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COMMUNICATION

Simple Rules for Efficient Assembly Predict the Layout of a Packaged Viral RNA

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Single-stranded RNA (ssRNA) viruses, which include major human pathogens, package their genomes as they assemble their capsids. We show here that the organization of the viral genomes within the capsids provides intriguing insights into the highly cooperative nature of the assembly process. A recent cryo-electron microscopy structure of bacteriophage MS2, determined with only 5-fold symmetry averaging, has revealed the asymmetric distribution of its encapsidated genome. Here we show that this RNA distribution is consistent with an assembly mechanism that follows two simple rules derived from experiment: (1) the binding of the MS2 maturation protein to the RNA constrains its conformation into a loop, and (2) the capsid must be built in an energetically favorable way. These results provide a new level of insight into the factors that drive efficient assembly of ssRNA viruses *in vivo*.

A cryo-electron microscopy (cryo-EM) analysis of the wild-type MS2 virus at ~9 Å resolution with icosahedral symmetry averaging previously revealed a characteristic polyhedral distribution of RNA close to the protein layer.[1] Based on this observation, we were able to narrow down the vast number of combinatorially possible ways in which the genome could organize itself within the capsid to just over 40,500 possibilities. The remarkable efficiency of virus assembly begs the question of whether all of these organizations are equally probable or whether there are further constraints that result in the RNA taking on an organization within the particle that favors a particular RNA fold or specific capsid assembly pathway.

Addressing this question becomes possible based on the data presented in this issue by Toropova *et al.* where they describe a cryo-EM reconstruction of bacteriophage MS2 bound to its receptor.[2] The contact between the virus and its receptor, the bacterial F-pilus, is mediated by a single copy of maturation protein,[3–5] which was found to sit at a 5-fold vertex of the $T=3$ capsid. This allowed a structure of the receptor-bound virus to be determined with C5 (5-fold rotational) symmetry. Density at lower radii than the coat protein layer of the virion is organized into two distinct shells, which must correspond to maturation protein, the genomic RNA, or a combination of the two. The outer shell is similar (albeit with different averaging) to *in vitro* assembled virus-like particles that lack maturation protein[6] and, therefore, most likely represents protein-bound RNA. Icosahedral symmetry averaging of the data determined with C5 symmetry[2] results in a

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Abbreviations used: ssRNA, single-stranded RNA; cryo-EM, cryo-electron microscopy; 1-D, one-dimensional.

distribution of material inside the protein layer that is very similar to the previously determined cryo-EM reconstructions of the wild-type virion and reassembled virus-like particles that have been reported using icosahedral symmetry averaging.[1,7] Since there is only one copy of maturation protein and since it is of significantly smaller mass than the RNA, this similarity in density adjacent to the coat protein layer must mostly reflect the interaction of the RNA with the coat protein subunit.

The density distribution obtained with C5 symmetry provides vital clues on how the RNA is distributed along the 5-fold axis on which the maturation protein is located. It allows us to predict, using a simple mathematical model, the asymmetric organization of the linear single-stranded RNA (ssRNA) molecule within its symmetric container. Strikingly, our results imply that only a very limited number of RNA configurations out of the over 40,500 ones identified in Ref. 1 are consistent with the 5-fold-averaged cryo-EM data, biochemical information regarding RNA interaction with maturation protein, and efficient capsid assembly. This provides new insights not only into the asymmetric organization of the genomic material but also into the sophisticated assembly strategies of the virus. For example, it shows that, besides having a role during infection, the maturation protein could also be used to vastly reduce the complexity of the assembly process by circularizing the genomic RNA.

