Crystal structures of bacteriophage MS2 coat protein mutants complexed with Qβ RNA stemloop operators – a proposed discrimination mechanism for the binding of RNA operators by related phages.

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Introduction

MS2 and Qβ are evolutionarily related T=3 icosahedral bacteriophages with single stranded RNA genomes that infect E. coli. Although the protein subunits of the MS2 and Qβ capsids share less than 25% sequence identity, the structures of the Qβ subunits are very similar to those of MS2. Subunits of both the MS2 and Qβ capsid shells exist as three distinct conformers (A, B and C) that associate to form AB and CC dimmers, which comprise the basic building blocks of both capsid shells. The structures of the MS2 and Qβ capsids have been determined via X-ray crystallography by our collaborators in Uppsala, Sweden.

The two bacteriophages both utilise a similar mechanism of translational repression. *In vivo*, a small RNA stemloop within the viral genomes binds to a specific site on a coat protein dimer, acting to inhibit viral replicase gene translation. The translational complex of MS2 has, for many years, been the paradigm for studying RNA/protein interactions at the atomic level.

The RNA stemloop operator binding site is located on a 10-stranded β sheet formed by AB and CC dimers within the capsid shells of both MS2 and Qβ. Many of the amino acid residues that have previously been shown to be important for high affinity binding of MS2 and Qβ stemloops are conserved between the two bacteriophages. Although the protein surfaces of the stemloop binding site of the two
bacteriophages display considerable similarity, profound differences exist in the sequence and secondary structures of the two stemloop operators (Fig. 1). In vivo, each bacteriophage preferentially discriminates against binding the stemloop operator of the other. Affinity binding studies have, however, identified specific coat protein mutants of MS2 at residues 87 and 89 that overcome this discrimination mechanism, some of the mutations allowing the binding of the Qβ RNA operator to MS2 mutant capsids with an affinity comparable with that of the wild type MS2 operator.

In order to gain new insight into this discrimination mechanism, Qβ RNA stemloop operators were soaked into pre-crystallised MS2 mutant capsids and the structure of the capsid/RNA complexes determined via X-ray crystallography.

Results
Diffraction data were collected for three different MS2 mutants (N87S, E89K and N87S, E89K) complexed with Qβ RNA stemloop operators at the SRS, Daresbury, UK. Although the electron density for the lower stem region of the RNA is weak in each of the complexes, unambiguous modelling of the loop and upper stem region of the RNA is possible (Fig. 2). In contrast to the four base loop observed in the MS2 stemloop-coat protein complex, the Qβ RNA maintains its three base loop topology on complex formation. The N87S mutation mediates the binding of Qβ RNA by its shorter sidechain allowing the stacking of A+7 onto the underside of TyrA85, an interaction that is not favoured by the bulky asparagine residue. The E89K mutation leads to the LysB89 sidechain being located between the phosphate groups at the P+2 and P+3 positions thus giving potential for the formation of hydrogen bond interactions which explains the increase in affinity for Qβ RNA displayed by capsids with this mutation. Thus the effects of these two mutations on binding affinity can be explained.

No crystal structure of the Qβ RNA stem-loop complexed to Qβ coat protein has yet been determined. However, using the crystallographic structure of the Qβ RNA complexed to the MS2 coat protein mutants as a model, and given the structural similarities between the MS2 and Qβ coat proteins, it has been possible to propose a model of the complex. A paper describing these crystallographic studies is currently in preparation.

Collaborators
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