FEBS 30039 FEBS Letters 579 (2005) 5669–5674

Revised structure of the AbrB N-terminal domain unifies a diverse superfamily of putative DNA-binding proteins

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Received 2 June 2005; revised 14 September 2005; accepted 19 September 2005

Available online 4 October 2005

Edited by Christian Griesinger

Abstract **New relationships found in the process of updating the structural classification of proteins (SCOP) database resulted in the revision of the structure of the N-terminal, DNA-binding domain of the transition state regulator AbrB. The dimeric AbrB domain shares a common fold with the addiction antidote MazE and the subunit of uncharacterized protein MraZ implicated in cell division and cell envelope formation. It has a detectable sequence similarity to both MazE and MraZ thus providing an evolutionary link between the two proteins. The putative DNA-binding site of AbrB is found on the same face as the DNA-binding site of MazE and appears similar, both in structure and sequence, to the exposed conserved region of MraZ. This strongly suggests that MraZ also binds DNA and allows for a consensus model of DNA recognition by the members of this novel protein superfamily.**

Keywords: NMR solution structure; Protein–DNA interactions; Structural genomics; Structural classification of proteins

1. Introduction

The transition state regulator AbrB from *Bacillus subtilis* is a transcription factor controlling the expression of more than 60 different genes, yet there is no apparent nucleotide consensus sequence [1]. It displays specificity within this target set by binding each promoter with a different affinity. It is thought that local variations of DNA structural parameters (e.g., propeller twist, opening, stretch) contribute to the differential binding proclivities of AbrB [1]. In both its free and DNA-bound states AbrB is a tetramer consisting of identical 94 residue monomers. Its DNA-binding function resides solely in the N-terminal domain (AbrBN) of 53 residues [1–4]. This domain also possesses a strong dimerization interface. Mutagenesis studies suggest that the role of the C-terminal domain is in forming multimers [5]. The N-terminal domains of very similar sequences are present in two more *B. subtilis* proteins, Abh and SpoVT, and their close homologues from other *Bacilli* and related firmicutes *Clostridia*. Like AbrBN, the SpoVT domain was shown to bind DNA [6]. The reported solution structure of the AbrBN dimer (PDB entry 1EKT) showed little similarity to any known DNA-binding protein supporting a non-classical explanation of its unusual target specificity [2,3].

More sensitive sequence similarity searches identified numerous SpoVT/AbrB-like domains in many sequenced bacterial and archaeal genomes (Pfam family 04014) [7]. These include a known DNA-binding protein MazE from *Escherichia coli* [8]. MazE, an antidote protein (82 residues) of the MazE/MazF addiction module, binds to and inhibits the toxin MazF, a ribonuclease [9]. It binds specifically to the *mazEF* operon repressing the expression of addiction module. Its DNA-binding function resides in the N-terminal domain, like AbrB [10]. In contrast to AbrB, the MazE known targets appear to be limited to three similar sequences in the *mazEF* promoter [8]. The crystal structures of MazE complexes with MazF and a camelid antibody have been determined recently [11,12]. MazE forms a homodimer consisting of a single DNA-binding domain made of the intertwined N-terminal 47 residue segments and two C-terminal arms that bind MazF. The MazE N-terminal domain revealed a similar secondary structure to AbrBN, but, despite the predicted homology, its overall fold appeared dissimilar to that of AbrBN.

There are two possible solutions to this paradox: either the two proteins are not related or their structural dissimilarity was due to an artifact. On one hand, it is not uncommon for small proteins to provide exceptions from the empirical rule that proteins of similar sequences have similar structures. By itself, a low pairwise sequence similarity of ~20% between the DNA-binding domains of AbrB and MazE is insufficient to guarantee overall structural similarity [13]. Indeed, on the basis of a comparable sequence similarity, it was predicted prior to structure determinations that MazE and AbrB dimers would adopt a β-barrel fold related to the double-ψ barrel fold of the VAT-Nn domain made of tandem repeat of two similar sequence motifs [14]. However, despite their sequence similarity to VAT-Nn repeats, neither MazE nor AbrBN displayed the predicted barrel fold. There was a local structural similarity of the MazE monomers to the VAT-Nn repeats corresponding

