Evidence that viral RNAs have evolved for efficient, two-stage packaging

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Introduction

Positive-sense, single-stranded (ss)RNA viruses are ubiquitous pathogens affecting human health and causing losses of livestock and crops. Therapeutic interventions are currently limited while vaccination is practical for only a few human and animal viruses. Understanding all steps in viral lifecycles will lead to novel therapeutic strategies. Packaging of genomic RNA is the prerequisite step in production of infectious virions. Many icosahedral protein capsids spontaneously co-assemble around their genomes, resulting in relatively high packing densities. It has been proposed that this is accomplished by a gradual condensation of RNA, driven primarily by electrostatic neutralisation of the nucleic acid charge by positive charges on the coat protein. However, the proposed mechanism cannot account for the observed specificity of packaging, i.e. discrimination between viral and the abundant non-viral RNA within cells. Here we took advantage of two model systems, bacteriophage MS2 and a plant virus, Satellite Tobacco Necrosis virus (STNV), for which *in vitro* assembly assays have been developed. The two viral coat protein architectures are distinct, the STNV CP having a positively charged N-terminal extension on a canonical jelly-roll globular domain, which is essential for assembly, whilst the MS2 CP dimer exhibits an RNA binding cleft. These differences allow us to identify conserved and distinct features of their assembly mechanisms and provide the basis for generalization.

Results

Single molecule fluorescence correlation spectroscopy (FCS) allowed us to selectively monitor coat protein (CP) or viral RNA components during assembly *in vitro* at low concentrations (<1 μM). This permitted detection of early, transient RNA:CP complexes. FCS is also excellent tool for hydrodynamic sizing of large RNA molecules. The viral, protein-free RNAs exist as an ensemble of differing conformers, most of which are larger than the capsids into which they must eventually fit. Remarkably, instead of gradual RNA condensation, which would be expected on the basis of the charge neutralisation mechanism, addition of CPs to their cognate RNAs results in a rapid collapse (<1 min, Fig. 1A & B) in their solution conformations. Collapse depends on protein-protein interactions and multiple specific RNA-CP interactions, since it does not occur on non-viral RNA or with the non-cognate viral RNA (Fig 1A & B). The collapsed state is smaller than the capsid and appears to consist of complexes with sub-stoichiometric amounts of coat proteins correctly organized to form a partial capsid shell of the correct size. The full complement of CPs are recruited in a second slower stage of assembly (Fig. 1C). Given that this two-stage assembly has been demonstrated for

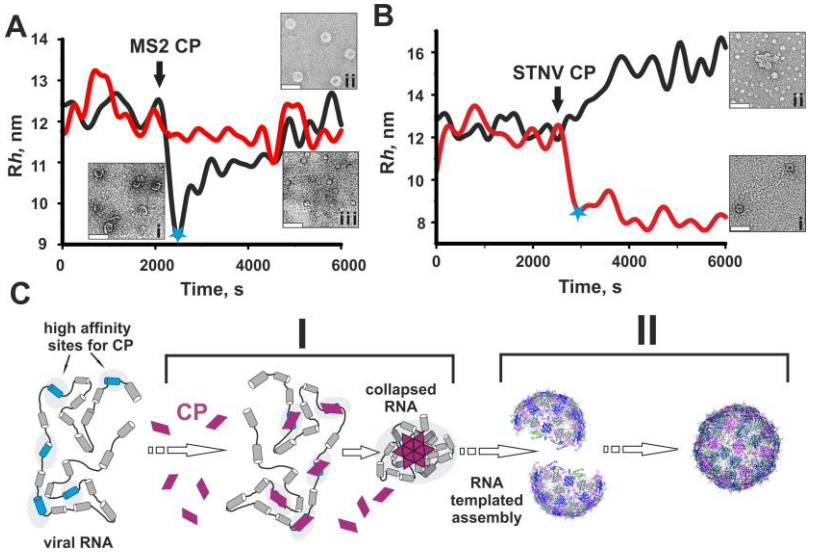

Figure 1: hydrodynamic radius (Rh) changes of viral RNAs (MS2-black, STNV-red) during assembly with MS2 coat **(a)** or STNV coat **(b)**. The initial collapse is indicated by blue star. **(c)** A two-stage mechanism of ssRNA virus assembly: Stage I-coat protein mediated rapid RNA collapse; Stage II-RNA-templated completion of capsids.

two unrelated viruses it may constitute a mechanism conserved among a large class of viral pathogens and provides a potential target for therapeutic intervention.

Publications

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