Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes

Project: Molecular Characterization of Host-virus Interactions, towards Novel Cellular Targets for Anti-viral Therapy

Ulrich Schubert - University of Erlangen-Nürnberg - ulrich.schubert@viro.med.uni-erlangen.de

Introduction

Understanding the very specific interaction of viral proteins with host cellular binding partners is an essential prerequisite for deciphering the pathomechanism of viral infection, and this knowledge in turn is essential to design strategies for anti-viral treatment and vaccination. The genomic structures of viruses, particularly small RNA viruses, are relatively easy to unravel. Most of the limited number of viral gene products have been identified and characterized at the molecular level especially for those virus species threatening the human population. However, a large discrepancy exists between the detailed knowledge of the viral genome and the encoded proteome as compared to the relatively poor understanding of the complex and intensive interactions these viral factors make with a wide variety of host cell proteins during the viral replication cycle.

By using our strategy, two major goals will be achieved: (i) the reduction of the probability that drug resistant viruses emerge under therapy, and (ii) the development of broadly active antiviral drugs. Therefore, the ultimate goal of the *human virogenomics* approach is the identification of cellular factors, including thus far uncharacterized novel human gene products that govern critical steps in replication cycles of different viruses. Based on this knowledge novel anti-viral drugs that interfere with the activity of these cellular targets will be designed and tested. It is obvious that such drugs are of high medical importance and economical value and therefore, offer the possibility for commercial exploitation and attracting industrial partners.

The ultimate goal of our project is the identification of cellular factors essential for the replication of different pathogenic human viruses. Based on this knowledge we will design and test anti-viral drugs that interfere with molecular aspects of host-virus interaction. Central to this project is the characterization of potential cellular targets for therapy of chronic as well as acute virus infections for which so far neither vaccines nor effective drug therapies exist. The major focus is on human immunodeficiency viruses (HIV). We will also investigate specific aspects of the Influenza A virus (IAV) and hepatitis C viruses (HCV), the latter on basis of the surrogate flavivirus model BVDV. As an example of a major cellular pathway involved in HIV replication we found that the ubiquitin-proteasome-system (UPS, see Fig. 1) is essential for propagation not only of HIV and other retro viruses, but also for a variety of completely unrelated viruses, such as hepatitis B and C, LASSA, pox, or even SARS-CoV. This strongly indicates that drug-mediated modulation of specific cellular pathways represents a conceivable antiviral strategy which potentially allows therapy of a variety of currently known and eventually even newly emerging viruses. Our pre- and clinical studies on proteasome inhibitors initiated during the NGFN-1 funding period support our assumption that this long-term goal of a so called '*broadly active virostatics*' can be achieved. Another advantage targeting the function of cellular proteins is the fact that these drugs should have a much lower risk, if any at all, to lead to selection of drug-resistant viruses. In contrast to our strategy, the currently used standard anti-retroviral treatment that target viral enzymes has the persistent potential of selection for therapy resistant HIV mutants, particularly if one considers the extremely high mutation and turn over rate of HIV *in vivo*. In continuation of our ongoing projects three specific examples of host-virus interaction will be intensively investigated during the NGFN-2 funding period.

1) components of the UPS, particular the 26 proteasome and specific ubiquitin ligases and their interactions with virus structure proteins;
2) molecular chaperones, particularly the cellular prolyl isomerase cyclophilin A (CypA) that interact with HIV-1 Gag and the regulator protein Vpr;
3) cellular factors that interact with the newly discovered Influenza A virus (IAV) regulator protein PB1-F2 which shares striking similarities to the HIV-1 pathogenic factor Vpr.

Central to our experimental strategy is the accessibility to full length biological active version of individual virus proteins which are used for both, structural and functional analyses as well as for binding studies with cellular counterparts. The availability of highly pure and specific 'bits' represents one of the most important prerequisite for efficient pull down experiments of cellular binding partners derived from organelle, cell, or complex organ extracts. Our comprehensive approach includes a wide variety of molecular and cell biology, including peptide synthesis and protein biochemistry; structural analyses by NMR, CD, DLS, BIAcore, and IFR spectroscopy; genome and proteome analyses; mass spectroscopy and sequence analyses; animal model; drug development and drug evaluation in animal and clinical studies. In general, the project will involve the use of proteomic, transcriptomic, and pharmagenomic approaches to find novel antiviral drugs which interfere with cell-virus protein interactions.

