The Multiple Roles of Genomic RNA in Small ssRNA Virions

Peter G. Stockley, Astbury Centre for Structural Molecular Biology, University of Leeds, Leeds, LS2 9JT, UK

Abstract:

Single-stranded (ss) RNA viruses are ubiquitous pathogens in all kingdoms of life and are disproportionally damaging to human, animal and crop plant health. We have been studying the roles of the genomic RNAs in small isometric members of this class of pathogens. In many cases *in vitro* reassembly from disassembled virion components is possible, implying that self-assembly is the dominant mechanism of virion formation in these systems. Many of these viruses will also assemble protein shells essentially identical to the virion around non-genomic RNAs, polyanions or even in the absence of any RNA. This has produced a protein-centric view of their assembly mechanisms. However, such non-cognate reactions are much less efficient than reactions containing genomic RNA, even *in vitro*, and cannot account for the high fidelity of specific RNA encapsidation and virion assembly seen *in vivo*. Recent work from our laboratory and those of our collaborators offers an alternative paradigm for the assembly process in which the cognate genomic RNA plays a number of major roles, both in virion assembly and subsequent uncoating at the start of the next round of infection. Using two model viral systems, Satellite Tobacco Necrosis Virus and bacteriophage MS2, which have T=1 and T=3 capsid architectures, respectively and very distinct coat protein folds, we have been able to identify common mechanistic aspects of assembly. Single molecule fluorescence correlation spectroscopy assembly assays show that viral RNA, but not realistic non-viral RNA controls, undergo a dramatic and rapid collapse in their solution conformations when exposed to the cognate coat protein. In both cases such a compaction is required for the RNAs to fit within the fully assembled capsid. The initial collapse pays the entropic cost of assembly in one process which appears to be the result of the formation of multiple coat protein-RNA and coat protein-coat protein contacts. The collapsed assembly intermediates then recruit additional coat protein subunits to complete their respective shells in a very faithful process. In contrast, the non-viral or non-cognate controls also support capsid assembly but very inefficiently and with the production of many malformed structures. In collaboration with the Twarock group we have identified the RNA sites underlying the cognate assembly process. For MS2, which uses these RNA contacts to regulate the quasi-conformers of incoming coat protein subunits, their positioning implies that the conformation of the genome within the virion is highly restricted. A recent asymmetric tomographic reconstruction of MS2 interacting with its natural receptor, the bacterial pilus, is fully consistent with this interpretation. The latest progress with these studies will be discussed.