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Filovirus replication and transcription

Elke Mühlberger

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The highly pathogenic filoviruses, Marburg and Ebola virus, belong to the nonsegmented negative-sense RNA viruses of the order Mononegavirales. The mode of replication and transcription is similar for these viruses. On one hand, the negative-sense RNA genome serves as a template for replication, to generate progeny genomes, and, on the other hand, for transcription, to produce mRNAs. Despite the similarities in the replication/transcription strategy, filoviruses have evolved structural and functional properties that are unique among the nonsegmented negative-sense RNA viruses. Moreover, there are also striking differences in the replication and transcription mechanisms of Marburg and Ebola virus. This includes nucleocapsid formation, the structure of the genomic replication promoter, the protein requirement for transcription and the use of mRNA editing. In this article, the current knowledge of the replication and transcription strategy of Marburg and Ebola virus is reviewed, with focus on the observed differences.

Marburg (MARV) and Ebola virus (EBOV) cause severe hemorrhagic fever in humans and nonhuman primates. With mortality rates as high as 90%, these viruses represent some of the most deadly human pathogens [1]. Owing to their high pathogenicity and the lack of either vaccines or effective therapies, MARV and EBOV are classified as biosafety level four agents.

MARV and EBOV comprise the family *Filoviridae* that, together with the paramyxo-, rhabdo- and borna-viruses, belong to the order Mononegavirales. All viruses within this order are enveloped and contain a nonsegmented negative-strand (NNS) RNA genome. The family *Filoviridae* is divided into two genera: *Ebolavirus,* with four distinct species (*Zaire*, *Sudan*, *Côte d'Ivoire* and *Reston ebolavirus*), and *Marburgvirus,* with the single species *Lake Victoria marburgvirus* [2].

Genome organization & viral proteins

The genome organization of the different filoviruses is rather similar. Seven viral genes are arranged tandemly on the negative-sense RNA genome, which is approximately 19 kb in length (Figure 1A). The genes contain the respective open reading frame (ORF) flanked by unusually long nontranslated sequences, ranging from 57 to 684 nucleotides. Among the NNS RNA viruses, this feature has only been observed for filoviruses and Nipah and Hendra viruses [3]. Thus far, the function of these long untranslated regions is not known. At the beginning and

end of each gene there are highly conserved transcription start and stop signals. The genes are either separated by short intergenic regions spanning 4–7 nonconserved nucleotides or they overlap. There is only one longer intergenic region located between the *GP* and *VP30* gene in the case of MARV and between the *VP30* and *VP24* gene in the case of the different EBOV species. The genome ends consist of short nontranscribed regions containing cis-acting signals important for replication, transcription initiation and encapsidation of the genomic RNA [4–7].
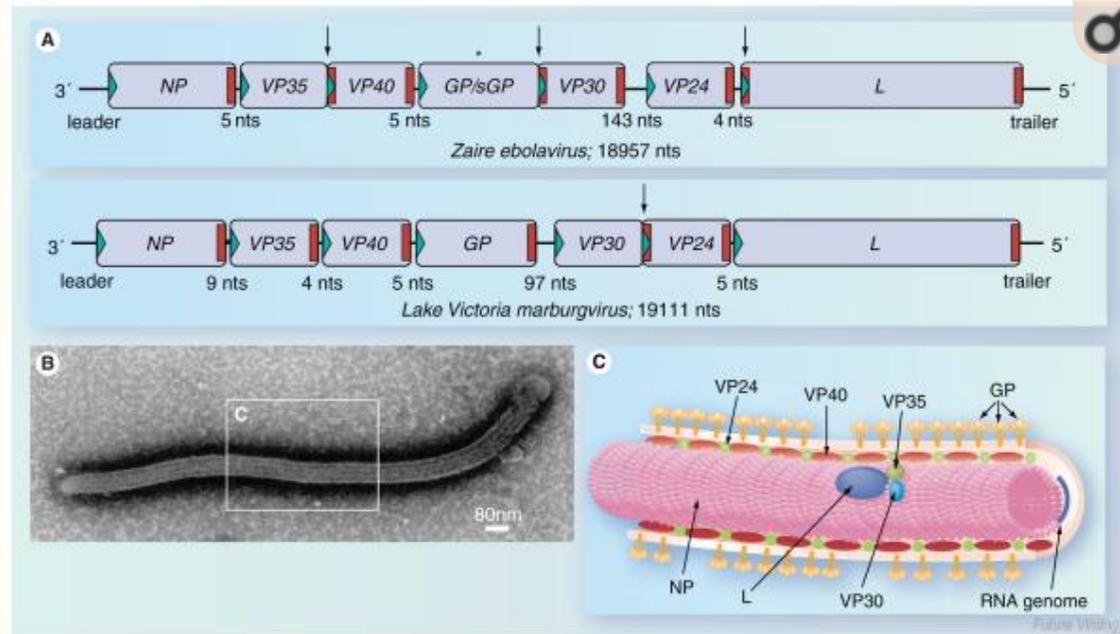
[Figure 1](#)

