Structural analysis of Hantavirus replication and transcription

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Hantaviruses are segmented negative stranded RNA viruses (sNSV) belonging to the *Bunyavirales* order. Spread worldwide, rodents are their natural reservoirs and man is occasionally infected resulting in severe diseases including meningitis, encephalitis and haemorrhagic fevers[1]. Of particular importance is Hantaan virus which causes haemorrhagic fever with renal syndrome and has a mortality rate of 30 to 40%. As is true for all bunyaviruses, no licenced drug is available to counteract its infection. It is thus crucial to analyse its viral cycle including two critical steps: replication, that gives rise to full-length copies of the genome, and transcription by cap-snatching, that gives rise to translation competent viral mRNAs. These reactions are catalyzed by the viral polymerase which interacts with the 3' and 5' ends of viral ribonucleic acid (RNA) segments and with nucleoproteins (NPs) to form ribonucleoprotein particles (RNP) that are the structural units of transcription and replication.

In this context, we are currently analysing proteins essential to the viral life cycle: the nucleoproteins. NPs coat RNA segments protecting them from the environment. They transiently allow RNA access to the viral polymerase and are thus essential for replication and transcription[2]. In hantaviruses, structures of fragments of NP have been determined[3 - 5]. However, it remains unknown how the RNA binds to NP and how NPs interact with each other to form a nucleocapsid (NC). Determining the structure of Hantaan recombinant NC would answer these questions and considerably enhance our knowledge on hantavirus replication. We are able to express NPs and purify them to homogeneity. OD260/280 clearly indicates the presence of RNA after purification. Inspection using negative stain microscopy shows formation of nearly rigid recombinant NC with length ranging from 100 nm to 500 nm (Figure 1.A). Cryo-electron microscopy dataset collection of Hantaan recombinant NC on a Polara microscope equipped with a K2 camera (IBS, Grenoble) (Figure 1.B) gives promising 2D class averages (Figure 2) with power spectra showing clear layer lines up to 9 Å. We are currently working on deciphering the symmetry of the complex.

Determination of this structure will reveal how Hantaviruses package their genome. This will be a key step towards an understanding of the replication and the transcription of this viral family. Considering that NC of other sNSV viruses (Influenza, La Crosse, Rift Valley Fever virus...) are too flexible to be analysed at high resolution, the structure of Hantaan recombinant NC would be a milestone in the field. On a broader level, it would also allow comparison with nucleocapsids of non-segmented NSV (Measles, Ebola, Rabies...)[6 - 8], thus deciphering similarities/differences of genome encapsidation between these important pathogens.

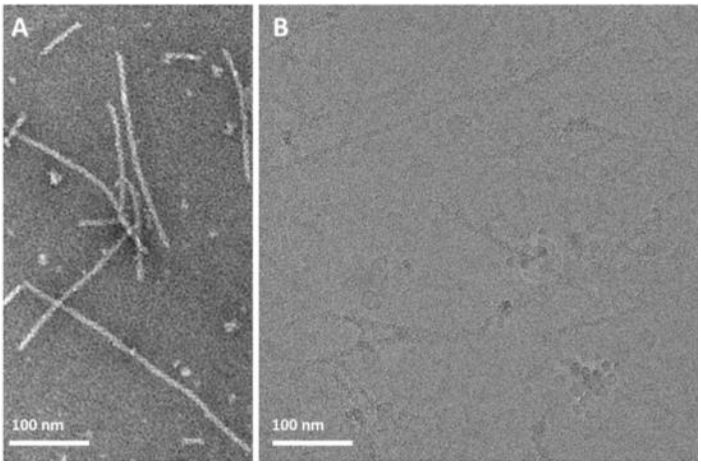

Figure 1: Micrographs of Hantaan virus recombinant nucleocapsid (A) Negative stain EM image collected on a T12 microscope (B) Cryo-EM image recorded on a Polara microscope equipped with a K2 detector.

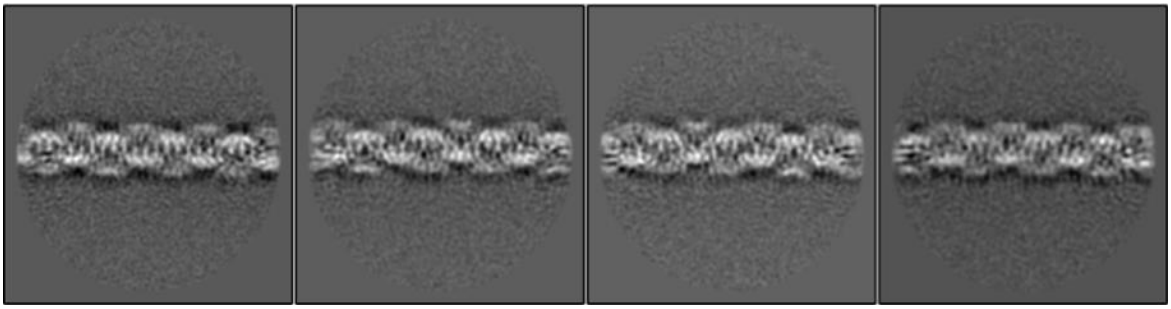

Figure 2: 2D class averages from cryo-EM data of Hantaan recombinant NC

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