How Poxviruses Evade the Sting of Our Basic Immune Defenses

One way that the innate immune system, our first line of defense against microbes, detects viruses is to detect viral DNA in our cells. Of course, we all have DNA in our cells, but it is neatly packaged into our nuclei and mitochondria. So, if DNA is detected outside of these areas, that is a reason for sounding the alarm. The way our cells do that is to have a messenger system that detects the viral DNA through an enzyme called cyclic GMP-AMP synthase (cGAS) that makes the small messenger molecule 2'3'-cGMP-AMP (cGAMP) carry the signal to the innate immune system through the receptor STING. This elegant system provides a way to activate immune responses to viral intruders, but also may present a target that viruses can exploit to evade our defenses by silencing the alarm. In fact, this idea was the basis for recent work in which researchers hypothesized that evolution would favor viruses that could disrupt the cGAS-STING signaling pathway and, thus, evade detection. The work, supported by research at the APS, identified a novel class of viral proteins and has implications for a wide variety of fields from vaccination and gene therapy to cancer therapeutics.

The first step in the project, conducted by researchers from the Harvard Medical School, Harvard University, the Dana Farber Cancer Institute, and the Dana-Farber/Brigham and Women's Cancer Center, was to develop an assay to detect viral evasion. cGAMP is extremely stable, making it both a good messenger and a good target for the assay. Reasoning that the best way to stop the message is to kill the messenger, the team developed a way to detect the degradation of cGAMP in cell lines that were infected with different viruses. After screening 24 different types of viruses, they had a candidate, vaccinia virus (VACV), a type of poxvirus. They named the viral factor poxvirus immune nuclease or poxin and discovered it was the product of the vaccinia B2R gene. Expression of the protein allowed them to purify and characterize the poxin biochemically. The VACV poxin is specific for cGAMP and acts to degrade this cyclic messenger molecule to a linear form of the molecule that is no longer recognized by STING, effectively blocking the signal. *In vivo*, they found that if they removed the poxin gene from vaccinia virus and then infected mice with it, it was attenuated by 40-fold compared to the wild-type virus.

In an effort to understand more about the molecular mechanism of the poxin protein, the researchers solved three crystal structures using data collected at the NE-CAT 24-ID-C beamline at the APS and at the Advanced Light Source beamline 8.2.1. They solved the structures for the unbound form, a pre-reactive form of the poxin bound to a non-hydrolyzable cGAMP analog, and a post-reactive form with natural cGAMP that showed the linear enzymatic product. The structures showed that the poxin protein forms a V-shaped homodimer in which two active sites are formed at the interface of the N-terminal protease-like domain and the C-terminal domain of opposing monomers (Fig. 1). The cGAMP lodges in a deep pocket between the N-terminus and C-terminus of the two monomers, and part of the C-terminal domain then recognizes the cGAMP substrate and forms a clamp that holds it in place while also moving into the active site the bond to be cut. The structures clearly show how the product aligns in the enzymatic pocket and how three specific amino acids perform the reaction.

These poxin genes appear to be conserved among viruses in the orthopoxvirus genus, but a search for proteins with similar structures did not identify any more distantly related poxin homologs in the structure database. To look for poxins in other organisms, the researchers utilized the Position-Specific Iterative Basic Local Alignment Search Tool, which can detect distant relationships between proteins. Interestingly, they found that insect viruses and some types of insects, including moths and