Filovirus genome organization and structure

(A) Schematic diagram of Ebola virus (EBOV) and Marburg virus (MARV) genomes. The genes are depicted as boxes and nontranscribed regions as black bars (leader, trailer and intergenic regions). Transcription start signals are depicted as green triangles and stop signals as red bars. Gene overlaps are marked by arrows. The mRNA editing site within the EBOV GP gene is indicated by an asterisk. The length of the intergenic regions is shown below the scheme. Note that the EBOV VP24 gene contains two transcription stop signals. **(B)** Electron micrograph of an EBOV particle. **(C)** Schematic presentation of EBOV structure. The RNA genome is encapsidated

by nucleocapsid proteins NP, VP35, VP30 and L. VP40 and VP24 are matrix proteins. GP trimers are inserted into the viral membrane.

GP: Glycoprotein; L: RNA-dependent RNA polymerase; NP: Nucleoprotein; VP: Viral protein.

Filoviruses encode seven structural proteins (Figure 1B & C). While five of these proteins, the nucleoprotein (NP), the viral proteins (VP) 35 and 40, the glycoprotein (GP) and the RNA-dependent RNA polymerase (L) are counterparts of proteins found in all NNS RNA viruses, VP24 appears to be unique to filoviruses, and a protein similar to VP30 is only described for the pneumoviruses [8].

The only surface protein, GP, is inserted into the viral membrane and mediates receptor binding and fusion during virus entry [9–11]. VP40 is the counterpart of the matrix proteins (M) of other NNS RNA viruses. It is a peripheral membrane protein located on the inner side of the membrane and plays a key role in the budding of viral particles (see below) [10,12]. The viral protein VP24 is presumed to be a minor matrix protein involved in nucleocapsid formation and assembly [13–17]. Since VP24 plays a crucial role in host tropism determination and is able to counteract the type I interferon response, it is considered to be an important virulence factor [18–20]. The four remaining proteins are tightly associated with the viral RNA genome, forming the nucleocapsid [21]. These are the NP, the polymerase cofactor VP35, the transcription activator VP30, and the major component of the RNA-dependent RNA polymerase, L (Figure 1C). The nucleocapsid proteins play a dual role in the viral replication cycle; they are structural components of the nucleocapsid complex and are, therefore, involved in viral morphogenesis and catalyze the replication and transcription of the RNA genome [22–25].

Additional functions have been determined for VP35, which has been shown to act as a type I interferon antagonist by interfering with retinoic acid inducible gene I-dependent activation of the interferon regulatory factor 3 and to block the activation of the double-stranded RNA-dependent protein kinase [26–28].

EBOV genomes code for an additional protein, the soluble GP (sGP). As with GP, sGP is encoded by the fourth gene but is translated from nonedited mRNA species [29]. sGP is not incorporated into viral particles but is secreted from infected cells. The function of the protein is not fully understood. A putative role of sGP in immune modulation has been discussed

Genetic diversity between human metapneumovirus subgroup [Virology. 2003]

Conserved receptor-binding domains of Lake Victoria marburg [J Biol Chem. 2006]

Review Biosynthesis and role of filoviral glycoproteins. [J Gen Virol. 2001]

controversially [30–32]. Recently, it has been shown to act as an anti-inflammatory factor by protecting the endothelial cell barrier function during infection [32].

Filovirus replication & transcription

Filovirus replication and transcription take place in the cytoplasm of the infected cells. The viruses encode their own RNA-dependent RNA polymerase, which recognizes the encapsidated negative-strand RNA genome as a template for replication and transcription. This replication and transcription strategy resembles that of other NNS RNA viruses [33]. However, there are some features unique to filoviruses that will be described in this review.

Nucleocapsid assembly

The driving force for RNA encapsidation is NP. MARV NP forms large helical structures resembling the nucleocapsids found in mature virions [22,34]. For EBOV, the minimal protein requirements for the formation of nucleocapsid-like structures are NP, VP35 and VP24 [17,35]. Recently, Noda and colleagues have proposed a model for EBOV nucleocapsid formation and subsequent budding [23]. According to this model, NP forms helical tubes which differ from nucleocapsid-like structures. VP35 and VP24 interact with the pre-formed NP-derived helices, resulting in the formation of nucleocapsid-like structures [36]. Both transport of the nucleocapsids to the plasma membrane and incorporation into virions are mediated by the interaction of NP with VP40 [23,37]. In addition, Johnson and colleagues have shown that EBOV VP35 interacts with the matrix protein VP40 and EBOV minigenomes, thus mediating the packaging of the minigenome RNA into virus-like particles [38].

Filovirus replication

Protein requirements Replication of the RNA genome begins with the synthesis of a positive-sense replicative intermediate, the antigenome, which is the reverse complement of the RNA genome. The antigenome, in turn, serves as a template for the generation of new genomes. Both the genomes and the antigenomes are encapsidated by the nucleocapsid proteins (Figure 2) [24,25].

