

Packaging host RNAs in small RNA viruses

An inevitable consequence of an error-prone polymerase?

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Viruses from all walks of life employ diverse strategies to ensure the specific encapsidation of their own viral genomes and to avoid the packaging of host nucleic acids. While the packaging of host nucleic acid may attenuate viral infectivity, this may also have extended biological implications. It is well-understood, for example, that the packaging of non-phage DNA by bacteriophages can lead to the horizontal transfer of genes between unrelated species of bacteria.

Using next-generation sequencing, we recently analyzed the RNA content of flock house virus (FHV), a eukaryotic, non-enveloped +ssRNA virus.¹ In addition to the viral genome, we found that approximately 1% of the RNA encapsidated by FHV virions was derived from the host cell. We found a diverse range of RNAs including mRNAs, rRNAs, retrotransposons and non-coding RNAs. The packaging of host RNA raises the possibility that small RNA viruses could be vectors in the horizontal transfer of genes between eukaryotic species.

To understand how viruses like FHV are able to package cellular RNA, it is first important to understand what strategies they employ to encapsidate their own RNA. Three sites in the capsid protein have been identified as being essential for the specific encapsidation of viral RNA (Fig. 1). Five arginines at the N terminus of the capsid protein between residues 6 and 14 direct the specific encapsidation of FHV RNA 2. Virions with point mutations in these residues package defective RNAs and cellular RNAs in place of FHV RNA 2.² Similarly, further downstream, between residues 32–50, lays the arginine-rich motif (ARM) containing 12 arginine residues. Mutation of these residues to

alanine yields virus particles that do not encapsidate FHV RNA 1.³ Finally, at the C terminus of the capsid protein, there are three conserved phenylalanine residues. Individual alanine substitutions strongly attenuate viral RNA packaging, while deletion of all three residues almost totally abolishes viral RNA encapsidation, resulting in particles that primary packaging cellular RNA.⁴

Each of these three sites are known to be in proximity to the RNA in mature virus particles.⁵ However, the altered RNA-packaging phenotypes are not thought to be due to changes in protein-RNA interactions, but rather due to altered trafficking of the nascent capsid polypeptides.³ FHV virions are assembled in invaginations in the mitochondria that are formed by arrays of interacting RNA-dependent RNA polymerases.⁶ This compartmentalizes the replicating viral RNA. Failure to traffic the capsid proteins to this site may result in the auto-assembly of virus-like particles in other regions of the cytoplasm, where viral RNAs are not available.

It has also been demonstrated that viral RNA replication is coupled to RNA packaging.⁷ In FHV RNA 2, cis-acting elements in the 5' and 3' UTRs and in a region between nucleotides 538 and 616, are required for RNA replication.⁸ Mutations at these sites attenuate replication. However, if a functional capsid protein is still expressed, virions are produced that package cellular RNA. In fact, this coupling is so robust that it is possible to simultaneously express two viral RNAs within a single cell, one replicating and one not, each of which produces distinct virus particles with distinct RNA-packaging properties.⁹

With this understanding of viral RNA encapsidation, we may rationalize the encapsidation of non-viral RNAs. In vivo, the mutations in the regions described above will inevitably appear due to the error-prone nature of the viral polymerase and will be present in a small fraction of viral RNAs that are packaged into authentic virus particles. If these mutant RNAs are successfully delivered to a cell, they will produce mutant virus particles that subsequently package host-RNAs.

From our deep sequencing analysis,¹ we measured the average mismatch frequency in RNA packaged into authentic FHV virions to be 9.8 mismatched nucleotides per 10,000 mapped nucleotides. From our data, we can thus infer the frequency of amino acid substitutions that occur in the FHV capsid protein and, in particular, at the three sites known to be important for directing the encapsidation of viral genomic RNA (Fig. 1). We found single amino substitutions in 1.3% of the five N-terminal arginines and 2.1% of the arginines in the arginine-rich motif. Similarly, we found 0.3% of the individual C-terminal phenylalanines to be mutated with a 0.25% chance of a premature stop codon appearing immediately upstream. While these numbers are small, altogether they may account for the host RNA that we observed to be encapsidated by 1% of authentic FHV virions.