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Abbreviations: SCOP, structural classification of proteins; CSI, chemical shift indices; TALOS, torsion angle likelihood obtained from shift and sequence similarity

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doi:10.1016/j.febslet.2005.09.045

to their sequence similarity, but these similar substructures were assembled in the MazE globular domain in a distinctly different way. On the other hand, the distant homology recognition by thoroughly calibrated multiple sequence alignment-based methods has proven quite reliable [7,15]. Homologous proteins are expected to share a common fold, and an intertwined MazE-like fold with an additional region of dimeric interface between β-strands 2 and 2′, not previously observed in AbrBN, helped explain the strong interaction seen between two AbrBN monomers (Fig. 1A).

The hypothesis of AbrBN probably adopting the MazE-like fold gained further support from the discovery of an unexpected structural relationship between MazE and MraZ, an uncharacterized protein encoded by an operon involved in cell envelope formation and cell division. The crystal structure of the MraZ homologue MPN314 from *Mycoplasma pneumoniae* revealed a ring-shaped octamer [16]. Its rim surface is positively charged and contains protrusions formed by a highly conserved sequence motif. In the original report, there was no local structural similarity to the MraZ conserved surface site found, and the subunit structure was also described as a novel fold, precluding the structure-based functional assignment [16]. However, when classifying the MraZ subunit structure in the structural classification of proteins (SCOP) database [17], we noted its striking similarity to the MazE dimeric domain (Fig. 1A and B). There are two repeats of similar structure and sequence. The N-terminal parts of both repeats are intertwined into a single domain of the MazE fold, whereas the extra C-terminal helical regions determine the oligomeric assembly.

