BACKGROUND

Herpes Simplex Virus type 1 (HSV-1) is a common human pathogen of considerable clinical interest. The ability to establish a latent infection is of crucial importance since in cases of cell-mediated immunity depression the virus can reactivate presenting significant clinical problems to immunocompromised individuals and certain transplant patients resulting in a life-threatening disease. HSV-1 latency is therefore a key component of its biological and clinical importance and as such, the underlying mechanisms have been a subject of considerable interest. The mechanism by which HSV-1 reactivates from latency in transplant recipients has not been well understood.

Human cytomegalovirus (HCMV), a ß-herpesvirus, is characterized by its strict host specificity. The virus has become widely appreciated as a cause of congenital infection but has recently also received medical attention for its role in pathogenesis in immunocompromised individuals like transplant recipients or AIDS-patients. HCMV has a linear double stranded DNA genome of high complexity and a coding capacity of up to 220 different genes, entitling it as the most complex of all known pathogenic human viruses. Little is known about the molecular mechanisms that govern pathogenesis in vivo and only recently has the viral genome become available to directed mutagenesis to study gene function in vitro.

The pathogenic and cytotoxic effects of viruses are largely due to the expression of viral gene products. Therefore, the determinants of these outcomes are the mechanisms underlying the expression of viral genes.

RESEARCH INTERESTS

The main interest of my group concerns the control of both HSV-1 and HCMV gene expression during lytic and quiescent infection and the analysis of the virus-host interactions at early stages of the infection.

These above questions are approached in a multidisciplinary way, using a variety of techniques of Cell Biology and most important of them, monitoring of live infected cells. Genetically engineered viruses are constructed, expressing autofluorescent proteins (e.g. EGFP) fused to viral proteins, enabling the visualisation of their dynamics. Furthermore, the experimental approaches utilise a variety of molecular genetical techniques such as gene cloning and expression, transient transfection assays and biochemical assays of enzyme activity.

1. Spatial organization and localization of HSV-1 genomes during quiescent infection and reactivation.

Herpes Simplex Virus type 1 (HSV-1) owes its evolutionary success and clinical importance to its ability to establish a latent infection and to reactivate. The balance between the lytic and latent status is controlled by both host and viral factors and among them, the HSV-1 immediate early protein ICP0. Upon HSV-1 infection, the distinct nuclear domains ND10 are disrupted at early times and the regulatory protein ICP0 is necessary and sufficient for this effect.
It has been proposed that ICP0 play a role in maintenance of the latent state of HSV-1 DNA and that its activity might be involved in viral gene expression or repression pathways. Considering that the environment of parental viral genomes in the nucleus and their interactions with viral and cellular proteins are important in transcriptional activation or silencing, it is particularly interesting that several HSV-1 transcripts are preferentially found in association with ND10 and a number of ND10 proteins or proteins with which they interact, have been implicated in repression. The current project investigates the localisation and dynamics of quiescent and reactivating viral genomes in the cell and explore the involvement of ICP0 in the molecular and cellular events that take place during reactivation of HSV-1 gene expression. Parental HSV-1 genomes are visualised by using engineered HSV-1 amplicon plasmids. The dynamics of quiescent parental amplicon genomes at early times of infection as well as their reactivation is monitored in live infected cells while interactions between the ICP0 and the host proteins such as ND10 and their functional consequences are studied by constructing fluorescent HSV-1 viruses expressing viral proteins and Baculoviruses expressing those proteins, respectively.

Figure: The development of an amplicon replication compartment in association with ND10. A cell containing several parental amplicon genomes (red) was selected early during infection, and a double-labelled image was captured to show two genomes initially juxtaposed with a PML body (green) in the lower right-hand portion of the cell. The inset shows this region of the cell at a higher magnification. The greyscale panels show the EYFP-labelled amplicon genomes in the same cell during the course of the infection. A replication compartment developed from the genomes initially associated with the ND10 site, but not from other genomes that were not associated with PML foci.

2. Dynamics of HCMV genomes and the Immediate-Early proteins -1 in live infected cells. This project investigates the principles governing the localisation and dynamics of HCMV genomes in the nucleus of live infected cells, and further analyse the importance of the IE1 regulatory protein during human cytomegalovirus infection. The HCMV Immediate-Early protein 1 (IE1), is one of two major products of the ie gene and is a predominant protein expressed during the immediate-early phase of HCMV infection. Recent data provide evidence that the lytic/latent balance is controlled by both host and viral factors and of the latter a crucial component is the regulatory protein IE1. Because IE1 is nonessential at high multiplicity of infection
(m.o.i.) in HF cells, the exact role of these processes in viral infection has been enigmatic. The study of interactions between HCMV virus - nuclear domains ND10 and constituent proteins, as well as the dynamics of cellular chromatin in response to viral infection are of great interest.

