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Structure of CbpA J-Domain Bound to the Regulatory Protein CbpM Explains Its Specificity and Suggests Evolutionary Link between CbpM and Transcriptional Regulators

Naghmeh S. Sarraf1,2,3, Rong Shi3,9, Laura McDonald1,2, Jason Baardsnes2, Linhua Zhang4, Miroslaw Cygler4,5*, Irena Ekiel1,2*

1 Department of Chemistry and Biochemistry, Concordia University, Montréal, Québec, Canada, 2 Life Sciences, National Research Council of Canada, Montréal, Québec, Canada, 3 Département de biochimie, de microbiologie et de bio-informatique, et l’Institut de biologie intégrative et des systèmes, et PROTEO, Université Laval, Québec City, Québec, Canada, 4 Department of Biochemistry, McGill University, Montréal, Québec, Canada, 5 Department of Biochemistry, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Abstract

CbpA is one of the six E. coli DnaJ/Hsp40 homologues of DnaK co-chaperones and the only one that is additionally regulated by a small protein CbpM, conserved in γ-proteobacteria. CbpM inhibits the co-chaperone and DNA binding activities of CbpA. This regulatory function of CbpM is accomplished through reversible interaction with the N-terminal J-domain of CbpA, which is essential for the interaction with DnaK. CbpM is highly specific for CbpA and does not bind DnaJ despite the high degree of structural and functional similarity between the J-domains of CbpA and DnaJ. Here we report the crystal structure of the complex of CbpM with the J-domain of CbpA. CbpM forms dimers and the J-domain of CbpA interacts with both CbpM subunits. The CbpM-binding surface of CbpA is highly overlapping with the CbpA interface for DnaK, providing a competitive model for regulation through forming mutually exclusive complexes. The structure also provides the explanation for the strict specificity of CbpM for CbpA, which we confirmed by making mutants of DnaJ that became regulated by CbpM. Interestingly, the structure of CbpM reveals a striking similarity to members of the MerR family of transcriptional regulators, suggesting an evolutionary connection between the functionally distinct bacterial co-chaperone regulator CbpM and the transcription regulator HspR.

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* Email: irena.ekiel@bri.nrc.ca (IE); miroslaw.cygler@usask.ca (MC)

† These authors contributed equally to this work.

Introduction

In response to environmental stress, including heat, all cells produce heat shock proteins (HSPs), the most important classes of which include chaperones and proteases. Many heat shock proteins are among the most conserved proteins known; however, they possess diverse regulatory mechanisms. Interestingly, in bacteria, HSP chaperones are often directly involved in regulation of transcription through interactions with transcription regulators or sigma factors [1–3]. This contribution can be either positive (e.g. involving sigma32 factors), or negative (e.g. involving transcription regulators such as HspR and HrcA) [1].

Molecular chaperones of the Hsp70 class bind and stabilize proteins at intermediate stages of folding, degradation, assembly and translocation across membranes. They are required for growth at normal temperatures, but their level of expression is enhanced under conditions of stress. The most important for bacterial viability and the most extensively characterized Hsp70 chaperone in Escherichia coli is DnaK [4]. The activity of DnaK/ Hsp70 chaperones is regulated by co-chaperones, members of the DnaJ/Hsp40 family [5–8]. DnaJ is composed of four domains. The N-terminal strongly conserved ~70 residue long so called J-domain [9], the central cysteine-rich domain and two C-terminal domains of similar fold. DnaJ stimulates ATPase activity of DnaK, through conformational change in DnaK from the ATP-bound state, which binds substrates weakly, to an ADP-bound state, which binds substrates tightly [4–7]. Biochemical studies on the E. coli DnaK–DnaJ system have shown that the J-domain, and in particular its H-P-D sequence motif, is important for both DnaK binding and ATPase stimulation [10,11]. These processes are mediated by direct interaction between the J-domain of a co-chaperone and the ATPase domain of DnaK/Hsp70 [7]. J-domains and their mechanism of chaperone regulation are highly conserved from bacteria to humans [8].
Materials and Methods

