Molecular interactions in the RNA bacteriophage MS2

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The simple RNA bacteriophages of E.coli have long been used as ideal model systems in which the details of RNA replication, translation and the control of gene expression can be studied in molecular detail1-3. In particular, the translational repression of the phage replicase cistron by coat protein subunits has attracted a great deal of attention and this has become a paradigm for sequence-specific RNA-protein interactions. In a series of pioneering experiments, Uhlenbeck and his colleagues were able to demonstrate that the key recognition determinants in the RNA lay entirely within a 19 nucleotide (nt) fragment (TR) capable of forming a stem-loop4,6. Further experiments suggested that this interaction was also the trigger of phage assembly in vitro and in vivo 7,8. Thus the specific interaction between the RNA and the phage coat protein subunits is used to regulate two distinct aspects of the phage life-cycle, translational repression of the replicase and assembly initiation.

Recently, the molecular basis of the sequence-specific interaction has been revealed by determination of the X-ray crystal structure of a C,C-variant TR-coat protein complex at 3.0 Å resolution7. Both C,C-variant and wild-type TR complexes have now been refined at 2.7 Å (Valegård et al., in progress). These complexes were prepared by soaking crystals of RNA-free T=3 capsids with TR fragments which were able to penetrate to the centre of the particles, presumably via the channels at the particle five-fold and/or three-fold axes. Each T=3 capsid consists of 60 coat protein dimers in the A/B conformation and 30 coat protein dimers in the C/C conformation. At saturating levels of TR RNA, each coat protein dimer within the capsid became bound to an RNA molecule. Perhaps surprisingly, the RNA bound in a unique orientation at the A/B protein dimer positions allowing the details of the protein-RNA interaction to be seen for the first time. Experiments with chemical variants of the TR sequence suggest that the complex which forms in solution between a coat protein core-shell and RNA-binding stabilises a quasi-equivalent protein dimer interaction. These data suggest that RNA-binding stabilises a quasi-equivalent protein dimer conformation, which acts as the assembly initiator. The extreme co-operativity of the subsequent reassembly reaction can, however, only be accounted for by the formation of a subsequent nucleation complex containing at least five coat protein dimers, equivalent to formation of a pentamer of the T=3 capsid. There are no kinetically significant intermediates between the nucleation complex and the final capsid.

References