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Assembly of trans-encapsidated recombinant viral vectors engineered from Tobacco mosaic virus and Semliki Forest virus and their evaluation as immunogens

Mark L. Smith, Tina Corbo [1], Jacqueline Bernales [2], John A. Lindbo [3], Gregory P. Pogue [4], Kenneth E. Palmer, Alison A. McCormick *

Large Scale Biology Corporation, 3333 Vaca Valley Parkway, Suite 1000, Vacaville, CA 95688, USA

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Abstract

RNA virus vectors are attractive vaccine delivery agents capable of directing high-level gene expression without integration into host cell DNA. However, delivery of non-encapsidated RNA viral vectors into animal cells is relatively inefficient. By introducing the tobacco mosaic virus (TMV) origin of assembly into the RNA genome of Semliki Forest virus (SFV), we generated an SFV expression vector that could be efficiently packaged (trans-encapsidated) *in vitro* by purified TMV coat protein (CP). Using cellular assays, pseudovirus disassembly, RNA replication and reporter gene expression were demonstrated. We also evaluated the immune response to trans-encapsidated recombinant SFV carrying a model antigen gene (β-galactosidase) in C57/B6 mice. Relative to RNA alone, vector encapsidation significantly improved the humoral and cellular immune responses. Furthermore, reassembly with recombinant TMV CPs permitted the display of peptide epitopes on the capsid surface as either genetic fusions or through chemical conjugation, to complement the immunoreactivity of the encapsidated RNA genetic payload. The SFV vector/TMV CP system described provides an alternative nucleic acid delivery mechanism that is safe, easy to manufacture *in vitro* and that also facilitates the generation of unique nucleic acid/protein antigen compositions.

Keywords: Tobacco mosaic virus; Reassembly; Semliki Forest virus; Pseudovirus; Antibody response; Cellular immune response; Alphavirus

Introduction

Genetic immunization against pathogen or tumor antigens has proven successful using DNA and modified virus vectors. As a result of intracellular protein expression and class I antigen presentation, such vaccines often demonstrate superior abilities to stimulate high levels of cellular immune responses, when compared with protein antigens of extracellular origin. The simplest approach to gene therapy is the injection of naked plasmid DNA, yet due to the inefficiency of cellular uptake (Nishikawa and Huang, 2001), a high dose of plasmid is required to stimulate immunity, which has posed a challenge for widespread use (Hoare et al., 2005; Levy et al., 2000). Concerns have also been raised regarding the possibility of genomic integration and resulting cellular transformation events. Extensive animal testing has indicated that the likelihood of plasmid incorporation into host DNA is low (Martin et al., 1999; Vilalta et al., 2005). However, a recent study (Wang et al., 2004) evaluating strategies for improved plasmid delivery resulted in multiple independent genomic integration events, suggesting that a balance between efficacy and safety must be considered.

One alternative to DNA vaccination are RNA virus vectors (Lundstrom, 2002) such as Semliki Forest virus (SFV) based

* Corresponding author. Fax: +1 707 446 3917.
E-mail address: alison.mccormick@myifp.com (A.A. McCormick).
[1] Present address: Genentech, 1 DNA Way, South San Francisco, CA 94080, USA.
[2] Present address: Alta Analytical Laboratory, Inc., 1100 Windfield Way, El Dorado Hills, CA 95762, USA.
[3] Present address: Department of Plant Pathology, The Ohio State University, Ohio Agricultural Research and Development Center, 24 Selby Hall, 1680 Madison Ave., Wooster, OH 44691, USA.
[4] Present address: Office of Technology Commercialization, University of Texas at Austin, 3925 W. Braker Lane, Suite 1.9A, Austin, TX 78759, USA.

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vectors (Zhou et al., 1994). Relative to DNA vectors, RNA virus vectors show transient persistence (Morris-Downes et al., 2001), have a comparable or superior ability to deliver pathogen and tumor antigen genes in animal models to either prevent or cure disease (Lundstrom, 2002; Lundstrom, 2005) and do not integrate into chromosomal DNA. For immunizations, the SFV RNA can be either used directly or packaged into recombinant replication-deficient SFV particles (Berglund et al., 1993). The current process of manufacturing SFV particles via transient transfection procedures is both difficult to scale and may result in replication competent virus, although improvements to the system have reduced the likelihood of recombination (Smerdou and Liljestrom, 1999). One recently reported alternative is to encapsidate SFV-based RNA vectors using the vesicular stomatitis virus glycoprotein (VSV-G) in place of the SFV structural proteins, as there is no sequence homology between VSV-G and the SFV vector genome. However, the culture conditions for this system remain problematic (Dorange et al., 2004). We therefore investigated alternative packaging strategies for the SFV replicon, by employing the capsid of a distantly related member of the alphavirus superfamily, Tobacco mosaic virus (TMV).