Protein Expression and Purification

All proteins were expressed in Luria-Bertani (LB) media at 22°C for 18 hrs. Recombinant CbpA, CbpM and CbpA\textsuperscript{Jdom} were purified by standard immobilized metal affinity chromatography using Ni\textsuperscript{2+}-NTA resin (Qiagen) using 50 mM TRIS-HCl, pH 8.0 with 250–300 mM NaCl and eluted with the same buffer containing 200–350 mM imidazole. DnaK, DnaJ and DnaJ\textsuperscript{Bom} were purified using the glutathione sepharose resin (GE Healthcare). The tags were cleaved using either thrombin or tobacco-etch virus protease (TEV), depending on the construct. Following cleavage, the tag was removed from the protein sample using Ni-NTA resin (New England BioLabs Inc). For CbpM and CbpA, the protein samples were also passed through amylose resin (New England Biolabs Inc). All proteins were further purified using size exclusion. For crystallization, the complex CbpM-CbpA\textsuperscript{Jdom} was loaded on a Superdex 75 column and separated from excess CbpA\textsuperscript{Jdom}. For ATPase assays, the proteins were purified on a Superdex 200 column equilibrated with 20 mM Tris pH 8, 150 mM NaCl, 1 mM MgCl\textsubscript{2} and 0.2 mM DTT. Magnesium was omitted for CbpM to avoid contaminated ATPase that co-eluted in the presence of MgCl\textsubscript{2}. No ATPase activity for the CbpM sample was seen when MgCl\textsubscript{2} was removed from the gel filtration buffer.

Surface Plasmon Resonance Assays

All SPR assays were performed using malachite PColorlock ALS Phosphate detection system (Innova Biosciences), a malachite green based dye. For phosphate detection, an equal volume of ALS mix was added to samples and absorbance at 635 nm was measured after 30 minutes (Spectromax 250 plate reader). Phosphate release was linear in the presence of DnaJ and CbpA over the time point studied.

Plasmids and Bacterial Strains

Con structs expressing CbpA\textsuperscript{Bom} were prepared by generating a PCR fragment coding for CbpA (residues 2–73 or 2–76) from E. coli K12 genomic DNA, which were ligated into the expression vector pFO1, a derivative of pET15b (Novagen), to obtain an N-terminal His\textsubscript{6}-tagged thrombin-cleavable construct. The CbpM PCR fragment (residues 2–101) amplified from Klebsiella pneumoniae (strain ATCC 700721/ MGH 78578) and E. coli K-12 genomic DNA as well as full length E. coli CbpA were ligated into pJW271, a derivative of the pMAL-c2X vector (New England BioLabs Inc) to obtain a TEV-protase cleavable, N-terminal His-MBP-fusion protein. E. coli DnaK, DnaJ and DnaJ\textsuperscript{Bom} (residues 2–79) were expressed from pRL652, a derivative of pGEX-4T1 vector (GE Healthcare) to obtain N-terminal GST-fusion constructs with TEV cleavage site. After verification by DNA sequencing, the constructs were transformed into E. coli Rosetta pLysS (Novagen) for protein expression. Site directed mutagenesis was done with the QuikChange Site-Directed Mutagenesis kit (Stratagene) as recommended by the manufacturer.
Data Collection and Refinement

Data collection was carried out at the CMCF1 beamline, Canadian Light Source. The Br-SAD data were collected to 2.6 Å resolution at a wavelength of 0.9197 Å. Crystals belong to space group \textit{P2}_1\textit{2}_1\textit{2}_1 with its unit cell dimensions \(a = 52.6\), \(b = 74.0\), \(c = 113.2\) Å. Native diffraction data were collected to 1.87 Å at a wavelength of 0.9795 Å with unit cell \(a = 52.8\), \(b = 77.1\), \(c = 111.2\) Å. Data processing and scaling were performed with HKL2000 [23]. The 18-site Br-substructure was determined with auto-SHARP [24]. Solvent flattening with RESOLVE [25] led to phases with a figure-of-merit of 0.70 and automated model-building completed 75% of the expected residues in the asymmetric unit. The resulting model was then used for molecular replacement with MolRep [26] using the higher resolution native dataset, non-isomorphous to the Br-soaked crystal. Several cycles of refinement using REFMAC5 [27] followed by model rebuilding with Coot [28] were carried out, resulting in the final model with \(R\)\textit{work} = 0.195, and \(R\)\textit{free} = 0.230 (Table 1).