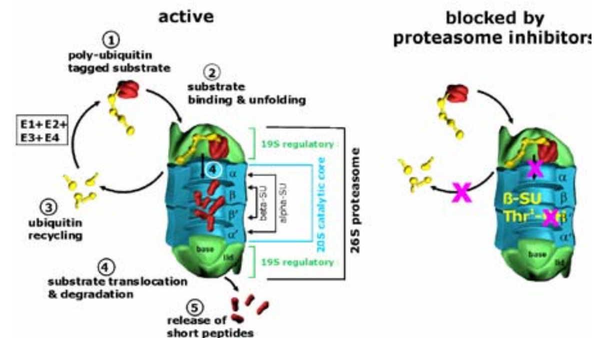


Fig 1: Function of the 26S proteasome and their inhibitors in context of the UPS.

The 26S proteasome is composed of the 20S core particle which is capped by a 19S regulatory subunit (SU) at both ends. The 20S proteasome represents the catalytic core and comprises 28 (14 different) SUs, each in the range of 21 to 31 kDa. The α-SUs (α1-α7) form the two outer ring structures which are organized in two heptameric staggered ring structures, while the β-SUs form the inner heptameric ring structures. Three of the β-USs (β1,β2, and β5) harbor the catalytic active Thr[1] residues, which together form 6 catalytic active sites within the 20S particle. The 20S unit can also associate with different activators, such as PA28. It can degrade mostly unfolded proteins in a non-selective fashion that occurs independently of the 19S SUs, and thus

NGFN Nationales Genomforschungsnetz
GEFÖRDERT VOM Bundesministerium für Bildung und Forschung

independently of poly-ubiquitinylation. The 19S SU consists of two SU-domains. The base connects the 19S SU to the α-SUs of the 20S particle, which harbors several ATPases. The base itself is attached to the lid, which consists of up to ten non-ATPase SUs, and is assumed to regulate substrate binding of the 26S proteasome. In its active state the 19S cap of the 26S proteasome recognizes poly-ubiquitin chains that must consist of at least 4 ubiquitin molecules. Ubiquitin itself is removed by de-ubiquitinating enzymes and reenters the pool of free ubiquitin. The substrate is unfolded and translocated into the digestive chamber of the 20S particle. Neutralizing the hydroxyl group of the Thr^1 within the catalytic sites of the β-SUs, which is the inhibitory mechanism of almost all of the known proteasome inhibitors, causes either reversible or irreversible inhibition of the 26S proteasome. Inactivation of the proteolytic chamber of the 26S proteasome also stalls other steps within the UPS. Major consequences are accumulation of poly-ubiquitinylated proteins, disturbance of the recycling of free ubiquitin. Limitation of the pool of free ubiquitin finally results in the de-ubiquitinylation of mono-ubiquitinylated proteins such as histones, or the $p6^{Gag}$ domain of HIV-1.

Results/Project Status

- We have solved the NMR-structure of the L-Domain containing HIV-1 p6 protein that is required for virus budding (Fig. 2)
- A novel RING finger E3 ubiquitin ligase, the Tsg101 associated ligase (Tal), that physically binds to, and selectively ubiquitinylates Tsg101 was recently discovered by the laboratory of Dr. Yosef Yarden from the Weizmann Institute of Science, Rehovot, Israel, in collaboration with colleagues from Proteologics, from the same area, and our laboratory. Most interestingly, Tal mediated ubiquitylation of the budding factor Tsg101 regulates its ability to sort endocytotic (cell surface receptors) and exocytotic (virus assembly) cargoes (Fig. 3).
- Another E3 ligase was discovered that regulate HIV-1 assembly and virus egress: the E3 ubiquitin ligase human POSH (Plenty Of SH3s). The homolog of murine POSH was described as a trans Golgi network (TGN) associated E3 enzyme that somehow targets assembly of HIV-1 Gag molecules to the plasma membrane (Fig. 3).

