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Viperin vs. Tick-borne Encephalitis Virus
Mechanism of a Potent Antiviral Protein

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie doktorsexamen framläggs till offentligt försvar i sal E04, byggnad 6A, fredagen den 7:e Oktober, kl. 09:00.
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Abstract
Tick-borne encephalitis virus (TBEV) is a very important virus medically, causing mild or severe encephalitis often with long-lasting sequelae. Treatment of tick-borne encephalitis is limited to supportive care, and antiviral drugs are much needed.
The type-I interferon (IFN) system is the first line of host defense against many viruses. Infected cells secrete type-I IFN to alert neighboring cells. These cells in turn upregulate the expression of antiviral proteins to protect themselves from the virus.
In this work, we found that the interferon-induced host protein viperin (virus-inhibitory protein, endoplasmic reticulum-associated, interferon-inducible) has a pronounced antiviral effect against TBEV.

Viperin is an evolutionarily conserved protein with three domains: the N-terminus, the radical S-adenosyl methionine (SAM) domain, and the C-terminus. Viperin shows antiviral activity against a broad spectrum of different viruses. However, its mode of action appears to be virus-specific.
We therefore concentrated on determining the antiviral mechanism of viperin against TBEV. The specific questions addressed in this thesis are: (1) which steps of the TBEV infectious cycle are targeted by viperin?, (2) which domains of viperin are responsible for its antiviral activity?, and (3) which interaction partners does viperin need in order to have an antiviral effect against TBEV?
First, we investigated which step(s) of the TBEV life cycle viperin targets by using several assays to examine the effects of viperin on virus binding, entry, genome replication, assembly, and release. We found that viperin inhibited the replication of positive-sense genomic RNA and also targeted particle release, selectively enhancing the release of membrane-associated capsid particles.
For inhibition of genome replication, viperin was dependent on the host cellular protein CIAO1 (cytosolic iron-sulfur assembly component 1). CIAO1 interacted with the C-terminus of viperin and was necessary for the maturation and stability of viperin, and also for loading of an iron-sulfur cluster onto the SAM domain. The SAM domain required this iron-sulfur cluster to perform its function as a radical SAM enzyme, which was required for the inhibition of TBEV genome replication. In addition to the SAM domain and the C-terminus, viperin needed its N-terminus in order to be fully antivirally active during late replication, since the N-terminus directed viperin to the endoplasmic reticulum, where genome replication takes place.
Furthermore, viperin targeted GBF1 (Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1), a host protein known to be involved in the secretory pathway. Interaction between the N-terminus of viperin and GBF1 appeared to induce an enhance release of capsid particles independently of the later steps of the classical secretory pathway. The enhanced secretion of capsid particles by viperin occurred at the expense of whole, infectious virions and is therefore a completely novel antiviral mechanism.

In summary, this work identified viperin as a very strong inhibitor of TBEV, and its antiviral mechanism was characterized in detail. Viperin was found to target multiple steps in the TBEV infectious cycle by both inhibiting viral RNA replication and inducing secretion of capsid particles. These findings provide new insights into the interplay between TBEV and viperin, and offer new approaches to our understanding of the molecular and cellular mechanisms of TBEV infection, which may contribute to the development of a treatment for TBEV.

Keywords
Tick-borne encephalitis virus, TBEV, type-I interferon, IFN, Interferon-stimulated gene, ISG, viperin, GBF1, CIAO1, antiviral protein, genome replication, particle release, capsid

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