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Abstract

The dramatic effectiveness of recent mRNA (mRNA)-based COVID vaccines delivered in lipid nanoparticles has highlighted the promise of mRNA therapeutics in general. In this report, we extend our earlier work on self-amplifying mRNAs delivered in spherical *in vitro* reconstituted virus-like particles (VLPs), and on drug delivery using cylindrical virus particles. In particular, we carry out separate *in vitro* assemblies of a self-amplifying mRNA gene in two different virus-like particles: one spherical, formed with the capsid protein of cowpea chlorotic mottle virus (CCMV), and the other cylindrical, formed from the capsid protein of tobacco mosaic virus (TMV). The mRNA gene is rendered self-amplifying by genetically fusing it to the RNA-dependent RNA polymerase (RdRp) of Nodamura virus, and the

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In Vivo Delivery of Spherical and Cylindrical *In Vitro* Reconstituted Virus-like Particles Containing the Same Self-Amplifying mRNA

Sweta Karan,[#] Ana Luisa Durán-Meza,[#] Abigail Chapman, Cheylene Tanimoto, Soo Khim Chan, Charles M. Knobler, William M. Gelbart,[*] and Nicole F. Steinmetz[*]

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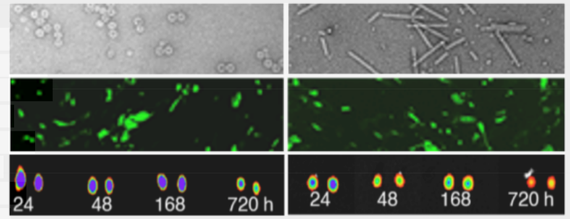
ABSTRACT: The dramatic effectiveness of recent mRNA (mRNA)-based COVID vaccines delivered in lipid nanoparticles has highlighted the promise of mRNA therapeutics in general. In this report, we extend our earlier work on self-amplifying mRNAs delivered in spherical *in vitro* reconstituted virus-like particles (VLPs), and on drug delivery using cylindrical virus particles. In particular, we carry out separate *in vitro* assemblies of a self-amplifying mRNA gene in two different virus-like particles: one spherical, formed with the capsid protein of cowpea chlorotic mottle virus (CCMV), and the other cylindrical, formed from the capsid protein of tobacco mosaic virus (TMV). The mRNA gene is rendered self-amplifying by genetically fusing it to the RNA-dependent RNA polymerase (RdRp) of Nodamura virus, and the relative efficacies of cell uptake and downstream protein expression resulting from their CCMV- and TMV-packaged forms are compared directly. This comparison is carried out by their transfections into cells in culture: expressions of two self-amplifying genes, enhanced yellow fluorescent protein (EYFP) and Renilla luciferase (Luc), packaged alternately in CCMV and TMV VLPs, are quantified by fluorescence and chemiluminescence levels, respectively, and relative numbers of the delivered mRNAs are measured by quantitative real-time PCR. The cellular uptake of both forms of these VLPs is further confirmed by confocal microscopy of transfected cells. Finally, VLP-mediated delivery of the self-amplifying-mRNA in mice following footpad injection is shown by *in vivo* fluorescence imaging to result in robust expression of EYFP in the draining lymph nodes, suggesting the potential of these plant virus-like particles as a promising mRNA gene and vaccine delivery modality. These results establish that both CCMV and TMV VLPs can deliver their *in vitro* packaged mRNA genes to immune cells and that their self-amplifying forms significantly enhance *in situ* expression. Choice of one VLP (CCMV or TMV) over the other will depend on which geometry of nucleocapsid is self-assembled more efficiently for a given length and sequence of RNA, and suggests that these plant VLP gene delivery systems will prove useful in a wide variety of medical applications, both preventive and therapeutic.



KEYWORDS: *self-amplifying mRNA, Nodamura replicon, tobacco mosaic virus (TMV), cowpea chlorotic mottle virus (CCMV)*