Abstract

Viruses are intracellular parasites that can replicate only within living cells and require cellular machinery to synthesize their own proteins. The essential stage of a viral infection is the transfer of newly synthesized viral particles to the next permissive cells. This process can occur by releasing mature viral particles into an extracellular space, from where the virus can spread to the surrounding cells. During this time, viral particles are exposed to the host immune system attack, such as secretion of interferon and other pro-inflammatory cytokines by cytotoxic T lymphocytes, activation of NK cells and the production of neutralizing antibodies.

Viruses have developed various strategies to avoid detection and elimination by the immune system. The avoidance of the extracellular environment is one of such strategies. Some viruses, instead of releasing newly synthesized viral particles outside the cell, transmit them directly to the neighbouring permissive cells via cellular connections. This process of direct transmission, named cell-to-cell spread, is used by many human and animal viruses, including alphaherpesviruses which were investigated in this PhD project. Effective viral infection by alphaherpesviruses requires travelling a long distance from the place of the primary infection to the site of latency. The infection begins in the mucous membranes, from where the virus must reach the peripheral nervous system, where it switches into the latent stage in the nerve ganglia. During reactivation from latency newly formed virus particles can be easily recognized by the immune response developed against the virus during the primary infection. For these reasons, the intracellular route is a very important way for herpesviruses to spread in an infected organism.

The aim of my work was to gain a better understanding of the mechanism of direct transmission of alphaherpesviruses between cells and, in particular, to thoroughly examine the ability of these viruses to exploit long cellular connections, named in English "tunneling nanotubes" (TNT). As there is no established Polish translation of this term , for the purpose of this work tunnelling nanotubes have been described as “międzykomórkowe nanorurki” interchangeably with English term TNT. The first stage of the work was to determine the TNT fixation method, which allowed for reproducible analysis of these connections. The characterization of nanotubes was first carried out on non-infected cells. Due to their

composition, these connections have been classified as containing F-actin and tubulin or only F-actin. Due to their length, I distinguished for two types: short (≤20 µm) and long (> 20 µm).

The next stage of the work was to describe the role of nanotubes during viral infection. For these experiments, the BoHV-1 virus (bovine type 1 herpesvirus) was used as a model virus. BoHV-1 is a good model for the study of herpesviruses due to its safety and its close biological relationship to other alphaherpesviruses, such as human viruses HSV-1 and VZV. I showed that viral proteins from different parts of BoHV-1 viral particle could be detected in nanotubes. Moreover, my further observations demonstrated that BoHV-1 infection stimulated the formation of TNTs between cells and the number of long nanotubes was increased. Then, using the fluorescent BoHV-1 mutants, I showed that the virus could infect distant cells through direct transmission via nanotubes, even in the presence of neutralising antibodies that prevented external infection. Such transmission occurred also between two different types of cells.

The next step was to investigate the role of Us3 protein in this process. The Us3 protein is a serine -threonine kinase, which has many functions during herpesvirus infection. It has been shown that Us3 kinase has the ability to rearrange cell cytoskeleton, and that this function depends on its kinase activity. To facilitate the study of Us3, I constructed two fluorescent BoHV-1 mutants: a variant containing wild type of Us3 protein and a kinase dead mutant Us3-K282A. These constructs have been characterized and used to analyse the influence of Us3 on the formation of TNT. I have shown that the lack of kinase activity of Us3 protein affected the number and length of the nanotubes: less nanotubes were formed and they were shorter than in case of wild type protein. This effect was manifested at the later stage of infection. A truncated form of Us3, containing the region of the kinase domain, showed no ability to rearrange the cytoskeleton, but partially retained the ability to phosphorylate its substrates.

Furthermore, the effect of Us3 activity was also analysed in neuronal cells. For this purpose, rat neurons have been transferred by plasmids containing Us3-WT and Us3-K282A sequences. In contrast to other cells used in this work, the expression of wild type Us3 in neurons resulted in cell death. Such effect was not observed for cells expressing Us3-K282A.

In summary, the results obtained in this work indicate the important role of nanotubes (TNT) in the process of direct intercellular transmission of alphaherpesviruses. Previously published studies on the role of TNT in viral infection related to RNA viruses (HIV, influenza virus) and here for the first time such observation was obtained for a DNA virus. The results of the work also bring new information on the important, though not fully explained, role of Us3 kinase in herpesviral infection.