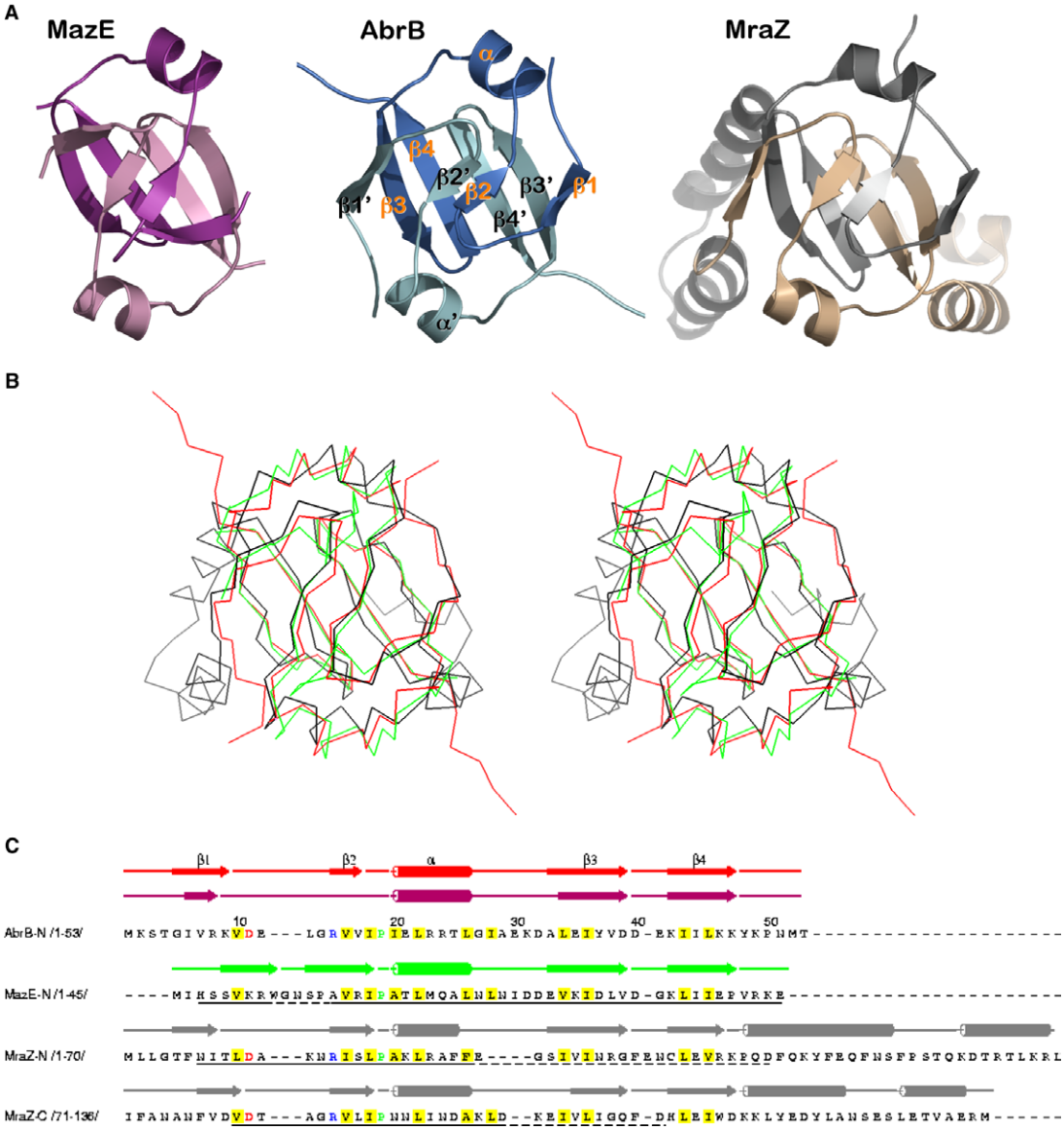

Fig. 1. Structural and sequence relationships in the AbrB/MazE/MraZ superfamily. (A) Common fold of MazE, AbrBN (secondary structures labeled) dimers, MraZ subunit. (B) Backbone superposition of AbrBN dimer (1Z0R, red), MazE dimer (1 MVF, green) and MraZ subunit (1N0E, grey) structures. The pairwise Cα-atom r.m.s. deviations are: MazE v MraZ – 1.4 Å (74 pairs); AbrB v MazE – 1.2 Å (82 pairs); and AbrB v MraZ – 1.5 Å (84 pairs). (C) Structure-based alignment of MazE and AbrB subunits with MraZ repeats, colors correspond to those in (B). PSI-BLAST hits to AbrB from the MazE and MraZ sequences are underlined, with solid line indicating correctly aligned regions. Parts A and B were produced by using PYMOL [40] and C was produced by using ALSCRIPT [41].

SCOP is a database of known structural and probable evolutionary relationships amongst proteins of known structure [17]. The structural similarity of MazE and MraZ, apparently undetected by automated structural similarity searches [16], is one of many new relationships routinely discovered during the classification of new protein structures in SCOP by human experts. The discovery of a new superfamily of evolutionary related proteins is always of special significance, as it allows the integration of known structural, biochemical and functional data on the constituent families, thereby assisting with functional predictions. To facilitate the discovery of new superfamilies, the SCOP classification procedure includes a thorough bioinformatics analysis of new structural relationships, looking for the evidence of a probable distant homology. Our analysis of the MazE/MraZ relationship resulted in the identification of AbrB as a probable evolutionary link between the two proteins and the finding that their common intertwined fold was fully consistent with the published NMR experimental data on the AbrBN dimer. Provided AbrBN indeed shared the common fold with MazE and MraZ, this would imply the possibility of a common structural basis for the DNA-binding function of AbrB and MazE and the structure-based functional assignment of MraZ.

Here, we present the results of our bioinformatics analysis of the AbrB, MazE and MraZ structures, highlighting some of the SCOP team's approaches to the detection and classification of new relationships. We also present the revision of the AbrBN structure, prompted by this analysis. The revised AbrBN structure has revealed additional similarities to the MazE and MraZ structures. It is supported well by our functional investigations and, as discussed, may provide insight into the general DNA-binding properties of this new superfamily.

2. Material and methods

2.1. Sequence analysis

Protein sequence comparison and database searches were performed with PSI-BLAST v.2.2.5. [15]. A two-step routine procedure was implemented for the pre-classification of new PDB entries in SCOP. In the first step, a PSSM was generated by searching the NCBI non-redundant protein database [18] (the database release dates were 09-May-2003 and 11-Oct-2003 for MazE and MraZ searches, respectively). The *E*-value cutoff for the inclusion of PSI-BLAST hits in the PSSM was 0.001. In the second step, the SCOP domain sequences [19] were scanned with this PSSM. Database searches were performed with a standalone BLAST program shortly after the release dates of the corresponding PDB entries – 1UB4 and 1MVF for MazE, and 1N0E, 1N0F and 1N0G for MraZ.

