SPATIOTEMPORAL DYNAMICS OF HOST CELL MODIFICATION DURING HERPESVIRUS REPLICATION

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Herpesviruses are large and complex DNA viruses that are composed of an icosahedral capsid, a proteinaceous layer termed the tegument, and a glycoprotein-rich lipid envelope. One important area of host-pathogen interaction that is still poorly understood is the extensive change to intracellular organelles and cellular morphology that occurs within the infected cell during active virus replication. In previous studies, immunofluorescence was used to label viral and cellular organelles and snapshots were taken at different times after infection (see e.g. [1]). However, the rearrangement of cell organelles is a dynamic process correlated to the stage of virus replication which can vary highly between individual cells.

In order to characterise the spatiotemporal dynamics of host cell remodelling caused by herpesvirus infection, we use novel multiparametric fluorescence microscopy methods compatible with live-cell imaging such as Structured Illumination Microscopy (SIM) and expansion microscopy to map 3D arrangement in great detail. We have constructed a recombinant reporter virus that expresses eYFP-tagged ICP0, a multifunctional immediate early tegument protein, as well as mCherry-tagged glycoprotein C (gC), a late protein that is a major component of the viral envelope. The sequential expression of these two viral proteins provides us with an intrinsic time stamp for the stage of virus infection in each cell.

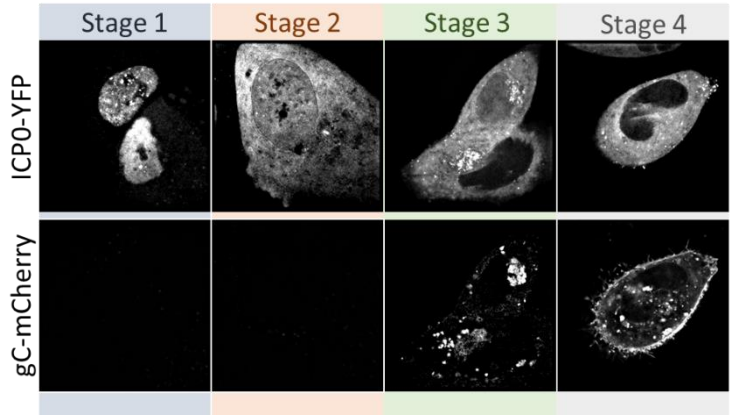

Figure 1: Time-stamping virus replication state: SIM images of HFF cells infected with an eYFP-ICP0/gC-mCherry HSV-1 mutant. Dependent on the cellular distribution of the marker proteins, four distinct stages in the infection cycle can be determined.

With this fluorescent reporter virus, we are able to describe the spatiotemporal remodelling of: (1) the three-dimensional architecture of microtubules and the actin network; (2) compartments of the secretory and endocytic pathways which are intimately linked to viral envelope protein synthesis, maturation and transport; and (3) key antiviral and inflammatory signalling platforms (mitochondria and peroxisomes).

[1] B. Norrild B, V.P. Lehto and I. Virtanen, “Organization of cytoskeleton elements during herpes simplex virus type 1 infection of human fibroblasts: an immunofluorescence study”, *J. Gen. Virol.*, 97-105 (1986).