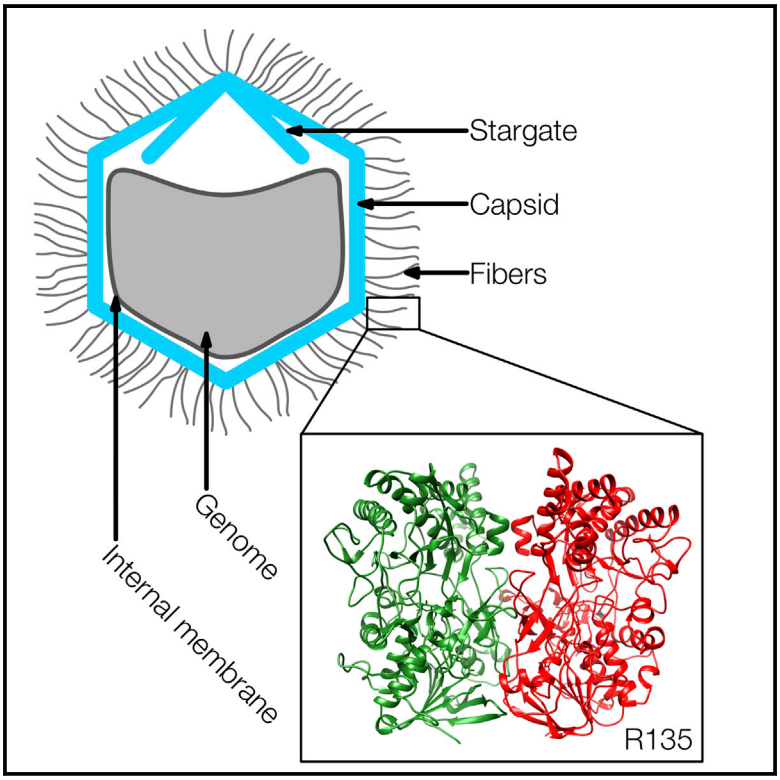
Article

Structure

A Mimivirus Enzyme that Participates in Viral Entry

Graphical Abstract



Authors

Thomas Klose, Dominik A. Herbst, ...,
Hilkka I. Kenttämaa,
Michael G. Rossmann

Correspondence

mr@purdue.edu

In Brief

Mimivirus is one of the largest viruses discovered to date, and little is known on how it infects its host. Klose et al. have determined the structure of R135, a protein that assists Mimivirus to enter its natural host.

Highlights

- Mimivirus fibers play an important role during infection
- First structure of a major component of the Mimivirus fiber
- Member of the GMC oxidoreductase family
- R135 is probably involved in the infection of alternative hosts

Accession Numbers

4Z24
4Z25
4Z26

Klose et al., 2015, Structure *23*, 1058–1065
June 2, 2015
http://dx.doi.org/10.1016/j.str.2015.03.023

CellPress

Structure
Article

A Mimivirus Enzyme that Participates in Viral Entry

Thomas Klose,[1] Dominik A. Herbst,[1,3] Hanyu Zhu,[2] Joann P. Max,[2] Hilkka I. Kenttämaa,[2] and Michael G. Rossmann[1,*]
[1]Department of Biological Sciences, Purdue University, 240 South Martin Jischke Drive, West Lafayette, IN 47907-2032, USA
[2]Department of Chemistry, Purdue University, West Lafayette, IN 47907, USA
[3]Present address: Biozentrum, University of Basel, Klingelbergstrasse 50/70, 4056 Basel, Switzerland
*Correspondence: mr@purdue.edu
http://dx.doi.org/10.1016/j.str.2015.03.023

SUMMARY

Mimivirus was initially identified as a bacterium because its dense, 125-nm-long fibers stained Gram-positively. These fibers probably play a role during the infection of some host cells. The normal hosts of Mimivirus are unknown, but in the laboratory Mimivirus is usually propagated in amoeba. The structure of R135, a major component of the fibrous outer layer of Mimivirus, has been determined to 2-Å resolution. The protein's structure is similar to that of members of the glucose-methanol-choline oxidoreductase family, which have an N-terminal FAD binding domain and a C-terminal substrate recognition domain. The closest homolog to R135 is an aryl-alcohol oxidase that participates in lignin biodegradation of plant cell walls. Thus R135 might participate in the degradation of their normal hosts, including some lignin-containing algae.

