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Report:

Bacterial plasmid replication is highly regulated because the number of copies has to be kept constant. In many multicopy bacterial plasmids, this is accomplished by the availability of the initiator of replication protein (Rep). In the promiscuous streptococcal 5536-bp plasmid pMV158, the synthesis of initiator RepB is stringently controlled by both a 50-nt antisense RNA and the 45-residue transcriptional repressor CopG (formerly RepA) the smallest naturally occurring transcriptional repressor described to date. CopG protein represses its own synthesis and that of the RepB protein by binding to the single *cop-rep* promoter region containing a 13-bp imperfect inverted repeat (IR). An intrinsic DNA curvature is observed around the CopG DNA target region, which includes several A-tracts spaced 10-bp apart in both directions of the IR. Upon addition of CopG this intrinsic curvature is greatly enhanced. Hydroxyl radical and DNase I footprint experiments of CopG at low protein:DNA ratios show a cooperative protection that extends in both directions from the IR. The protected sequences cover at least four successive major grooves, two on each side of the two-fold axis of the IR. The protected sites are located on the same face of the double helix and cover the promoter -35 and -10 regions. Binding of CopG thus could hinder host RNA polymerase binding. At high protein:DNA ratios, the protected region can extend up to 100 bp (our unpublished results). These observations indicate formation of a multiprotein complex nucleating from the CopG 13-bp element and extending several helical turns of DNA. Formation of these complexes apparently requires DNA binding, since the free protein remains dimeric up to 780 μM concentrations as revealed by analytical ultracentrifugation analysis.

The unliganded CopG structure reveals a ribbon-helix-helix (RHH) topology. The polypeptide chain commences with a segment in extended conformation (strand 1) reaching until Leu9. At Glu11, helix A starts with its axis rotated for about seventy degrees away from the direction of strand 1. This helix finishes with Met24, then enters a glycine-mediated turn leading to helix B, which begins with Lys28 and extends to the C-terminal end. This a helical axis of B is rotated about ninety degrees with respect to helix A, the direction

change permitted by the presence of Gly25 and a 1-5 main chain hydrogen bond (Arg22 N-Leu26 O). This topology is the same as that found in proteins with helix-turn-helix (HTH) motifs, and formed the basis of the hypothesis for many years that the structure of CopG and related plasmid repressors resemble that of the Cro repressor proteins of bacteriophages 1 and 434. Circular dicroism measurements of CopG indicated a consensus average content of more than 50 % a-helix and 10-35 % b-strands and turns, consistent with the expectation of the hypothesis. In the crystal structure, however, two CopG monomers (termed A and B) associate in a symmetrical manner (via a local two-fold axis) over a common surface of 1615 $Å^2$. This monomer-monomer association leads to the formation of a two-stranded twisted antiparallel sheet or β-ribbon comprising the N-terminal segments of each molecule and based on 10 inter main chain hydrogen bonds from Met1 to Ser10. This β-ribbon is observed to interact with the major groove of the target DNA (see below) and is presented to the exterior from a hydrophobic and dimeric protein core.

A third protein monomer (C) is present in the asymmetric unit of the unliganded crystal structure that, via a crystallographic dyad, forms a further dimer of type molecule C-molecule C, similar to the first. Therefore, the establishment of dimers is observed to occur in our structure by either a local (molecule A-molecule B) or a crystallographic axis. Each AB dimer interacts on one side with a crystallographically related second AB-type dimer with almost equivalent occluded surface. The dimers in the unliganded crystal displays a polymerization behaviour that generates a helical structure.

The structure of CopG in complex with a 19-bp dsDNA containing the 13-bp pseudosymmetric element reveals a tetramer composed of two AB dimers as observed in the unliganded structure. CopG interacts with DNA bases via the N-terminal β-ribbon using residues Arg4, Thr6, and Thr8 of each monomer and with backbone phosphate groups via residues Lys28 and Ser29 (of each protomer) of the protein segment connecting helices A and B, inducing a bend of 60 degrees. The bend is produced by the marked compression of both minor and major grooves facing the protein. The minor groove located at the center of the operator becomes extremely narrow (1.9 Å). The major grooves are also remarkably tighter (8.6 Å) than regular B-DNA (11.7 Å) at the area interacting with the β-ribbon. The DNA backbone follows, in general, a smooth path, except for the zone close to the center of the operator, where the minor groove is compressed and the base pairs are rather inclined.

Electrophoretic mobility shift assays (EMSA) showed four discrete DNA-repressor complexes, indicating that at least four CopG dimers bind to the whole target region, which agrees with stoichiometry determinations. A model is proposed, in which four dimers are required to protect four consecutive major grooves on one face of the DNA, where the target DNA is bent for about 110 degrees, based on the results of EMSA, electron microscopy and footprinting assays and the structural data described here. In this model, AB-AB types of interdimer interactions have been assumed to generate the four protein dimers.

In order to further investigate the bending behaviour of CopG, several complexes with dsDNA of distinct sequence and length have been set up for crystallization. Among these, a complex of CopG with a 19-bp dsDNA of palindromic sequence interestingly rendered crystals of a new spacegroup, primitive orthorrhombic (instead of the tetragonal space group of the complex reported above). The cell constant of these crystals are a= 59.4 Å, b= 70.9 Å, c= 110.8 Å. As initial molecular replacement calculations using the known structure of the CopG/19-bp dsDNA as a searching model did not produce the expected success, a mercury derivative obtained by soaking in a mercury acetate conatining solution of proper composition was measured at ID02B. These diffraction data range up to 2.95 Å resolution, display an Rmerge of 0.089 and a completeness of 96.1 % for the whole range (110.8 – 2.95 Å) and of 83.8 % for the last shell (3.11 – 2.95 Å). Difference Patterson calculations to determine putative mercury positions are currently under way.