Genome-regulated Assembly of a ssRNA Virus May Also Prepare It for Infection

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1. Abstract

Many single-stranded, positive-sense RNA viruses regulate assembly of their infectious virions by forming multiple, cognate coat protein (CP)-genome contacts at sites termed Packaging Signals (PSs). We have determined the secondary structures of the bacteriophage MS2 ssRNA genome (gRNA) frozen in defined states using constraints from X-ray synchrotron footprinting (XRF). Comparison of the footprints from phage and transcript confirms the presence of multiple PSs in contact with CP dimers in the former. This is also true for a virus-like particle (VLP) assembled around the gRNA in vitro in the absence of the single- copy Maturation Protein (MP) found in phage. Since PS folds are present at many sites across gRNA tran- scripts, it appears that this genome has evolved to facilitate this mechanism of assembly regulation. There are striking differences between the gRNA-CP contacts seen in phage and the VLP, suggesting that the latter are inappropriate surrogates for aspects of phage structure/function. Roughly 50% of potential PS sites in the gRNA are not in contact with the protein shell of phage. However, many of these sit adjacent to, albeit not in contact with, PS-binding sites on CP dimers. We hypothesize that these act as PSs tran- siently during assembly but subsequently dissociate. Combining the XRF data with PS locations from an asymmetric cryo-EM reconstruction suggests that the genome positions of such dissociations are non- random and may facilitate infection. The loss of many PS-CP interactions towards the 30 end of the gRNA would allow this part of the genome to transit more easily through the narrow basal body of the pilus extruding machinery. This is the known first step in phage infection. In addition, each PS-CP dissociation event leaves the protein partner trapped in a non-lowest free-energy conformation. This destabilizes the protein shell which must disassemble during infection, further facilitating this stage of the life-cycle.