E. coli persists against antibiotics through HipA-induced dormancy

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Bacteria hunker down and survive antibiotic attack when a protein flips a chemical switch that throws them into a dormant state until treatment abates, researchers at The University of Texas M. D. Anderson Cancer Center report in the Jan.16 edition of *Science*.

"For antibiotics to work, bacteria have to be growing. Dormancy stops everything, allowing some bacteria to persist after treatment," said senior author Richard Brennan, Ph.D., professor in M. D. Anderson's Department of Biochemistry and Molecular Biology.

By demonstrating in detail how the HipA protein freezes bacterial activity, the researchers have opened the possibility of adding a new class of drugs to therapy against chronic and multidrug resistant bacterial infection.

Working in Escherichia coli, the team solved the structure of HipA and several of its protein complexes down to the atomic level, confirming that HipA is a protein kinase - an enzyme that works by transferring phosphate groups to its target molecules.

HipA is a type of protein kinase that is uncommon in bacteria, said lead author Maria Schumacher, Ph.D., associate professor of biochemistry and molecular biology. While other types of phosphorylation occur in bacteria, HipA phosphorylates proteins at their serine or threonine amino acids. This kinase activity is more commonly associated with eukaryotic cells, which make up animals, plants and fungi, and are generally thought

to be more complex.

"These 'simple bacteria' are so complex. We're finding that life is sophisticated at all levels," Schumacher said. HipA is active in other types of gram-negative bacteria, which cause significant human bacterial infections.

Inhibitor could make persistent cells 'vanish'

A number of cancer drugs inhibit kinase activity in specific targets.

"If you stop HipA from working, there essentially is no persistence," Brennan said. "We need to see whether kinase inhibitors will bind to and block HipA's active site. If they work, persistent cells, which are already rare, would vanish." Persistent cells are a one-in-a-million-cells occurrence because HipA is normally kept in check by a protein called HipB.

Persistence is common in "biofilms," bacterial colonies that become attached to a surface in a supportive matrix. Drug-resistant biofilms cause about 60 percent of infections in the developed world, the researchers note.

Overexpression of HipA previously had been associated with cell dormancy and bacterial persistence. Evidence had pointed to kinase activity.

Schumacher, Brennan and colleagues demonstrated the molecular details of HipA's role in multidrug tolerance and HipB's role keeping HipA under wraps in a series of experiments:

• Using X-ray crystallography to determine and then compare the structures of several HipA complexes, they showed that HipA has a

serine/threonine protein kinase fold and that it binds tightly to adenosine triphosphate (ATP), a common characteristic of kinases. Phosphorylation occurs when an enzyme binds to both ATP and to its target protein.

• Assays of candidate proteins to identify a target for HipA found that EF-Tu interacts strongly with HipA in the presence of ATP. EF-Tu is the most abundant protein in E.coli and plays an essential role in protein synthesis.

• Subsequent experiments and structural analysis of a HipA/EF-Tu peptide complex indicated that HipA phosphorylates EF-Tu, freezing up the bacteria's protein-making machinery and inducing dormancy.

• To analyze how HipB normally prevents HipA's function, the team solved the structure of the HipB/DNA/HipA complex. HipB tightly binds two HipA molecules in a sandwich-like structure.

• HipB does not block HipA's active site, but inactivates it by forcing it into an "open" position. "Proteins move a lot to function, they open and close - think of a clam shell, for example," Brennan explains. To function, a protein must be able to close down on its target molecules - called substrates. The closed state is the active state.

• HipB also might physically sequester HipA from EF-Tu because the HipA/HipB/DNA complex is located in E. coli's nucleoid, far from the bacteria's membrane where EF-Tu is mainly found.

HipA is free to cause trouble when its ties to HipB are broken; an infrequent occurrence which the authors note is likely caused by proteases tugging the smaller and structurally vulnerable HipB protein out of the complex.

Protein kinases often bind to more than one protein, so there are likely multiple targets for the protein in E. coli and other gram-negative bacteria, Schumacher and Brennan said.

Future research will focus on finding other HipA targets in E. coli, and kinase inhibitors will be examined for their ability to affect HipA function. If a promising inhibitor is found, its structure will be solved to clarify its binding mode and how it might be tweaked to bind HipA even better. "Structure-based drug design should provide the best chance at formulating highly specific and effective drugs against HipA," Schumacher said.

Source: University of Texas M. D. Anderson Cancer Center