Replication of and Protein Synthesis by TT Viruses

L. Kakkola, K. Hedman, J. Qiu, D. Pintel, and M. Söderlund-Venermo(✉)

Contents

Abstract The host cells and the events in the cells during Torque teno (TT) virus infection are at present unknown. Replicating TT virus DNA has been detected in liver, in peripheral blood mononuclear cells (PBMC), and in bone marrow. By alternative splicing this small virus generates three mRNA species, from which by alternative translation initiation at least six proteins are produced. The functions of the proteins are not yet fully understood. However, functions associated with, e.g., DNA replication, immunomodulation, and apoptosis have been suggested to reside in the multifunctional proteins of anelloviruses.

Replication of TTV

Torque teno virus (TTV) DNA is present in several organs and tissues, reflecting a potentially wide host cell tropism for TTV. Identification of a host cell has been attempted by detecting the presence of TTV DNA (*in situ* methods or DNA quantification) in certain cells, or by detecting replicating TTV DNA [gel separation methods, strand-specific primer extension, or full-length polymerase chain reaction (PCR)] in tissues. With the latter methods, double-stranded, circular, and potentially replicating TTV DNA has been detected in a variety of tissues: e.g., lung,

M. Söderlund-Venermo
Department of Virology, Haartman Institute, University of Helsinki and Helsinki University Central Hospital Laboratory Finland
maria.soderlund-venermo@helsinki.fi

E.-M. de Villiers, H. zur Hausen (eds.), *TT Viruses: The Still Elusive Human Pathogens,*

stimulated peripheral blood mononuclear cells (PBMC), bone marrow, liver, lymph node, thyroid gland, spleen, pancreas, and kidney (Bando et al. 2001; Mariscal et al. 2002; Okamoto et al. 2000b; Okamoto et al. 2000c; Okamoto et al. 2001). In experimental TTV infections of Rhesus monkeys, viral DNA was detected in various organs, whereas replicating TTV DNA was detected only in liver, bone marrow, and the small intestine (Luo et al. 2000; Xiao et al. 2002). Could all these cell types be the hosts for TTVs? As noted by Takahashi and co-workers, since TTV is present in the cells of the immune system that invade multiple tissues in inflammation, it should be carefully determined whether TTV really harbors in the cells inherent in each of the tissues (Takahashi et al. 2002).

Cells circulating in blood and originating from the hematopoietic compartment have been strong candidates for TTV host cells, and the presence of TTV in PBMC was confirmed by fluorescent *in situ* hybridization (Lopez-Alcorocho et al. 2000; Mariscal et al. 2002; Zhong et al. 2002). Furthermore, PBMC can be infected with TTV, and when stimulated, the cells produce infectious TTVs (Maggi et al. 2001a; Mariscal et al. 2002). It has been suggested that some genotypes could have preference for PBMC (Okamoto et al. 1999) and that PBMC could serve as a reservoir for TTV (Chan et al. 2001a; Garbuglia et al. 2003; Maggi et al. 2001b). The precursors for hematopoietic cells reside in bone marrow. The observation that during myelosuppression in bone marrow-transplant recipients the levels of TTV DNA are decreased, led to a suggestion that hematopoietic cells could sustain TTV replication (Kanda et al. 1999). Further evidence showing replicating TTV DNA in bone marrow (Okamoto et al. 2000b; Yu et al. 2002; Zhong et al. 2002) and clearance of TTV after bone marrow transplantation (Chan et al. 2001b) support the hypothesis that the hematological compartment could be the site of replication and/or sustained persistence of TTV.

In addition to the hematopoietic cells, the presence of high TTV DNA levels in saliva (Deng et al. 2000; Gallian et al. 2000) has led to investigations of oropharyngeal tissue as a putative target/host tissue for TTV infection. TTV DNA has been detected by *in situ* hybridization in the cytoplasm of oral epithelial cells (Rodriguez-Inigo et al. 2001), and TTV DNA loads in the nasal cavity exceed those in serum, suggesting that the nasal cavity could be the primary site of TTV infection (Maggi et al. 2003).

Due to the history of TTV as a potential hepatitis virus, liver as a main target organ for TTV infection has also been studied extensively. TTV is found by *in situ* methods in the nucleus and/or the cytoplasm of hepatocytes in patients with liver damage (Cheng et al. 2000; Comar et al. 2002; Jiang et al. 2000) without any cytopathological changes (Ohbayashi et al. 2001; Rodriguez-Inigo et al. 2000).

Infection and replication of TTV has been examined also in various cell lines. Desai and co-workers infected a liver cell line, Chang, a B lymphoblast cell line, Raji, and stimulated PBMCs with TTV genotype 1 (genogroup 1)-positive sera. The infected Chang cells produced constant, low amounts of TTV, whereas in PBMC and Raji cells the release of viruses was transient (Desai et al. 2005). Leppik and co-workers transfected a Hodgkin's lymphoma-derived cell line (L428) with a full-length TTV clone of tth8-isolate (genogroup 5), whereas Kakkola and co-workers

transfected monkey kidney-, human erythroid-, liver-, and kidney-derived cell lines (Cos-1, KU812Ep6, Huh7, Chang liver, UT/Epo-S1, 293T, and 293) with a full-length TTV clone of isolate HEL32 of genotype 6, a member of genogroup 1 (Kakkola et al. 2007; Leppik et al. 2007). Both studies showed, by *Dpn*I restriction enzyme and Southern analysis, the recirculation and replication of TTV DNA in human-derived cell types.

