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Review

Development and application of reporter-expressing mononegaviruses: Current challenges and perspectives

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ABSTRACT

Reverse genetics allows the generation of recombinant viruses entirely from cDNA. One application of this technology is the creation of reporter-expressing viruses, which greatly increase the detail and ease with which these viruses can be studied. However, there are a number of challenges when working with reporter-expressing viruses. Both the reporter protein itself as well as the genetic manipulations within the viral genome required for expression of this reporter can result in altered biological properties of the recombinant virus, and lead to attenuation *in vitro* and/or *in vivo*. Further, instability of reporter expression and purging of the genetic information encoding for the reporter from the viral genome can be an issue. Finally, a practical challenge for *in vivo* studies lies in the attenuation of light signals when traversing tissues. Novel expression strategies and the continued development of brighter, red and far-red shifted reporters and the increased use of bioluminescent reporters for *in vivo* applications promise to overcome some of these limitations in future. However, a "one size fits all" approach to the design of reporter-expressing viruses has thus far not been possible. Rather, a reporter suited to the intended application must be selected and an appropriate expression strategy and location for the reporter in the viral genome chosen. Still, attenuating effects of the reporter on viral fitness are difficult to predict and have to be carefully assessed with respect to the intended application. Despite these limitations the generation of suitable reporter-expressing viruses will become more common as technology and our understanding of the intricacies of viral gene expression and regulation improves, allowing deeper insight into virus biology both in living cells and in animals.

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Abbreviations: ATU, additional transcriptional unit; GFP, (enhanced) green fluorescent protein; IRES, internal ribosomal entry site; RSV, respiratory syncytial virus; VSV, vesicular stomatitis virus.

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1. Introduction

Reverse genetics systems are based on the generation and subsequent replication and transcription of full-length virus RNA genomes or truncated genome analogues from cDNA. They encompass both life cycle modelling systems, which allow the modelling and dissection of discrete parts of the virus life cycle, and full-length clone systems, which allow the generation of recombinant viruses from cDNA (Hoenen et al., 2011). Among negative-sense RNA viruses, the first virus to be rescued entirely from cDNA was a recombinant Rabies virus in 1994 (Schnell et al., 1994). Since then, full-length clone systems have become available for almost all negative-sense RNA viruses. An obvious application for these systems is the generation of mutated viruses in order to study the impact of those mutations on virus biology. For example, the first recombinant Rabies viruses generated were used to investigate the role of the Rabies ψ pseudogene region (Schnell et al., 1994). Also a common application of full-length clone systems is to create chimeric viruses in order to identify pathogenic determinants. For example, the role of the ebolavirus glycoprotein GP as a pathogenic determinant has recently been investigated by exchanging the GP gene between different ebolavirus species of varying pathogenicity (Groseth et al., 2012). Another application, which will be discussed in this review, is the generation of viruses that express foreign reporter proteins. These viruses can be used to investigate virus biology; however, they also have tremendous potential in the screening of antivirals, the visualization of infection in animal models in order to increase our understanding of pathogenesis and transmission, and for the rational attenuation of viruses for vaccine purposes, as well as for use as oncolytic agents. This review will discuss the current state of the art for reporter-expressing negative-sense RNA viruses, as well as challenges in their development and perspectives to overcome these challenges, with a special focus on the order *Mononegavirales*.

2. Genome structures of mononegaviruses

There are four families within the order *Mononegavirales*: *Rhabdoviridae*, *Paramyxoviridae*, *Filoviridae* and *Bornaviridae* (in addition, *Nyaminiviridae* has been proposed as a fifth family within this order). Despite the fact that these families encompass

