An up-close view of bacterial 'motors'

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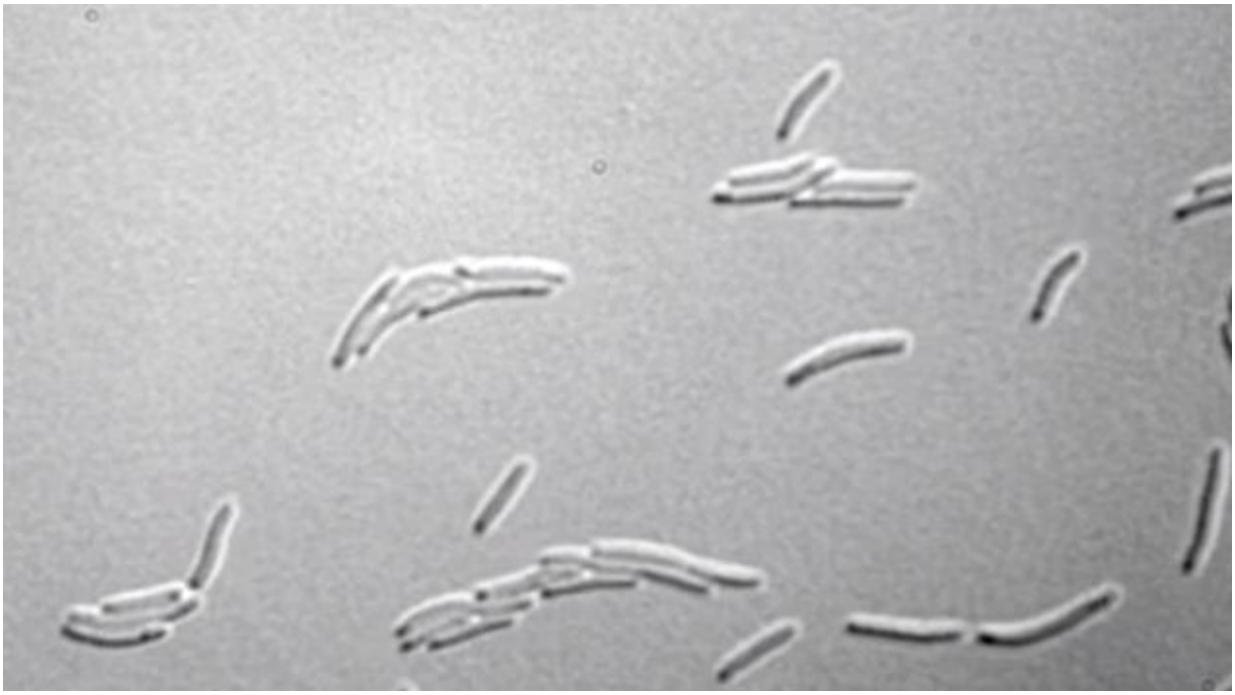

Bacteria are the most abundant form of life on Earth, and they are capable of living in diverse habitats ranging from the surface of rocks to the insides of our intestines. Over millennia, these adaptable little organisms have evolved a variety of specialized mechanisms to move themselves through their particular environments. In two recent Caltech studies, researchers used a state-of-the-art imaging technique to capture, for the first time, three-dimensional views of this tiny complicated

machinery in bacteria.

"Bacteria are widely considered to be 'simple' cells; however, this assumption is a reflection of our limitations, not theirs," says Grant Jensen, a professor of biophysics and biology at Caltech and an investigator with the Howard Hughes Medical Institute (HHMI). "In the past, we simply didn't have technology that could reveal the full glory of the nanomachines—huge complexes comprising many copies of a dozen or more unique proteins—that carry out sophisticated functions."

