Structural Rearrangement in Ebola Virus Protein VP40 Creates Multiple Functions

As x-ray crystallographers, we often assume that when we build a model into an electron density map, that we can assign the known biological functions of the protein to the structure we see in the map. But what if the protein can make more than one equally valid structure? Zachary Bornholdt, Erica Ollmann Saphire, and colleagues at The Scripps Research Institute and the University of Wisconsin–Madison recently revealed that a protein of the Ebola virus, termed VP40, arranges itself into three very different structures to achieve three very different functions in the virus life cycle.

Viruses can be under tremendous evolutionary pressure for economy of genomic information. Ebola virus, which causes hemorrhagic fever with up to 90% lethality, for example, encodes only seven genes, the protein products of which must achieve all of the different steps of the virus life cycle. As a result of this genomic economy, each of the proteins it encodes is essential and commonly multifunctional. How exactly a viral protein achieves multiple functions is not well understood.

One of these multifunctional proteins is VP40, the viral matrix protein that builds the protein shell underneath the viral membrane to assemble and release progeny viruses form the infected cell. VP40 alone is necessary and sufficient for assembly and release of Ebola virus-looking particles from transfected cells. The first crystal structures of Ebola virus VP40 were determined in 2000 and 2003. These structures revealed the general fold of VP40 which has weakly associated N- and C-terminal domains [1], and a ring-like arrangement made by the N-terminal domains alone [2]. Curiously, RNA was observed bound to the ring in the electron density map. An open question in the field for the next decade was whether or not this ring structure or something like it represented how VP40 assembled the viral matrix, and if not, then what assembly did?

Zachary Bornholdt and Dafna Abelson in the Saphire lab expressed VP40 for another reason, but noted when purifying VP40 that it was dimeric, not monomeric as originally thought. This dimeric VP40 grew multiple crystal forms in space groups C2, $P6_2$, and

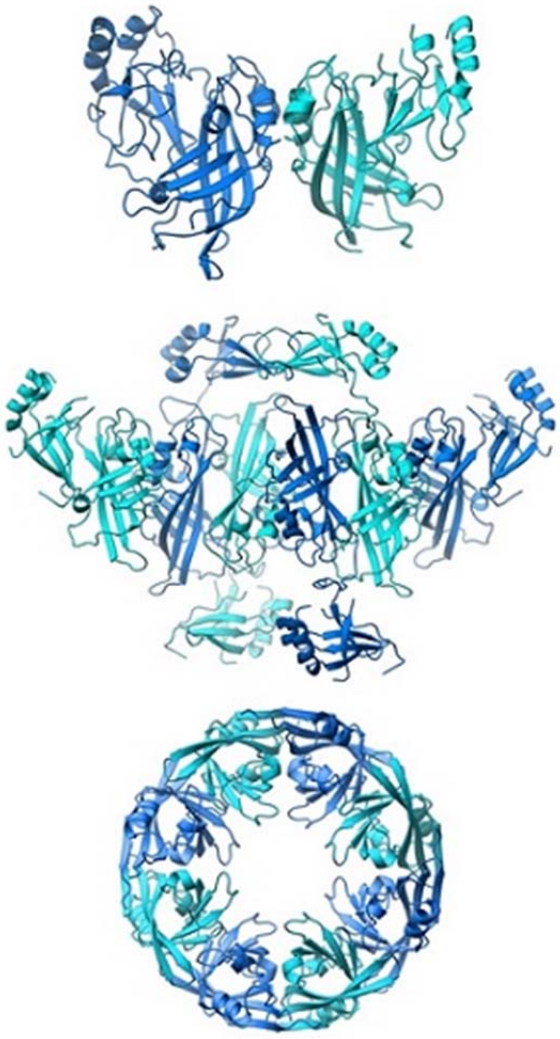

Figure 1. Three structures of VP40. Top, a butterfly-shaped dimer structure critical for membrane trafficking. Middle, a rearranged hexameric structure essential for building and releasing nascent virions. Bottom, an RNA-binding octameric ring that controls transcription in infected cells.

$P6_{422}$. No matter what space group the crystals belonged to, or what species of Ebola virus was analyzed, the team noted that the VP40 dimers assembled end to end in identical linear filaments. The filaments were assembled by protein–protein interfaces that were distinct from those that assembled the RNA-binding ring. The striking conservation of these filaments led the team to wonder if they were a possible model for how VP40 assembled in the viral matrix. By designing a great many point mutations in the filament-building and ring-building interfaces, and testing the function of these mutations in virus assembly, the team, with collaborator Yoshihiro Kawaoka of the University of Wisconsin–Madison and The University of Tokyo, showed that the linear filament that assembled the crystals was essential for virus assembly. By contrast, the RNA-binding ring assembly of VP40 was not involved in virus assembly.

Further studies by the Saphire lab revealed how the filaments undergo electrostatically driven rearrangements and polymerization to build and bud Ebola virus virions. Atomic models built from their structures closely matched the VP40 matrix present in authentic viruses observed by electron tomography [3].

The RNA-binding ring structure remained a puzzle. Mutations that prevented ring formation had no effect on virus assembly or budding, but were nonetheless lethal [4]. No Ebola virus could be propagated with a mutation to the RNA-binding ring. Hence, the ring structure of VP40 must perform an essential function during the virus life cycle. During the course of these studies, an additional function was discovered for VP40: control of viral transcription inside the infected cells. Perhaps this function was what the ring was for? The Saphire lab developed point mutations that would either drive VP40 into assembling exclusively rings, or no rings at all. The Kawaoka lab tested each of them for their ability to control viral transcription. Indeed, ring-only VP40 can recapitulate the transcriptional control function better than wild-type VP40, but ring-blocked VP40 cannot.

In summary, the team found that wild-type, unmodified VP40 could assemble a butterfly-shaped dimer for membrane trafficking, an RNA-binding ring structure for viral transcription, and a filamentous oligomeric assembly to build and bud new virions. VP40 thus makes not one 3D structure, but three with each structure conferring a separate and essential function in the virus life cycle (Figure 1).

They call VP40 a “transformer” to reflect its ability to refold its structure to achieve new functions. One or two other transformers have been identified, such as RfaH, or some of the morpheein proteins [5], but it is likely that many more transformer proteins exist in biology, but remain unknown.

This body of work encompassed ~20 mutations, cellular microscopy and the determination of eight crystal structures. Automated sample mounting and screening facilities available at SSRL’s Beam Lines 11-1 and 12-2, as well as support of the beam line staff were essential for the analysis and data collection processes associated with the crystal structures.

Primary Citation

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