2.2. NMR Spectroscopy

AbrBN expression and purification were preformed as previously described [4]. All NMR experiments were performed at 305 K on a Varian INOVA 600. 1.0–2.0 mM protein samples in the following buffer: 90%:10% or 1%:99% H_2O:D_2O, 15 mM KH_2PO_4, pH 5.8, 10 mM KCl, 1 mM EDTA and 1 mM DTT. Sequential assignments were made from HNCACB, CBCACONH, HNCA, HNCOCA, HNCO and HNCACO experiments [20–24]. Side-chains were assigned from H(CCO)NH, (H)C(CO)NH and HCCH-TOCSY experiments [20,22,23]. Exchange protected amides were monitored by sequentially recording 100 12-min 2D ^{1}H–^{15}N HSQC experiments over a 24-h period. HNHA, chemical shift indices (CSI) and torsion angle likelihood obtained from shift and sequence similarity (TALOS) experiments were used to determine coupling constants for assigning backbone ϕ and ψ angles [25]. NOE experiments that were analyzed include a 120 ms and 150 ms mixing time ^{15}N-NOESY-HSQC and ^{13}C-NOESY-HSQC. Structures were calculated with NOEs, hydrogen bond restraints (CSI predictions and amide exchange experiments) and ϕ and ψ angles (TALOS predictions). ARIA, version 1.2, and CNS, version 1.1, programs were used to compute the solution structure starting from an extended structure with random side-chain conformations [26,27]. The CNS protocols used simulated annealing with torsion angle and Cartesian space dynamics using the default parameters. Manually assigned inter- and intramolecular NOEs were input to ARIA as unassigned and uncalibrated with respect to distance. The total number of ambiguous NOE restraints allowed for each peak in the NOESY spectra was set to 20. Distance restraints, derived from the manually assigned NOEs, were set to 1.8–6.0 Å. The dihedral angle restraints were taken to be ±2 standard deviations or at least ±20 from the average values predicted by TALOS [23]. Here, the dihedral angles were restrained to $\phi = -70°$ (±50°) and $\psi = -50°$ (±50°) for the helical regions. Non-crystallographic symmetry energy term (NCS) was used to keep the Cα atoms of the monomers superimposable and distance symmetry potential was used to ensure that the relative orientations of all the Cα atoms of the monomers were symmetric [28]. The spectra were processed with NMRPIPE and analyzed with NMRVIEW on LINUX workstations running Fedora Core 1 [29,30]. Molecules were visualized and aligned with MOLMOL [31]. The seven lowest energy structures were further water refined with ARIA. Analysis of the Ramachandran plot, from the robust structure analysis and validation program MolProbity [32], showed that 99.3% of modeled residues were in allowed or favored regions with a clash score of 31.97, indicative of a well comprised solution structure.

3. Results and discussion

3.1. Structure-based sequence analysis

PSI-BLAST searches with MazE and MraZ sequences failed to detect their relationship but both gave hits to the AbrBN sequence. At the time of analysis, the similarity of MazE and AbrBN sequences was detected with *E*-value 0.002, and the sequence similarity of the MraZ repeats and AbrBN with *E*-values 0.72 and 0.65, for the N- and C-terminal repeats, respectively. This suggested that AbrB might be an evolutionary link between MazE and MraZ. However, this sequence-based hypothesis was not confirmed by examination of the published structure of AbrBN, where the overall fold was dissimilar.

