# Pathogen turns protein into a virulence factor in one easy step

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To infect its host, the respiratory pathogen *Pseudomonas aeruginosa* takes an ordinary protein usually involved in making other proteins and adds three small molecules to turn it into a key for gaining access to human cells. In a study to be published May 7 in *mBio*, the online open-access journal of the American Society for Microbiology, scientists at Emory University School of Medicine, the University of Virginia, and Universidad de las Islas Baleares in Mallorca, Spain, uncover this previously unknown virulence factor in *P. aeruginosa*, one of the most common causes of hospital-acquired pneumonia.

Co-author Joanna Goldberg of Emory says scientists have long thought *P. aeruginosa* mostly uses this protein called elongation factor-Tu (EF-Tu) inside the cell, but she and her collaborators have learned that as a virulence factor, it could represent a vulnerability for the bacterium. "EF-Tu is presumed to be an essential protein, and it's performing these moonlighting functions as well. If we figured out how it was doing that, we could devise strategies to inhibit it," says Goldberg.

*P. aeruginosa* pneumonia is a big problem in the hospital setting, where it is a frequent cause of hospital-acquired pneumonia and is the leading cause of death among critically ill patients whose airways have been damaged by ventilation, trauma, or other infections. The pathogen is also a contributor to disease in the lungs of cystic fibrosis patients and forms thick biofilms that are difficult or impossible to treat with antibiotics. Goldberg and her co-author Sebastian Alberti and their colleagues study the molecular events that enable the bacterium to infect human cells in

the hopes of finding ways to prevent *P. aeruginosa* pneumonia.

In their earlier work, Goldberg and Alberti found that *P. aeruginosa* takes the protein EF-Tu, which was generally thought to exist only inside the cell, and decorates the exterior of the cell with it, but in a modified form. This modified EF-Tu is recognized by antibodies to the common bacterial epitope phosphorylcholine (ChoP), indicating that the EF-Tu is modified somehow to mimic ChoP, allowing *P. aeruginosa* to enjoy the benefits of ChoP. By interacting with receptors on human cells, ChoP carries out a crucial step for setting up an infection for a number of different types of respiratory pathogens.

But how is EF-Tu modified, they wondered? And does it help *P. aeruginosa* establish an infection? This study answers those questions.

Using a host of techniques, including mass spectrometry, site directed mutagenesis of key residues in the protein, and genetic loss of function/gain of function studies, they found that *P. aeruginosa* only makes small changes to EF-Tu to get it to mimic this powerful ligand. *P. aeruginosa* transfers three methyl groups to a lysine on EF-Tu, giving it a structure similar to ChoP and allowing it to fit in the PAFR receptor in the way ChoP does.

But the modified EF-Tu not only looks like ChoP, in many ways it works like ChoP: testing in cultures of human airway cells shows that the modification of EF-Tu enables the bacterium to adhere to human cells.

"It allows [*P. aeruginosa*] to adhere to the cells and invade," says Goldberg. "And it seems to be involved in virulence in mouse models. It might also impact persistence in the lung."

As an environmental pathogen, *P. aeruginosa* lives in soil, water, and other environments outside the body, a fact that may offer a clue why it

uses this re-purposed protein as a virulence factor. Proteins that can be put to work in both worlds - in the environment and the in the human host - would be useful to *P. aeruginosa* in much the way a spork can allow you to enjoy both the coleslaw and the pudding in your take out dinner.

"Its interaction with humans is accidental. It's an opportunist. The fact that it has this novel modification on this protein that is inherent in the bacterium that enables it to attach and persist and cause disease is exciting," says Goldberg.

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