Review Filovirus budding.
[Virus Res. 2004]

Review Filovirus assembly and budding.
[Virology. 2006]

VP24 of Marburg virus influences formation of infectious particl [J Virol. 2005]

Nucleocapsid-like structures of Ebola virus reconstructed using ε [J Vet Med Sci. 2005]

Molecular characterization of guinea pig-adapted variants of Ebola v [Virology. 2000]

Ultrastructural organization of recombinant Marburg virus nucleoprotein: [J Virol. 2000]

Ebola virus secretory glycoprotein (sGP) diminishes Fc gamma Rl [J Immunol. 2000]

Role of Ebola virus secreted glycoproteins and virus-like particles in acti [J Virol. 2005]

Review Transcription and replication of nonseg [Curr Top Microbiol Immunol. 2004]
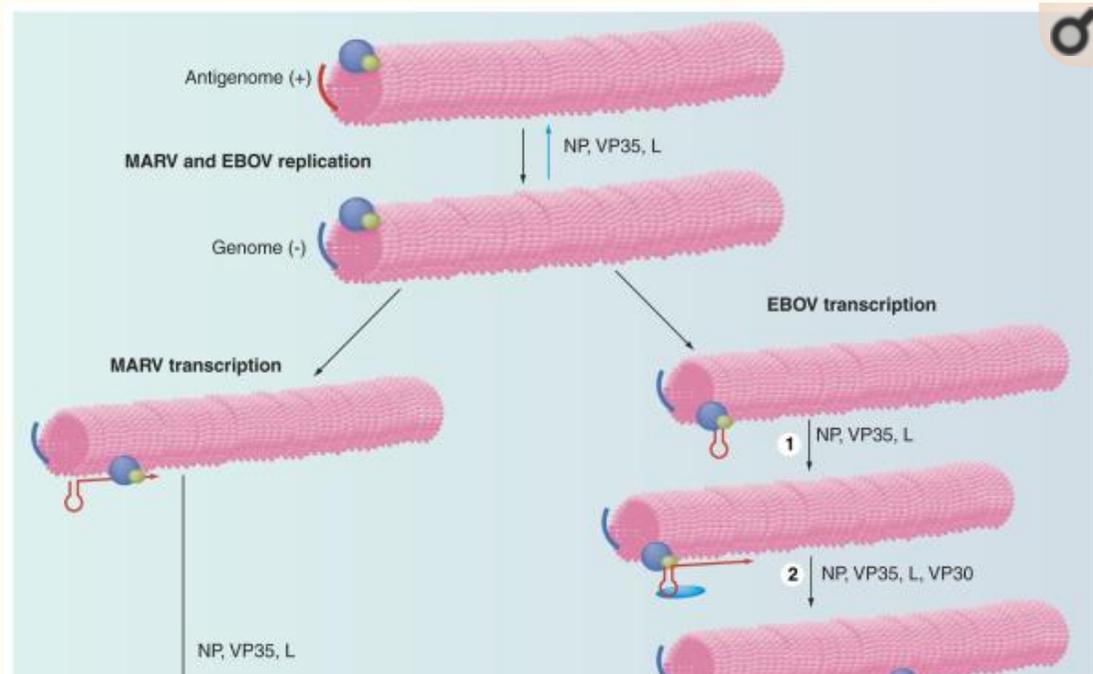
Ultrastructural organization of recombinant Marburg virus nucleoprotein: [J Virol. 2000]

Morphology of Marburg virus NP-RNA. [Virology. 2002]

Nucleocapsid-like structures of Ebola virus reconstructed using e [J Vet Med Sci. 2005]

The assembly of Ebola virus nucleocapsid requires virion-associated [Mol Cell. 2002]

Functional mapping of the nucleoprotein of Ebola virus. [J Virol. 2006]

Mapping of the VP40-binding regions of the nucleoprotein of Ebola vi [J Virol. 2007]

Ebola virus VP35-VP40 interaction is sufficient for packaging 3E-5 [J Virol. 2006]

Figure 2

Hypothetical model of filovirus replication and transcription

Transcription results in the synthesis of seven monocistronic mRNAs that are capped and polyadenylated. The mRNAs are not encapsidated. For MARV transcription, NP, VP35 and L are sufficient. By contrast, EBOV transcription is dependent on the transcription activator VP30. Hypothetical model for EBOV transcription: **(1)** Transcription initiation: it is proposed that the polymerase consisting of L and VP35 initially binds to a single promoter site within the leader region and initiates transcription at the transcription start site of the first gene, the *NP* gene. Immediately after transcription initiation, the first 23 nucleotides of the nascent mRNA form a stable secondary structure (Figure 4A). In the absence of VP30, this RNA structure hampers movement of the polymerase along the RNA template and, consequently, the transcription complex pauses. **(2)** Transcription antitermination: the transcription block caused by the RNA secondary structure is abrogated by VP30 by a mechanism that is as yet unknown. It may be speculated that VP30 resolves the RNA structure or directs additional cofactors to the folded RNA. **(3)** Elongation and transcription reinitiation: transcription elongation and reinitiation at the following genes takes place independently of VP30, although each transcription start signal is