The strategies employed by FHV to direct the encapsidation of its own genome are by no means unique. For example, the coupling of replication to RNA packaging has also been demonstrated in poliovirus.¹⁰ When considering the erring nature of viral polymerases, the generation of mutant virions that fail to select their viral genomes for encapsidation almost seems

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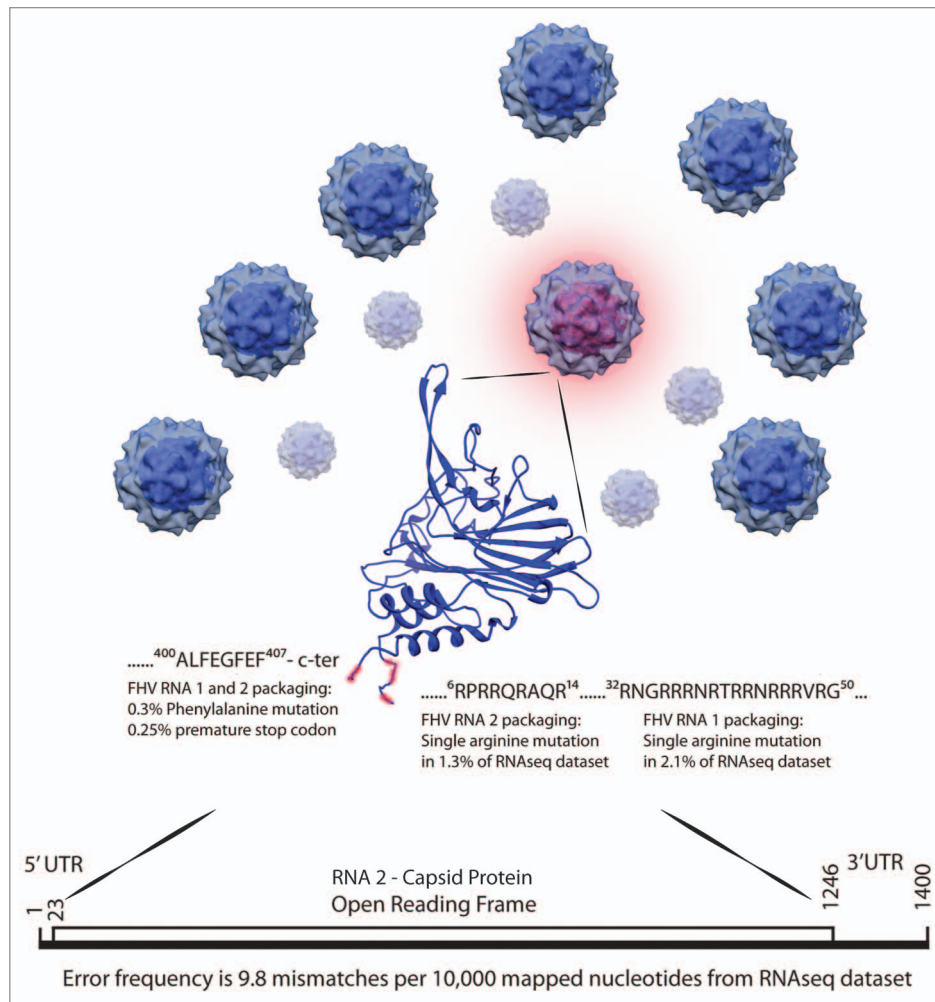


Figure 1. Schematic representation of the capsid protein of flock house virus. Three sites have been previously identified as being critical for correct packaging of the viral genome into virus particles. These sites are highlighted in red in the crystal structure of the FHV capsid protein. Each of these sites is mutated to some degree (as indicated) within a population of virus particles due to the error-prone nature of the viral polymerase.

inevitable. Consequently, the packaging of host transcripts may be a wide-spread phenomenon. A remaining question is whether a particle that carries non-viral RNA will still be able to deliver its cargo to a host cell. If so, then small RNA viruses may be routinely shuttling host RNA transcripts between host cells.

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