INTRODUCTION

Acanthamoeba polyphaga Mimivirus, the prototypic member of the family of Mimiviridae, was isolated from a water tower in Bradford, UK. It initially was identified as a Gram-positive bacterium due to a highly glycosylated, dense layer of fibers (La Scola et al., 2003). A subsequent study recognized the viral nature of this particle, placing Mimivirus into the group of nucleocytoplasmic double-stranded DNA viruses (NCLDVs) (La Scola et al., 2003). Its genome of 1.2 Mbp (Raoult, 2004) encodes 979 open reading frames, of which 42 are common genes in other NCLDVs. About 24% of the Mimivirus genes have homologs in bacteria, archaea, and eukaryotes. However, almost half of the Mimivirus genes encode proteins with no known homologs. Furthermore, no homologous sequences can be found for 39% of the proteins that can be isolated from the mature virions (Renesto et al., 2006).

Structural studies of Mimivirus show that the virus has an overall icosahedral shape, with a unique 5-fold structure termed the stargate (Xiao et al., 2005; 2009) because it is probably where the genome exits the capsid when the virus infects a host (Zauberman et al., 2008). A dense layer of 125-nm-long fibers covers the whole virus except for the stargate (Kuznetsov et al., 2010; Xiao et al., 2009). Each fiber is capped by a small protein of unknown function (Kuznetsov et al., 2010). The fibers are resistant to protease treatment unless the virus is first treated with lysozyme (Xiao et al., 2009).

The role of the fibers in the life cycle of Mimivirus is not well understood, but because the fibers mimic the peptidoglycan layers found in Gram-positive bacteria, they may aid entry of the virus into the amoeba host (Kuznetsov et al., 2010; La Scola et al., 2003; Raoult, 2004; Xiao et al., 2009). Boyer et al. (2011) have shown that R135, L725, and L829 are components of the fibers. None of these proteins are essential to maintain infectivity under laboratory conditions, but their deletion during serial passage leads to a loss of fibers on the particles (Boyer et al., 2011). This fiberless variant of Mimivirus, M4, does not allow replication of the Sputnik virophage. Furthermore, R135 is found in association with Sputnik on isolating it from amoeba (La Scola et al., 2008). Thus R135 is the protein that Sputnik uses to attach to Mimivirus for coinfection. R135 and L829 have been identified as major antigens of Mimivirus (Raoult et al., 2007). However, the M4 fiberless variant of Mimivirus showed no reactivity with sera from human patients (Boyer et al., 2011), confirming that these proteins are missing in M4.

R135 has homology to members of the glucose-methanol-choline (GMC) oxidoreductases, which utilize FAD to carry out a wide variety of oxidation/reduction reactions. These proteins were found to have common structural features, such as a conserved FAD binding domain and a variable substrate binding domain, as is also the case for other enzymes that depend on a bound nucleotide (Rossmann et al., 1974). R135 is a part of a gene cluster in the Mimivirus genome involved in glycosylation of viral surface proteins (Piacente et al., 2012), but the precise function of R135 remains unknown. Here we describe the structure of R135, the first Mimivirus protein structure to be determined that is related to capsid assembly and host infection.

RESULTS AND DISCUSSION

Structure of R135

The R135 protein has a molecular weight of 75 kDa and consists of 702 amino acids. The protein was successfully expressed in its apo form in *Escherichia coli*, and ran as an apparent monomer on a size-exclusion column but failed to crystallize. Secondary predictions showed that the first 50 amino acids were likely to be disordered. Therefore, these residues were removed by generating a deletion construct, R135_50.

The truncated protein crystallized in three different space groups, P1, $P2_1$, and $P2_12_12_1$. The Matthews coefficient

