Recombinant Influenza Vectors with a Pol II Promoter and Ribozymes for Vaccines and Gene Therapy

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Technology description

Recombinant, infectious influenza viruses can be used as vaccine and gene therapy vectors; however, producing influenza virus particles is difficult because the influenza genome consists of negative-sense RNA molecules that cannot directly serve as templates in protein synthesis. Virus production normally requires a helper virus that provides needed structural proteins and polymerases. UW-Madison resarchers have developed an improved reverse genetics system for producing influenza virus in vertebrate cells in the absence of helper virus. The system starts with a set of plasmids containing viral genome cDNAs flanked by ribozymes. Each plasmid carries a cDNA for one of the eight influenza A viral RNA segments. On each plasmid, the cDNA sits between a polymerase II promoter and a poly-A addition signal at the 3-prime end. When the plasmids are transfected into a vertebrate cell, the host cell’ s RNA polymerase II transcribes each construct into a capped viral RNA with a proper poly-A tail. The flanking ribozyme RNAs then undergo site-specific, self-catalyzed cleavage to precisely trim each end of the viral RNA. Next, viral polymerase, which is provided by a protein expression plasmid, acts upon the viral RNAs, resulting in replication and mRNA synthesis. This system does not require a helper virus and allows the creation of transfectants with mutations in any gene segment.
The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing an improved reverse genetics system for producing influenza virus in vertebrate cells in the absence of helper virus.

Application area

Vaccine production
Viral mutagenesis studies
Gene therapy

Advantages

Offers a superior method for producing influenza virus for use in vaccines
Unlike previous methods using RNA polymerase I, this system allows the production of influenza virus in any vertebrate cell.

Viral RNAs are generated from cellular RNA polymerase II, eliminating need to provide polymerase in trans .
Generates a precise 3-prime RNA terminus, which is essential for efficient production of virus
Results in increased viral production as compared to current methods
Allows easy manipulation of influenza viruses, such as through the introduction of attenuating mutations into the viral genome

Institution

Wisconsin Alumni Research Foundation

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