To evaluate this approach, we incorporated the TMV origin of assembly (Oa) into a SFV replicon containing the beta-galactosidase (β-gal) reporter gene. Packaging of the RNA then occurs simply by mixing purified coat protein (CP) with modified SFV RNA *in vitro*. RNA-directed self-assembly results in the efficient *in vitro* manufacturing of trans-encapsidated SFV pseudovirus in amounts directly proportional to RNA concentration when CP is in excess. To further expand this delivery system, we considered encapsidation with recombinant CPs, to introduce functionality to the pseudovirion capsid. As examples, the SFV replicon was combined with a TMV CP displaying an integrin-binding motif and a CP displaying a surface-exposed lysine. The latter facilitates the conjugation and co-delivery of peptides or whole protein (Smith et al., 2006) with the genetic payload, to create "bi-functional" pseudovirion vaccine compositions. For one such bi-functional vaccine, we complemented the encapsidated RNA with a surface-displayed T-cell peptide epitope derived from β-gal. Interestingly, the immune response to the combination of nucleic acid and protein antigen was skewed completely to the Th1 cellular response. The methods described can be easily extended to RNA encoding any biological activity including cytokines, chemokines, multiple target RNAs, encapsidated by any number of CP modifications with the desired immunomodulatory function(s).

Results

Assembly and characterization of pseudovirions and virus-like particles

TMV capsid formation is initiated by the interaction of CP subassemblies with a ~300 nt stem–loop structure internal to the genomic RNA (the origin of assembly; Oa) that has been well defined (reviewed in Butler, 1999). The CP is incorporated predominantly as two-ring 'disks' during elongation towards the 5′ RNA terminus (illustrated schematically in Fig. 1A), while elongation towards the 3′ terminus employs smaller aggregates.

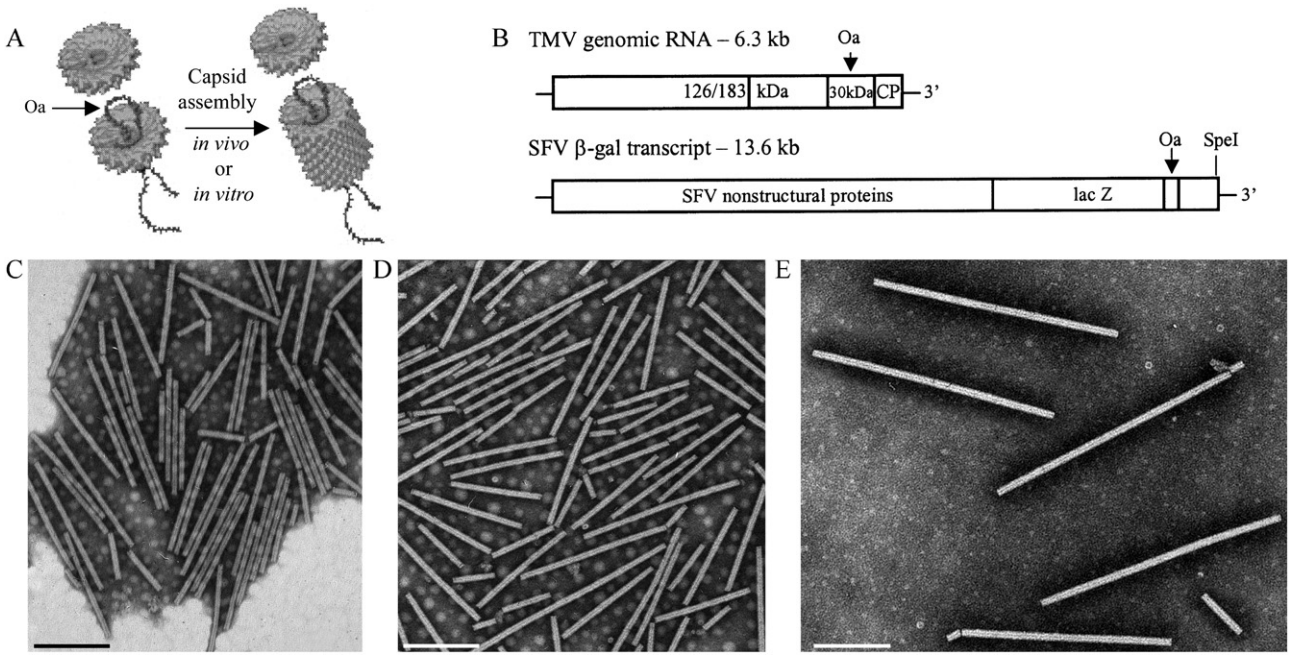

Fig. 1. RNA scaffolds compatible with TMV CP reassembly. (A) Simplified schematic representation of TMV CP reassembly onto RNA containing the origin of assembly (Oa; Reproduced courtesy of G. Stubbs). (B) Size and structure of the + sense RNA transcripts employed as scaffolds; TMV genomic RNA and SFV lacZ construct expressing β-galactosidase (SFV β-gal). (C to E) Representative electron micrographs of the reassembly product obtained when the transcripts in panel B were combined with various TMV CP preparations. (C) TMV genomic RNA with U1 CP. (D) TMV genomic RNA with RGD CP. (E) SFV β-gal transcript with the lysine modified CP (LSB 1295.10). Scale bars represent 200 nm.