In addition to the presence of TTV DNA in certain cell types, the enhancer activity of the TTV promoter of isolate VT416 (genotype 1) has been investigated in various cell lines. The activity was highest in K562 human erythroleukemia cells, and also in HepG2 human hepatocellular carcinoma cells (Kamada et al. 2004), which further support that these two cell types are targets of TTV infection. Taken together, the best candidates at the moment for TTV host cells are cells of hepatic and erythroid origin, as well as the epithelium of the respiratory tract. However, it is also possible that only a specific subset of those cells or yet unidentified cell types could be the main targets for TTV infection and replication. In addition, the various TTV genotypes can differ in host cell tropism thereby further complicating the picture.

Replication Mechanism

The majority of small DNA viruses depend on the host cell replication machinery for virus replication. Viruses either infect actively dividing cells or induce their host cells to enter S-phase, and subsequently prevent apoptosis. Based on sequence analysis TTV is not assumed to encode a DNA polymerase for replication, but instead it would use cellular polymerases. Indeed, in the presence of aphidicolin, a drug blocking the cellular DNA polymerase, DNA replication of HEL32 TTV (genotype 6) did not occur, indicating that TTV utilizes for DNA replication the cellular replication machinery (Kakkola et al. 2007).

The exact mechanism of TTV replication is unknown. It is assumed, based on similarities with other circular single-stranded (ss)DNA viruses, that TTV could use the rolling circle mechanism (Mushahwar et al. 1999). Probably, as has been shown with the TTV-related circoviruses, viral proteins are needed to interact with cellular proteins for the initiation of replication. For this task circoviruses encode replication-associated proteins with specific Rep-motifs that bind to the replication initiation site (Mankertz et al. 1998; Niagro et al. 1998). Based on the amino acid sequence, TTV open reading frame (ORF)1 seems to contain similar Rep-motifs (Erker et al. 1999; Mushahwar et al. 1999; Tanaka et al. 2001). In addition to replication-associated proteins, animal circoviruses have conserved sequences and genomic structures that are involved in replication (Mankertz et al. 2004; Niagro et al. 1998; Todd et al. 2004). The untranslated region (UTR) of TTV also contains sequences that could form similar structures (Hijikata et al. 1999; Mushahwar et al. 1999; Peng et al. 2002). Whether these structures and proteins of TTV are used in viral replication is currently unknown.

RNA Processing of TTV

Due to the lack of an efficient culture system to support TTV replication, the transcription profile of TTV has been largely gained from cells transfected with TTV plasmids. Kamahora and co-workers analyzed in transfected Cos-1 cells the TTV mRNAs transcribed from a construct that contained a linearized TTV genome (VT416, genotype 1) (Kamahora et al. 2000). Subsequently, Okamoto and co-workers detected TTV mRNAs (of TYM9-isolate; genogroup 3) in human bone marrow cells from a TTV-infected patient with acute myeloblastic leukemia, in which TTV was actively replicating (Okamoto et al. 2000a). More recently, the detailed TTV transcription profile was characterized by transfection of a clone of the TTV genotype 6, HEL32 isolate in human 293 cells (Qiu et al. 2005). In addition, Leppik and co-workers showed the presence of additional splice variants in L428 Hodgkin's lymphoma cells transfected with tth7 and tth8 isolates (genogroup 5) (Leppik et al. 2007).

Three species of TTV mRNAs (2.8–3.0 kb, 1.2 kb, and 1.0 kb) have been detected in transfected Cos-1, 293 and L428 cells, as well as in infected bone marrow cells (Kamahora et al. 2000; Leppik et al. 2007; Okamoto et al. 2000a; Qiu et al. 2005). The genetic map of TTV generated by transfection of a clone of TTV genotype 6 is shown in Fig. 1 (Qiu et al. 2005), with the nucleotide numbers referring to GenBank accession number AY666122 (Kakkola et al. 2002). All the three TTV mRNAs are transcribed from a single promoter, which is located in the region −154/−76 (the RNA initiation site is denoted as position +1) (Kamada et al. 2004), and are polyadenylated at a single site at nt 2978 in HEL32. Therefore, all three mRNAs have the same 5′- and 3′-ends in common. All the mRNAs splice out a small intron (~100 nt) located approx. 70 nt from the RNA initiation site. The two short mRNAs (1.2 kb and 1.0 kb) have further been spliced approx. 400 nt downstream of the small intron, excising another larger intron with alternative 3′ splice sites 2A1 (nt 2315) and 2A2 (nt 2505) (Qiu et al. 2005). The large 2.8-kb mRNA is unspliced in the region of the large intron. All the splice junctions detected in transfected cells or infected bone marrow cells are at their corresponding locations in all TTV genomes tested (Kamahora et al. 2000; Leppik et al. 2007; Okamoto et al. 2000a; Qiu et al. 2005).