Accession Numbers

Coordinates and structure factors have been deposited with the RCSB PDB under accession code 3UCS.

### Table 1. X-ray data collection and refinement statistics.

| Data set                     | Br-soaked (SAD) | CbpM-CbpA(Jdom) |
|------------------------------|-----------------|------------------|
| Space group                  | \(P2_1,2_1\)    | \(P2_1,2_1\)     |
| \(a, b, c\) (Å)              | 52.6, 74.0, 113.2 | 52.8, 77.1, 111.2 |
| Wavelength (Å)               | 0.9197          | 0.9795           |
| Resolution (Å)               | 50–2.50 (2.59–2.50) | 50–1.87 (1.94–1.87) |
| Observed hkls                | 162,723         | 309,898          |
| Unique hkls                  | 29,765*         | 38,222           |
| Redundancy                   | 5.5 (5.1)       | 8.1 (7.6)        |
| Completeness (%)             | 100.0 (99.9)    | 99.7 (98.7)      |
| \(I/\langle I \rangle\)      | 0.116 (0.497)   | 0.062 (0.489)    |
| Wilson B (Å\(^2\))           | 38.3            | 28.2             |
| \(R\)\textit{work} (hkls)   | 0.196 (36253)   | 0.232 (1911)     |
| \(R\)\textit{free} (hkls)   | 0.195 (36253)   | 0.232 (1911)     |
| Protein B-factors (# atoms)  | 31.0 (2814)     | 35.5 (450)       |
| Solvent B-factors (# atoms)  | 31.0 (2814)     | 35.5 (450)       |
| Ligands                      |                 |                  |
| Ramachandran allowed (%)     | 100%            |                  |
| Ramachandran generous (%)    | 0               |                  |
| Ramachandran disallowed (%)  | 0               |                  |
| RMSd's                        |                 |                  |
| Bonds (Å)                    | 0.012           |                  |
| Angles (°)                   | 1.15            |                  |
| PDB code                     | 3UCS            |                  |

*Friedel pairs were unmerged.

\(R\)\textit{work} = \sqrt{(\langle I_{\text{obs}} - I_{\text{calc}}\rangle^2)}/\langle I_{\text{obs}}\rangle.

\(R\)\textit{free} = \sqrt{(\langle F_{\text{obs}} - F_{\text{calc}}\rangle^2)}/\langle F_{\text{obs}}\rangle.

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Results

### Overall Structure of the CbpM-CbpA\(^{Jdom}\) Complex

Initial attempts to obtain crystals of the \textit{E. coli} CbpM-CbpA\(^{Jdom}\) complex were unsuccessful and as a result we expressed and purified protein orthologues from \textit{Klebsiella pneumoniae} that show high sequence identity to their \textit{E. coli} counterparts (71% and 89% identity for CbpM and CbpA\(^{Jdom}\), respectively). While this complex also resisted crystallization, a mixed complex containing \textit{K. pneumoniae} CbpM and \textit{E. coli} CbpA\(^{Jdom}\) led to well-diffracting crystals. This heterologous complex behaved better during purification and eluted as a single peak from size exclusion column.

