

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

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Introduction

MS2 and Q β are evolutionarily related T=3 icosahedral bacteriophages with single stranded RNA genomes that infect *E. coli*. Although the protein subunits of the MS2 and Q β capsids share less than 25% sequence identity, the structures of the Q β subunits are very similar to those of MS2. Subunits of both the MS2 and Q β 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 Q β 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 Q β . Many of the amino acid residues that have previously been shown to be important for high affinity binding of MS2 and Q β stemloops are conserved between the two bacteriophages. Although the protein surfaces of the stemloop binding site of the two

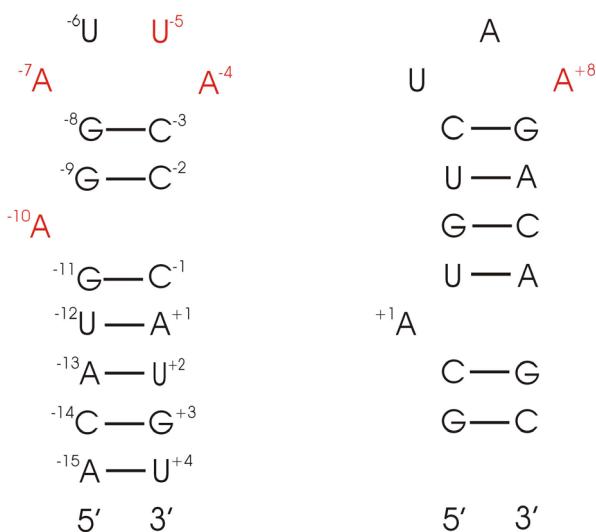
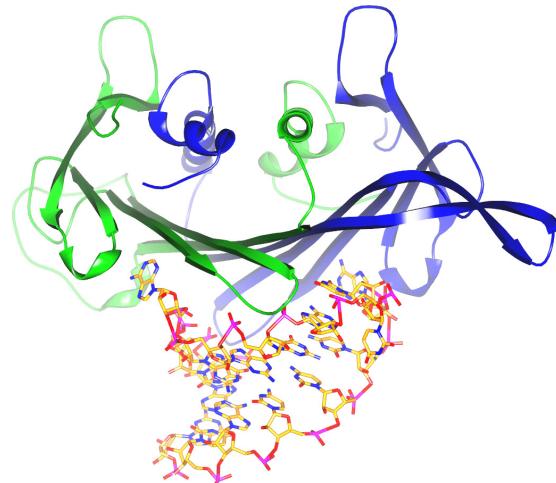


Fig. 1

(Top) Ribbon diagram of an AB coat protein dimer from the MS2 phage (subunit A in blue, B in green) complexed with the wild-type MS2 RNA stem-loop operator (shown in stick format).

(Bottom) Diagram of the secondary structure of the MS2 (left) and Q β (right) stemloop operators. Residues that have been shown to be important for high affinity binding to capsid protein are highlighted in red.

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 Q β 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, Q β 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 Q β 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 Q β RNA maintains its three base loop topology on complex formation. The N87S mutation mediates the binding of Q β 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 Q β 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 Q β RNA stem-loop complexed to Q β coat protein has yet been determined. However, using the crystallographic structure of the Q β RNA complexed to the MS2 coat protein mutants as a model, and given the structural similarities between the MS2 and Q β 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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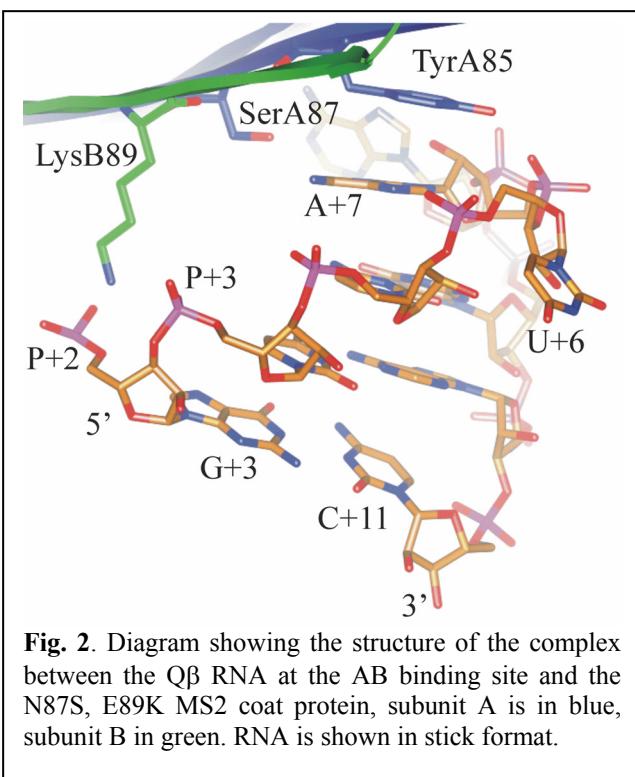


Fig. 2. Diagram showing the structure of the complex between the Q β RNA at the AB binding site and the N87S, E89K MS2 coat protein, subunit A is in blue, subunit B is in green. RNA is shown in stick format.