Approximately half of the TTV mRNAs of HEL32 (genotype 6) are unspliced at the second intron, which generates the abundant 2.8-kb mRNA. The ratio of mRNAs spliced at 2A1 relative to mRNAs spliced at 2A2 is approx. 1:10. The 2.8-, 1.2-, and 1.0-kb mRNAs comprise approx. 60%, 5%, and 35% of the total TTV RNA, respectively (Qiu et al. 2005). Consistent with this observation, it has been shown that during TTV genotype-1 infection of bone marrow cells, the large mRNA was the predominant mRNA (Okamoto et al. 2000a).

The polyadenylation signal (AUUAAA) at nt 2978 of the HEL32 TTV isolate is nonconsensus but functional (Qiu et al. 2005), while the isolates VT416 and TYM9 use a canonical polyadenylation site (Kamahora et al. 2000; Okamoto

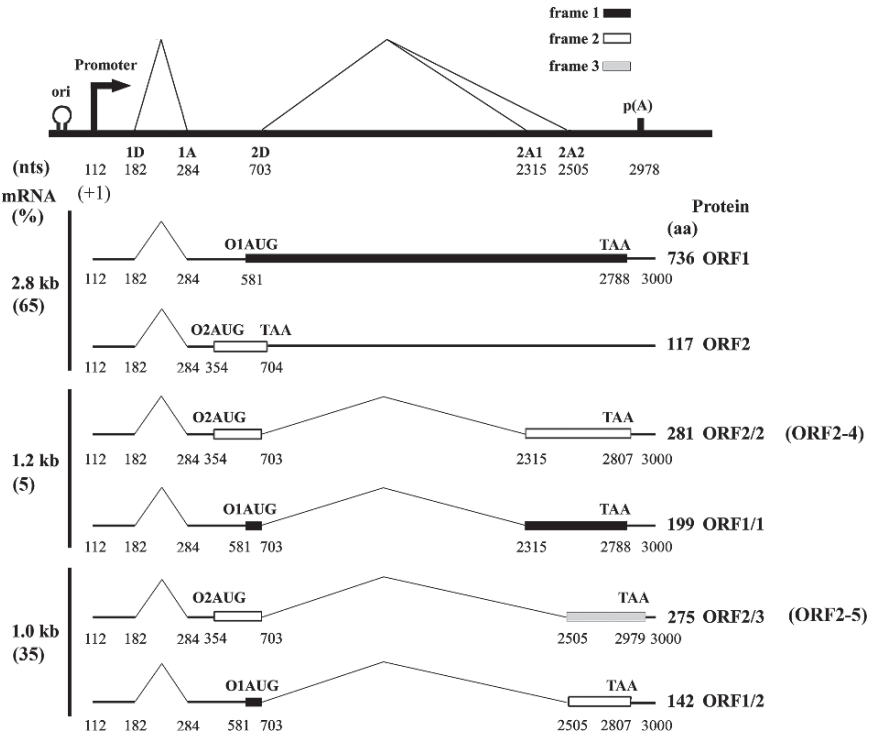

Fig. 1 The genetic map of TTV genotype 6. The RNA initiation site (*+1*), splice donors (*D*), and acceptors (*A*), and the polyadenylation signal, *p(A)*, are shown with nucleotide numbers (GenBank accession number AY666122). The three species of TTV mRNA are shown with their relative sizes and abundance (% of total) on the *left*. Six ORF expression strategies are diagrammed in the map. The ORF1 and ORF2 proteins are encoded from the singly spliced 2.8-kb mRNA by alternative translation, using O1AUG at nt 581 and O2AUG at nt 354, respectively. The ORF2/2 and ORF2/3 proteins (referred to as *ORF2–4* and *ORF2–5* proteins, respectively, by Kamahora et al. 2000) are initiated at the O2AUG, and are translated from the doubly spliced 1.2- and 1.0-kb mRNAs, respectively. ORF1/1 and ORF1/2 proteins are encoded by the 1.2- and 1.0-kb mRNAs, respectively, using the O1AUG at nt 581. The different reading frames are indicated by different *fill patterns*. (Adapted from Qiu et al. 2005)

et al. 2000a). All the TTV RNAs are cleaved at the same site at approx. nt 3000 (Qiu et al. 2005).

The transcription map of the TTV genotype 1 was determined by transfection of a clone of VT416, and is shown in Fig. 2. Analysis of the sequences of the donor and acceptor sites in 16 TTVs from various genotypes retrieved from the database showed that all the splice sites use the conserved GT–AG donor and acceptor sequences (Kamahora et al. 2000).