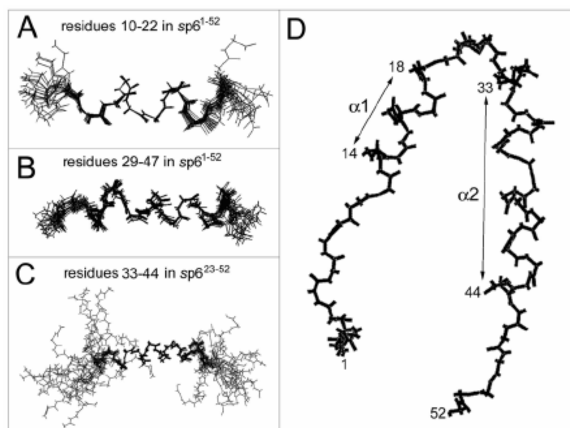
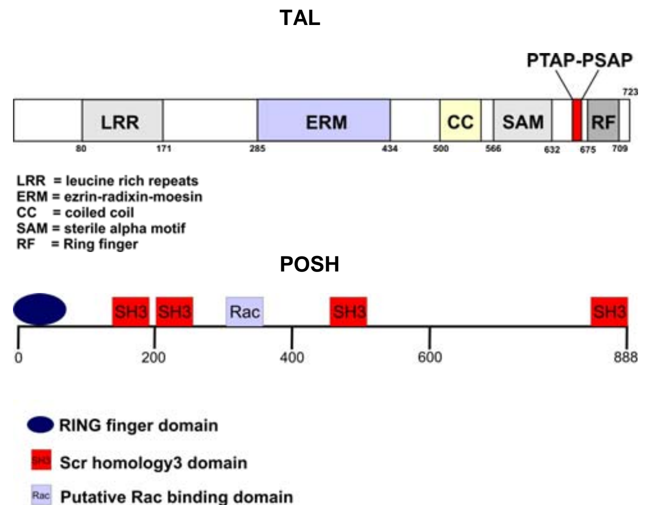


***Fig 2:** ^{1}**H NMR analysis of HIV-1 p6**. (A) Supposition of the 20 best final restrained structures of $sp6^{1-52}$ after alignment of the backbone atoms of residues Glu-13 to Gly-18. Shown are structures comprising residues Pro-10 to Thr-22. (B) Supposition of the 20 best final structures of $sp6^{1-52}$ after alignment of the backbone atoms of residues Lys-33 to Ser-43. Shown are structures comprising residues Glu-29 to Ser-47. (C) Supposition of the 20 best final structures of $sp6^{23-52}$ after alignment of the backbone atoms of residues Lys-33 to Leu-44. Structures comprising all residues are shown. (D) Central structure of $sp6^{1-52}$ as represented by the structure that shows the lowest average rmsd value to all other final structures.*



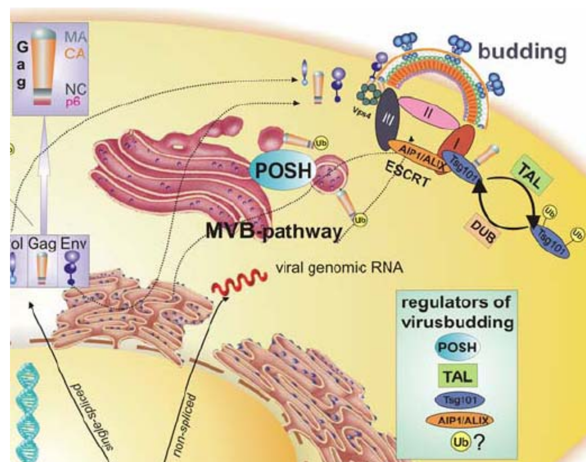


***Fig 3:** Domain structure of ubiquitin ligases Tal and POSH, and their function in HIV-1 budding.*

Outlook

We will continue in our search for novel cellular targets suitable in anti-viral therapy. Our main focus is directed on targets that regulate general mechanism(s) in virus replication. As a primary example for this strategy we will continue our studies with proteasome inhibitors in various virus systems. Major focus during early phase of NGFN-2 funding period will be the continuation of our work with UPS inhibitors targeting the 26S proteasome and ubiquitin ligases. To identify additional cellular we will follow three major steps: 1st, the identification of a potential cellular target by proteomics and functional genomics; 2nd, the validation of a target by using inhibitors or methods of siRNA gene silencing; 3rd, the development of specific inhibitors and their evaluation in pre-clinical as well as clinical studies. The principal of this so called *translational* or *bench-to-bedside* biomedical research has been already demonstrated by our ongoing phase 1 dose escalation study proteasome inhibitors in HCC patients. Further success in our strategy of cellular antiviral targets critical depends on the continuation in funding as well as close collaboration with other NFGN-2 centers.

Lit.: 1. Nature 404:770. 2. Proc. Natl. Acad. Sci. USA 97:13057. 3. J. Virol. 76:3038; J Virol.,77:3384. 4. J. Biol. Chem. 278:43202. 5. J. Biol. Chem. 278:43188. 6. Proc. Natl. Acad. Sci. USA. 102:1478. 7. Genes Dev. 18:1737.