Both matrix proteins of Ebola virus contribute to the regulation of viral genome replication and transcription

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ABSTRACT

Ebola virus (EBOV) causes severe hemorrhagic fevers in humans and non-human primates. While the role of the EBOV major matrix protein VP40 in morphogenesis is well understood, nothing is known about its contributions to the regulation of viral genome replication and/or transcription. Similarly, while it was reported that the minor matrix protein VP24 impairs viral genome replication, it remains unclear whether it also regulates transcription, since all common experimental systems measure the combined products of replication and transcription. We have developed systems that allow the independent monitoring of viral transcription and replication, based on qRT-PCR and a replication-deficient minigenome. Using these systems we show that VP24 regulates not only viral genome replication, but also transcription. Further, we show for the first time that VP40 is also involved in regulating these processes. These functions are conserved among EBOV species and, in the case of VP40, independent of its budding or RNA-binding functions.

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Introduction

Ebola virus (EBOV) is a member of the family *Filoviridae* in the order *Mononegavirales* (Sanchez et al., 2007). It is the causative agent of severe hemorrhagic fevers in human and non-human primates with high case fatality rates. Currently, there is neither a specific therapy nor a licensed vaccine available, and EBOV is classified as a biosafety level (BSL) 4 agent (Gene et al., 2009). Virus particles show a characteristic thread-like appearance and consist of a central ribonucleoprotein (RNP) complex containing the viral RNA (vRNA) genome complexed with the nucleoprotein NP, the viral polymerase L, the polymerase cofactor VP35 and the transcriptional activator VP30. The RNP is surrounded by a matrix space, containing the matrix proteins VP40 and VP24, and a host cell-derived membrane, in which the surface glycoprotein GP is embedded (Sanchez et al., 2007).

It is known that the RNP components alone are sufficient for viral genome replication and transcription, and these processes have been intensively studied using minigenome assays. Minigenomes are miniature versions of the viral genome, in which all viral open reading frames are deleted, which makes their study feasible under reduced biosafety conditions. Classical minigenomes consist of a

reporter gene (e.g. Renilla luciferase) flanked by the non-coding leader and trailer regions, which contain the minimal signals necessary for replication and transcription (Muhlberger et al., 1998, 1999) (Fig. 1A). This cassette is usually cloned under the control of a T7 promoter to allow generation of a vRNA-minigenome following transcription by a T7 RNA-polymerase, although RNA Polymerase I has also been successfully used for this purpose in filoviral minigenome systems (Groseth et al., 2005). The resulting vRNA-minigenome is recognized by the RNP complex components NP, VP35 and L and replicated using a complementary cRNA-minigenome as an intermediate. If the transcriptional activator VP30, which has been implicated in overcoming a secondary RNA structure inhibiting viral transcription but not replication (Weik et al., 2002), is also present, the vRNA-minigenomes can be further transcribed into mRNAs, which then lead to reporter activity. It is important to note that the number of vRNA-minigenome templates available for use in transcription is dependent on viral genome replication (Fig. 1A). An increase in viral genome replication leads to an increase in templates available for transcription and, thus, can lead to an increase in both mRNA levels and reporter activity. Therefore, in minigenome systems reporter activity as well as the amount of mRNA reflects not only viral transcription, but also viral genome replication.

In addition to defining the viral components necessary for replication and transcription, minigenomes have also been used to investigate the details of these processes, e.g. the role of the

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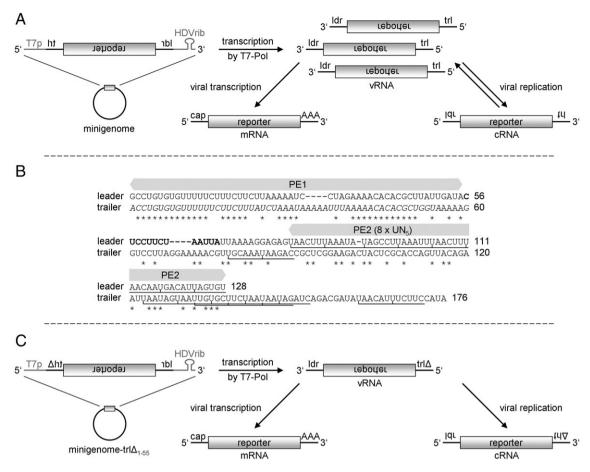 E-mail address: becker@staff.uni-marburg.de (S. Becker).