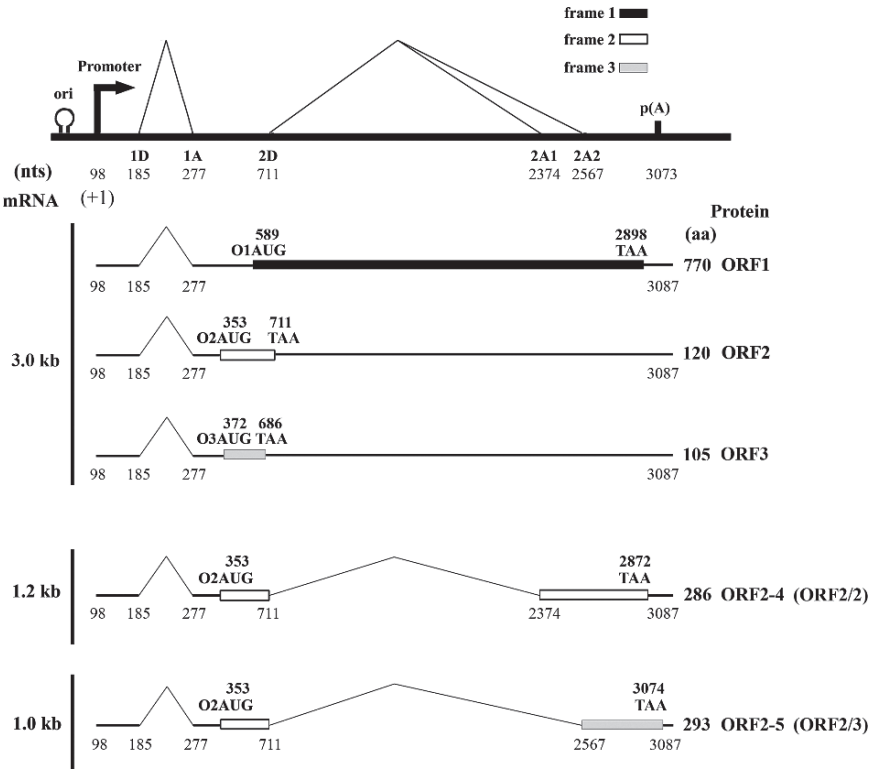

Fig. 2 The genetic map of TTV genotype 1. The RNA initiation site, splice junctions, and the polyadenylation signal are shown with nucleotide numbers (GenBank accession number NC_002076). The three species of TTV mRNAs with the relative sizes are shown on the *left*. Five predicted ORF expression strategies are diagrammed in the map. The ORF1, ORF2, and ORF3 proteins are predicted to be encoded from the singly spliced 3.0-kb mRNA by alternative translation, using O1AUG at nt 589, O2AUG at nt 353 and O3AUG at nt 372, respectively. The *ORF2–4* and *ORF2–5* proteins are equivalent to the *ORF2/2* and *ORF2/3* proteins, respectively, of genotype 6 as diagrammed in Fig. 1. The proteins are predicted from the coding capabilities of the genomes of TTVs (TA278 and VT416, genotype 1). (Adapted from Kamahora et al. 2000)

Analysis of RNA transcripts produced in the L428 Hodgkin's lymphoma cells transfected with isolates tth7 and tth8 (genogroup 5) confirmed the presence of the above-described TTV mRNAs (Leppik et al. 2007). However, 12 additional transcripts were also identified by RT-PCR. These transcripts were suggested to have resulted from additional splicing events and also from intragenomic rearrangements (leading to subgenomic isolates) within the TTV genome. Transfection of T cell leukemia-, Burkitt's lymphoma-, kidney-, and liver-derived cell lines (Jurkat, HSB2, BJAB, Raji, 293, HepG2) resulted in similar transcription profiles with both tth7 and tth8 isolates. Interestingly, differences between cell lines were observed; the T cell leukemia cell line HSB2 showed no TTV transcription, and 2/12 transcripts were produced specifically only in L428 cells (Leppik et al. 2007). This

variation in transcription, possibly resulting in translation of a variety of proteins from different TTV strains with different cell types, could have implications on the biological and/or pathogenetic differences of the TTV strains.

Protein Expression of TTV

The genome of TTV is a circular single-stranded negative-sense DNA of approx. 3,800 nt in length. Thus expression of TTV mRNA must employ efficient strategies, including both alternative splicing and alternative translation initiation. All three TTV mRNAs use alternative AUGs for translation, and therefore at least six TTV proteins are expressed in genotype 6 (Qiu et al. 2005). Antibodies in TTV-infected individuals have been detected toward all six proteins of the HEL32 isolate of genotype 6 (Kakkola et al. 2002; Kakkola et al. in press), and toward the N- and C-termini of the ORF1-encoded protein of genotype-1 isolates (Handa et al. 2000; Ott et al. 2000).