The replication and transcription processes of MARV and EBOV (species *Zaire ebolavirus* and *Reston ebolavirus*) have been investigated using reconstituted replication and transcription systems based on the simultaneous expression of the nucleocapsid protein genes and a virus-specific minireplicon in transfected cells (Box 1 & Figure 3) [24,25,39,40]. With the use of these systems it was demonstrated that three of the four nucleocapsid proteins were sufficient to support MARV and EBOV replication: NP, VP35 and L. For paramyxo- and rhabdovirus replication, similar minimum protein requirements have been described, consisting of the NP, the phosphoprotein (P) and the large protein (L) [33,41]. Thus, it is evident that the filoviral VP35 is the counterpart of the phosphoprotein of other NNS RNA viruses. The active polymerase complex is presumably composed of VP35 and L [25]. VP35 forms homo-oligomers, and interacts with L and NP [42–44]. While homo-oligomerization mediated by an aminoterminally located coiled-coil motif is a prerequisite for L binding, and, therefore, for an active polymerase complex, destruction of the oligomerization domain does not affect binding to NP-derived inclusion bodies [43]. In addition, triple complexes consisting of NP, VP35 and L have been identified, indicating that VP35 directs L to the encapsidated RNA genomes by acting as a linker protein between NP and L [42]. The L proteins of NNS RNA viruses are thought to contain all catalytic functions required for replication and transcription, such as polymerization, polyadenylation and methylation [45].
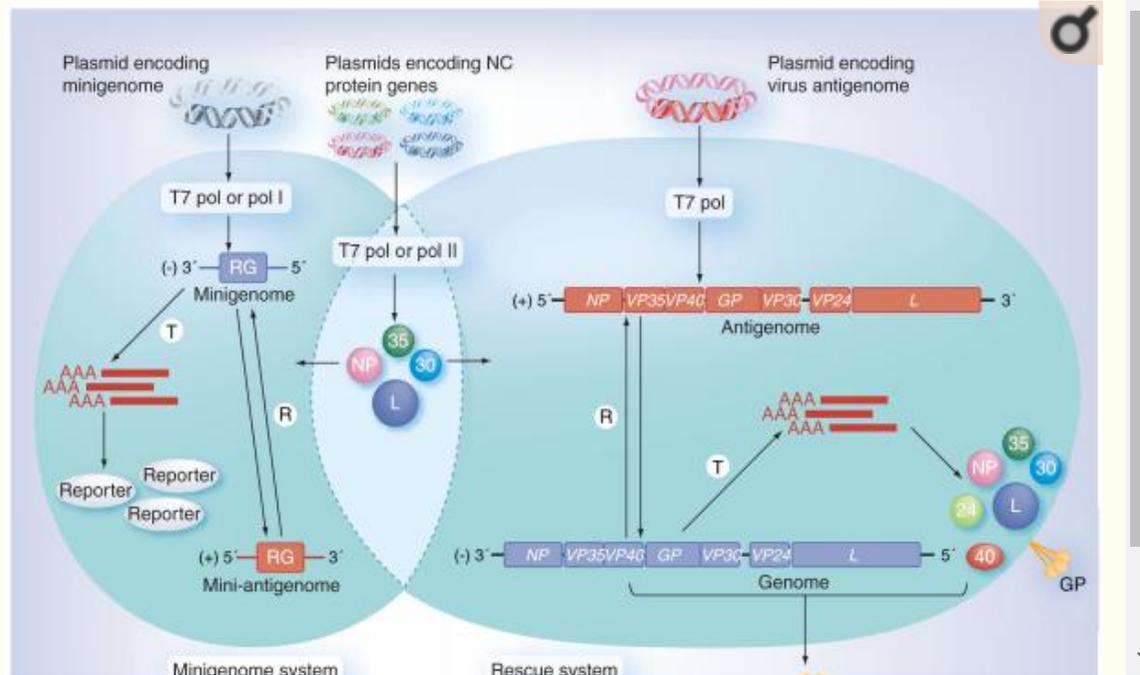
Figure 3

Schematic diagram of filovirus reverse genetics systems

Minigenome system (left): cells are transfected with plasmids encoding the nucleocapsid proteins along with a plasmid encoding a virus-specific minigenome. The minigenome consists of a RG flanked by the 3′ and 5′ ends of the viral genome containing the signals for replication, encapsidation and transcription. The minigenome is T and R by the nucleocapsid proteins, leading to RG expression (reporter). Rescue system (right): here, cells are transfected with plasmids encoding the nucleocapsid proteins, along with a plasmid encoding a full-length copy of the positive-sense viral antigenome. The antigenome is first R by the nucleocapsid proteins resulting in the generation of a negative-sense genome. The genome serves as a template for replication and transcription, leading to viral mRNA production and subsequent protein synthesis. Finally, the genomes are packaged by the viral proteins and released into mature infectious virus particles. The nucleocapsid protein genes, minigenomes and full-length antigenomes can be cloned under the control of different promoters (T7 pol, pol I and pol II). Negative-sense RNA is indicated in blue, positive-sense RNA in red.