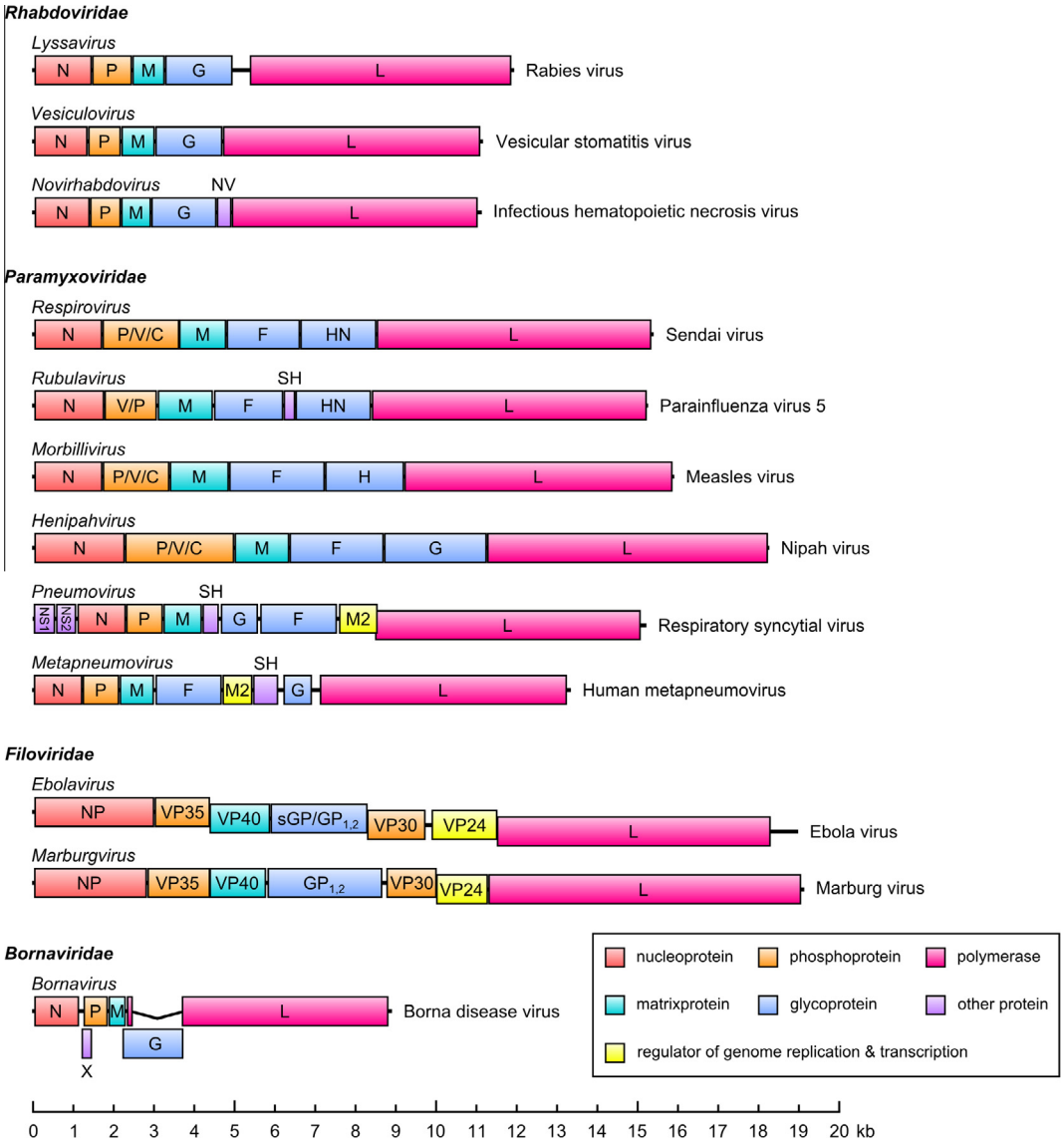

Fig. 1. Genome structure of mononegaviruses. The genes (or in the case of Borna disease virus the coding sequences) of representative members of virus genera are shown as boxes, with the gene names indicated. Non-transcribed regions are shown as a black bar. Gene overlaps are indicated as steps. Genes and genomes are drawn to scale, with the exception of the first part of the Borna disease virus L coding sequence (before the splice site), which is enlarged for the purposes of clarity.