The abundant 2.8-kb mRNA of HEL32 expresses the ORF1 and ORF2 proteins by alternative AUG usage. The TTV ORF1 protein (~81 kDa) is the largest encoded by TTV mRNA (Fig. 1), and (for genotype 6) is predicted to be 736 amino acids (aa) in length, initiating at the AUG at nt 581 (O1AUG). The ORF2 protein (~13 kDa; Fig. 1) is encoded by the 2.8-kb mRNA in the second ORF, initiating at the AUG at nt 354 (O2AUG) and extending for 117aa (Qiu et al. 2005). Although the Kozak translation signal of the O1AUG (ORF1) in most of the TTV isolates represents more of a consensus sequence (a/gcc AUG g) than that of the O2AUG (Kamahora et al. 2000), the relative usage of these two AUGs has not been conclusively determined.

The small 1.2-kb mRNA of HEL32 expresses TTV proteins ORF2/2 and ORF1/1 with sizes of 31 and 22 kDa, respectively; the smallest 1.0-kb mRNA expresses TTV proteins ORF2/3 and ORF1/2 with sizes of 30 and 16 kDa respectively. Expressions of these proteins were confirmed by transfection of the TTV clone in 293 cells (Qiu et al. 2005). The presence of ORF2 (nt 2315–2807) and ORF3 (nt 2502–2979) at the 3′ termini of both the 1.2- and 1.0-kb mRNAs in virtually all TTV sequences in the database supports their usage during the viral life cycle. The 1.0-kb spliced mRNAs of HEL32, which use the 2A2 splice acceptor and encode the ORF2/3 and ORF1/2 proteins, are approx. 7 times more abundant than the 1.2-kb spliced mRNAs that use the 2A1 acceptor and encode the ORF2/2 and ORF1/1 proteins (Fig. 1). Whether these differences can be seen also in the corresponding protein abundances is unknown. The transcription maps of the three different isolates, VT416 of genotype 1, HEL32 of genotype 6 (both belonging to genogroup 1), and isolate TYM9 (belonging to genogroup 3) are similar (Kamahora et al. 2000; Okamoto et al. 2000a; Qiu et al. 2005), and this is likely to hold true also for the other TTV genotypes. Indeed, similarities have been shown also in representatives of genogroup 5 (isolates tth7 and tth8) in which, however, additional transcripts—produced by variant splice sites and by intragenomic rearrangements—have been detected (Leppik et al. 2007).

Functions of TTV Proteins

The functions of the TTV proteins are poorly understood since neither a virus culture system nor an animal model is available. Furthermore, there are as yet no reports of TTV purification, whereby the structural proteins forming the virus capsid have not been identified. An animal circovirus, chicken anemia virus (CAV), which causes severe immunosuppression, anemia, and thrombocytopenia (Noteborn et al. 1991), has one structural protein, VP1. The TTV ORF1 and ORF2 encoded proteins have been shown (for the HEL32 isolate) to be predominantly localized in the cytoplasm (Qiu et al. 2005), and the former is assumed to be a structural and replication-associated protein based on similarities with CAV VP1, i.e., possessing an arginine-rich N-terminus and Rep-motifs (Erker et al. 1999; Mushahwar et al. 1999; Tanaka et al. 2001). The expression level of ORF1 was, for an unknown reason, extremely low following transfection of the full-length clone of genotype 6 into 293 cells (Qiu et al. 2005). In HEL32 the two ORF1-related proteins, ORF1/1 and ORF1/2, are distributed evenly both in the cytoplasm and the nucleus, and when compared to the ORF2-containing genes (described below), are transcribed at a low level (Qiu et al. 2005). Interestingly, TTV (isolate TRM1, genotype 1) *ORF1*-transgenic mice have morphological changes in renal epithelial cells. The expression of the ORF1 gene in mice affected the maturation of renal epithelial cells in a dose-dependent manner. However, it was not determined which of the three ORF1-containing proteins was responsible for the changes, as small spliced mRNAs with a potential to express ORF1/1 or ORF1/2 proteins were also detected (Yokoyama et al. 2002).

The TTV ORF2 protein (SANBAN of genogroup 3) has been studied in cells transfected with ORF2 complementary DNA (cDNA) (Zheng et al. 2007). Following transfection into human cell lines the TTV ORF2 protein was shown to suppress both the canonical and the noncanonical nuclear factor (NF)-κB pathways by interacting with the catalytic subunits (inhibitor of κB[1κB] kinase) IKKα and IKKβ of the IκBα kinase complex, thus inhibiting its degradation. By hindering NF-κB from reaching the nucleus the TTV ORF2 protein also indirectly decreased the expression of the inflammation factors interleukin (IL)-6, IL-8, and cyclo-oxygenase (COX)-2. NF-κB activation is a protective response of the host to viral pathogens. This suggests that the TTV ORF2 protein may be involved in the regulation of the host innate and adaptive immunity. Thus, the role of the ORF2 protein in immune evasion may be an important aspect of TTV biology.

