[MS9-04] Structural Studies of Rolling Circle Replication Initiator Proteins

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pT181 family plasmids confer antibiotic resistance to theri host cell and replicate by a rolling-circle mechanism. This is initiated by a plasmid-encoded Rep initiator protein, which has sequence-specific DNA nicking and religation activity. The replication origin is nicked by Rep, which binds covalently to one DNA strand via an active site tyrosine, initiating rolling circle replication and religating the strand at the end of the cycle. Rep proteins also associate with PcrA helicase to form a highly processive complex. We have determined the structure of the Rep protein from cryptic plasmid pSTK1 of *Geobacillus stearothermophilus (Gst)*, and several variants of RepD from *Staphylococcus aureus (Sau)*, representing the first structural information on this class of initiator proteins. Cloning and expression of a construct derived from the pSTK1 Rep yielded a product that relaxed plasmid substrates encoding an inverted repeat sequence from pSTK1, which resembles the replication origin of the pT181 family, and activated the cognate Gst PcrA helicase. The crystal structure for the 31 kDa fragment of *Gst* Rep has been solved at 2.3 Å, showing a novel, ring-shaped dimer with a 20Å diameter pore. The inner surface is formed by an 20-stranded β-sheet, while the outer surface is decorated with 18 α-helices. The protein has a novel fold, but the extended sheet exhibits similarities to that in TATA-binding protein (TBP). The active site Tyr179residues, one from each subunit, lie 26 Å apart across the pore, with a nearby catalytic magnesium ion co-ordinated by three carboxylate side-chains. Crystal structures for the *Sau* Rep variants RepDE, RepDN and RepDC have been solved by molecular replacement using the *Gst* Rep as a model, showing similar structural features in the catalytic domains, but with additional DNA-binding domains (DBDs). The DBDs resemble bacterial sigma factors and recognise a target sequence in dsDNA adjacent to a putative cruciform structure at the replication origin, allowing cleavage of one DNA strand in a loop, and covalent attachment via the catalytic tyrosine. This is followed by recruitment of the PcrA helicase, processive replication of one strand of the plasmid and a final ligation step via the second catalytic tyrosine to generate a single strand product. The implications for the mechanism of rolling circle replication will be discussed in the light of extensive functional data available for *Sau* RepD.

Keywords – antibiotic resistance, rolling-circle replication, PcrA helicase