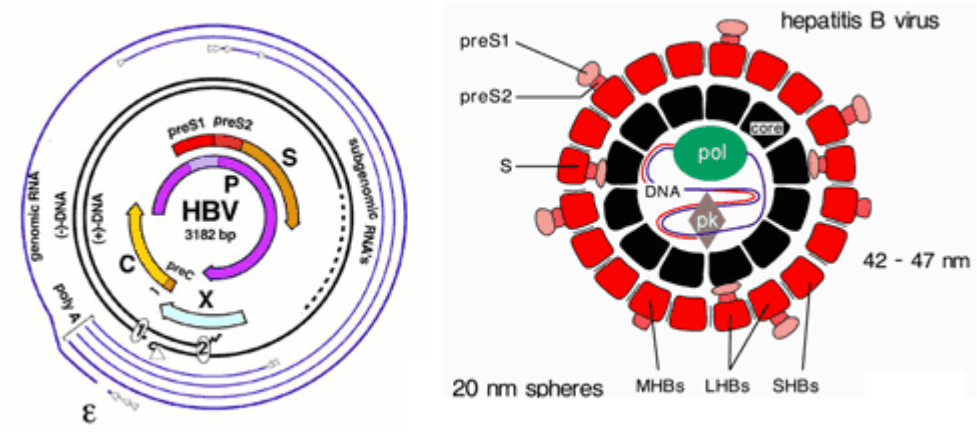
Background – Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV)

Hepatitis B Virus (HBV)

Hepadnaviridae are subdivided into mammalian and avian-hepadnaviruses. The mammalian hepadnaviruses include human hepatitis B virus (HBV), woodchuck hepatitis virus (WHV) and the ground squirrel hepatitis B virus (GSHV). The duck hepatitis B virus (DHBV) and the heron hepatitis B Virus (HHBV) belong to the avian-hepadnaviruses. The hepadnaviridae share the following features:

- An only partially double stranded genomic DNA comprising a complete coding strand (negative strand) and an incomplete non-coding strand (positive strand)
- A RNA-dependent DNA polymerase
- Replication through a pre-genomic RNA template
- A high degree of species and tissue specificity

The partially double stranded DNA genome of HBV is about 3.2 kb in size. The viral genome uses all three reading frames and contains at least four different open reading frames, coding for the viral polymerase, the HBc and HBe antigen, the regulatory protein HBx and the preS/S gene encoding the three surface antigens (LHBs, MHBs and SHBs).



Hepatitis B virus: structure and genomeSource: PEI

Infection by hepatitis B virus (HBV) can cause an acute inflammation of the liver. In addition, HBV is a major causative agent for the development of hepatocellular carcinoma (HCC). In the light of this, many studies have focused on the identification of potential viral oncogenic products. With the discovery that HBV encodes at least two transcriptional activators, HBx (Twu and Schloemer, 1987; Wollersheim et al., 1989) and the PreS2 activators large surface protein/middle surface protein (LHBs/MHBs) (Kekule et al., 1990; Hildt et al., 1996), attention focused on the identification of cellular targets which, upon activation, might lead to transformation of the infected cell (for reviews, see Hildt and Hofschneider, 1998; Andrisani and Barnabas, 1999; Lupberger et Hildt, 2006). The relevance of the activator function for the viral life cycle, however, remained more or less enigmatic.

In the case of WHV, it was observed that the productive viral life cycle requires the presence of functional WHX protein (Zoulim et al., 1994); in the case of HBV the relevance of HBx for the viral life cycle is suggested (Bouchard et al., 2001). There are reports describing that HBx is dispensable for HBV replication (Stockl et al., 2003, Hafner et al., 2003). HBx is a 154 aa-sized activator, with an apparent molecular weight of about 17 kDa. A variety of different signal transduction pathways was described to be HBx-dependent activated. Mechanisms mediating HBx-dependent activator function include the Ras-dependent activation of c-Raf-1/MEK/Erk2 and MEKK-1/JNK cascades, leading to the induction of several transcription factors, for example, AP-1 or NF-kB (Benn and Schneider, 1994; Su and Schneider, 1996; Klein and Schneider, 1997; Su et al., 2001; Hafner et al., 2003).