Fig. 1. Replication-competent and replication-deficient minigenomes. A) Replication-competent minigenome system. A minigenome consisting of a reporter open reading frame and the viral leader (ldr) and trailer (trl) regions is cloned into an expression plasmid flanked by a promoter for the T7 RNA polymerase (T7p) and a Hepatitis delta virus ribozyme (HDVRib). Upon coexpression with T7 RNA polymerase (T7-Pol) viral RNA minigenomes (vRNA) are synthesized, which can serve as templates for viral genome replication using complementary anti-minigenomes (cRNA) as an intermediate, as well as for viral transcription to produce reporter mRNAs. B) Alignment of the termini of the leader region of the genome and the trailer region of the antigenome. The 128 terminal bases of the leader, containing the replication promoter, and the 176 terminal bases of the trailer are shown. The known promoter elements 1 and 2 (PE1 and PE2) in the leader are indicated. The UN_5 repeats of the genomic PE2 and UN_5 repeats that could serve as an antigenomic PE2 are underlined and the U residues indicated. The transcription start signal is written in bold face, and the 55 bases that are deleted in the replication-deficient minigenome are italicized. Stars indicate conserved bases. C) Replication-deficient minigenome system. Fifty-five bases in the trailer region of a replication-competent minigenome were deleted (indicated in panel B) in order to destroy the antigenomic replication promoter. The genomic replication promoter and viral transcription promoter remained unchanged. The resulting vRNA-minigenomes could serve as template for cRNA-production and transcription of mRNA; however, no vRNA could be synthesized from the cRNA intermediates, rendering transcription efficiency in this assay independent of replication.

transcriptional activator VP30 (Hartlieb et al., 2003; Martinez et al., 2008; Modrof et al., 2002; Weik et al., 2002), the genomic regions serving as replication promoters (Enterlein et al., 2009; Weik et al., 2005), and differences in the transcription/replication capacities of RNPs from different EBOV species (Groseth et al., 2005). In addition, minigenomes have been used as screening tools for antiviral approaches (Groseth et al., 2007), as well as forming the basis for more complex infectious virus-like particle systems that allow the study of virtually all steps of the viral life cycle without the need for BSL-4 facilities (Hoenen et al., 2006; Watanabe et al., 2004; Wenigenrath et al., 2010).

A role for matrix proteins in the regulation of viral genome replication and transcription has been shown for a number of negative strand RNA viruses, including Vesicular Stomatitis Virus, where a regulatory role of M was first shown more than 30 years ago (Clinton et al., 1978), Influenza virus (Watanabe et al., 1996), Tacaribe virus (Lopez et al., 2001), Rabies virus (Finke et al., 2003), Lassa virus (Hass et al., 2004), and most recently Measles virus (Iwasaki et al., 2009). Interestingly, it has also been shown that VP40, the major matrix protein of EBOV, binds to RNA in a sequence specific manner (Gomis-Ruth et al., 2003), although the nature of the bound RNA remains unknown. Recently, a similar phenomenon was described for the matrix protein of Borna disease virus, and it was suggested that this

might contribute to RNP condensation and, thus, regulation of transcription and replication (Neumann et al., 2009). Nevertheless, except for one study showing that the EBOV minor matrix protein VP24 impacts on viral genome replication, and possibly transcription (Watanabe et al., 2007), no studies have been performed investigating a regulatory role for the filoviral matrix proteins in viral genome replication and transcription. Therefore, this study aimed to investigate the effect of the EBOV matrix proteins on viral genome replication and transcription using a replication-deficient minigenome, which allows the independent study of these processes in filoviruses for the first time.

Results

Establishment of assays to independently measure replication and transcription

In order to study viral transcription independently of viral genome replication, a replication-deficient minigenome was designed in which the antigenomic replication promoter was deleted. Until now the only information available about the antigenomic promoter is that the last 176 nucleotides of the trailer are sufficient to allow replication of DI particles and, thus, should contain the antigenomic promoter