Jensen and his colleagues used a technique called electron cryotomography to study the complexity of these cell motility nanomachines. The technique allows them to capture 3-D images of intact cells at macromolecular resolution—specifically, with a resolution that ranges from 2 to 5 nanometers (for comparison, a whole cell can be several thousand nanometers in diameter). First, the cells are instantaneously frozen so that water molecules do not have time to rearrange to form ice crystals; this locks the cells in place without damaging their structure. Then, using a transmission electron microscope, the researchers image the cells from different angles, producing a series of 2-D images that—like a computed tomography, or CT, scan—can be digitally reconstructed into a 3-D picture of the cell's structures. Jensen's laboratory is one of only a few in the entire world that can do this type of imaging.

In a paper published in the March 11 issue of the journal *Science*, the Caltech team used this technique to analyze the cell motility machinery that involves a structure called the type IVa pilus machine (T4PM). This mechanism allows a bacterium to move through its environment in much the same way that Spider-Man travels between skyscrapers; the T4PM assembles a long fiber (the pilus) that attaches to a surface like a grappling hook and subsequently retracts, thus pulling the cell forward.

Although this method of movement is used by many types of bacteria, including several human pathogens, Jensen and his team used electron cryotomography to visualize this cell motility mechanism in intact Myxococcus xanthus—a type of soil bacterium. The researchers found that the structure is made up of several parts, including a pore on the outer membrane of the cell, four interconnected ring structures, and a stemlike structure. By systematically imaging mutants, each of which lacked one of the 10 T4PM core components, and comparing these mutants with normal M. xanthus cells, they mapped the locations of all 10 T4PM core components, providing insights into pilus assembly, structure, and function.

"In this study, we revealed the beautiful complexity of this machine that may be the strongest motor known in nature. The machine lets M. xanthus, a predatory bacterium, move across a field to form a 'wolf pack' with other M. xanthus cells, and hunt together for other bacteria on which to prey," Jensen says.

Another way that bacteria move about their environment is by employing a flagellum—a long whiplike structure that extends outward from the cell. The flagellum is spun by cellular machinery, creating a sort of propeller that motors the bacterium through a substrate. However, cells that must push through the thick mucus of the intestine, for example, need more powerful versions of these motors, compared to cells that only need enough propeller power to travel through a pool of water.

In a second paper, published in the online early edition of the *Proceedings of the National Academy of Sciences* (*PNAS*) on March 14, Jensen and his colleagues again used electron cryotomography to study the differences between these heavy-duty and light-duty versions of the bacterial propeller. The 3-D images they captured showed that the varying levels of propeller power among several different species of

bacteria can be explained by structural differences in these tiny motors.

In order for the flagellum to act as a propeller, structures in the cell's motor must apply torque—the force needed to cause an object to rotate—to the flagellum. The researchers found that the high-power motors have additional torque-generating protein complexes that are found at a relatively wide radius from the flagellum. This extra distance provides greater leverage to rotate the flagellum, thus generating greater torque. The strength of the cell's motor was directly correlated with the number of these torque-generating complexes in the cell.

"These two studies establish a technique for solving the complete structures of large macromolecular complexes in situ, or inside intact cells," Jensen says. "Other structure determination methods, such as X-ray crystallography, require complexes to be purified out of cells, resulting in loss of components and possible contamination. On the other hand, traditional 2-D imaging alone doesn't let you see where individual protein pieces fit in the complete structure. Our electron cryotomography technique is a good solution because it can be used to look at the whole cell, providing a complete picture of the architecture and location of these structures."

The work involving the type IVa pilus machinery was published in a *Science* paper titled "Architecture of the type IVa pilus machine."

Work involving the flagellum machinery was published in a *PNAS* paper titled "Diverse high-torque bacterial flagellar motors assemble wider stator rings using a conserved protein scaffold."

More information: Y.-W. Chang et al. Architecture of the type IVa pilus machine, *Science* (2016). DOI: 10.1126/science.aad2001

Morgan Beeby et al. Diverse high-torque bacterial flagellar motors

assemble wider stator rings using a conserved protein scaffold, *Proceedings of the National Academy of Sciences* (2016). DOI: 10.1073/pnas.1518952113