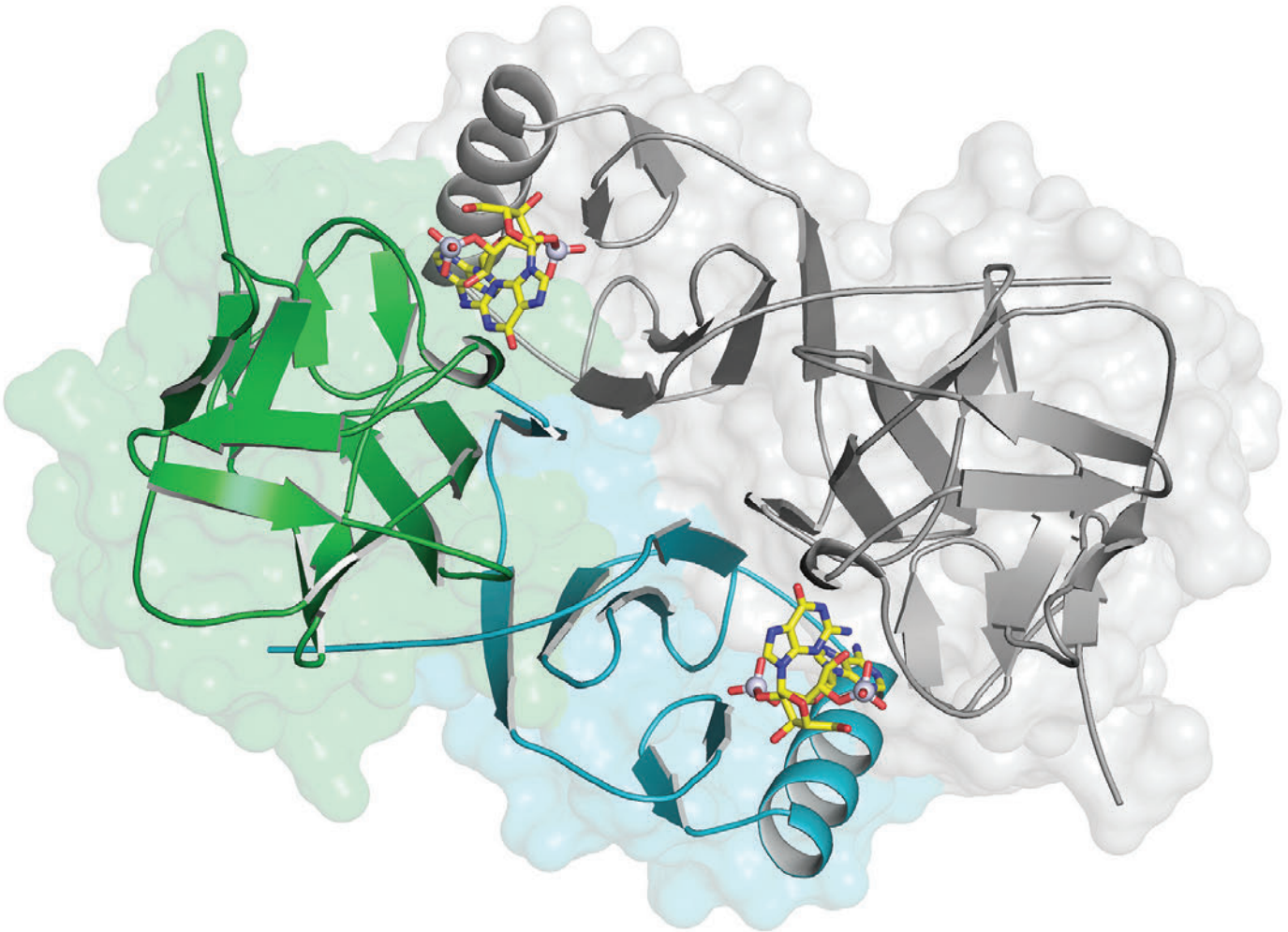

Fig. 1. Crystal structure of the VACV poxin. This protein is a homodimer with two active sites for binding cGAMP (shown in stick representation) between the N-terminal domain of one monomer (N-terminal domain in green, C-terminal domain in cyan) and the C-terminal domain of the other monomer (shown in gray).

butterflies, contain similar genes, suggesting an ancient origin for these poxin enzymes.

For the present, the team hopes their discovery will provide valuable new information that may impact the work of others who use poxviruses in a wide array of medical applications and for researchers who study the fascinating world of immune detection and evasion.

— Sandy Field

See: James B. Eaglesham[1,2,3], Youdong Pan[1], Thomas S. Kupper[1,4], and Philip J. Kranzusch[1,2,3]*, "Viral and metazoan poxins are cGAMP-specific nucleases that restrict cGAS–STING signalling," Nature **566**, 259 (14 February 2019).
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Author affiliations: [1]Harvard Medical School, [2]Dana-Farber Cancer Institute, [3]Harvard University, [4]Dana-Farber/Brigham and Women's Cancer Center
Correspondence: * philip_kranzusch@dfci.harvard.edu

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