To investigate the possibility of AbrBN actually having the MazE/MraZ fold, PSI-BLAST hits were combined with the structural alignment of MazE subunit with MraZ repeats. The AbrBN secondary structure, mapped onto the resulting alignment, showed a strong correlation with MazE and MraZ secondary structures with the exception of the aforementioned "missing" β-strand 2 (Fig. 1C). Subsequently, a 3D model of the intertwined AbrBN dimer was generated from this alignment. In this model and indeed in the revised structure, many of the original intra-molecular structural contacts have become inter-molecular contacts (Fig. 2). Despite differences at the atomic level, many elements of secondary structure and their interactions remain. For example, previously suggested intramolecular interactions between the N-terminal elements (β-strand 1 and α-helix) and the C-terminal elements (β-strands 3 and 4) have become inter-molecular interactions. On the other hand, the extensive dimeric six-stranded β-sheet retains the same "2D" structure albeit twisted into a more barrel-like shape. Concomitant with the adjustment of the β-sheet, the α-helices move apart to the barrel ends, whereas at the molecular 2-fold axis, the formation of new dimeric interface between β-strands 2 and 2′ occurs.

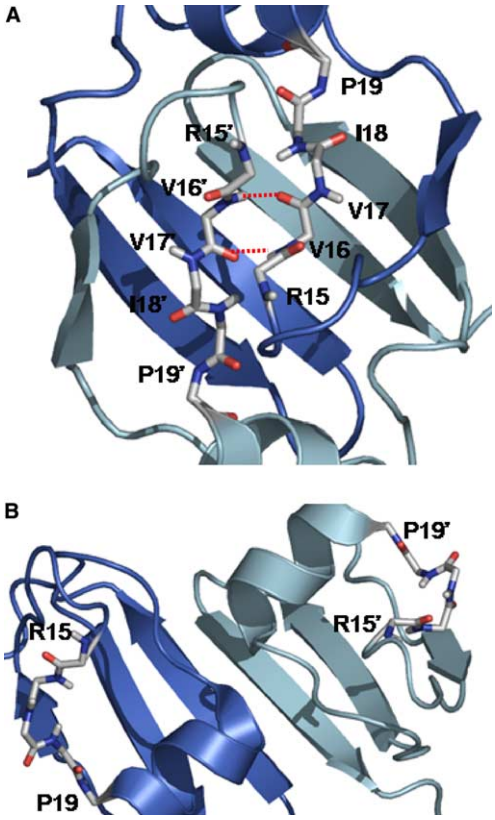

Fig. 2. Revised dimerization interface of AbrBN. (A) Main chain interactions between the β-strands 2 and 2′ are detailed (see the text); hydrogen bonds between V16 and V16′ are shown with red dashed lines for the revised AbrBN structure (1Z0R). (B) Main chain atoms of the β-strands 2 and 2′ regions in detail for the original AbrBN structure (1EKT). This figure was produced by using PYMOL [40].

3.2. Revised structure of AbrBN

A complete and additional set of NMR experiments were performed on AbrBN for assignment and structure purposes. These included all sequential assignment experiments as well as 100 sequential 12-min ^{15}N-HSQC's to unequivocally determine hydrogen bonds. This was performed particularly to verify the predicted existence/interaction of β-strands 2 and 2′. CSI values, TALOS predictions and $3J_{N\alpha}$ coupling constants suggested that a short, loose, β-strand exists between residues 15–18. Hydrogen exchange data confirm that the amide proton of V16 is involved in a dimeric interface hydrogen bond. The previously collected data for V16 was correct but was unfortunately misrepresented as being in fast rather than slow exchange, suggestive of no hydrogen bond. Unambiguous inter-molecular NOE connectivities between R15 and V17 (R15 to V17′ and vice versa) are also observed (Fig. 2). We comprehensively defined the complete dimerization interface by ~1000 constraints. The resulting AbrBN dimer structure (1Z0R) displays a fold similar to the MazE dimer/MraZ subunit (Fig. 1). While MazE and MraZ structures differ in some regions, AbrBN possesses additional conformational similarity to one or the other. AbrBN and MazE retain their similarity in the region between α-helix and β-strand 3, while AbrBN and MraZ are similar in the region between β-strands 1 and 2. Thus the hypothesis that AbrB evolutionary links MazE and MraZ is bolstered through both sequence and structure. This suggests the possibility of a common structural basis for the DNA-binding function of AbrB and MazE and infers analogous function for MraZ.