CAV VP2 possesses a functional protein-tyrosine phosphatase (PTPase)-like domain (Peters et al. 2002; Takahashi et al. 2000). The ORF2 proteins of TTV, SAV/TT midi virus (TTMDV) and Torque teno minivirus (TTMV) also share a high similarity with the CAV VP2 (Andreoli et al. 2006; Biagini et al. 2001; Takahashi et al. 2000), of which the latter has been shown to exert phosphatase activity (Peters et al. 2002). Protein phosphatases are known to have important functions in the regulation of gene transcription, signal transduction, and mitogenesis, and in cytokine responses of lymphocytes (Ong et al. 1997; Schievella et al. 1993). The CAV VP2 PTPase activity may regulate these events in infected lymphocytes, which could lead to profound immunosuppression and anemia in chickens

(Noteborn et al. 1991; Peters et al. 2002). The five TTV ORF2 coding sequences of the TTV SANBAN group (genogroup 3) have a high amino acid similarity (53%–55%), with an identity of 22%–26%, with the CAV VP2 (Peters et al. 2002). However, the sequence variation of TTV ORF2 may interfere with the PTPase function, which could contribute to the pathogenesis of different anelloviruses.

The ORF2/2 and ORF2/3 proteins of HEL32, genotype 6 [corresponding to the respective ORF2-ORF4 and ORF2-ORF5 proteins of genotype 1 (Kamahora et al. 2000)] are exclusively localized in discrete foci in the nucleus (Qiu et al. 2005) implying roles in genome expression and replication. Both contain the ORF2 protein at the N-termini, with the ORF2/3 transcript being the most abundant (Qiu et al. 2005). Considering the high expression level of the ORF2-containing TTV transcripts of HEL32 (ORF2, ORF2/2 and ORF2/3), it will be interesting to determine whether all of these corresponding proteins have PTPase activities and to understand their roles in TTV pathogenesis. The ORF2/2 protein not only shares the N-terminus with the ORF2 protein (Kamahora et al. 2000; Qiu et al. 2005), but also has a serine-rich domain at the C-terminus (Asabe et al. 2001), capable of generating different phosphorylation sites, similar to hepatitis C virus (HCV) NS5A (Tellinghuisen et al. 2008). This suggests that a phosphorylated version of the ORF2/2 protein may play a similar role in binding the DNA template as does the NS5A of HCV in viral replication.

In TTV genotype 1, isolate TA278, an additional protein at nt 372–686 in reading frame 3 was proposed to be encoded from the N-terminus of the 3.0-kb mRNA (Fig. 2). Due to the similarity to apoptin, the main apoptosis-inducing agent of CAV (Kooistra et al. 2004; Miyata et al. 1999), this putative 105-aa protein (~12 kDa) was termed TTV-derived apoptosis-inducing protein (TAIP). Both TAIP and apoptin induced p53-independent apoptosis in human hepatocellular carcinoma (HCC) cell lines; however, unlike apoptin, TAIP was only weakly apoptotic in other human cancer cell lines (Kooistra et al. 2004). Interestingly, the 5′-end of the ORF3-encoding region in e.g., HEL32 (genotype 6) is interrupted with stop codons, whereby the TAIP-related ORF3 protein is not expressed from TTV genotype 6. The activity of TAIP, coupled with the heterogeneity of TTV isolates, could contribute to some extent to the putatively variable pathogenesis of TTV.

Overall, our knowledge of the function of TTV proteins is to a large extent limited by the lack of an efficient cell culture system and by restricted availability of the virus itself. However, it is possible that, for example, the multifunctional nature of TTV ORF2-containing proteins could confer pathogenetic diversity to this large genus of anelloviruses.

References

Andreoli E, Maggi F, Pistello M, et al (2006) Small anellovirus in hepatitis C patients and healthy controls. Emerg Infect Dis 12:1175–1176

Asabe S, Nishizawa T, Iwanari H, Okamoto H (2001) Phosphorylation of serine-rich protein encoded by open reading frame 3 of the TT virus genome. Biochem Biophys Res Commun 286:298–304

Bando M, Ohno S, Oshikawa K, et al (2001) Infection of TT virus in patients with idiopathic pulmonary fibrosis. Respir Med 95:935–942

Biagini P, Gallian P, Attoui H, et al (2001) Genetic analysis of full-length genomes and subgenomic sequences of TT virus-like mini virus human isolates. J Gen Virol 82:379–383

Chan PK, Chik KW, Li CK, et al (2001a) Prevalence and genotype distribution of TT virus in various specimen types from thalassaemic patients. J Viral Hepat 8:304–309

Chan PK, Chik KW, To KF, et al (2001b) Clearance of TT virus after allogeneic bone marrow transplantation. J Pediatr Hematol Oncol 23:57–58

Cheng J, Hada T, Liu W, et al (2000) Investigation of TTV by in situ hybridization in patients with chronic hepatitis. Hepatol Res 18:43–53

Comar M, Ansaldi F, Morandi L, et al (2002) In situ polymerase chain reaction detection of transfusion-transmitted virus in liver biopsy. J Viral Hepat 9:123–127

