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Review

Reverse genetics technology for Rift Valley fever virus: Current and future applications for the development of therapeutics and vaccines

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ABSTRACT

The advent of reverse genetics technology has revolutionized the study of RNA viruses, making it possible to manipulate their genomes and evaluate the effects of these changes on their biology and pathogenesis. The fundamental insights gleaned from reverse genetics-based studies over the last several years provide a new momentum for the development of designed therapies for the control and prevention of these viral pathogens. This review summarizes the successes and stumbling blocks in the development of reverse genetics technologies for Rift Valley fever virus and their application to the further dissection of its pathogenesis and the design of new therapeutics and safe and effective vaccines.

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1. Introduction

Several bunyaviruses including Rift Valley fever virus (RVFV), Crimean-Congo hemorrhagic fever and Hantaan viruses are responsible for potentially lethal hemorrhagic fevers and therefore are of significant medical and public health importance. Unfortunately, there are no FDA-approved vaccines available for any of these viruses. The real threat posed by RVFV, coupled with the fact that there currently is no effective licensed vaccine for human use, clearly illustrates the need for more RVFV vaccine research and development. A better understanding of bunyavirus-associated pathogenesis remains critical to the development of effective vaccines and antiviral compounds to combat hemorrhagic fevers. Many vaccine candidates and therapeutics developed through traditional methods either fail to provide protection or result in unacceptable adverse effects. This problem clearly requires the application of new technologies, such as reverse genetics systems, for the design and generation of safe and efficacious vaccine candidates and therapeutics based on the use of genetically manipulated viruses.

2. Bunyaviruses

Viruses within the *Bunyaviridae* family are classified into five genera: *Orthobunyavirus*, *Nairovirus*, *Phlebovirus*, *Hantavirus* and *Tospovirus* (Elliott, 1996; Schmaljohn and Hooper, 2001). Bunyaviruses are characterized by a tripartite negative-stranded RNA genome comprised of Large (L), Medium (M), and Small (S) segments (Schmaljohn and Hooper, 2001). The L segment encodes for the viral RNA-dependent RNA polymerase—RdRp (L), the M segment for a protein precursor which is post-translationally processed into the mature glycoproteins G_N and G_C and the S segment for the nucleoprotein (N). Some bunyaviruses express additional non-structural proteins from the S and M segments, NSs and NSm, respectively (see Fig. 1A). General features of viruses classified in the *Bunyaviridae* family are similar to other segmented negative-strand RNA virus families (Elliott, 1996).

Following envelope glycoprotein-mediated virus binding to a receptor on a permissive cell, viral and plasma membranes fuse and viral RNA is transferred into the cytoplasm. The immediate early primary transcription step then begins, allowing the viral genomic RNAs (vRNA), in association with N, to be transcribed into mRNAs by the RNP-associated viral RNA polymerase L (Bellocq and Kolakofsky, 1987; Ikegami et al., 2005b; Kolakofsky et al., 1987; Vialat et al., 2000). Genome replication takes place in the cytoplasm via a complementary full-length template RNA (cRNA), also called antigenomic RNA. The vRNAs generated from this replication step provide the template for additional mRNA synthesis (secondary transcription step) and serve as genome segments for progeny virions which are released by budding into Golgi vesicles (Elliott, 1996; Gerrard and Nichol, 2002, 2007). Maturation and budding of particles through the Golgi apparatus is a property of bunyaviruses. mRNA synthesis is initiated using capped oligonucleotides, captured from cellular mRNAs through a cap-snatching mechanism, similar to the one described for orthomyxoviruses except that all steps take place in the cytoplasm. In contrast with most mRNAs, bunyavirus mRNAs are not polyadenylated and represent an incomplete copy of the vRNA template.

3. Rift Valley fever virus

RVFV is an arthropod-borne member of the *Phlebovirus* genus that causes recurrent outbreaks affecting humans and ruminants predominantly in Subsaharan Africa, but spread to Egypt in 1977 and to the Arabian Peninsula, including Saudi Arabia and Yemen in 2000 (Al-Hazmi et al., 2003; Anonymous, 2000; Balkhy and Memish, 2003; Madani et al., 2003; Shoemaker et al., 2002), and re-emerged in four Egyptian Governorates in 2003 (Balkhy and Memish, 2003).

The largest RVFV epidemic-epizootic outbreak affected Egypt along the Nile River (1977–1979), afflicting approximately 200,000 persons with 594 deaths (Meegan, 1979; Meegan et al., 1979), followed by recurrent epidemics in 1993 and 1997 (Abd el-Rahim et al., 1999; Abu-Elyazeed et al., 1996; Arthur et al., 1993). Other severe outbreaks in the past twenty years have affected West African nations in Senegal-Mauritania (1987) where an outbreak affected an estimated 89,000 victims, resulting in 220 deaths

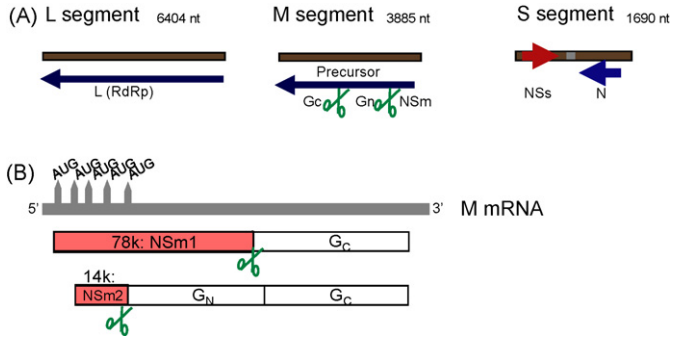

Fig. 1. RVFV genome organization. (A) Schematic representation of the three genomic segments and coding strategy. The arrows indicate the open reading frames in each segment with the cleavage sites generating the mature glycoproteins. (B) RVFV M segment-based mRNA with the five in frame AUG start codons at its 5′ terminus. The proteins expressed from the first and the second AUG are displayed.