The crystals of the CbpA\(^{Jdom}\)-CbpM complex contain two CbpM and two CbpA\(^{Jdom}\) molecules in the asymmetric unit. The CbpM forms a tight dimer and the CbpA\(^{Jdom}\) subunits are bound to this dimer on opposite sides, near the globular domains (Fig. 1A). Thus CbpM and CbpA\(^{Jdom}\) form a heterotetramer with a 2:2 stoichiometry. Size exclusion chromatography and dynamic light scattering indicate that a heterotetramer is also present in solution, strongly suggesting that this is the biological unit. Upon complex formation, each CbpA\(^{Jdom}\) molecule buries 740 Å\(^2\) or 13.5% of its total surface (≈740 Å\(^2\)) while the buried surface of the CbpM dimer is ≈700 Å\(^2\) or 5.5% of the total solvent-accessible surface (12,700 Å\(^2\)).
CbpM Structure

The structure of K. pneumoniae CbpM is the first reported structure of a bacterial co-chaperone regulator. Each subunit of the CbpM dimer contains an N-terminal globular domain comprising three \( \alpha \)-helices (residues 2–63) followed by a nine-turn helix, \( \alpha_4 \) (residues 67–99) that extends away from the globular domain (Fig. 1A). The dimer is formed through a parallel coiled-coil of the helix \( \alpha_4 \) with its \( \alpha_4 \) counterpart from the other subunit. The parallel arrangement of coiled-coils in CbpM is stabilized by the presence of two asparagines (Asn80 and Asn87) in the \( \alpha_4 \) positions in the heptad repeats. Asparagines present in these positions are known to influence the helix orientation [29].

CbpA \( ^{\text{Jdom}} \). The structure of CbpA \( ^{\text{Jdom}} \) is similar to that previously determined by NMR [22] (PDB 2KQX) with four helices arranged as an orthogonal bundle, although the NMR model is more compact along the length of the bundle (Fig. 1B). The loop between helices \( \alpha_2 \) and \( \alpha_3 \) is usually structurally flexible in J-domains as shown by several NMR structures [22,30–32]. We observe that in the CbpM-CbpA \( ^{\text{Jdom}} \) complex structure, the N-terminal part of this loop participates in binding of CbpM and, in contrast to the free J-domain, this loop assumes a well-defined conformation.

Molecular Interactions at the CbpA \( ^{\text{Jdom}} \) and CbpM Interface

Each CbpA \( ^{\text{Jdom}} \) binds the CbpM dimer at the crevice between the two subunits, making contact with both subunits, and the contacting region is contiguous on the CbpM dimer surface. The crystal structure reveals extensive interactions between CbpA \( ^{\text{Jdom}} \) and CbpM involving sixteen residues from CbpA \( ^{\text{Jdom}} \) and twenty residues from CbpM. The conserved J-domain residues that were shown to be essential for DnaK binding, including His33 and Asp35 from the conserved H-P-D motif [33], as well as the neighboring residue Val36, are located in the center of the interface and interact with both molecules of CbpM (Fig. 2A). Helix \( \alpha_2 \) of CbpA \( ^{\text{Jdom}} \) provides the majority of the interacting residues, with additional contributions from the loop \( \alpha_2/\alpha_3 \) and helix \( \alpha_3 \) (Fig. 2A).

