Abstract

The most important step in the virus replication cycle is the release of progeny virions to infect other cells or hosts. A typical route of transmission for all viruses is the cell-free entry (CFE), in which viruses infect target cells from the extracellular environment by binding specific receptors. To evade physicochemical, kinetic, and immunological barriers in the host organism, apart from CFE viruses developed a more complex type of transmission - cell-to-cell spread (CTC). The CTC process allows the direct transmission of virions form infected cells to adjacent cells in the presence of neutralizing antibodies. To date, nine CTC mechanisms have been described in the scientific literature, six of which are related to alphaherpesvirus infection. The envelope glycoproteins and the US3 kinase play a key role in the CTC transmission of viruses from this subfamily.

The aim of this PhD thesis was better understanding of the role of the gE/gI glycoprotein complex and the US3 kinase in the CTC process of alphaherpesviruses. The model virus in these studies was bovine herpesvirus type 1 (BHV-1), which due to its similarity to human alphaherpesviruses, is an excellent and safe model for studying the modification of the cellular environment by viral infection.

The research was divided into two stages - live analysis of the spread of fluorescent viral recombinants by CTC in cell cultures and the study of the interaction of the gE/gI complex with cellular proteins in this process.

In the first step, a universal test was developed to determine the rate of the CTC process of fluorescent viral recombinants, which was based on the real-time counting of single infected cells. Unlike the classical viral plaque size assay, this analysis provided the space-time context of the CTC process and a reliable and reproducible results. Furthermore, microscopic observations provided new information on the role of US3 kinase in the initial stages of BHV-1 infection.

Additionally, two viral recombinants were constructed, both carrying the gene encoding a small capsid protein VP26 fused to the mCherry fluorophore - BHV-1 VP26-mCherry and BHV-1 gE-GFP VP26-mCherry. The fluorescent recombinants were used for direct studies of the transport of structural elements of the virus in intercellular connections.

In the second step of the research, seventeen proteins potentially interacting with the gE/gI complex were identified by mass- spectrometry utilizing Stable Isotope Labeling by

Amino Acids in Culture (SILAC). Among the cellular interactome, the highest enrichment ratio was found for the α catalytic subunit of protein phosphatase 1 (PP1α). It was confirmed in further experiments that PP1α protein co-immunoprecipitated with gE and gI glycoproteins in three types of infected cells, and that the gE/gI complex and PP1α co-localized within the cell membrane and tunneling nanotubes. However, a direct interaction of gE and PP1α was excluded, which indicates that the presence of glycoprotein gI or of other viral proteins is necessary for this interaction.

Overall, the research presented in this thesis contributed to a more detailed understanding of the BHV-1 CTC process, in particular its dynamics and the role of viral and cellular proteins in this type of transmission.