How Ebola virus Evades the Immune System

The human immune system, which has evolved in the presence of wily immune invaders such as bacteria and viruses, has very specific and effective means of recognizing these intruders and eliminating them before they can cause too much harm. In response, some invaders have developed ways to get around the immune system, sometimes with terrible consequences for the human hosts. One of these is the Ebola virus, which causes an often fatal hemorrhagic fever in humans. Recent studies have suggested that the interferon inhibitory domain (IID) of the Zaire ebolavirus protein VP35 (Fig. 1) is responsible for this evasion through its ability to subvert the immune system's recognition of double stranded (ds) RNA molecules that alert the immune system to viral invasion. Structural studies carried out at the SBC-CAT 19-ID beamline at the APS explain how VP35 binds to dsRNA, which amino acids are important in these interactions, and how this inhibits the immune system. These fundamental details about the way viruses evade the immune system and important information about *ebolavirus* infection can be used to design treatments or vaccines against this virus that is highly pathogenic and a potential tool for bioterrorism.

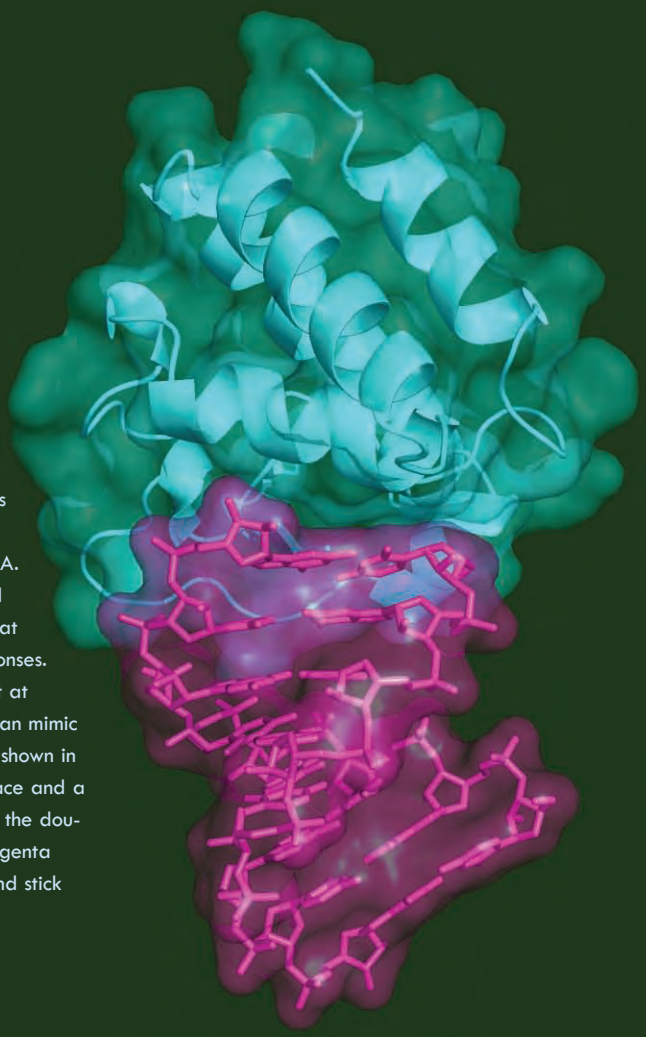

Fig. 1. *Ebolavirus* VP35 protein mimics RNA recognition by cellular RIG-I like receptors. VP35 interferon inhibitory domain forms an "end-cap" that binds at the blunt end of double-stranded RNA. A similar interaction mode is used by cellular RIG-I like receptors that activate host innate immune responses. New structural studies carried out at Sector 19 show that viral VP35 can mimic cellular proteins. VP35 protein is shown in cyan (transparent molecular surface and a cartoon secondary structure) and the double stranded RNA is shown in magenta (transparent molecular surface and stick representation).

