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Enzyme Structure Offers Smallpox Drug Target

Researchers searching for ways to combat the highly contagious, often lethal smallpox virus have a powerful new tool: a detailed picture of the enzyme the pathogen uses to maintain its DNA in a usable form. Researchers can now see exactly how the enzyme tightly wraps itself around smallpox DNA as it aids in the virus's mission to multiply inside infected cells. Understanding this structure opens the way to developing the first drugs against smallpox, which, although eradicated worldwide, is still considered a potential bioterrorism threat.

The researchers, led by Howard Hughes Medical Institute investigator Gregory D. Van Duyne and colleague Frederic Bushman, both at the University of Pennsylvania, published their findings in the August 4, 2006, issue of the journal *Molecular Cell*. Kay Perry in the Van Duyne laboratory and Young Hwang in the Bushman laboratory were co-authors.

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The focus of the study was the smallpox version of an enzyme called topoisomerase. The enzyme relieves strain on smallpox DNA that comes from overtwisting, or supercoiling, of the molecule. Supercoiling creates knots in DNA much like the kinks in an overtight telephone cord - leaving a tangled mess that cannot be read by the smallpox machinery that transcribes the DNA to produce essential proteins. To eliminate this problem - not just in smallpox, but in humans and other organisms — topoisomerase snips one strand of the two-stranded DNA, allowing the molecule to untwist into a more relaxed position, and then repairs the snip.

Biochemists have amassed extensive data on the function of both viral and human topoisomerases. However, said Van Duyne, The details of how this

viral topoisomerase works, and in particular why it so specifically targets a particular viral DNA sequence, remain unknown and are being very actively explored.

To get a closer look at smallpox topoisomerase in action, Van Duyne, Bushman, and their colleagues created crystals of the enzyme linked to DNA at two stages of the process. They then used x-ray crystallography to obtain high-resolution structures of the enzyme-DNA complexes. In this widely used analytical technique, researchers direct x-rays through crystals of a molecule, creating a pattern of diffraction that they can analyze to deduce the molecule's structure. In this way, the researchers created snapshots of two moments during topoisomerase's processing of the DNA strand.

The resulting structure dramatically enhanced the scientists' understanding of how the smallpox enzyme recognizes and clamps itself onto a specific DNA sequence, as well as how its recognition of that sequence actually switches the enzyme on, Van Duyne said. The related human topoisomerase is not sequence-specific, and we now understand that this is because it does not grasp the DNA bases that determine the sequence nearly as intimately as the viral enzyme does, he said. We anticipate that this new structural information will greatly help in revealing the secrets of the specificity of this enzyme and its role in viral DNA replication and transcription.

Importantly, said Van Duyne, the new structure may enable pharmacologists to design drugs that trap the enzyme attached to its viral DNA, preventing the DNA from being read and ultimately killing the virus.

The structure of the viral topoisomerase-DNA complex also reveals why the human anticancer drug camptothecin, which kills cancer cells by targeting their topoisomerase, does not affect the viral enzyme. This structure really illustrates beautifully why camptothecin doesn't work on the viral topoisomerase, he said.

Now that we have an experimental framework for this complex, one of the hopes is that structure-based drug design can be used to develop homologs of camptothecin, or other drugs that can target the smallpox topoisomerase. I think the next step is for computational chemists to try to design molecules that are specific for the viral protein-DNA complex and target that intermediate the same way camptothecin does the human enzyme complex, he said. To aid that drug development, Van Duyne and his colleagues plan to develop detailed structures of the topoisomerase-DNA complex bound by such inhibiting molecules.