Subsequent to the release of 1Z0R, Coles et al. corroborated this revised structure [33]. The two independently solved structures are in excellent agreement with only minor local conformational differences that may be the result of well known dynamic fluctuations in the AbrBN structure [3] or due to minor differences in the parameters/implementation of symmetry constraints during the calculation process. It is worth noting, that there are structural variations on a similar scale in MazE that displays multiple conformations in the crystal structure of the MazF complex (1UB4). There are occasional flips of peptide groups between the two structures, for example, between residues 30 and 31. In the structure determined by Coles et al. (1YSF), this peptide appears to make a hydrogen bond, resulting in a local turn of type II. There is an equivalent turn and probable H-bond in the MazE structure. Our hydrogen exchange data, however, did not support this H-bond; therefore the preference for a type I turn in this region was defined by the significant amount of NOEs (see Table 1). This region is suggested to have a significant role in binding DNA [3].

3.3. Consensus model of DNA recognition

A model of the MazE–DNA complex, supported by mutational data, has been proposed [12]. It aligns the dyad of the DNA double strand with the molecular 2-fold axis of the MazE dimer facing DNA with β-strands 2 and 2′. Despite sequence and structural variations, the equivalent surface of AbrB is similar, allowing for a comparable docking model (Fig. 3). This surface has considerable positive electrostatic

Table 1
NMR and refinement statistics

NMR distance and dihedral constraints	AbrBN dimer
Distance constraints	
Total	3247
Intramolecular	2110 (65%)[a]
Intermolecular	1136 (35%)[a]
Hydrogen bonds	48
Total dihedral angle restraints	
ϕ	70
ψ	70
Structure statistics	
Average violations per structure	
NOEs and/or H-bonds	0.57 ± 0.73[b]
Dihedrals	0
Violations (mean and S.D.)	
Distance constraints (Å)	0.044 ± 0.004
H-bonds (Å)	0.049 ± 0.006
Dihedral angle constraints (°)	0.78 ± 0.10
Deviations from idealized geometry	
Bond lengths (Å)	0.00584 ± 0.00022
Bond angles (°)	0.66 ± 0.029
Impropers (°)	1.70 ± 0.16
Average pairwise r.m.s.[c] (Å)	
Secondary structure (backbone)	0.21 ± 0.05
Secondary structure (heavy)	0.63 ± 0.06
Backbone	0.52 ± 0.14
Heavy atoms	0.89 ± 0.12
Clash score	31.97

[a]Percentage of total NOEs.
[b]One NOE violation for the ensemble of seven lowest energy structures.
[c]Pairwise r.m.s. deviation was calculated among the seven lowest energy refined structures for residues 1–53.

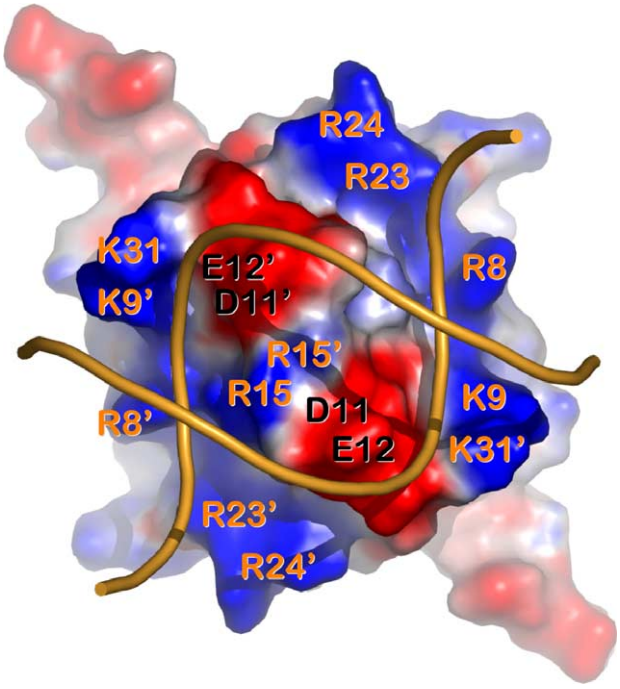

Fig. 3. Putative DNA-binding site of AbrB. Conserved residues remain on same face but are rearranged around the central β2/β2′ pair. The transformed surface is complementary to DNA in shape and charge. This figure was produced by using PYMOL [40].

