Transgenic expression of PB2 in 293T cells increases avian influenza virus packaging in reverse genetic systems

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March 07, 2024

Abstract

Plasmid-based reverse genetics has transformed influenza virus research by enabling the production of recombinant influenza viruses from cloned cDNA copies of viral genome segments. Reverse genetic production of influenza viruses requires cellular expression of influenza proteins polymerase basic 1, polymerase basic 2, polymerase acidic and nucleoprotein which collectively allow for transcription of viral mRNA and synthesis of new negative-sense genomic RNA, thus enabling synthesis of all components needed to assemble infectious virus from transfected cell lines. Given the importance of these proteins in the generation of influenza viruses via reverse genetics, we sought to explore how transgenic expression of mammalian-adapted PB1, PB2, PA, or NP in the 293T packaging cell line may impact the recovery of recombinant influenza viruses. We constructed four transgenic 293T cell lines expressing PB1, PB2, PA or NP derived from the mouse-adapted 2009 pandemic influenza A virus, UI182. Transgenic expression of UI182 PB2 in 293T cells enhanced recovery of replication-competent avian influenza viruses generated by reverse genetics relative to levels achieved in unmodified 293T cells. Virus recovered from PB2-expressing 293T cells replicated with kinetics that were indistinguishable from viruses recovered from unmodified 293T cells. Provision of UI182 PB2 protein via transgenic expression in 293T cells resulted in enhanced viral polymerase activity as measured by a minigenome assay, which may account for the improved efficiency of viral packaging relative to unmodified 293T cells. Transgenic expression of mammalian-adapted PB2 in 293T cells may serve as an important tool for enhancing influenza virus recovery in reverse genetic systems.

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