Multidose Transient Transfection of HEK293 Cells Modulates rAAV2/5 Rep Protein Expression and Influences the Enrichment Fraction of Filled Capsids

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Abstract

Recombinant adeno-associated virus (rAAV) is a commonly used *in vivo* gene therapy vector because of its non-pathogenicity, long-term transgene expression, broad tropism, and ability to transduce both dividing and non-dividing cells. However, rAAV vector production via transient transfection of mammalian cells typically yields a low fraction of filled-to-total capsids (˜1–30% of total capsids produced). Analysis of our previously developed mechanistic model for rAAV2/5 production attributed these low fill fractions to a poorly coordinated timeline between capsid synthesis and viral DNA replication and the repression of later phase capsid formation by Rep proteins. Here, we extend the model by quantifying the expression dynamics of total Rep proteins and their influence on the key steps of rAAV2/5 production using a multiple dosing transfection of human embryonic kidney 293 (HEK293) cells. We report that the availability of pre-formed empty capsids and viral DNA copies per cell are not limiting to the capsid filling reaction. However, optimal expression of Rep proteins ($< 240 \pm 13$ ag per cell) enables enrichment of the filled capsid population ($> 12\%$ of total capsids/cell) upstream. Our analysis suggests increased enrichment of filled capsids via regulating the expression of Rep proteins is possible but at the expense of per cell capsid titer in a triple plasmid transfection. Our study reveals an intrinsic limitation of scaling rAAV2/5 vector genome (vg) production and underscores the need for approaches that allow for regulating the expression of Rep proteins to maximize vg titer per cell upstream.

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