The monitoring of the dynamics of the IE1 protein became feasible after the construction of recombinant HCMV viruses expressing IE1 fused to EGFP (HCMV/IE1-EGFP). Coupled with infection of primary human fibroblasts expressing Sp100, an ND10 constituent, in fusion with autofluorescent proteins, live-cell imaging experiments are performed. This revealed that IE1 protein is recruited onto intense foci at very early times after infection which progressively are diffused into the nucleus of the infected cells. During HCMV/IE1-EGFP infection, the viral regulatory protein IE1 precisely colocalizes with nuclear domains ND10 and rapidly induces the degradation of PML or Sp100, thereby disrupting ND10 and dispersing their constituent proteins. Direct evidence that IE1 molecules are juxtaposed next to viral DNA early after HCMV infection has been obtained, similarly to the HCMV DNA-ND10 association.

Figure: HCMV IE1 is recruited onto metaphase chromatin after transient expression in HeLa cells during mitosis

3. The role of Human Cytomegalovirus (HCMV) infection in the activation of the Ras signalling pathway.

The objective of this project is to address the question of whether permissiveness to Human Cytomegalovirus is enhanced by the activation of the ras signalling pathway. Experimental data have shown that H-ras transformed cells demonstrate increased permissiveness to HCMV compared to their parental non-transformed cells. Specifically, both progeny viral titres and expression levels of the HCMV proteins are elevated in the H-ras transformed infected cells, in contrast to the non-transformed cells. Inhibition of key molecules of the ras signalling pathway effectively suppresses HCMV infection in H-ras transformed cells. Two crucial molecules of the Ras signalling pathway, MEK1/2 and ERK1/2 have been both found to be activated by phosphorylation upon HCMV infection. At the cellular level, infection of H-ras transformed cells with HCMV shows a higher proliferation rate compared to the non-transformed cells as well as the formation of a significantly higher number of cellular foci compared to uninfected cells, suggesting a rather enhancing role for the virus towards transformation. Viral entry alone is insufficient to induce cell proliferation but active HCMV gene expression is required.

Figure: Striking recruitment of the viral pp65 protein onto the cellular foci in H-ras transformed fibroblasts. Arrows indicate the formation of foci (left image, bright field) and the localization of HCMV pp65 in the corresponding foci (right image, immunofluorescence).
In summary, we propose that HCMV employs the oncogenic ras pathway to induce cellular and/or viral gene expression for the promotion of viral replication. HCMV employs several host-cell signalling pathways to increase the viral output. These novel findings provide detailed knowledge on viral entry and amplification mechanisms in transformed cells, allowing the discovery of new target molecules for the treatment of the diseases associated with HCMV infection.

4. Study of HCMV microRNAs (miRNAs).

MicroRNAs constitute a family of non-coding RNA molecules. They are transcribed as long transcripts and undergo a maturation process, which gives rise to mature miRNAs, approximately 22 nucleotides long. The latter base-pair usually with the 3' UTR of mRNAs and affect the cellular mRNA and/or protein levels of their targets. Herpesviruses express miRNAs, whose maturation is mediated by the same enzymes as the cellular miRNAs. So far, HCMV is known to produce 11 miRNAs, each one giving rise to 1 or 2 mature miRNAs. Currently, there is substantial information for only one of the cellular targets of HCMV miRNAs; protein MICB is downregulated by hcmv-miR-UL112 and this results in reduced NK killing of HCMV infected cells. Therefore, the impact of viral miRNAs on cellular processes and cell fate can be crucial. The study of HCMV miRNAs is a new field of research and is expected to substantially contribute to the delineation of HCMV infection and possibly provide the background for novel antiviral therapeutic approaches. The aim of our recently initiated miRNA-focused experiments is to verify computational predictions regarding HCMV miRNAs and potential cellular targets, using luciferase assays as a first step, and to try expressing some of these miRNAs with lentiviral constructs for further investigation.

**REPRESENTATIVE PUBLICATIONS**


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