Tracking of Intracellular Assembly of Hantavirus Glycoprotein Complex by a Toolbox of Fluorescence Microscopy Techniques

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Hantaviruses (HVs) pose a global health threat [1]. They are considered emerging pathogens which have the potential to cause major outbreaks in human populations [2]. Depending on the HV species, infections can lead to HV cardiopulmonary syndrome or hemorrhagic fever with renal syndrome [3]. The former is caused by New World HVs, and occurs in America, the latter origins from Old World HVs, found in Europe and Asia. Most infections in Europe are caused by the Old World species Puumala virus (PUUV) [3].

HVs are enveloped viruses with a single stranded RNA genome. The two envelope glycoproteins Gn and Gc play key roles in virus entry, assembly and budding [4]. Although it is known, that Gn and Gc assemble together the spike complex of the viral envelope, the genesis of the complex in the host cell and its determinants are not well characterized. To address this topic, we tagged wild-type glycoproteins of PUUV and respective mutants with fluorescent proteins and investigated their trafficking and interaction in host cells [5]. We analyzed their intracellular distribution, co-localization and oligomerization, applying comprehensive live single-cell fluorescence techniques, including confocal microscopy, imaging flow cytometry, anisotropy imaging and Number&Brightness analysis. We demonstrate that Gc is significantly enriched in the Golgi apparatus in absence of other viral components, while Gn is mainly restricted to the endoplasmic reticulum. Importantly, upon co-expression both glycoproteins were found in the Golgi apparatus. Furthermore, we show that an intact cytoplasmic tail of Gc is necessary for efficient Golgi localization. Finally, we found that Gn assembles into higher-order homo-oligomers, mainly dimers and tetramers, in the endoplasmic reticulum while Gc was present as mixture of monomers and dimers within the Golgi apparatus. Our findings suggest that PUUV Gc is the driving factor of the targeting of Gc and Gn to the Golgi region, while Gn possesses a significantly stronger self-association potential. Taken together, Gn and Gc have complementary biological activities that enable a tightly controlled organization of the intracellular targeting of the glycoproteins and the assembly of the homomeric subunits.

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