Reverse genetics of rotaviruses: A method for better understanding virus biology.

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Abstract

Rotaviruses (RVs) are one of the most common causes of viral gastroenteritis in newborns and young children around the world. The lack of an adequate reverse genetics (RG) technology to make recombinant rotaviruses and examine the precise roles of viral proteins in the setting of RV infection has hampered studies on rotavirus biology until recently. Recently, a fully traceable plasmid-only RG system for rescuing recombinant rotaviruses was devised, resulting in a major milestone in the RV sector. Since then, the reproducibility and advancements in this technology have permitted the creation of many recombinant rotaviruses with various gene segment changes, allowing for the manipulation of viral genes to characterise the specific roles of viral proteins during the RV replication cycle alternatively, foreign proteins can be encoded for a variety of functions. Through direct editing of the viral genome, reverse genetics (RG) technology allows researchers to examine rotavirus gene structure and function. Several facets and critical concerns of virus biology can be addressed and resolved, ranging from infection to replication, assembly, contrast to cellular immunity, and so on. RG systems have been successfully established for most RNA viruses, including segmented viruses, and have led to the discovery of new activities of viral proteins as well as the production of next generation vaccines.

Keywords: Reoviridae, Plasmid, Polymerase, Cytosolic transcription.

Introduction

The Reoviridae family of recombinant viruses, Replication begins when newly transcribed mRNAs from core particles enter the cell cytosol, and the virus completes the whole replication cycle in the cytoplasm, including viral protein production, de novo synthesis of the viral genome, and assembly of infective particles [1]. As a result, the cytosolic production of virus-like mRNAs has formed the foundation of all reverse genetic technology efforts. A successful plasmid-based RG system for orthoreoviruses was developed using a recombinant Vaccinia Virus system, which provides the T7 RNA polymerase to drive transcription of appropriate transfected plasmid constructs, resulting in single-stranded (ss)(+)RNAs of all genomic segments in the cytosol of transfected cells. The 3' end of viral mRNAs was made certain by using the antigenomic hepatitis-virus (HDV) ribozyme's autocleavage activity [2].

The first approach relies on T7 RNA polymerase-driven cytosolic transcription of a transfected cDNA encoding the structural protein VP4 (gs4) and a rotavirus helper from a strain other than the foreign segment [3]. Antibodies that specifically neutralised the helper-virus VP4 protein provided the strong selective pressure needed to favour the rescue of recombinant rotavirus and compete with the native helper virus in this system; thus, the generated recombinant viruses carrying the genetically modified VP4 gene segment were the only viral particles not neutralised and thus able to infect cells and propagate. As a result of the selection, the recombinant virus's rescue efficiency was very poor, and it was limited to sequences encoding the structural proteins VP4 and VP7, since the selection of recombinant viruses was based on the neutralisation of helper virus infection by specific neutralising antibodies.

In the rotavirus area, an RG system based solely on the transfection of 11 plasmids encoding RV RNA viral segments of the SA11 strain in BHK cells that constitutively produce T7 RNA polymerase has been developed [4]. Because no helper virus is involved, this approach does not require selection pressure. This RG system works by inserting 11 plasmids encoding RV genome segments between a T7 RNA polymerase promoter at the 5′end and the antigenomic hepatitis-virus ribozyme at the 3′end of a T7 RNA polymerase promoter. Two plasmids encoding the two subunits of the VV capping enzyme (D1R and D12L) were co-transfected with the rescue plasmids in order to stabilise the viral (+)ssRNAs generated in the cytoplasm. Despite the fact that this technique allowed for transcription and The recovery of infectious RV progeny was unsuccessful due to the lack of stability of authentic full-length viral (+)ssRNA transcripts [5].

intriguing, raising crucial issues about virus packing and reproduction. For example, a construct containing the Csy4

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target sequence within the EGFP ORF of an NSP3-EGFP fusion in genome segment 7 revealed that the targeted ssRNA was not duplicated to dsRNA only after Csy4 cleavage, despite all other newly replicated segments being identified as dsRNA.

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