The interface on CbpM involves residues mainly from helices \( \alpha_2 \) and \( \alpha_4 \) of one subunit and helices \( \alpha_3 \) and \( \alpha_4 \) of the other subunit. Since almost half of the residues of CbpM contacting CbpA \( ^{\text{Jdom}} \) are located on helix \( \alpha_4 \), the coiled-coil region not only contributes to the dimerization of CbpM, but also plays a critical role in binding to CbpA \( ^{\text{Jdom}} \) (Fig. 2A). We have previously shown using mutagenesis that residues Tyr25, Arg26, Ala29, His33, Val36 and Phe46 of CbpA \( ^{\text{Jdom}} \) are important for CbpM binding [22]. The crystal structure of the complex shows that these residues are all directly involved in contacting CbpM either through hydrogen bonds or van der Waals interactions (Fig. 2A). Additional ten residues of CbpA \( ^{\text{Jdom}} \) (Lys19, Lys22-Ala24, Arg27, Arg30, Pro34-Asp35, Lys38 and Trp53; Fig. 2B) participate in the interface, proving it to be much more extensive than originally anticipated [22]. In CbpM, a highly conserved patch of hydrophobic residues (Leu59, Leu63, Ala71, Val72, Leu76 and Leu77) constitutes a central region of the interface and is surrounded by much less conserved polar residues (Fig. 2C).
Figure 2. Interactions between CbpA and CbpM. A) The interacting residues of CbpM (in green) and CbpA$_{Jdom}$ (in beige) on the complex interface. The most critical residues for the specificity of interaction are shown as sticks; B) The view on the CbpA$_{Jdom}$ surface facing CbpM, showing sidechains of all residues interacting with CbpM; C) The conserved hydrophobic patch on CbpM surface (cyan, Leu59, Leu63, Ala71, Val72, Leu76 and Leu77) at the interface with CbpA$_{Jdom}$ and surrounded by an acidic patch (red, Glu22, Glu62, Glu64, Asp66 and Glu79). CbpA$_{Jdom}$ is shown as a cartoon colored beige with seven basic residues from (Lys19, Lys22, Arg26, Arg27, Arg30, His33 and Lys38) shown in stick mode.

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Figure 3. Sequence alignment of J-domains of DnaJ and CbpA. The amino acids mutated are highlighted in gray for DnaJ. The CbpA regions inserted in chimeric DnaJ are shown schematically in gray above the sequences. The CbpA region involved in CbpM binding is boxed, with residues on the interface underlined. DnaJ residues interacting with DnaK are overlined. Secondary structure elements are indicated above the sequences.

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only highly conserved CbpM polar residue facing CbpA is Glu62. This residue forms a hydrogen bond with His33CbpA of the conserved H-P-D sequence motif and was recently shown to be essential for CbpA binding [18]. In addition to Glu62, the CbpM interface involves four other acidic residues (Glu22, Glu64, Asp66 and Glu79) while CbpA Jdom contributes seven basic residues (Lys19, Lys22, Arg26, Arg27, Arg30, His33 and Lys38) (Fig. 2C). This asymmetric charge distribution contributes to the initial electrostatic attraction driving complex formation. The tips of the acidic sidechains of CbpM residues involved in CbpA binding are solvent exposed, while their hydrophobic parts participate in van der Waals interactions with β- and γ-CH2 moieties on CbpA. This architecture of CbpM explains why many polar residues on the interface are generally maintaining their polar characteristics but are not otherwise conserved in γ-protobacteria.

Figure 4. Characterization of the interaction between CbpM and engineered variants of DnaJ Jdom by Surface Plasmon Resonance. A) Representative sensorgrams for binding of WT DnaJ Jdom and the mutants to immobilized CbpM. All mutants have DnaJ residues replaced by structurally equivalent residues of CbpA. DnaJ Jdom(25–47) denotes a chimeric protein (25YKRLAMKYHPDRNQGDKEAEARKF–25YRRLARYHPDVSKEPDÆARF46) with an incorporated central region of the CbpA Jdom that binds to CbpM into DnaJ Jdom. DnaJ Jdom(36–42) represents a chimera (36RNQGDKE42->36VSKEPD41), in which the loop between helices α2 and α3 in DnaJ Jdom was replaced with equivalent loop in CbpA Jdom. The steady state assay was used to analyze the results and double referencing was used to subtract the buffer effects; B) The binding isotherms. Clearly, WT DnaJ Jdom does not bind to CbpM, but all mutants containing CbpA residues show some binding. The chimera DnaJ Jdom(25–47) has the highest affinity (K_d = 26 μM).

