Structure, function and evolution of the Crimean Congo hemorrhagic fever nucleocapsid protein

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Introduction

Recent research in our lab has focused on the Crimean-Congo hemorrhagic fever virus (CCHFV). CCHFV is a member of the *Bunyaviridae* family, and together with members of the *Arenaviridae* and *Orthomyxoviridae* families these viruses are known as segmented negative stranded RNA viruses (sNSV) by virtue of their multistranded genomes.

While these viruses are extremely diverse in their disease causing ability, they possess one common structural characteristic that is at the core of their respective life cycles; a ribonucleocapsid assembly (RNP). This is an association of the RNA genome with a virus-encoded nucleocapsid (N) protein, and its formation is essential for several fundamental aspects of the virus replication cycle including gene expression and virus assembly.

One aspect of our research is to try to understand how the structure of the CCHFV N RNP protein dictates and relates to its function. Towards this aim, we have solved the crystal structure of the CCHFV N protein to 2.1 Å. Our work describes the N protein structure, and the high degree of structural homology between the CCHFV N protein and the N protein from another sNSV member Lassa virus (LASV), which is an arenavirus. Furthermore the crystal structure of the CCHFV N protein guided site-directed mutagenesis of specific N protein residues, which delineate how N binds RNA and thus forms a competent RNP template for both RNA replication and mRNA transcription.

Results

The CCHFV N is composed of a globular core comprising 15 alpha helices (α1-8; α14-20) and a prominent additional structural element we termed the 'arm' comprising two long alpha helices (α9-10) extending away from the core, presenting an exposed loop at its apex, and supported by a small three helix bundle (α11-13; Figure 1A). Electrostatic surface potential suggests a possible RNA binding 'platform' adjacent to the arm. Additional results from CCHFV mini-replicon system show some of these residues are important in CCHFV-specific RNA synthesis.

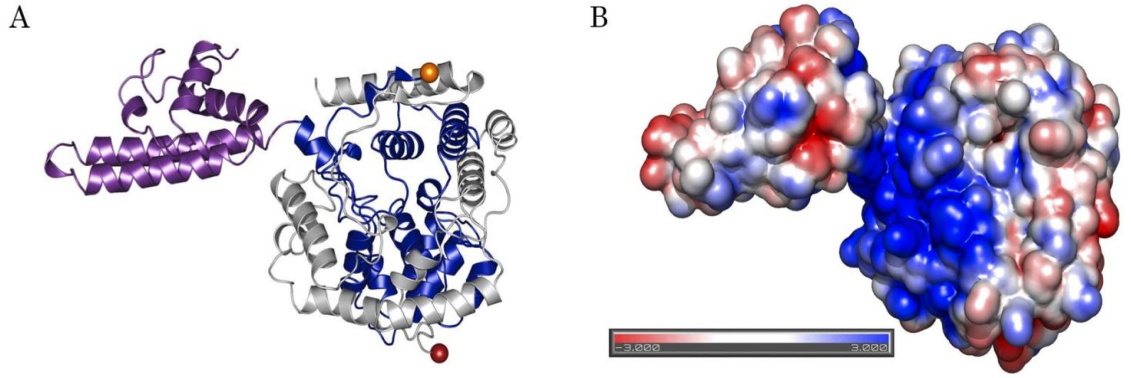

Figure 1: (a) Structure of CCHFV N monomer in ribbons representation. Helices are shown in silver for the N-terminal and dark blue for the C-terminal contribution to the globular domain, purple for the arm, which is in the middle of the primary sequence. **(b)** The electrostatic surface potential (generated with APBS). The scale bar shows the contour levels for the electrostatic potential at the solvent-exposed surface in kT/e.

Structural comparisons indicate that the CCHFV N globular domain exhibits a high degree of structural homology with the N-terminal domain of LASV N, a member of the *Arenaviridae*

family, whilst essentially structurally un-related to Rift Valley fever virus (RVFV), a member of the *Bunyaviridae* family. This data suggests the taxonony of two and three segmented RNA viruses may need re-examining.

The structural alignment provided additional clues about the RNA binding mechanism. The helix equivalent to LASV N α5 (yellow, Figure 2) is the helix in CCHFV N, α11, which precedes the long flexible loop at the base of the arm structure. This may represent a strong candidate for providing a similar RNA gating mechanism to that proposed for LASV N; RNA gating is mediated by a movement of α5 in the LASV N. Electrostatic surface of CCHFV N revealed an additional RNA binding 'pocket' equivalent to the LASV N RNA binding surface.

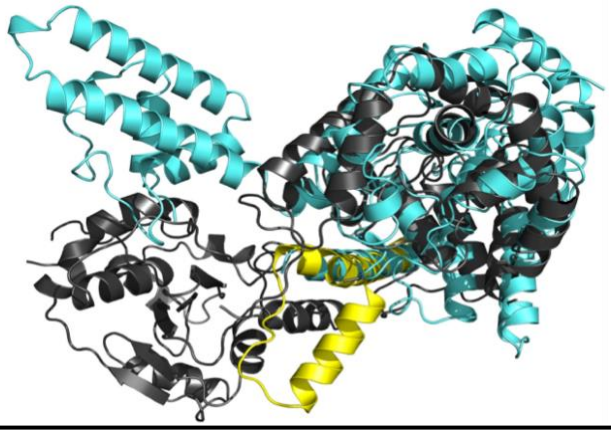

Figure 2: structural homology with LASV N. Structural alignment of CCHFV and LASV structures. The CCHFV N protein is shown in aquamarine, with most of the LASV N structure in grey. Highlighted in yellow are the LASV N elements that move upon RNA binding (α5, loop, α6). Note that there is no structural equivalent of the α6 RNA gate in CCHFV N.

Publications

Carter, S., Barr, J. & Edwards, T. (2012) Expression, purification and crystallization of the crimean-congo haemorrhagic fever virus nucleocapsid protein. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* **68:** 569-573.

Carter, S., Surtees, R., Walter, C., Ariza, A., Bergeron, E., Nichol, S., Hiscox, J., Edwards, T. & Barr, J. (2012) Structure, function, and evolution of the crimean-congo hemorrhagic fever virus nucleocapsid protein. *J. Virol.* **86:** 10914-10923.

Collaborators

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