# Structure of a plasmid replication initiator protein: first example of the *Rep_Trans* family displays a novel fold

Stephen Carr, Simon Phillips and Chris Thomas

## Introduction

Plasmids of the pT181 family are widespread among the staphylococci, typically carrying antibiotic resistance genes in a mobilisable context. As such they contribute the ongoing problem of "superbugs" such as MRSA. These plasmids replicate by a rolling circle mechanism, requiring the action of a plasmid-encoded replication initiator protein, Rep. Rep proteins have both DNA-binding and nicking/closing (or DNA-relaxing) activities, and also serve to recruit cellular replication components (such as the PcrA helicase) to the origin.

Similar Rep proteins are involved in virus replication and conjugative DNA transfer. All known examples require a conserved tyrosine residue for nicking/closing activity. Such relaxases can be broadly divided into two groups: those possessing a histidine-hydrophobic-histidine (HuH) amino acid motif, and those without (such as the pT181 family proteins). Sequence conservation among the latter is represented by the *Rep_trans* motif (pfam 02486). Structural data for examples of the HuH family is already available, but (despite over 20 years of effort) the structural characterisation of a *Rep_trans* example has until now remained elusive.

## Results

Our studies of *Rep_trans* proteins have included RepSTK, encoded by plasmid pSTK1 which was isolated from the thermophile *Geobacillus stearothermophilus*. Previous work has identified that a 42 kDa N-terminally extended translation product is necessary for site-specific nicking and closing of DNA *in vitro*. Derived from this product, a 33 kDa C-terminally truncated form (RepSTK-b) retains both activity and stability at high temperature.

Crystals of RepSTK-b yielded diffraction data to 2.3 Å, phase information being obtained using heavy metal derivatives. Four monomers were present in the asymmetric unit, arranged as two dimers. Little variation was evident between monomers; the dimer represented by molecules a and c is presented in Figure 1.

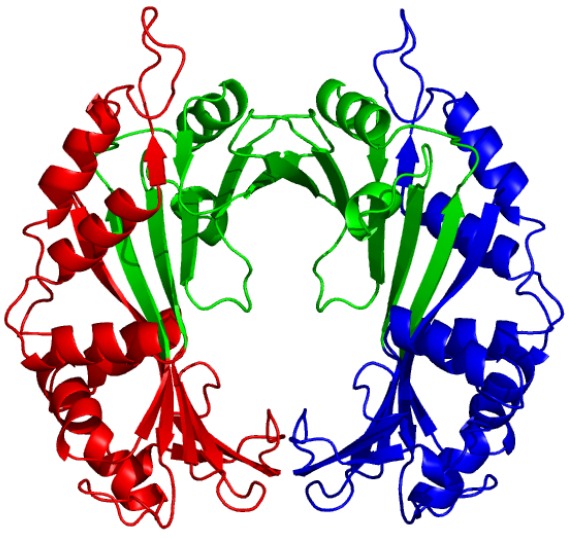

**Figure 1:** overall architecture of RepSTK-b, with *Rep_trans* motif in green.

Each monomer is crescent-shaped, with the dimer presenting a near-continuous antiparallel beta sheet lining a central hole roughly 20 Å diameter. The dimer interfaces are at the top and bottom in the orientation shown. Residues making up the active site are included within the span coloured in green in Figure 1, corresponding to the *Rep_trans* motif.

The side chains of conserved and active site residues project inward towards the centre of the structure, clustered around the essential nucleophilic tyrosine. The arrangement within one monomer is shown in Figure 2. HuH and *Rep_trans* motifs are fundamentally different: the active site of *Rep_trans* contains no histidine residues, nor does it utilise a nucleophilic

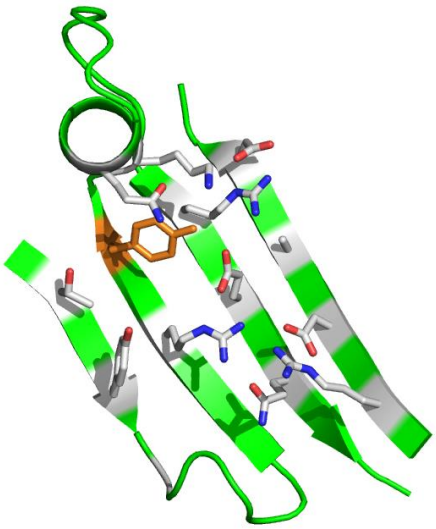

**Figure 2:** arrangement of active site residues surrounding the tyrosine nucleophile (shown in gold).

tyrosine approaching from a separate helical structure. We have also been unable to find any significant structural similarities to coordinates deposited within the PDB; we therefore believe the fold to be new.

The structure presents many useful clues regarding physical characteristics of Rep proteins and their likely mechanism of action. For example, the N-terminal extension to RepSTK required for isolation of a viable relaxase is an integral part of the beta sheet structure. Furthermore, the accommodation of a continuous DNA strand running through the central pore would account for the processivity conferred on PcrA helicases by such Rep proteins.

We are currently using these coordinates to solve diffraction data obtained from several staphylococcal Rep proteins of the pT181 family, and work is already well advanced on the structural determination of variants based on RepD, RepN and RepE.