

Cholera pathogen reveals how bacteria generate energy to live

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Researchers at Rensselaer Polytechnic Institute have discovered new details about how bacteria generate energy to live. In two recently published papers, the scientists add key specifics to the molecular mechanism behind the pathogen that causes cholera. The work could provide a better understanding of this pathogen, while also offering insight into how cells transform energy from the environment into the forms required to sustain life.

As a single-cell organism, *Vibrio cholerae* depends on resources in its immediate environment to sustain itself. Blanca Barquera, assistant professor of biology at Rensselaer and principal investigator for the project, studies an enzyme that resides in the membrane that encapsulates *V. cholerae*. This enzyme, known as NQR, pumps sodium ions out of the bacteria to generate a difference in concentrations between outside and inside. This gradient acts like a battery that powers essential cell functions, such as the movement of the bacterium's tail, the flagellum.

Most cells, including human cells, use gradients of protons for this energy conservation function, but enzymes that work with sodium ions are ideal for experimental study, according to Barquera. Sodium is easier to trace and its concentration can be changed without affecting pH, which is a complication with protons. "It's a very good system to understand this very basic mechanism charging this battery to create energy," she said.

In order to learn how the enzyme works, researchers are trying to get an idea of its three-dimensional structure. "The enzyme is like two machines together — imagine the turbine and generator of a hydroelectric dam. One is the source of energy; the other uses this energy to pump ions out of the cell," Barquera said. How the two machines are connected is one key question.

In the first paper, published in the *Journal of Bacteriology*, Barquera tackled the question of how the structure of the enzyme is organized with respect to the two sides of the membrane. The problem is that the enzyme is not amenable to standard methods of determining structure. Since an ion pump needs to carry ions from one side of the bacterial membrane to the other, the enzyme has to reach all the way from the water-like medium inside the cell, through the oily membrane interior, to the water-like environment outside the cell. For this reason, the enzyme is made up of water-soluble and oil-soluble components within a single entity, so it can't hold its shape in any one solvent.

Using a stepwise process, Barquera attached labels at significant points along the length of the protein and then determined whether these labels appeared inside or outside the envelope of the cell membrane. The results showed that the cofactors — important parts of the enzyme's machinery — are all located on the inner side of the membrane, which corresponds to the "intake" port of the ion pump.

The second paper was published in the *Journal of Biological Chemistry*. In this study, Barquera focused on structures, known as flavins, within the enzyme that carry the electric current that drives the ion pump. Using an interdisciplinary approach that combined genetic methods — to modify the enzyme structure — with an analytical technique known as Electron Paramagnetic Resonance Spectroscopy, which observes electron spin, she and her co-worker Mark Nilges at the University of Illinois analyzed the properties of the flavin molecules, and mapped

these functional properties to specific points in the protein structure.

NQR is only one of several sodium pumping enzymes that Barquera plans to study. Because these enzymes are significantly different from human proteins that do similar work, some of them might be targeted by novel antibiotics. "An inhibitor or drug would be specific," she said. "You could kill the bacteria without doing anything to the human host."

But Barquera believes that the most important benefits of her research could develop in ways that cannot be foreseen: "From the basic science point of view, the more you know, the better," Barquera said. "It's basic science that will take us to unexpected places."

One of those unexpected places in Barquera's career has been her developing interest in the physiology and life cycle of *V. cholerae* itself. Much of what is known about *V. cholerae* is from study of the organism when it is in the body, yet the bacteria spend most of their lives outside their hosts. Study of the rest of the life cycle could be important in disease prevention.

"We have to know the enemy," Barquera said. As it stands, "We are trying to kill our enemies with very little knowledge."

Source: Rensselaer Polytechnic Institute

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