Cryo-EM data imply a dimer switching model for virus assembly

Icosahedrally averaged cryo-EM reconstructions of Leviviridae show that the outer shells of their genomic RNAs form distinct, cage-like structures.[1,7] An example is bacteriophage MS2, with a cage akin to the polyhedron in Fig. 1a. This polyhedron is formed from two distinct types of edges (Fig. 1b): 60 short edges, occurring in groups of five around the particle 5-fold axes, and 30 long edges, which cross underneath the 2-fold axes and connect the short edges of two neighboring 5-fold axes. Previous work

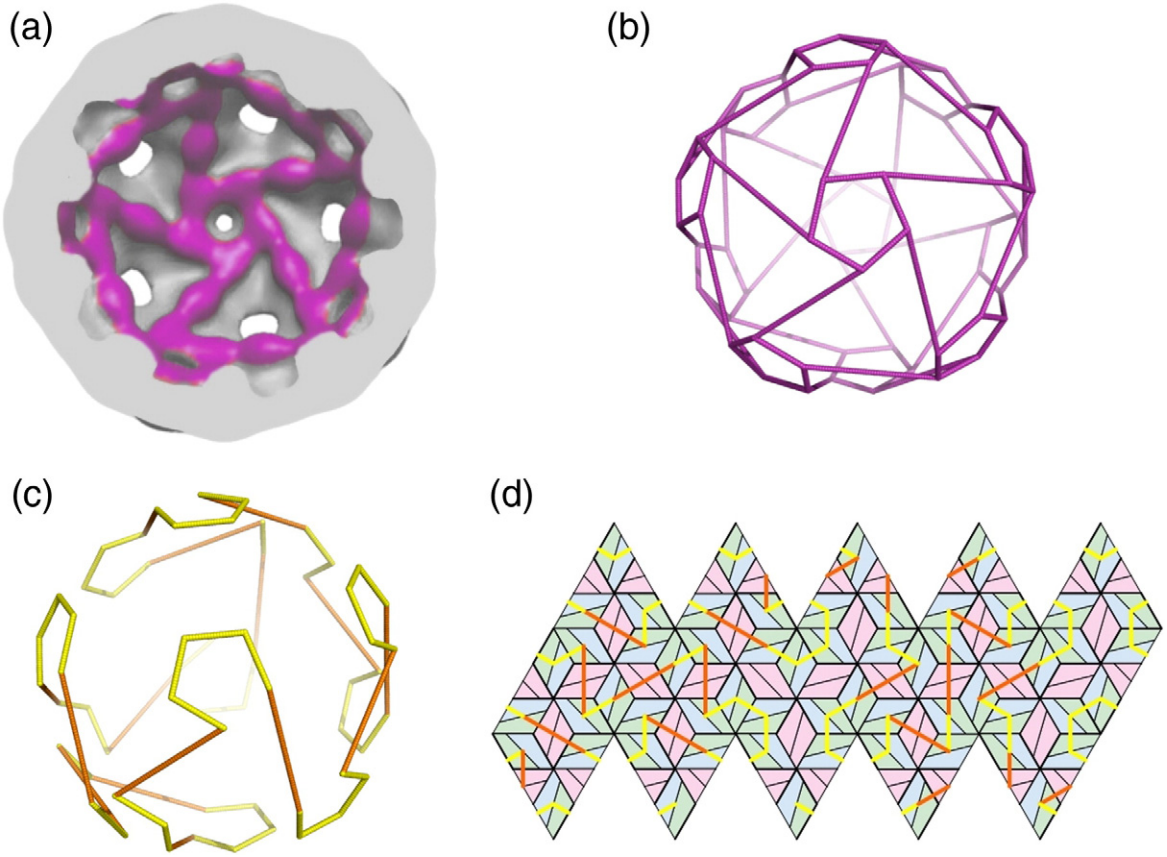

Fig. 1. The polyhedral cage of MS2 RNA density can be described as a Hamiltonian path. (a) A cryo-EM reconstruction of the outer RNA shell of bacteriophage MS2 (depicted in magenta) based on an image at ~17 Å resolution adapted from Van den Worm *et al.*[7] (b) A representation of this RNA shell as a polyhedral cage. (c) A three-dimensional view of a single Hamiltonian path, which meets every vertex of the polyhedron exactly once by moving along the short (yellow) and long (orange) edges of the polyhedral cage. (d) A planar net representation of the Hamiltonian path shown in (c) and its relation to the A (blue), B (green), and C (pink) quasi-equivalent subunits of the MS2 capsid.