A reconstituted replication and transcription system for Ebc [Virology. 2005]

Review Transcription and replication of nonseg [Curr Top Microbiol Immunol. 2004]

Review Reverse genetics of monone [Curr Top Microbiol Immunol. 2004]

Interactions of Marburg virus nucleocapsid proteins. [Virology. 1998]

GP: Glycoprotein; L: RNA-dependent RNA polymerase; NP: Nucleoprotein; pol I: RNA polymerase I; pol II: RNA polymerase II; R: Replicated; RG: Reporter gene; T7 pol: T7 RNA polymerase; T: Transcribed; VP: Viral protein.

Homo-oligomerization facilitates the interferon-antagonist activit [Virology. 2005]

Homo-oligomerization of Marburgvirus VP35 is essential for its funct [J Virol. 2005]

Sequence comparison of five polymerases (L proteins) of unsegme [J Gen Virol. 1990]

Genomic replication promoter The 3′ termini of filovirus genomes and anti-genomes contain the replication promoters for plus- and minus-sense RNA synthesis. Defective interfering particles generated by serial passaging of *Zaire ebolavirus* revealed a minimal promoter length of 155 and 176 nucleotides for the genomic and antigenomic RNA 3′ termini, respectively [46]. Since 13–23 nucleotides of the 3′ and 5′ termini of the filovirus genomes show a high degree of complementarity, base-pairing of the respective nucleotides could lead to the formation of a panhandle structure. Notably, the leader and trailer regions contain a second stretch of nucleotides complementary to the very end of the genome, approximately 34–40 nucleotides from either terminus, allowing the formation of an alternative internal hairpin loop [47]. Indeed, chemical modification assays performed with *in vitro* transcribed EBOV-specific RNA suggested the formation of an internal stem-loop structure within the leader region (Figure 4A) [48]. However, it remains to be elucidated if RNA secondary structure formation of the encapsidated genome really occurs and if it is necessary for replication [47].

Cis-acting elements on filovirus genomes

(A) RNA secondary structure formation at the 3′ terminus of MARV and EBOV genomes. The first predicted RNA secondary structure is located within the leader region of each genome, the second RNA stem-loop structure is formed by the respective first transcription start site and downstream-located sequences. The red lines running along the side of the stem-loops identify the transcription start signals. Predicted panhandle structures formed by complementary regions of the 3′ and 5′ ends are not shown. Nucleotide numbers refer to the genomic RNA of *Zaire ebolavirus*, strain Mayinga (accession number AF086833) and *Lake Victoria marburgvirus*, strain Musoke (accession number DQ217792), respectively. (B) Structure of the *Zaire ebolavirus* genomic replication promoter. The promoter is located at the 3′ end of the genome. It is bipartite consisting of PE 1 and 2. PE 1 and 2 are depicted as blue boxes. PE 1 and 2 are separated by a spacer (light blue) that contains the transcription start signal of the *NP* gene. PE 1 spans the leader region, PE 2 is located within the nontranslated region of the *NP* gene and consists of a stretch of eight UN_5 hexamers. The ORF of the *NP* gene is depicted in light gray. The nucleotide sequence of PE 2 is shown below the scheme. Conserved hexameric U residues are indicated in red. (C)

Ebola virus defective interfering particles and persistent infection. [Virology. 1999]

Analysis of the role of predicted RNA secondary structures in Eb [Virology. 2003]

Two typical gene boundaries within the EBOV genome. Transcription start signals are shown in green, transcription stop signals in red. Intergenic region is shown in blue, gene overlap in purple. A conserved pentamer, which is part of the transcription start signals as well as of the transcription stop signals, is boxed.

EBOV: Ebola virus; MARV: Marburgvirus; NP: Nucleoprotein; ORF: Open-reading frame; PE: Promoter element; tss: Transcription start signal; VP: Viral protein.

