7 Summary

The Torque Teno Virus (TTV) has been isolated from a patient with hepatitis of unknown etiology. Further investigations have shown, that in immunosuppressed individuals, as well as in patients with multiple infections of e.g. HIV or HCV, an increase in TTV titer was observed. Therefore, a putative link of TTV to induction of diseases or its aggravation of effects by interaction with other viruses has to date not been excluded.

TTV is characterized by a highly divergent genome of approximately 3700 nt. Until now 39 genotypes have been described. Since the sequence varies up to 60 %, variability of TTV genotypes may give rise to viruses with distinct molecular characteristics. Consequently, changes in genotypes may result in variable pathogenicity. To receive additional information about molecular and virological features of TTV, isolate P/1C1 obtained from a patient with non A-G hepatitis, was thoroughly analysed.

It was shown that four instead of three mRNA-species were transcribed from the P/1C1-genome with sizes of 2.8 kb, 1.2 kb, 1.0 kb, and 0.6 kb. Despite of the limited coding capacity of TTV, seven proteins were expressed due to alternative splicing (ORF1, ORF1/1, ORF1/2, ORF2, ORF2/2, ORF3, and ORF4). Thereby, the newly identified protein ORF4, which is expressed from the 0.6 kb transcript was shown for the first time.

With the exception of ORF2, all viral proteins have been found in the nucleus, whereas ORF1, ORF1/1 and ORF1/2 were predominantly localized in the nucleoli. Both, the N-terminus and the C-terminus of ORF1 carry nuclear localization sequences, which are responsible for localization in the nucleus. Moreover, the ORF1 N-terminus, which is characterised by an arginine-rich stretch of 68 aa, possesses a NuLS sufficient for localization of ORF1 protein in the nucleoli.

In general, circular single-stranded DNA-viruses replicate via the rolling circle mechanism (RCM). In this process, at least one virus-encoded enzyme initiates viral replication by introduction of a DNA single-strand break, whereas the following steps are performed by host factors. In this study, ORF1-protein was identified as the trans-activating replicase of TTV and expression was performed with an ORF1-specific antiserum for the first time.

This enzyme is translated from the 2.8 kb transcript and carries two of four motifs characteristic for replicases mediating the rolling circle replication. Motif I (FTL) is localized at position aa 124-126, whereas motif III (YXXK) was found twice at position aa 349-352

and position aa 430-433. The origin of replication is the cis-acting element for viral replication and was mapped to position 3205-103 nt of the UTR. Moreover, the assumption expressed in the literature that TTV may be able to replicate in different cell lines was confirmed in the study presented here. Furthermore, the replicase of TTV isolate JB106 (genopgroup 5) was able to initiate replication of TTV isolate P/1C1 (genogroup 1) thereby confirming crosstalk between distinct TTV-isolates. This outcome is very important, because multiple infections by different TTV-isolates have been described in several publications. Additionally the results presented here maintain the thesis, that TTV replicates in a RCM related manner.

In contrast to the other TTV-encoded proteins located in the nucleus, ORF3 and its spliced variant ORF4 resided in the nucleoplasm. A Luciferaseassay demonstrated that these proteins transactivate the viral promotor located in the UTR. In addition, the detection of homologous and heterologous interaction in the yeast two-hybrid assay suggests formation of multimeric protein complexes.

The results presented in this Ph.D. thesis indicate that molecular variability of TTV produces differences of the expression pattern of single TTV isolates. In case of isolate P/1C1, differences result in a new transcript and the expression of another new protein. Since the correlation between virus and pathogenicity is not finally resolved, identification of new protein and its further analysis seems of particular importance. Moreover, the functional analysis of the connection between sequence deviation and pathogenicity by molecular investigation is strongly required.