Deng X, Terunuma H, Handema R, et al (2000) Higher prevalence and viral load of TT virus in saliva than in the corresponding serum: another possible transmission route and replication site of TT virus. J Med Virol 62:531–537

Desai M, Pal R, Deshmukh R, Banker D (2005) Replication of TT virus in hepatocyte and leucocyte cell lines. J Med Virol 77:136–143

Erker JC, Leary TP, Desai SM, et al (1999) Analyses of TT virus full-length genomic sequences. J Gen Virol 80:1743–1750

Gallian P, Biagini P, Zhong S, et al (2000) TT virus: a study of molecular epidemiology and transmission of genotypes 1, 2 and 3. J Clin Virol 17:43–49

Garbuglia AR, Iezzi T, Capobianchi MR, et al (2003) Detection of TT virus in lymph node biopsies of B-cell lymphoma and Hodgkin's disease, and its association with EBV infection. Int J Immunopathol Pharmacol 16:109–118

Handa A, Dickstein B, Young NS, Brown KE (2000) Prevalence of the newly described human circovirus, TTV, in United States blood donors. Transfusion 40:245–251

Hijikata M, Takahashi K, Mishiro S (1999) Complete circular DNA genome of a TT virus variant (isolate name SANBAN) and 44 partial ORF2 sequences implicating a great degree of diversity beyond genotypes. Virology 260:17–22

Jiang XJ, Luo KX, He HT (2000) Intrahepatic transfusion-transmitted virus detected by in situ hybridization in patients with liver diseases. J Viral Hepat 7:292–296

Kakkola L, Hedman K, Vanrobaeys H, et al (2002) Cloning and sequencing of TT virus genotype 6 and expression of antigenic open reading frame 2 proteins. J Gen Virol 83:979–990

Kakkola L, Tommiska J, Boele LC, et al (2007) Construction and biological activity of a full-length molecular clone of human Torque teno virus (TTV) genotype 6. FEBS J 274:4719–4730

Kakkola L, Bondén H, Hedman L, et al (in press) Expression of all six human Torque teno virus (TTV) proteins in bacteria and in insect cells, and analysis of their ig6 responses. Virology.

Kamada K, Kamahora T, Kabat P, Hino S (2004) Transcriptional regulation of TT virus: promoter and enhancer regions in the 1.2-kb noncoding region. Virology 321:341–348

Kamahora T, Hino S, Miyata H (2000) Three spliced mRNAs of TT virus transcribed from a plasmid containing the entire genome in COS1 cells. J Virol 74:9980–9986

Kanda Y, Tanaka Y, Kami M, et al (1999) TT virus in bone marrow transplant recipients. Blood 93:2485–2490

Kooistra K, Zhang YH, Henriquez NV, et al (2004) TT virus-derived apoptosis-inducing protein induces apoptosis preferentially in hepatocellular carcinoma-derived cells. J Gen Virol 85:1445–1450

Leppik L, Gunst K, Lehtinen M, et al (2007) In vivo and in vitro intragenomic rearrangement of TT viruses. J Virol 81:9346–9356

Lopez-Alcorocho JM, Mariscal LF, de Lucas S, et al (2000) Presence of TTV DNA in serum, liver and peripheral blood mononuclear cells from patients with chronic hepatitis. J Viral Hepat 7:440–447

Luo K, Liang W, He H, et al (2000) Experimental infection of nonenveloped DNA virus (TTV) in rhesus monkey. J Med Virol 61:159–164

Maggi F, Fornai C, Zaccaro L, et al (2001a) TT virus (TTV) loads associated with different peripheral blood cell types and evidence for TTV replication in activated mononuclear cells. J Med Virol 64:190–194

Maggi F, Pistello M, Vatteroni M, et al (2001b) Dynamics of persistent TT virus infection, as determined in patients treated with alpha interferon for concomitant hepatitis C virus infection. J Virol 75:11999–12004

Maggi F, Pifferi M, Fornai C, et al (2003) TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity. J Virol 77:2418–2425

Mankertz A, Mankertz J, Wolf K, Buhk HJ (1998) Identification of a protein essential for replication of porcine circovirus. J Gen Virol 79:381–384

Mankertz A, Caliskan R, Hattermann K, et al (2004) Molecular biology of Porcine circovirus: analyses of gene expression and viral replication. Vet Microbiol 98:81–88

Mariscal LF, Lopez-Alcorocho JM, Rodriguez-Inigo E, et al (2002) TT virus replicates in stimulated but not in nonstimulated peripheral blood mononuclear cells. Virology 301:121–129

Miyata H, Tsunoda H, Kazi A, et al (1999) Identification of a novel GC-rich 113-nucleotide region to complete the circular, single-stranded DNA genome of TT virus, the first human circovirus. J Virol 73:3582–3586

Mushahwar IK, Erker JC, Muerhoff AS, et al (1999) Molecular and biophysical characterization of TT virus: evidence for a new virus family infecting humans. Proc Natl Acad Sci U S A 96:3177–3182