Concerning their promoter structure, NNS RNA viruses can be broadly divided into two groups. The genomic replication promoters of *Rhabdoviridae* and *Pneumovirinae* are short monopartite elements located entirely within the leader [33,49]. By contrast, the genomic replication promoters of the *Paramyxovirinae* subfamily are bipartite, consisting of a first promoter element located within the leader and a second promoter element contained within the nontranslated region of the first gene. The second promoter element consists of three consecutive hexamers [50]. While the total genome length of rhabdo- and pneumo-viruses does not appear to influence replication efficiency, the genome length of the *Paramyxovirinae* must be a multiple of six to allow for efficient replication [50]. A genome length rule, such as the 'rule of six', was neither found for filovirus genomes nor for naturally occurring EBOV-specific defective interfering particles [46]. Nevertheless, the *Zaire ebola-virus* genomic replication promoter is a bipartite structure consisting of a first promoter element spanning the leader region (nucleotides 1–55), and a second promoter element comprising nucleotides 81–128 within the nontranslated region of the first gene, the *NP* gene (Figure 4B). The two promoter elements are separated by a spacer region consisting of the transcription start signal of the *NP* gene and a downstream located region involved in secondary structure formation and transcription initiation (see later). While proper spacing of the two promoter elements is critical for replication, the spacer sequence itself is not important. The structure of the second promoter element is unique among the NNS RNA viruses and consists of a stretch of eight consecutive UN_5 hexamers, where N is any nucleotide (Figure 4B). Only nucleotide insertions or deletions of six or a multiple of six in the spacer region are tolerated, indicating that the genomic replication promoter obeys the rule of six whereas the total genome length does not [48].

Filovirus transcription

Transcription start & stop signals

During transcription, the negative-strand RNA genome is transcribed into seven monocistronic mRNA species. These mRNAs are capped and polyadenylated [51,52]. It is believed that for all NNS RNA viruses, the polymerase gains access to the viral genes through a single polymerase binding site at the 3′ end of the genome. Once bound, the polymerase complex proceeds along the RNA template by stopping and reinitiating at each gene junction and thereby transcribing the individual genes sequentially in their 3′ to 5′ order (Figure 2) [33]. Owing to the release of the polymerase complex from the RNA template, reinitiation at downstream located genes is attenuated. Accordingly, the first gene, *NP*, is transcribed at the highest levels, while the last gene, *L*, is transcribed at the lowest [53].

Filovirus genes are flanked by highly conserved transcription start and stop signals. The transcription start signals comprise of a stretch of 12 nucleotides and vary slightly between MARV (3′ CUUCUU^A/$_G$GUAAUU) and EBOV (3′ CU^C/$_A$CUUCUAAUU). Analysis of the 5′ ends of the viral mRNAs revealed that transcription starts precisely with the first nucleotide of the respective transcription start signal [52]. The consensus stop signal contains the sequence 3′ UAAUUC followed by a stretch of five to six uridine residues that serves as a template for the addition of the poly-A tail [4,5]. With analogy to other NNS RNA viruses, it is believed that polyadenylation of the mRNAs occurs through the mechanism of transcriptional stuttering [33]. A highly conserved pentamer, 3′ UAAUU, is located at the 5′end of each start site and the 3′ end of each stop signal (Figure 4C). Notably, the same pentamer can be shared by the transcription stop signal of the downstream and the start signal of the upstream-located gene, leading to gene overlaps whose structure is unique among the NNS RNA viruses (Figure 4C). Genes that do not overlap are separated by short intergenic regions varying in length and nucleotide composition (Figure 1A).

A unique feature of filovirus transcription start signals is that they are predicted to form stable RNA secondary structures [52,54]. The function of most predicted RNA structures remains to be determined. However, the secondary structure of the start site of the EBOV *NP* gene at least was found to play an important role during viral transcription (see later).

Protein requirements Typically, transcription of the NNS RNA virus is performed by NP, P and L [41]. The homologous filovirus proteins, namely NP, VP35 and L, were sufficient to support the transcription of MARV minigenomes in a reconstituted replication/transcription system [24]. By contrast, transcription of EBOV minigenomes was strongly dependent on the presence of the fourth nucleocapsid protein VP30 (Figure 2) [25,39,40]. In addition, rescue of full-length EBOV clones was only possible when VP30 was added as the helper plasmid [29,55,56].

Review Transcription and replication of nonseg [Curr Top Microbiol Immunol. 2004]

Review Unravelling the complexities of respiratory syncytial viru [J Gen Virol. 2006]

Review Paramyxovirus mRNA editing, the "rule of six" and error ca [J Gen Virol. 2005]

EBOV transcription factor VP30 Besides its function as a transcription activator, EBOV VP30 is involved in nucleocapsid assembly by binding to NP-derived inclusion bodies [21]. VP30 is a P that contains a zinc-binding domain and forms hexamers [57–60]. Phosphorylation of VP30 is presumed to be an important regulatory factor by determining whether the protein is involved in transcription activation or assembly [57]. The aminoterminal part of the protein contains an unconventional Cys_3–His zinc-binding domain that was also found in MARV VP30 and in the M2–1 proteins of pneumoviruses [58,61]. Destruction of the zinc-binding domain led to the loss of its function as a transcription factor, but VP30 was still able to bind NP inclusions [58]. Recently, the crystal structure of the C-terminal domain of EBOV VP30 has been determined [60]. The C-terminal part of the protein mediates the formation of dimers, which assemble to form hexamers by a leucine-rich oligomerization domain located in the N-terminal part of the protein. The hexamers are incorporated into virions [59,60]. Hexamerization is essential for transcription activation but dispensable for interacting with NP inclusion bodies. Interestingly, the C-terminal part of the protein is sufficient for the incorporation of nucleocapsid-associated VP30 into virions, whereas the N-terminal part of the protein is not required for this process [59].