A major pathway of HBx-dependent signaling is activation of Ras or further upstream of Src (Klein and Schneider, 1997; Klein et al., 1999). Recently, activation of Pyk2 and subsequent activation of src by HBx was described (Bouchard et al., 2001). The sequence encoding for PreS2 activators is localized in the HBV surface gene. The surface gene is separated by three in frame ATG-codons in the preS1-, preS2- and S-region. The LHBs encompasses the Pres1-, PreS2- and S-domain, the (MHBs) PreS2- and S-domain and the small (SHBs) surface protein encompasses the S-domain. The activator function of surface protein requires the integrity of the PreS2-domain and its cytoplasmic orientation as in the case of a fraction of LHBs (Bruss et al., 1994; Hildt et al., 1995, 1996). The PreS2-domain binds to PKCα/β leading to a Ras-independent, PKC-dependent activation of the c-Raf-1/MEK signal transduction cascade (Hildt et al., 2002, Friedrich et al., 2005, Schaedler et al., 2011).

In contrast to these steps of the HBV life cycle encompassing the viral gene expression and morphogenesis that can be investigated based on stable or transient expression systems, the first steps of virus entry have not well been characterized due to the lack of an efficient in vitro infection system for a long time. Recent reports, however, describe promising new systems that could enable an analysis of the early steps of hepadnaviral infection (Walter et al., 1996; Gripon et al., 2002).

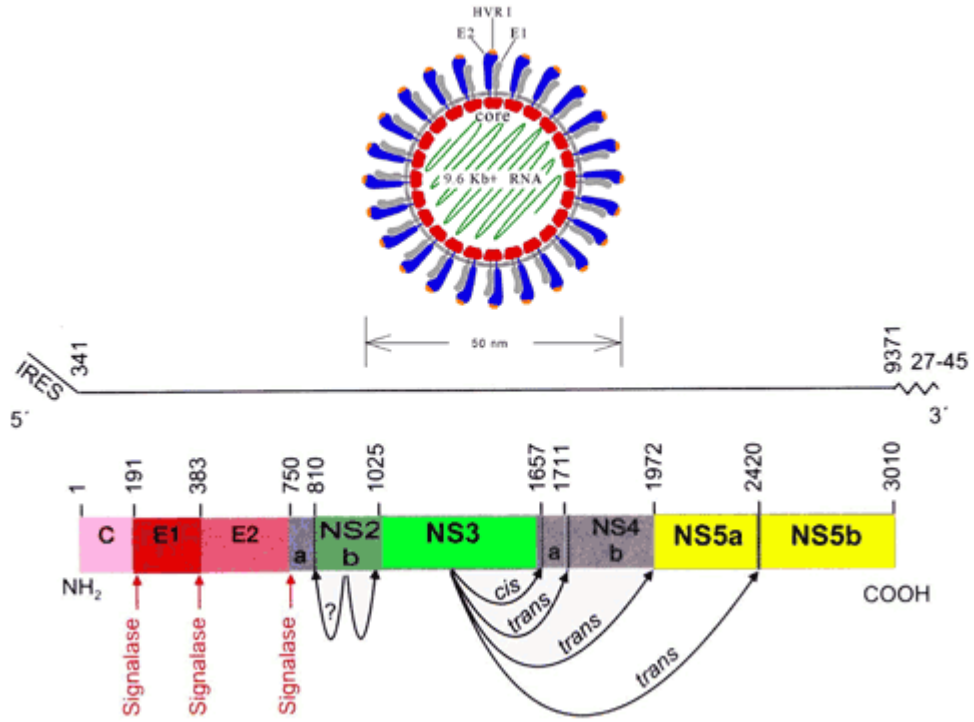
One open question concerns the fate of the viral nucleocapsid after the virus has entered the cell. There is evidence that the virus enters the cell by receptor mediated endocytosis and at the end of this process the nucleocapsid is released into the cytoplasm (Kock et al., 1996; Stoeckl et al., 2006; Funk et al., 2006). Productive viral infection requires the transport of the HBV genome into the nucleus where the conversion into cccDNA occurs (Nassal, 2008).

