

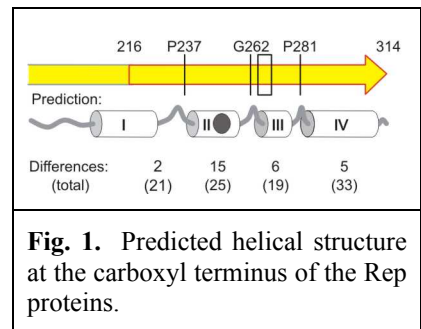
# Dimer specificity in a replication initiator protein

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## Background

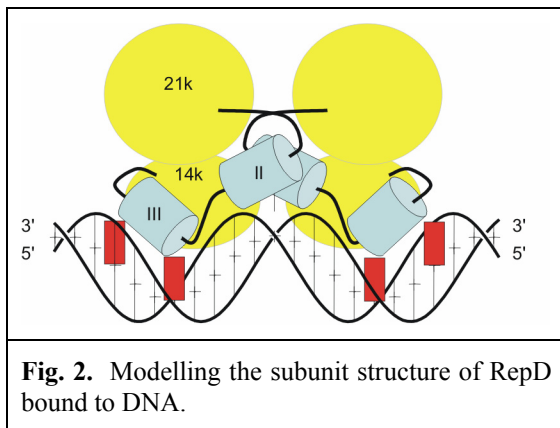
Replication of staphylococcal plasmids such as pT181 and pC221 requires the plasmid-encoded Rep proteins RepC and RepD. These proteins are over 80% identical in primary sequence, yet retain DNA binding specificity for their cognate plasmid replication origins due to a small divergent region of six amino acids within the carboxyl terminus.

The 34kDa fusion protein RepDC contains residues 35-216 of RepD and 217-314 of RepC, corresponding to the 21kDa and 14kDa fragments obtained by partial proteolysis of these proteins. RepDC displays DNA sequence specificity for pT181 but cannot form heterodimers with RepD, despite sharing over 80% amino acid sequence identity. Dissection of the C-terminal domain previously correlated this dimerisation specificity with residues 237-262, which is adjacent to (but not overlapping) the DNA binding determinant at 265-270. This dimerisation determinant includes the second of four predicted alpha-helical regions (I - IV) as shown in Fig. 1.



## Recent findings

Dimerisation studies have been conducted using a wide range of 34kDa variants, with sequences exchanged in each of the four indicated regions. Each has now been tested for dimerisation against both RepD and RepC. In each case, dimerisation specificity is conferred solely by the sequence at region II. No additional specificity is contributed by the rest of the protein: for example, a RepD variant altered only by possessing the 15 RepC-specific amino acids in region II will form heterodimers with RepC protein.



Dimerisation trials have also been conducted using fusion proteins based on other Rep proteins of the pT181 family. For example, RepDN (which substitutes residues 217-314 with those of the pCW7 RepN protein) demonstrates dimerisation against RepC but not RepD. From such results the contribution of individual residues within region II can be deduced, and the likely arrangement of helices and loops at the dimer interface modelled as in Fig. 2.

Based on this model, point mutations are currently being designed to create novel dimerisation interfaces. These will allow the construction of obligate heterodimers, with asymmetric DNA binding and nicking characteristics.

## Acknowledgements

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