The precise mechanism of VP30-regulated transcription is not yet fully understood. It is known that VP30 regulates a very early step of transcription, most likely transcription initiation or early antitermination. Interestingly, VP30-dependent transcription is regulated by a RNA hairpin loop formed by the promoter–proximal transcription start signal of the *NP* gene and a downstream located sequence (Figure 4A). When the formation of the RNA secondary structure was impaired, transcription took place independently of VP30. Although each EBOV-specific transcription start signal is predicted to form a RNA hairpin loop, VP30 is not required for transcription reinitiation at the internally located gene start sites [51]. A hypothetical model of VP30-dependent transcription is depicted in Figure 2.

Among the other members of the order Mononegavirales, a comparable process has not yet been described. Whether MARV VP30 is involved in transcription activation remains elusive. Although transcription took place independently of VP30 in a reconstituted MARV minigenome system, a full-length MARV clone could only be recovered when MARV VP30 was added as a helper plasmid [62]. In addition, RNA interference targeting MARV VP30 led to the down-regulation of the intracellular levels of all other viral proteins in infected cells, thereby suggesting that VP30 plays an essential role in transcription/replication [63]. However, in contrast to EBOV, a MARV VP30 mutant containing a destroyed zinc-binding motif was

Ebola virus defective interfering particles and persistent infection.   [Virology. 1999]

Termini of all mRNA species of Marburg virus: sequence and secon [Virology. 1996]

Review Transcription and replication of nonseg [Curr Top Microbiol Immunol. 2004]

able to mediate the rescue of a full-length MARV clone [62]. Thus, it appears that MARV VP30 is essential for the viral life cycle but probably not involved in transcription activation.

Besides filoviruses, the only other NNS RNA viruses possessing a fourth nucleocapsid protein are the pneumoviruses. The fourth nucleocapsid protein of respiratory syncytial virus, M2–1, acts in a later stage of transcription as a processivity factor [49].

mRNA editing There are two overlapping ORFs within the EBOV *GP* gene and neither of these are able to encode the virion-associated GP. By contrast, sGP is directly encoded by the first ORF. During transcription, an additional nontemplated adenosine residue is inserted into the nascent mRNA chain within a stretch of seven adenosines via the mRNA editing mechanism. Approximately 20% of GP-specific mRNA is edited [29,64,65]. Due to the insertion of the adenosine residue, the reading frame is shifted, leading to the expression of the membrane-anchored GP. High levels of GP were produced in cells infected with a recombinant EBOV clone encoding GP by a continuous ORF, whereas sGP was not expressed. Interestingly, the mutant was significantly more cytopathogenic than the wild-type virus, indicating that GP mRNA editing is an important biological mechanism for the control and down-regulation of the expression of the cytotoxic GP [29,66].

Future perspective

Go to: ☑

Replication and transcription of the RNA genome are crucial processes in the filovirus life-cycle. Since filoviruses encode their own replication/transcription machinery, the components involved in replication/transcription; cis-acting elements on the genomes and VPs mediating replication/transcription, are ideal targets for antiviral strategies. Indeed, initial approaches to use viral genes and proteins involved in replication/transcription as targets for antiviral compounds were quite promising [59,63,67–70]. Thus, a deeper understanding of the way in which filoviruses replicate and transcribe their genomes will lead to potential benefits in controlling filovirus infections. The reverse genetics systems now available for MARV and EBOV are especially powerful tools in the development of antiviral therapies and vaccines.

Another focus of future filovirus research will be the correlation of viral replication and antiviral response. It is already known for the polymerase cofactor VP35 that it interferes with the innate immune system [26–28]. Within the same vein, bioinformatic analysis has revealed that filovirus species differing in their replication efficiency also differ in their ability to evade the cellular antiviral response [71].

Termini of all mRNA species of Marburg virus: sequence and secon [Virology. 1996]

Marburgvirus genomics and association with a large hemorrhagic fev [J Virol. 2006]

Complete genome sequence of an Ebola virus (Sudan species) res [Virus Res. 2005]

Review Transcription and replication of nonseg [Curr Top Microbiol Immunol. 2004]

Termini of all mRNA species of Marburg virus: sequence and secon [Virology. 1996]

Sequence analysis of the Ebola virus genome: organization, ge [Virus Res. 1993]

Conclusion

In this review, the replication and transcription strategy of MARV and EBOV has been described. Although in many aspects, filoviruses follow the general mechanisms reported for NNS RNA virus replication/transcription, they have evolved individual molecular mechanisms that differ from all other NNS RNA viruses. These unique features include the structure of the genomic replication promoter, the structure of overlapping genes, the need for an additional nucleocapsid protein, VP30, as a transcription activation factor, and the use of mRNA editing to regulate the expression of the cytotoxic EBOV GP.