As with all important studies, this work was the culmination of many years of work by a number of laboratories. For example, it was already known that VP35 was important for antagonizing the innate immune system, bound to dsRNA, and that a single amino acid change could inhibit VP35's ability to antagonize the immune system. The single amino acid, Arginine(Arg) 312, was then shown to be crucial for dsRNA binding and the structure of VP35, solved in 2009 by this group, showed that Arg 312 was in a region of the protein containing a patch of basic amino acids (Fig. 2). Recent evidence suggested that the host pathway inhibited by Ebola was the RIG-I-like receptor pathway that recognizes dsRNA and activates the production of interferon to alert the immune system. With all of this evidence for the role of dsRNA binding by VP35 in host immune evasion, the group set out to prove how the virus does it.

The structure of the VP35 IID in complex with a short stretch of dsRNA was solved by molecular replacement. This demonstrated conclusively that the central basic region in the β-sheet structure of VP35 IID was important for dsRNA binding. Identification of important amino acids in this region involved in binding to the dsRNA backbone led them to also solve the structures for

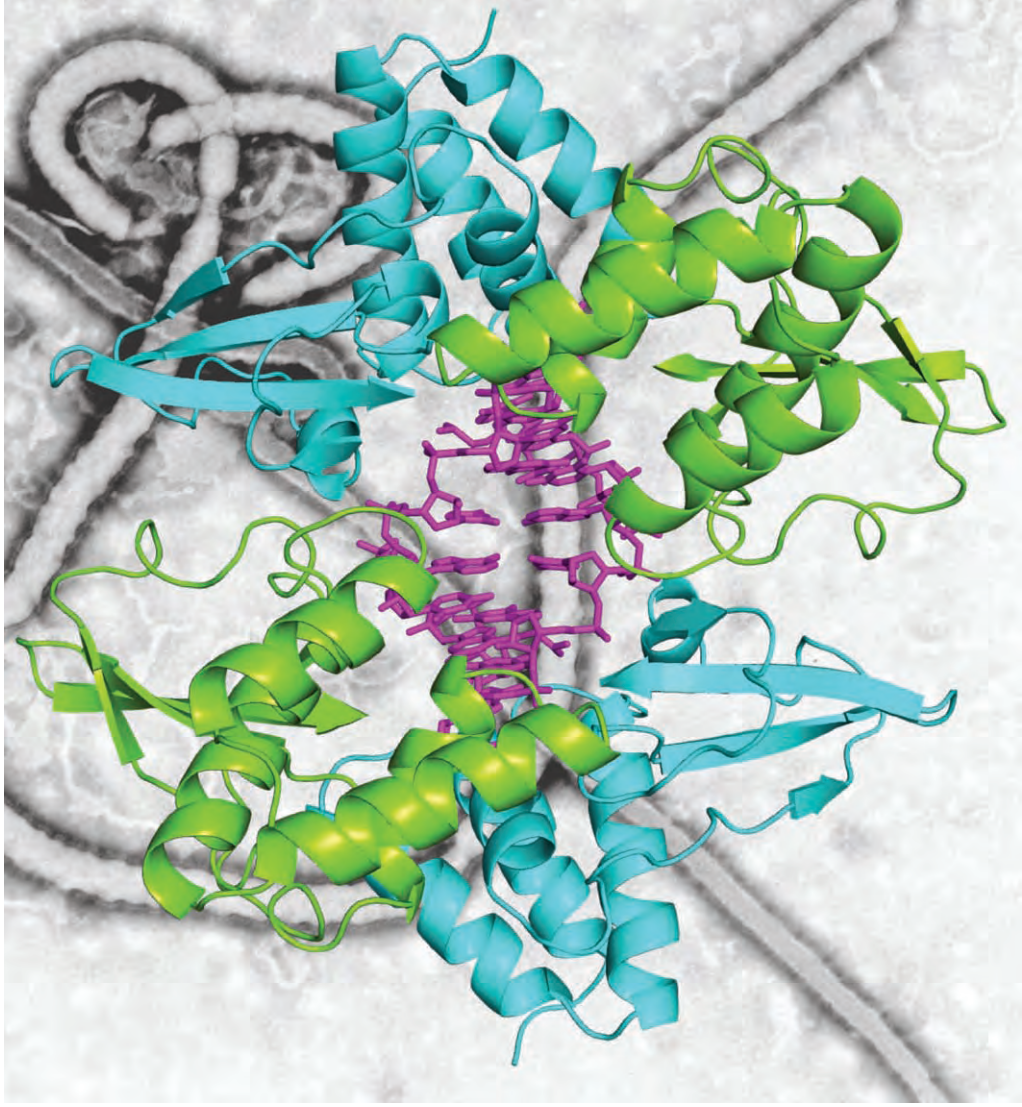

Fig. 2. *Ebolavirus* VP35 is a multifunctional virulence factor. Structure of Zaire Ebolavirus VP35 interferon inhibitory domain bound to double-stranded RNA reveals the structural basis of VP35-mediated immune evasion mechanisms (PBD: 3L25). Background: Transmission electron micrograph of Ebola virus virion. Image source: CDC/Frederick A. Murphy, Public Health Image Library #1833.

mutants of VP35 at two crucial sites, Arg312, and Lysine(Lys) 339. The structures of these two mutants showed that the basic surface of VP35 was not disrupted by the amino acid changes but that electrostatic distribution was changed significantly and this was the basis for disruption of dsRNA binding.

The structure also revealed that the blunt-end of the dsRNA is "capped" by VP35 in a hydrophobic pocket that mimics the binding of blunt-ended dsRNA by the RIG-I-like receptor of the host immune system. Mutation of one of these hydrophobic amino acids, Phenylalanine (Phe) 239, abolished dsRNA binding in RNA binding assays.

