Recoding mRNA therapeutic cargoes for efficient viral packaging

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1. Abstract

The efficient delivery of therapeutic genetic material into host cells is a prerequisite for successful gene therapy [1]. Here we introduce a novel approach to the development of virus like particle (VLP)-based gene delivery vectors. We have shown that many single-stranded (ss) RNA viruses/VLPs assemble by having their coat proteins form a capsid around their genomic RNA (gRNA) [2]. This is achieved through the action of multiple sequence-specific RNA/protein interactions, which define the assembly pathway and may even be responsible for the early steps of infection [3]. The RNA sequences/motifs (packaging signals; PSs) involved in these contacts are dispersed throughout the gRNAs, and vary in their affinity for cognate coat proteins (CP) by variation of CP recognition motif and the folding propensity of the RNA secondary structures in which they occur. These gRNA/CP interactions, therefore, have profound implications for virology. We have successfully grafted a subset of PSs found naturally in the ss pre-genomic RNA of the Hepatitis B virus (HBV) genome into a series of potentially therapeutic/diagnostic mRNAs. This relies on a computational synonymous recoding approach that is based on a bioinformatic analysis of the propensity by which each PS site folds into its active conformation, both in its natural and therapeutic contexts. In vitro reassembly of the modified mRNAs suggests that the HBV CP still recognises its cognate PSs in these altered contexts, conferring increased assembly efficiency upon them. We further describe the outcomes of in vitro reassembly with such artificial assembly substrates. These findings may have implications for the future of gene editing using the VLP delivery approach.