Team determines structure of a molecular machine that targets viral DNA for destruction

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With a featured publication in the Aug. 7 issue of *Science*, Montana State University researchers have made a significant contribution to the understanding of a new field of DNA research, with the acronym CRISPR, that holds enormous promise for fighting infectious diseases and genetic disorders.

The MSU-led research provides the first detailed blueprint of a multi-subunit "molecular machinery" that bacteria use to detect and destroy invading viruses.

"We generally think of bacteria as making us sick, but rarely do we consider what happens when the bacteria themselves get sick. Viruses that infect bacteria are the most abundant biological agents on the planet, outnumbering their bacterial hosts 10 to 1," said Blake Wiedenheft, senior author of the paper and assistant professor in MSU's Department of Microbiology and Immunology.

"Bacteria have evolved sophisticated immune systems to fend off viruses. We now have a precise molecular blueprint of a surveillance machine that is critical for viral defense," Wiedenheft said.

These immune systems rely on a repetitive piece of DNA in the bacterial genome called a CRISPR. CRISPR is an acronym that stands for Clustered Regularly Interspaced Short Palindromic Repeats. These

repetitive elements maintain a molecular memory of viral infection by inserting short segments of invading viral DNA into the DNA of the "defending" bacteria. This information is then used to guide the bacteria's immune system to destroy the invading viral DNA.

The molecular blueprint of the surveillance complex was determined by a team of scientists in Wiedenheft's lab at MSU using a technique called X-ray crystallography. Ryan Jackson, a postdoctoral fellow in the Wiedenheft lab, collected X-ray diffraction data from synchrotron radiation sources located in Chicago, Berkeley, and Stanford.

"Interpreting these X-ray diffraction patterns is a complex mathematical problem and Ryan is one of a few people in the world capable of interpreting this data," Wiedenheft said.

To help determine the structure, Wiedenheft sent Jackson to Duke University for a biannual meeting on X-ray crystallography. At the meeting, Jackson sat between "two of the greatest minds in the field of X-ray crystallography"– Randy Read from the University of Cambridge and Thomas Terwilliger from Los Alamos National Lab—whose expertise facilitated the computational analysis of the data, which was critical for determining the structure.

"The structure of this biological machine is conceptually similar to an engineer's blueprint, and it explains how each of the parts in this complex assemble into a functional complex that efficiently identifies viral DNA when it enters the cell," Wiedenheft said. "This surveillance machine consists of 12 different parts and each part of the machine has a distinct job. If we're missing one part of the machine, it doesn't work."

Understanding how these machines work is leading to unanticipated new innovations in medicine and biotechnology and agriculture. These CRISPR-associated machines are programmable nucleases (molecular

scissors) that are now being exploited for precisely altering the DNA sequence of almost any cell type of interest.

"In nature these immune system evolved to protect bacteria form viruses, but we are now repurposing these systems to cut viral DNA out of human cells infected with HIV. You can think of this as a form of DNA surgery. Therapies that were unimaginable may be possible in the future," Wiedenheft said.

"We know the genetic basis for many plant, animal, and human diseases, and these CRISRP-associated nucleases are now being used in research settings to surgically remove or repair defective genes," Wiedenheft said. "This technology is revolutionizing how molecular genetics is done and MSU has a large group of researchers that are at the cutting edge of this technological development."

Wiedenheft, a native of Fort Peck, Mont., was recently recruited by MSU from UC-Berkeley. Wiedenheft explained that the research environment, colleagues and support at MSU is second to none and the opportunity to move back to this great state was a "no-brainer."

More information: "Crystal structure of the CRISPR RNA-guided surveillance complex from Escherichia coli" *Science*, 2014. www.sciencemag.org/lookup/doi/ … 1126/science.1256328

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