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Regulation of the DnaK ATPase Activity by CbpM

The structure of the CbpA Jdom–CbpM complex allowed us to propose the key residues that define the specificity of the J-domain for CbpM. To test this hypothesis we selected the J-domain of DnaJ, which shares 38% of amino acids with J-domain of CbpA and yet does not bind CbpM. We mutated these key residues of DnaJ to match those of CbpA and tested its binding to CbpM. In the first DnaJ construct we have replaced the entire central region of DnaJ Jdom for that of CbpA Jdom (25YKRLAMKYHPDRNQGDKEAEARKF–25YRRLARYHPDVSKEPDÆARF46) (Fig. 3). In the second construct only the loop between helices α2 and α3 was replaced (36RNQGDKE42->36VSKEPD41). The final construct had only three mutations (K26R/M30R/R36V). The DnaJ Jdom constructs were expressed and their binding to CbpM was evaluated by Surface Plasmon Resonance (SPR) experiments. Wild-type DnaJ Jdom clearly does not bind to CbpM. However the chimeric DnaJ Jdom with the amino acid fragment Y25-F47,
representing the central region of the interface, replaced with the corresponding region of CbpA\textsuperscript{Jdom}, binds CbpM with \(K_D\) of 26 \textmu M (Fig. 4). The second DnaJ construct with replacement of loop 36–41 binds CbpM more weakly (Fig. 4). Finally, even the triple DnaJ\textsuperscript{Jdom} mutant shows weak binding to CbpM (Fig. 4).

Similarly to what was previously observed for CbpA\textsuperscript{Jdom} [22], DnaJ\textsuperscript{Jdom} mutants bind CbpM with fast on and off rates. Additionally, functional assays were used to show that the interface in the CbpM-CbpA\textsuperscript{Jdom} complex is important in regulating chaperone activity. This regulatory activity was detected through assays measuring ATPase activity of DnaK (Fig. 5). As expected, the full-length CbpA and full-length DnaJ co-chaperones up-regulate DnaK ATPase activity. The J-domain of CbpA also stimulates ATPase activity of DnaK, however higher concentrations are required for similar potency as the full-length CbpA. Our data confirm that CbpM decreases stimulatory activity of CbpA but has no effect on activity of DnaJ. CbpM regulates CbpA\textsuperscript{Jdom} in a similar way to the full-length CbpA.

To further show that the interface between the J-domain and CbpM has important functional role in controlling chaperone function, we tested the responsiveness of the full-length DnaJ[Y25-F46] chimera to the presence of CbpM using the DnaK ATPase assay. To this end, the sequence Y25-F47 in DnaJ was replaced with the corresponding fragment from CbpA and assayed in conjunction with DnaK and CbpM. The assay confirmed that the chimeric DnaJ becomes regulated by CbpM (Fig. 5). Thus, the CbpM-binding interface on CbpA\textsuperscript{Jdom} is functionally important and provides differential specificity of CbpM for CbpA, and not DnaJ.

**Discussion**

**Dimers vs. Monomers**

We have shown previously using size-exclusion chromatography (SEC) that CbpM [11.5 kDa protein] elutes with apparent MW of \(\sim 36\) kDa, corresponding either to a globular trimer or to an elongated dimer [22]. The oligomeric state was also suggested in earlier studies [15] based on a broad SEC peak corresponding to 15–60 kDa. Although in the cross-linking experiments [15] the majority of CbpM treated with crosslinking agent migrated in the SDS-PAGE with an apparent molecular mass of \(\sim 8\) kDa; a minor species with an apparent molecular mass of 27 kDa (dimeric CbpM) was also present [13]. The crystal structure of CbpM sugests why these cross-linking experiments were not effective at detecting its oligomeric state. The reagent used in these experiments targets mainly side chains of lysines and N-terminal amino groups. \textit{E coli} CbpM contains only one lysine, Lys84 (Thr84 in \textit{Klebsiella} CbpM in the C-terminal coiled-coil (Gly69-Leu98) segment. This lysine faces Leu83 of the second CbpM molecule and its Lys84 is located on the opposite side of the coiled-coil, making it difficult to bridge by the crosslinker. The N-terminal amino groups of the dimer are far from each other and from Lys84. Therefore, low efficiency of cross-linking is not surprising. The large interface area of \(\sim 960\) \(\text{Å}^2\) for each CbpM subunit strongly support predominance in solution of CbpM dimers although equilibrium between the monomers and dimers cannot be excluded.

