Structure of a bacterial Type-I DNA methyltransferase with a bound antirestriction protein from phage T7 using electron microscopy

Christopher K. Kennaway and John Trinick

Introduction

Bacteria protect themselves from invading foreign genetic material (from phage, plasmids, cosmids, etc.) by several means, one of which is the type I Restriction Modification (R-M) system. R-M systems act to limit the spread of pathogenic traits such as antibiotic resistance by horizontal gene transfer. The bacterium's own DNA is modified by addition of methyl groups at specific sites, and when DNA lacking any modification is encountered the 'foreign' DNA is destroyed by an endonuclease. The double stranded breaks are distant from the initial recognition site due to the action of a DNA translocase. The DNA methylation, translocation and restriction functions are all carried out by a type I complex made of three types of subunits: S (specificity), M (methyltransferase) and R (restriction endonuclease and translocase), assembling as $R_2M_2S_1$. DNA methylation alone requires a M_2S_1 complex. Type I R-Ms are specifically inhibited by DNA mimic proteins produced by phage. In this work a complex of $M_2S_1$with Ocr, an antirestriction protein from phage T7, was studied by electron microscopy (EM).

Results

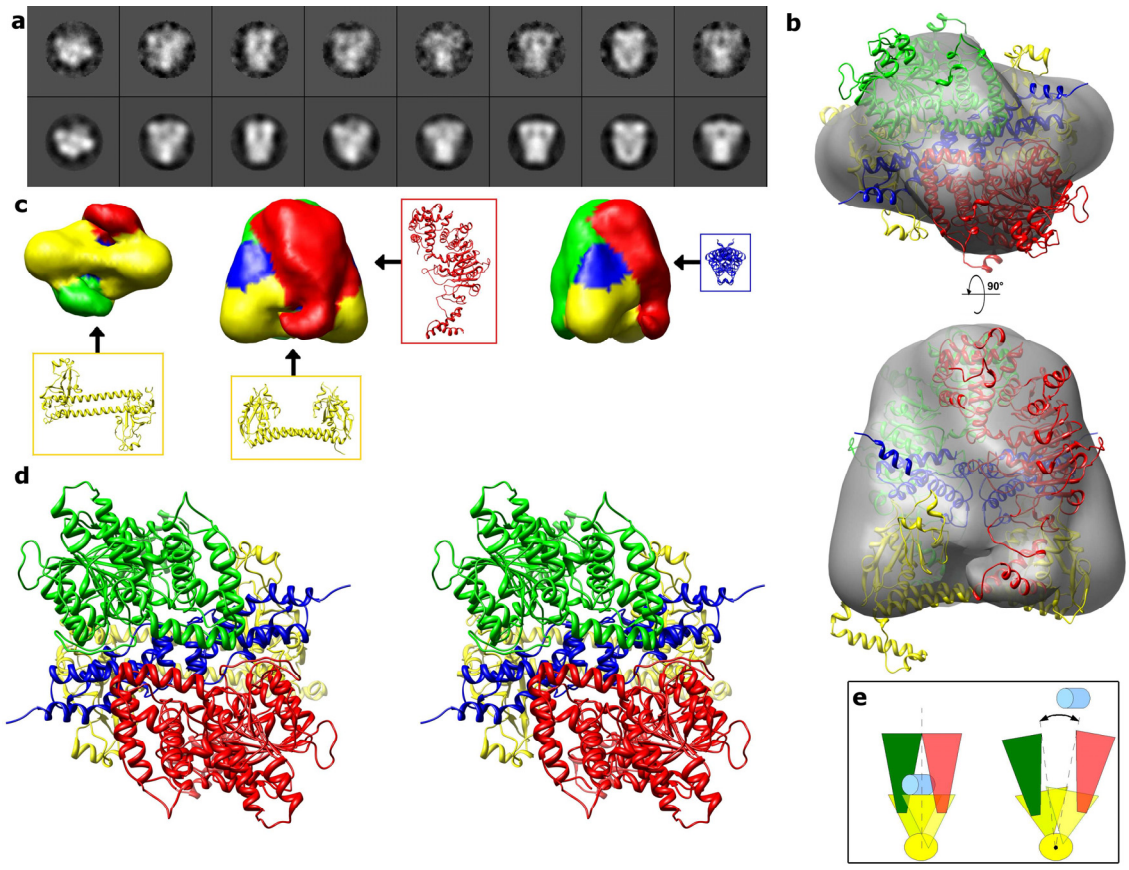

Figure 1. EM data, 3D reconstructions and model for the M_2S_1-ocr complex. **a**: Eight selected EM class average images (top row) with their corresponding reprojections of the EM map (second row), showing a range of views. **b**: Two orthogonal semi-transparent surface representations of the EM 3D reconstruction each with a view of the modelled coordinates fitted as a rigid body (green and red – M; blue – ocr dimer; yellow – S). **c**: 3 surface views of the M_2S_1-ocr EM 3D reconstruction coloured according to proximity to the fitted coordinates, with the positions of individual protein chains indicated with arrows. **d**: A stereo view of the M_2S_1-ocr atomic model. **e**: Schematic diagram of proposed mechanism of clamping and release of DNA substrate (light blue) facilitated by twisting of the coiled-coil (yellow, viewed end-on).

Negative stain electron microscopy of M_2S_1-Ocr assemblies (~200 kDa) showed a homogeneous array of particles with a variety of orientations visible on the carbon substrate. Single particle analysis and three dimensional reconstruction image processing techniques resulted in a 3D density map, which was judged by Fourier shell correlation to be about 18 Å resolution. X-ray crystallographic structures of individual subunit proteins were modified using homology modelling methods and automated docking programs. The resulting atomic assembly matched the EM density when fitted either manually or computationally. Similar atomic models were also constructed for the M_2S_1-DNA complex, giving a complete explanation of how the proteins come together and bind their target sequence. This model also encompassed bending of the DNA backbone, flipping out the adenine bases, sequence recognition, and catalytic site formation.

Conclusions

The new EM structure and computational model of M_2S_1-Ocr rationalise, for the first time, a large body of experimental data obtained using many different methods over many years. A mechanistic explanation of the Type I methyltransferase enzymes is suggested by the model, which clearly indicates locations for further analyses such as the M-S and M-M interfaces. When combined with data on M.EcoR124I**,** the model also suggests that a dynamic opening and closing of the protein, driven by a flexing and twisting of the conserved coiled-coil region within S, is required to open up the M-M interface to allow either DNA binding or attack of the Ocr antirestriction protein, a protein that "disguises itself" as DNA.

Publications

Kennaway CK, Obarska-Kosinska A, White JH, Tuszynska I, Cooper LP, Bujnicki JM, Trinick J, & Dryden DT. (2009) The structure of M.EcoKI Type I DNA methyltransferase with a DNA mimic antirestriction protein. *Nucleic Acids Res.* **37**, 762-70.

Collaborators

A Obarska-Kosinska, I Tuszynska and J Bujnicki (Poznan) and J White, L Cooper and D Dryden (Edinburgh)

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