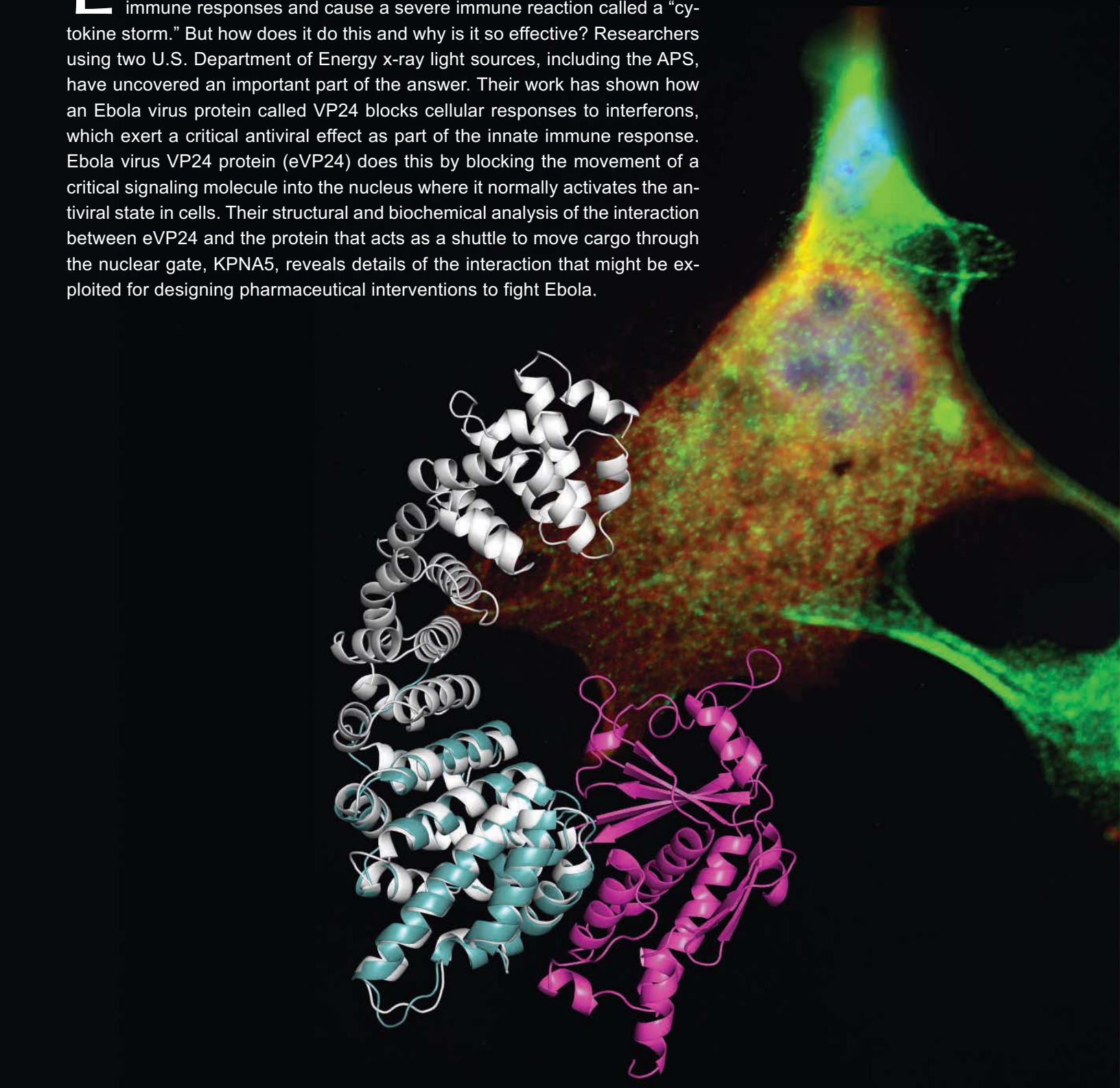
How Ebola Silences Those Who Would Sound the Alarm

Ebola virus causes a deadly hemorrhagic disease with case fatality rates up to 90%. This is due to the virus' ability to rapidly shut down innate immune responses and cause a severe immune reaction called a "cytokine storm." But how does it do this and why is it so effective? Researchers using two U.S. Department of Energy x-ray light sources, including the APS, have uncovered an important part of the answer. Their work has shown how an Ebola virus protein called VP24 blocks cellular responses to interferons, which exert a critical antiviral effect as part of the innate immune response. Ebola virus VP24 protein (eVP24) does this by blocking the movement of a critical signaling molecule into the nucleus where it normally activates the antiviral state in cells. Their structural and biochemical analysis of the interaction between eVP24 and the protein that acts as a shuttle to move cargo through the nuclear gate, KPNA5, reveals details of the interaction that might be exploited for designing pharmaceutical interventions to fight Ebola.