character, consisting of the conserved arginine residues (R8, R15, R23 and R24) previously identified as essential for binding DNA [2,5]. In this model, residues in the centre of this site are crucial for AbrB's ability to recognize different nucleotide sequences within the major groove. Indeed, a pair of arginines on the molecular 2-fold axis, R15 and R15′, can hydrogen bond to the acceptor groups of all base pair types, preferably with guanine and thymine bases. Restrained by interactions with conserved aspartate residues D11/D11′, R15/R15′ pair retains some conformational variability. There can be alternative

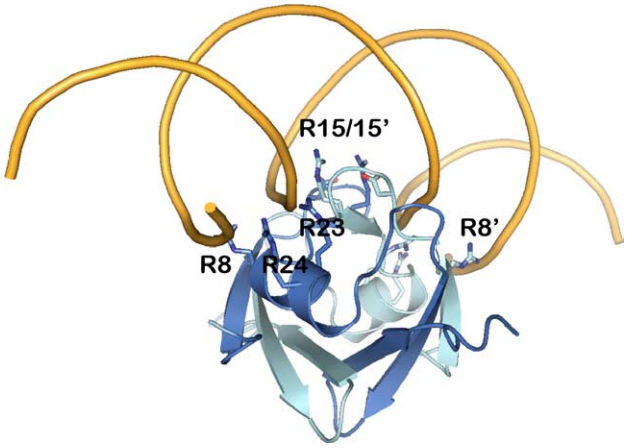

Fig. 4. Model of AbrB interaction with a bent DNA. The DNA-binding surface of the AbrBN dimer was docked manually into the major groove so that the molecular 2-fold axis of the dimer was aligned with a local dyad of double-stranded DNA. A symmetrically bent DNA conformation was constructed by using a known DNA bend (from the SRF core complex; 1SRS). This figure was produced by using PYMOL [40].

pairings of each arginine with either one aspartate or both of them, shifting the positions of its hydrogen donor groups. This model is supported by NMR dynamics data [3] that suggest R15, R23 and R24 have a propensity to alter conformation. Similarly, alternative conformations of the equivalent MazE segment (residues 14–26) are seen in the structure of MazE–MazF complex [11]. DNA flexibility also has been shown to be a contributing factor in the ability of AbrB to bind its targets [11]. These data connect the proposed model with mutational data that identified R8, R15, R23 and R24 as being involved in binding DNA [2,5] (Fig. 4).

Since AbrBN shares sequence and structural similarity to the MraZ subunit, the model can be extended to MraZ. R15 and D11 are conserved in both MraZ repeats, while R23, but not R24, is also fairly conserved. This suggests that MraZ, like AbrB, may bind different DNA sequences. In the MraZ octamer, adjacent subunit sites are suitably oriented and spaced for docking in the major groove of consecutive DNA turns. This allows the hypotheses that the ring-shaped MraZ octamer may wrap a length of DNA along its rim, or, alternatively, MraZ subunits may assemble into longer polymers on the DNA surface. Indeed, a recent crystallization report presents evidence that the *E. coli* MraZ homologue exists in a different oligomeric state, probably forming a dodecamer [34]. The putative DNA-binding function of MraZ suggests a role in the organization of chromosomal DNA and/or the regulation of gene expression during cell division.

Knowledge of the AbrB/MazE/MraZ superfamily common fold and DNA-binding surface will facilitate structural and functional characterization of its other probable members widespread in bacteria and archaea [7,35]. Some of these proteins are shown to be antidote proteins of two-component addiction systems analogous to the MazE/MazF system but containing unrelated toxin proteins [36,37]. There are many more related genes found in similar two-gene cassettes possibly encoding for analogous addiction systems [35]. Mutation in one such cassette, *ntrPR*, has a pleiotropic effect on gene expression in *Sinorhizobium meliloti* [38]. Other addiction systems were shown to participate in the stress response and programmed cell death in bacteria [35,39]. Discovery of new biological roles of such systems warrants further research of protein–DNA interactions in this superfamily.

3.4. Protein Data Bank deposition

The AbrBN coordinates have been deposited in the Protein Data Bank (entry 1Z0R).

Acknowledgement: This work was supported by NIH Grant GM55769 (J.C.). This research was supported in part by MRC Grant G0100305 and the Intramural Research Program of the NIH, National Institute of Environmental Health Sciences.

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