SUMMARY

The human pathogenic herpesviruses, human cytomegalovirus (HCMV) and herpes simplex virus (HSV) both encode inhibitors of the peptide transporter TAP, i.e. gpUS6 and ICP47, respectively, to block antigen presentation to CD8+ T cells. While gpUS6 is an ER-resident type I transmembrane glycoprotein of 21 kDa, the soluble 8,5 kDa ICP47 inhibitor is located to the cytosol. TAP is a dimeric protein built by the subunits TAP1 and TAP2, each consisting of one transmembrane domain (TMD) and one cytosolic nucleotide binding domain (NBD). The NBDs utilize ATP energy for translocation of the peptide across the ER membrane. The membrane topology of the pore forming TMDs, the number of their transmembrane segments (TMSs) and the functional coupling of the processes by the NBDs with the movements of both TMDs required to complete the transport cycle, are not understood.

This thesis comprehends gpUS6 and ICP47 as molecular tools for the investigation of TAP structure and function. Despite their profound biochemical differences, gpUS6 and ICP47 share phenotypical similarities, i.e. a physical interaction with preformed TAP complexes and a species-restricted mode of TAP inactivation. Taking advantage of these characteristics, mixed–species transporters and a large set of human/rat TAP chimeras were constructed, which allowed the identification of explicit and cryptic interaction domains for ICP47 and gpUS6 on TAP1 and TAP2. A dominant binding forwarding the inhibitory function of gpUS6 was delimited to the most C-terminal lumenal loop of the TAP1 TMD. This finding provided for the first time evidence for a model of TAP1 topology with 10 TMSs. Independent gpUS6 interaction with TAP2 is a prerequisite for efficient inhibition of TAP. For initial ICP47 contact to TAP human sequences of the N-terminus of TAP2 were found to be indispensable, but not sufficient for optimal binding. Further sequence requirements to maintain ICP47 interaction were mapped to the C-terminus of TAP2 in addition to undefined parts of TAP1.

A second set of human TAP1 and TAP2 constructs mutated in the Walker A domain of the NBDs, which are responsible for ATP hydrolysis, allowed a detailed analysis of the cytosolic NBDs. This approach revealed a direct transmission of conformational changes to the TMDs which are sensored by gpUS6. The findings are integrated into a four step model for gpUS6 interaction with TAP in which initial ATP binding to the NBD of TAP2 results in a conformational change of the TMD. This forms the gpUS6 binding domain on TAP1 before association of gpUS6 to specific sites on TAP2 and TAP1 interrupts the peptide translocation

cycle. Unlike gpUS6, ICP47 binding to TAP was found to be ATP independent. However, gpUS6 and ICP47 binding is mutually exclusive, indicating that ICP47 recognizes a distinct cytosolic TAP conformation. Furthermore, while ICP47 binding to TAP is inhibited by gpUS6, whereas peptide binding to TAP is not, this finding indicates that ICP47 acts through conformational constraints rather than competing out peptides for binding as deduced from earlier studies.