**Biological Implications Derived from the Structure of the CbpM-CbpA\textsuperscript{Jdom} Complex**

The structure of the CbpM-CbpA\textsuperscript{Jdom} complex supports a competitive model of regulation of CbpA by CbpM, in which CbpA binds to DnaK or to CbpM in a mutually exclusive fashion. Although structural data for a DnaK-CbpA complex are not available yet, high sequence similarity between J-domains in co-chaperones allows using a better characterized DnaK/DnaJ system as a model for interpreting our results. The J-domain residues critical for co-chaperone activity and binding to DnaK include Tyr25, Arg26, His33-Pro34-Asp35-Arg36-Asn37 and Phe47 [33] (DnaJ numbering). Since most of these residues are conserved between the J-domains of CbpA and DnaJ, the two
co-chaperones most likely bind DnaK in the same fashion, using the same surfaces as an interface. The structure of the CbpM-CbpA_{dom} complex reveals that nearly all of the CbpA residues expected to participate in DnaK binding are also involved in binding to CbpM. The importance of this region in CbpA for modulation by CbpM was evaluated through site-directed mutagenesis of DnaJ, focusing on residues, which are different in DnaJ and CbpA (Fig. 3), including amino acids Arg26, Arg30, Val36 and the loop between helices α2 and α3. Interestingly, even though the wild-type DnaJ_{dom} does not bind CbpM, mutations of these residues to their CbpA equivalents, induces weak interactions. The chimeric DnaJ, in which residues 25–47 were replaced by equivalent residues in CbpA that constitute the interface for CbpM, becomes responsive to CbpM in ATPase assays. Thus, the specificity of CbpM for CbpA, but not DnaJ, results from subtle sequence differences between the two J-domains. These sequence differences in the interface region between CbpA and DnaJ are conserved in γ-proteobacteria. The unique CbpA_{dom} residues that contribute to the specificity of recognition participate in an extensive network of interactions with CbpM. Arg26^{CbpA}_{dom} is involved in hydrogen bonding with Glu64 and Glu66 of CbpM; the sidechain of Arg36^{CbpA} interacts with Thr75 and Leu30 of CbpM; finally, Val36^{CbpA} is located in a hydrophobic region in the CbpA-CbpM complex and forms van der Waals contacts with highly conserved residues Val72^{CbpM} and Leu76^{CbpM}. Corresponding DnaJ residues could not support such interactions. Instead of Arg26^{CbpA}_{dom} DnaJ has a lysine sidechain, which would not be able to form hydrogen bonds with both Glu64^{CbpM} and Glu66^{CbpM}. A hydrophobic Val36^{CbpA}_{dom} is replaced in DnaJ by an arginine. In contrast, sequence differences in this region of the J-domain have negligible influence on interactions with DnaK, as both co-chaperones stimulate ATPase activity to a similar extent (Fig. 5).

Relationship between CbpM and MerR-like Transcription Regulators

The structure of CbpM reveals a striking similarity to the members of the MerR family of transcription regulators. Proteins from this family are usually involved in regulation of transcription in response to a variety of stresses [1, 34]. They have a common architecture, with an N-terminal DNA-binding helix-turn-helix domain followed by a coiled-coil region and a C-terminal effector-binding domain. The two most similar structures to CbpM, as identified by Dal [35] are MtaN from Bacillus Subtilis (PDB code 1R8D) and CueR from E. coli (PDB code 1Q06). Both CbpM and MerR-like regulators form dimers associated through their coiled-coil segments, however, while in CbpM the coiled-coils are parallel, in all the known structures of the MerR family regulators the coiled-coils are antiparallel, leading to a completely different orientation of the N-terminal globular domains within respective dimers (Fig. 6A). An antiparallel topology plays a key role in the function of transcription regulators [36, 37].

The region of the highest similarity between CbpM and MerR-like regulators comprises the N-terminal ~70 residues, corre-
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