Niagro FD, Forsthoefel AN, Lawther RP, et al (1998) Beak and feather disease virus and porcine circovirus genomes: intermediates between the geminiviruses and plant circoviruses. Arch Virol 143:1723–1744

Noteborn MH, de Boer GF, van Roozelaar DJ, et al (1991) Characterization of cloned chicken anemia virus DNA that contains all elements for the infectious replication cycle. J Virol 65:3131–3139

Ohbayashi H, Tanaka Y, Ohoka S, et al (2001) TT virus is shown in the liver by in situ hybridization with a PCR-generated probe from the serum TTV-DNA. J Gastroenterol Hepatol 16:424–428

Okamoto H, Kato N, Iizuka H, et al (1999) Distinct genotypes of a nonenveloped DNA virus associated with posttransfusion non-A to G hepatitis (TT virus) in plasma and peripheral blood mononuclear cells. J Med Virol 57:252–258

Okamoto H, Nishizawa T, Tawara A, et al (2000a) TT virus mRNAs detected in the bone marrow cells from an infected individual. Biochem Biophys Res Commun 279:700–707

Okamoto H, Takahashi M, Nishizawa T, et al (2000b) Replicative forms of TT virus DNA in bone marrow cells. Biochem Biophys Res Commun 270:657–662

Okamoto H, Ukita M, Nishizawa T, et al (2000c) Circular double-stranded forms of TT virus DNA in the liver. J Virol 74:5161–5167

Okamoto H, Nishizawa T, Takahashi M, et al (2001) Heterogeneous distribution of TT virus of distinct genotypes in multiple tissues from infected humans. Virology 288:358–368

Ong CJ, Dutz JP, Chui D, et al (1997) CD45 enhances positive selection and is expressed at a high level in large, cycling, positively selected CD4+CD8+ thymocytes. Immunology 91:95–103

Ott C, Duret L, Chemin I, et al (2000) Use of a TT virus ORF1 recombinant protein to detect anti-TT virus antibodies in human sera. J Gen Virol 81:2949–2958

Peng YH, Nishizawa T, Takahashi M, et al (2002) Analysis of the entire genomes of thirteen TT virus variants classifiable into the fourth and fifth genetic groups, isolated from viremic infants. Arch Virol 147:21–41

Peters MA, Jackson DC, Crabb BS, Browning GF (2002) Chicken anemia virus VP2 is a novel dual specificity protein phosphatase. J Biol Chem 277:39566–39573

Qiu J, Kakkola L, Cheng F, et al (2005) Human circovirus TT virus genotype 6 expresses six proteins following transfection of a full-length clone. J Virol 79:6505–6510

Rodriguez-Inigo E, Casqueiro M, Bartolome J, et al (2000) Detection of TT virus DNA in liver biopsies by in situ hybridization. Am J Pathol 156:1227–1234

Rodriguez-Inigo E, Arrieta JJ, Casqueiro M, et al (2001) TT virus detection in oral lichen planus lesions. J Med Virol 64:183–189

Schievella AR, Paige LA, Johnson KA, et al (1993) Protein tyrosine phosphatase 1B undergoes mitosis-specific phosphorylation on serine. Cell Growth Differ 4:239–246

Takahashi K, Hijikata M, Samokhvalov EI, Mishiro S (2000) Full or near full length nucleotide sequences of TT virus variants (Types SANBAN and YONBAN) and the TT virus-like mini virus. Intervirology 43:119–123

Takahashi M, Asabe S, Gotanda Y, et al (2002) TT virus is distributed in various leukocyte subpopulations at distinct levels, with the highest viral load in granulocytes. Biochem Biophys Res Commun 290:242–248

Tanaka Y, Primi D, Wang RY, et al (2001) Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family. J Infect Dis 183:359–367

Tellinghuisen TL, Foss KL, Treadaway J (2008) Regulation of hepatitis C virion production via phosphorylation of the NS5A protein. PLoS Pathog 4:e1000032

Todd D, Bendinelli M, Biagini P, et al (2004) Circoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds) Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London

Xiao H, Luo K, Yang S, et al (2002) Tissue tropism of the TTV in experimentally infected rhesus monkeys. Chin Med J (Engl) 115:1088–1090

Yokoyama H, Yasuda J, Okamoto H, Iwakura Y (2002) Pathological changes of renal epithelial cells in mice transgenic for the TT virus ORF1 gene. J Gen Virol 83:141–150

Yu Q, Shiramizu B, Nerurkar VR, et al (2002) TT virus: preferential distribution in CD19(+) peripheral blood mononuclear cells and lack of viral integration. J Med Virol 66:276–284

Zheng H, Ye L, Fang X, et al (2007) Torque teno virus (SANBAN isolate) ORF2 protein suppresses NF-kappaB pathways via interaction with IkappaB kinases. J Virol 81:11917–11924

Zhong S, Yeo W, Tang M, et al (2002) Frequent detection of the replicative form of TT virus DNA in peripheral blood mononuclear cells and bone marrow cells in cancer patients. J Med Virol 66:428–434