This phase of nuclear entry is still enigmatic. It is discussed that the intact viral capsid shuttles the genome-polymerase complex into the nucleus (Kann et al., 1997) or that a partial disassembly of the capsid within the nuclear pore complex or in a perinuclear domain leads to a release of the polymerase-linked genome (Brandenburg et al., 2005). The polymerase-genome complex is too big for free diffusion through the nuclear pore complex. Recently we could identify that the HBV-polymease harbors a bipartite nuclear localization signal. The integrity of this NLS is crucial for the establishment of the viral infection.

Hepatitis C Virus (HCV)

Hepatitis C virus (HCV) infection results in chronic hepatitis in more than 70% of the infected individuals. At present more than 170 million people are persistently infected with HCV worldwide. Persistent HCV infection is associated with chronic inflammation of the liver (hepatitis), which can progress to liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Levrero et al., 2006; Longerich et al., 2005). Since the discovery of HCV as the causative agent

for non-A, non B-hepatitis in 1989 (Choo et al., 1989), considerable progress has been made with respect to treatment strategies, but for a large proportion of patients, specifically those infected with HCV genotypes 1a/1b, the currently available therapy with pegylated interferon α and ribavirin is ineffective. Moreover, there is no prophylactic vaccine in sight.



Hepatitis C virus: structure and genomeSource: PEI

HCV is the sole member of the genus hepacivirus that belongs to the flaviridae family. The HCV genome is a single-stranded positive-sense RNA molecule of approx. 9600 bases length. The viral RNA codes for a large polyprotein of approx. 3100 amino acids, which is posttranslationally processed by cellular and viral proteases. The N-terminus encompasses the structural proteins core, E1, and E2, the C-terminus, the p7 protein and the nonstructural (NS) proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B (Hijikata et al., 1993; Penin et al., 2004; Tellinghuisen et al., 2004). The mature NS5A protein is generated by the action of the viral NS3/NS4A serine protease. Subsequently, NS5A associates with the cytoplasmic face of the endoplasmatic reticulum (ER) via an amphipathic alpha-helix. In this association NS5A is an integral part of the replicon complex (Moradpour et al., 2004) and required for viral morphogenesis (Appel et al., 2008; Tellinhuisen et al., 2008). NS5A is a phosphoprotein that exists in a basal or in a hyperphosphorylated state (p56 and p58) (Tellinghuisen et al., 2004; Appel et al., 2005; Neddermann et al., 2004). Moreover, as an integral part of the HCV replicon complex, NS5A is able to interfere with viral proteins (Moradpour et al., 2003) as well as with a variety of cellular proteins (Shi et al., 2002; Sklan et al., 2007; Burckstummer et al., 2006; Taguwa et al., 2008).

Some of these interaction partners seem to trigger a deregulation of the host cell signal transduction, such as Grb2, PI3K, p53, or c-Raf. Overall, NS5A consists of three discreet domains I - III: Based on the crystal structure, it is assumed that domain I is involved in homodimer formation, generating a large putative RNA binding groove located at the interface of the monomers. Domains II and III are less well characterized. However, genetic mapping has shown that domain II is crucial for HCV RNA replication. With respect to c-Raf, we could recently demonstrate that the interaction with NS5A is mediated by domain II, and eventually

leads to activation of c-Raf (Burckstummer et al., 2006; Himmelsbach et al., 2009; Sauter et al., 2009). In addition, recent work identified a small deletion in domain III of NS5A that disrupts the production of infectious viral particles without affecting RNA replication (Appel et al., 2008; Tellinghuisen et al., 2008). Based on recently developed tissue culture model systems, it is now possible to study the complete HCV life cycle in vitro, thus allowing the detailed analysis of virus-cell interactions and assessment of substances with respect to their potential effect on HCV replication (Wakita et al., 2005; Zhong et al., 2005; Lindenbach et al., 2005).