When the body encounters a virus, it sends out signals, activating a cascade of events that sound the alarm and produce an antiviral state in cells. This state is triggered by interferon (IFN), which activates the transcription factor STAT1. Once activated, STAT1 is able to access a special entrance into the nucleus through transporters via a non-classical pathway. The non-classical pathway is a secondary pathway that is distinct from the classical transport pathways through which most protein traffic enters the nucleus. STAT1's access to the non-classical pathway is facilitated by several nuclear transporters from the NPI-1 subfamily, including KPNA5, which was the protein used in this study. Once inside the nucleus, STAT1 activates a group of genes that gets the antiviral arsenal up and running.

The researchers in this study from Washington University School of Medicine in St. Louis, Icahn School of Medicine at Mount Sinai, the University of Texas Southwestern Medical Center at Dallas, and Washington University in St. Louis started their investigation on the basis of previous work. They knew that Ebola blocks the entry of STAT1 into the nucleus, effectively blocking antiviral gene transcription, and they knew that it did it by blocking STAT1's access to KPNA5 with eVP24. What they didn't know was the molecular details of the eVP24-KPNA5 interaction that might suggest a way to block eVP24 with pharmaceutical interventions. For this they needed structural information.

< Fig. 1. Effects of Ebola VP24 in cells and structural interaction with KPNA5. A cell expressing fluorescent Ebola VP24 (red) and labeled for STAT1 (green) after interferon activation shows that VP24 effectively blocks STAT1 entry into the nucleus (overlap of two proteins in yellow). Ribbon diagram shows structural interactions between eVP24 (magenta) and the C-terminal end of KPNA (cyan) (PDB: 4U2X). Full-length KPNA5 is overlaid into the image in white (PDB: 1BK5). Image courtesy of Daisy W. Leung (Washington University School of Medicine in St. Louis) and Christopher F. Basler (Icahn School of Medicine at Mount Sinai)

The team's first step was to define the interaction between KPNA5 and eVP24. They determined that eVP24 binds just to one end of KPNA5, allowing them to use this smaller region of the protein for crystallization. The crystal structure of eVP24 bound to the fragment of KPNA5 was obtained at the SBC-CAT beamline 19-ID-D at the APS and the Advanced Light Source beamline 4.2.2 at Lawrence Berkeley Laboratory. The structure revealed a number of important features of the interaction (Fig.1). First, the binding site for eVP24 on KPNA5 is unique and, while it overlaps the known site for STAT1, it does not resemble that for any other cargo that come in through the non-classical pathway. Also, while neither protein changes conformation much in the binding interaction, eVP24 contributes a large binding interface to the interaction involving several amino acids from various loops on the structure.

Biochemical analysis of eVP24 proteins with these amino acids mutated showed that the loss of single contacts only resulted in slightly impaired binding while loss of several contacts resulted in completely abolished binding. Interestingly, the specificity of the interaction is mediated mostly by eVP24 amino acids that are different from those in the same region in the related Marburg virus VP24 (mVP24) protein. In fact, despite significant protein sequence similarity to eVP24, mVP24 adopts a different structure in this region. This may explain why Marburg does not block interferon signaling using the same mechanism.

Further analysis of eVP24 proteins possessing mutations in the KPNA5 binding domain showed that eVP24 mutants with reduced KPNA5 binding also have reduced ability to block STAT1 nuclear transport and IFN-induced gene activation. This supports a model in which the eVP24 interaction with KPNA5 is critical to its ability to block innate immune system activation by IFN. Similar assays also show that eVP24 does not block the classical transport pathway. This is important because it allows Ebola to block progress to the antiviral state while at the same time keeping cellular machinery up and running to churn out viral proteins needed to propagate its deadly progeny.

The research team hopes that this work will provide clues that will allow them to identify new or existing drugs that can block the interaction between eVP24 with KPNA5 while leaving STAT1 signaling intact. *— Sandy Field*

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