machinery, thereby conferring hereditary features to recipient cells [7••,10]. Contact-dependent synergistic molecular exchange was recently demonstrated to occur among different bacterial species, and was featured by both Gram positive and negative bacteria. Utilizing nanotube-like structures, the Gram negative bacteria *Acinetobacter baylyi* and *Escherichia coli*, as well as the Gram

Figure 1



Nanotube visualization. **(a)** *B. subtilis* (Δ*hag*) cells, lacking flagella, were grown on solid LB medium for 3 h and subjected to HR-SEM analysis with gold coating. The formation of intercellular nanotubes is exemplified. **(b)** *B. subtilis* (*wapA*-HA, Δ*hag*, Δ*ymdB*, $P_{hyperspank}$-*ymdB*) cells, were spotted over an EM grid. The grid was placed on solid LB medium for 3 h and the cells were subjected to immuno HR-SEM, without coating, using gold conjugated antibodies. White dots correspond to WapA molecules localized to an intercellular nanotube and to the cell surface. Adapted from Ref. [14]. **(c)** *B. subtilis* (Δ*hag*) cells, lacking flagella, were spotted over an EM grid at low cell density. The grid was placed on solid LB medium for 2 h and subjected to HR-SEM with gold coating. The formation of extending nanotubes by a single cell is exemplified. **(d)** *B. subtilis* (Δ*hag*) cells were treated as in (c). Image demonstrates that extending nanotubes can become intercellular. Adapted from [10]. **(e)** *B. subtilis* (Δ*hag*) cells were spotted at low density onto an ITO-coated coverslip and incubated in LB medium for 2 h. Cells were subjected to HR-SEM without coating. Intercellular and extending nanotubes are visible. **(f)** *B. subtilis* (Δ*hag*) cells were incubated in LB supplemented with a fluorescent membrane dye and visualized by fluorescence microscopy. *B. subtilis* cells producing nanotubes are manifested. Adapted from Ref. [10]. **(g)** *B. subtilis* (Δ*hag*, Δ*ymdB*, $P_{hyperspank}$-*ymdB*) cells, were incubated in LB for 2 h, and visualized by Cryo-EM. Shown is a nanotube having a constricted pattern. Adapted from Ref. [10]. **(h)** *B. subtilis* (Δ*hag*) cells were spotted over an EM grid that was placed on solid LB medium for 3 h, and visualized by HR-SEM without coating. Shown is a nanotube having a constricted pattern similar to (g). Yellow and white arrows indicate intercellular and extending nanotubes, respectively. Scale bars 250 nm.

positive *Clostridium acetobutylicum* and Gram negative *Desulfovibrio vulgaris*, were able to cross feed each other, and exchange nutrients and metabolic substances, allowing them to survive. Interestingly, the trade of vital molecules was coupled with substantial swap of cytoplasmic fluorescent proteins [8••,9••]. These findings suggest that at least for some species, nanotube formation can overcome nutrient deprivation by establishing molecular collaborations with nearby species. Notably, although the phenomenon of cross feeding among bacterial cells