Study points to potential new target for antibiotics against E. coli, other bugs

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Scientists have identified a protein that is essential to the survival of *E. coli* bacteria, and consider the protein a potential new target for antibiotics.

In the study, the researchers confirmed that this protein, called MurJ, flips a fatty molecule from one side of a bacterial cell membrane to the other. If that molecule isn't flipped, the cell cannot construct a critical layer that keeps pressurized contents of the cell contained. If those contents aren't contained, the cell bursts.

E. coli is part of the gram-negative family of bacteria, characterized by having an extra membrane, called the outer membrane, that reduces the chances for a drug to penetrate the cell to kill it. Inhibiting MurJ, however, would require getting past just one of the two membranes, meaning it could be an attractive new target for antibiotics in this age of resistant pathogens.

"We have proof of principle that MurJ is actually a valid target because we showed that if we stop it from working, the cells will die within 10 minutes – very quickly," said Natividad Ruiz, assistant professor of microbiology at The Ohio State University and a co-lead author of the study.

"If you want to develop an antibiotic, it's important to know a protein's function. Defining the activity associated with MurJ is a big step forward toward possibly designing antibiotics that could target it."

Ruiz co-led the study with Thomas Bernhardt, associate professor of microbiology and immunobiology at Harvard Medical School. The research is published in the July 11, 2014, issue of the journal *Science*.

This work zeroes in on trying to stop construction of a bacterial cell layer called peptidoglycan, a mesh-like structure that, in gram-negative bacteria like *E. coli*, rests between the inner and outer cell membranes. Without this layer, *E. coli* cells can't survive.

Scientists have long known most of the steps behind the creation of this layer, which consists of sugars and amino acids cross-linked with each other. But one detail has remained elusive: which protein could get a specific lipid required for building the peptidoglycan layer to change its location, from the inside of the inner membrane to the outside of that membrane, where the peptidoglycan construction is under way.

Lipids contain fat and other substances and serve as part of a cell membrane's infrastructure. The mystery protein has been referred to as a flippase because of its function: flipping the lipid.

About 25 years ago, other groups of scientists proposed two likely proteins that fulfilled this role based on their locations in the bacterial cell. The proteins were known to contribute to construction of the peptidoglycan, but their specific function was never demonstrated.

While investigating cell membranes as a postdoctoral researcher, Ruiz narrowed in on the potential of the MurJ protein to serve as the *E. coli* flippase.

Ruiz and colleagues have previously shown that MurJ has several features that point to this possibility: A model of its structure shows the characteristic cavity that a flippase needs to have; eliminating the protein showed that cells wouldn't make the peptidoglycan layer; and it was

demonstrated to be related to other flipping proteins.

In this new work, the labs led by Ruiz and Bernhardt combined to take the additional steps needed to confirm MurJ's function.

One step that was important to Ruiz was being able to stop MurJ's work in the cells and see the immediate effects of that inhibition. With most research like this, scientists lower protein levels by suppressing activation of the genes that make the protein – which takes time and doesn't necessarily fully eliminate the protein's presence.

Ruiz's lab instead used a synthetic chemical to bind to hotspots on MurJ in cells in ways that immediately stopped the protein from functioning.

"The idea is to inhibit the protein, and then – boom – analyze it and see whether you're stopping the flipping," Ruiz said. "It's the equivalent to using an antibiotic that would kill the protein by not allowing it to work when it binds."

Bernhardt's lab then developed a way to further test the effects of inhibiting MurJ. The researchers used a toxin some cells release that is known to "eat" the flipped lipid shortly after it appears on the outside of its inner membrane, effectively halting construction of the peptidoglycan.

In normal cells, very little of the target lipid could be detected when the toxin was inserted into the cells, meaning the lipid was being flipped and immediately consumed by the toxin. But when MurJ was inhibited in those cells and the toxin was added, the scientists detected a buildup of the lipid that the toxin could not eat – meaning that the lipid never got flipped because the activity of MurJ was gone.

"We showed these cells will die if we inhibit MurJ and we showed that

MurJ is required for flipping to occur. If the cells are dying because the flipping doesn't occur, then nobody else is doing that job. This is the one," Ruiz said, explaining that MurJ is the mystery flippase.

More information: "MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis," by L.-T. Shamwww.sciencemag.org/lookup/doi/ … 1126/science.1254522

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