Box 1

Tools to investigate filovirus replication and transcription

- For a long time, research on Marburg (MARV) and Ebola virus (EBOV) replication was hampered by the requirement for biosafety level four containment facilities. Then, in 1998 and 1999, respectively, the first transfection-based filovirus-specific minigenome systems were described, allowing for the investigation of the replication and transcription strategy of these dangerous viruses under lower biosafety conditions [24,25]. The central element of these systems is a virus-specific minigenome consisting of the 3′ and 5′ ends of the filoviral genome containing the cis-acting signals required for replication/transcription initiation and RNA packaging. In this construct, all viral genes are replaced by a reporter gene. The minigenome was cloned under the control of the T7 RNA polymerase promoter and used to transfect cells expressing the T7 RNA polymerase. The same cells were transfected with plasmids containing the viral nucleocapsid protein genes under the control of the T7 RNA polymerase promoter. Recognition and subsequent replication and transcription of the minigenome by the nucleocapsid proteins could be determined by the detection of the replicated and transcribed RNA species or by reporter gene assays (Figure 3). Meanwhile, alternative minigenome systems exist. Groseth and colleagues have described an RNA polymerase I- and II-driven minigenome system in which the minigenome is supplied by RNA polymerase I and the supporting nucleocapsid proteins by RNA polymerase II (Figure 3) [40].

Review Reverse genetics of mononeg [Curr Top Microbiol Immunol. 2004]

A reconstituted replication and transcription system for Ebo [Virology. 2005]

Rescue of Ebola virus from cDNA using heterologous support pro [Virus Res. 2004]

Phosphorylation of VP30 impairs ebola virus transcription. [J Biol Chem. 2002]

Ebola virus transcription activator VP30 is a zinc-binding protein. [J Virol. 2003]

The Cys(3)-His(1) motif of the respiratory syncytial virus M2-1 protein i [J Virol. 2000]

Oligomerization of Ebola virus VP30 is essential for viral trans [J Biol Chem. 2003]

- In order to generate infectious EBOV-like particles containing a minigenome RNA, the plasmid-based minigenome system was extended with the inclusion of the viral genes needed for assembly [16,72].

- Based on the minigenome system, rescue systems have been established for MARV and EBOV, allowing the recovery of complete recombinant viruses entirely from cDNA (Figure 3) [29,55,62,73].

Execcutive summary

Genome organization & viral proteins

- The nonsegmented negative-sense RNA genome of Marburg (MARV) and Ebola viruses (EBOV) are 19 kb in length and contain seven genes (Figure 1A).

- At the 3′ and 5′ ends of the genome are short extragenic regions containing signals required for replication, transcription initiation and packaging of the RNA genome.

- Genes are flanked by highly conserved transcription start and stop signals.

- Genes are either separated by short intergenic regions or they overlap (Figure 4C).

- Filoviruses encode seven structural proteins (Figure 1B).

- The only surface protein, glycoprotein (GP) binds to the cellular receptor and mediates fusion. Viral protein (VP)-40 is the major matrix protein and is required for the transport of the nucleocapsids to the plasma membrane and for virus budding. VP24 is presumed to be a minor matrix protein involved in nucleocapsid formation and assembly. It is considered to be a virulence factor.

- In contrast to most other Mononegavirales, filoviruses possess four nucleocapsid proteins. These are nucleoprotein (NP), polymerase cofactor VP35, transcription activator VP30 and RNA-dependent RNA polymerase (L).

- The nucleocapsid proteins are tightly associated with the RNA genome.

Filovirus replication

Inhibition of Marburg virus protein expression and viral rele [J Gen Virol. 2005]

Review Unravelling the complexities of respiratory syncytial viru [J Gen Virol. 2006]

Ebola virus glycoprotein GP is not cytotoxic when expresse [J Gen Virol. 2006]

- Replication of the RNA genome starts with the synthesis of a positive-sense reverse complement of the genome, the antigenome. The antigenome, in turn, serves as a template for the generation of new genomes (Figure 2).

- The minimal protein requirement for filovirus replication is NP, VP35 and L.

- The structure of the genomic replication promoter of EBOV is unique among nonsegmented negative-sense RNA viruses (Figure 4B).

Filovirus transcription

- During transcription, the negative-strand RNA genome is transcribed into seven monocistronic mRNA species. These mRNAs are capped and polyadenylated by the viral RNA-dependent RNA polymerase (Figure 2).

- A unique feature of filovirus transcription start signals is that they are predicted to form stable RNA secondary structures.

- NP, VP35 and L are sufficient to support the transcription of MARV minigenomes. By contrast, the minimal protein requirement for EBOV transcription is NP, VP35, L and VP30.

- The EBOV transcription factor, VP30, regulates a very early step of transcription, most likely transcription initiation or early antitermination.

- VP30-dependent transcription is regulated by an RNA hair-pin loop formed by the transcription start signal of the first gene, the *NP* gene.

- EBOV GP mRNA is subjected to mRNA editing to regulate the expression of cytotoxic GP. mRNA editing has not been observed with MARV transcription.

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