Next, the group tested their hypothesis in a cell culture assay for inhibition of immune activation. While the native VP35 was able to suppress immune activation, the mutants of VP35 at sites important for recognizing dsRNA ends and those with mutations in the basic patch important for dsRNA backbone binding were unable to suppress the immune responses. Moreover, the complex structure also identified residues important for protein-protein interactions, which may function through inhibition of signaling events downstream of RIG-I-like receptors, suggesting that VP35 may play a role in dsRNA-independent suppression of immune responses as well.

Further elucidation of these dsRNA-independent mechanisms and exploitation of the knowledge derived here for vaccine and antiviral development will be the topic of future studies.

— Sandy Field

See: Daisy W. Leung[1], Kathleen C. Prins[2], Dominika M. Borek[3], Mina Farahbakhsh[1], JoAnn M. Tufariello[2], Parameshwaran Ramanan[1], Jay C. Nix[4], Luke A. Helgeson[1], Zbyszek Otwinowski[3], Richard B. Honzatko[1], Christopher F. Basler[2], and Gaya K. Amarasinghe[1]*, "Structural basis for dsRNA recognition and interferon antagonism by Ebola VP35," Nat. Struct. Mol. Biol. **17**(2), 165 (February 2010). DOI: 10.1038/nsmb.1765

Author affiliations: [1]Iowa State University, [2]Mount Sinai School of Medicine, [3]University of Texas Southwestern Medical Center, [4]Lawrence Berkeley National Laboratory

Correspondence:
*amarasin@iastate.edu

Use of the Argonne National Laboratory SBC-CAT beamlines at the APS was supported by the U.S. Department of Energy (DOE) under Contract No. DE-AC02-06CH11357. This work is supported by U.S. National Institutes of Health grants (1F32AI084324 to D.W.L., R01GM053163 to Z.O., R01NS010546 to R.B.H., R01AI059536 and AI057158 (Northeast Biodefense Center-Lipkin) to C.F.B. and R01AI081914 to G.K.A.), a Midwest Regional Center of Excellence Developmental grant (U54AI057160-Virgin(PI) to G.K.A.) and the Roy J. Carver Charitable Trust (09-3271 to G.K.A.). Use of the Advanced Photon Source, an Office of Science User Facility operated for the U.S. DOE Office of Science by Argonne National Laboratory, was supported by the U.S. DOE under Contract No. DE-AC02-06CH11357.

19-ID • SBC-CAT • Life sciences • Large unit cell crystallography, macromolecular crystallography, microbeam, multi-wavelength anomalous dispersion, single-wavelength anomalous dispersion, subatomic (<0.85 Å) resolution, ultra-low-temperature (15K) • 6.5-19.5 keV • On-site, remote, mail-in • Accepting general users