The Structural Mechanism for Transcription Activation by MerR Family Member Multidrug Transporter Activation, N Terminus*

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Transcription regulators of the MerR family respond to myriad stress signals to activate $\sigma^{32}/\sigma^{54}$-targeted genes, which contain suboptimal 19-bp spacers between their $−35$ and $−10$ promoter elements. The crystal structure of a BmrR-TPP$^+$/DNA complex provided initial insight into the transcription activation mechanism of the MerR family, which involves base pair distortion, DNA undertwisting and shortening of the spacer, and re-arrangement of the $−35$ and $−10$ boxes. Here, we describe the crystal structure of MerR family member MtaN bound to the $mta$ promoter. Although the global DNA binding modes of MtaN and BmrR differ somewhat, homologous protein-DNA interactions are maintained. Moreover, despite their different sequences, the $mta$ promoter conformation is essentially identical to that of the BmrR-TPP$^+$-bound $bmr$ promoter, indicating that this DNA distortion mechanism is common to the entire MerR family. Interestingly, DNA binding experiments reveal that the identity of the two central bases of the $mta$ and $bmr$ promoters, which are conserved as either a thymidine or an adenine in nearly all MerR promoters, is not important for DNA affinity. Comparison of the free and DNA-bound MtaN structures reveals that a conformational hinge, centered at residues N-terminal to the ubiquitous coiled coil, is key for $mta$ promoter binding. Analysis of the structures of BmrR, CueR, and ZntR indicates that this hinge may be common to all MerR family members.

MerR family members are dimeric proteins that display homologous N-terminal DNA binding domains, which are linked by variable length coiled coils to ligand-specific C-terminal “coactivator” binding domains. These transcriptional regulators are activated in response to stress signals in bacteria, such as exposure to oxygen radicals, heavy metals, or cytotoxic compounds (1). MerR family members activate transcription from $\sigma^{32}$/targeted promoters in Gram-negative bacteria and $\sigma^{54}$/targeted promoters in Gram-positive bacteria that contain suboptimal 19-bp spacers between their $−35$ and $−10$ promoter elements, which results in the misalignment of these promoter elements and preclusion of an open complex formation by RNA polymerase (2–4). The typical spacer length of most $\sigma^{32}$- and $\sigma^{54}$/regulated promoters is 17 bp (5, 6). Insight into the transcription activation mechanism of the MerR family was obtained by the crystal structure of a BmrR-TPP$^+$/DNA$^1$ complex, which revealed that the center base pair of its pseudopalindromic promoter binding site was dramatically bent and twisted, thus shortening the distance between the $−10$ and $−35$ promoter hexamers, as well as repositioning them for recognition of the $−10$ box by RNA polymerase (7).

The Bacillus subtilis MerR family member, MtaN (multidrug transporter activation, N terminus), is a 109-residue truncation mutant that retains only the N-terminal DNA binding domain and dimerization helix of Mta (8). In the absence of the coactivator binding domain, MtaN constitutively activates transcription of the $mta$ gene and thus provides a model for the minimal structure needed for transcription activation by the MerR family. The crystal structure of MtaN has been determined recently and reveals a dimeric structure that is unsuitable for binding B-DNA or the distorted DNA revealed in the BmrR-TPP$^+$/bmr promoter complex (9). This indicated that if MtaN were to utilize the DNA distortion mechanism of transcription activation of BmrR, both protein and DNA must undergo significant conformational changes. Thus, whether all MerR family members utilize the same DNA distortion mechanism as BmrR to activate transcription is unknown, as are the protein structural changes necessary for DNA binding. Here, we present the 2.70-Å resolution crystal structure of MtaN bound to the $mta$ promoter as well as a higher resolution structure of the transcription-activated BmrR-DNA complex, which has been extended to 2.4-Å resolution. The structure of the MtaN-$mta$ promoter complex reveals that MtaN uses a DNA distortion mechanism that is identical to that of BmrR to activate transcription, but with differences between the structures of each protein dimer. Moreover, the DNA binding specificity of each of these MerR family members is discerned with the surprising result that the two most central sequences of these promoters, which are nearly universally conserved among all MerR family promoter sites, plays a minor role in binding affinity. Furthermore, analysis of the structure of MtaN and MtaN-DNA reveals a conformational hinge that acts in concert with the DNA to reconfigure the DNA for transcription activation.

EXPERIMENTAL PROCEDURES

MtaN-DNA Structure Determination and Refinement—MtaN was expressed and purified as described previously (10). The MtaN-DNA complex was prepared by mixing the MtaN protein in a 1:1 molar ratio with a 26-bp $mta$ promoter, which was composed of the $mta$ 19-bp spacer region, the 6-bp $−35$ element, and 1 bp of the $−10$ element, and contains 5′-AT overhangs (see Fig. 1 and Fig. 2a). Two $\mu$L of 0.25–0.35 mm MtaN-DNA complex was mixed with 2 $\mu$L of reservoir solution

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The abbreviations used are: TPP$^+$, tetraphenylphosphonium; MtaN, multidrug transporter activation, N terminus; PIPES, 1,4-piperazinediethanesulfonic acid; MES, 4-morpholineethanesulfonic acid; r.m.s.d., root mean squared deviation.

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containing 30% polyethylene glycol 1450, 0.2 M Li$_2$SO$_4$, 0.1 M PIPES, pH 6.5. Crystals were grown by the hanging drop vapor diffusion method at 21 °C, appearing overnight and reaching final dimensions of 0.2 x 0.2 x 1.0 mm in approximately 1 week.

Cryoprotecting conditions were developed by soaking crystals for

### Table I

| Selected crystallographic statistics | MtaN-DNA | BmrR-TPP−DNA |
|-------------------------------------|----------|---------------|
| Data collection                     |          |               |
| Resolution (Å)                      | 35.86-2.70 | 86.40-2.40    |
| Outer shell (Å)                     | 2.85-2.70  | 2.46-2.40     |
| Observed reflections                | 162,404   | 249,956       |
| Unique reflections                  | 21,769    | 33,677        |
| Completeness (%) (outer shell)      | 98.9 (99.4) | 99.4 (99.4)   |
| $I/\sigma(I)$ (outer shell)         | 9.7 (1.8)  | 9.4 (1.8)     |
| $R_{merge}$ (%) (outer shell)Å      | 6.1 (42.3) | 5.1 (40.9)    |

Refinement statistics

| Resolution (Å)                      | 20.0-2.70  | 86.4-2.40     |
| Data collection                     | 19,572/2,161 | 31,919/1,694 |
| Protein atoms                       | 1,753      | 2,255         |
| DNA atoms                           | 1,039      | 467           |
| Solvent molecules                   | 36         | 153           |
| $R_{merge}/R_{free}$ (%)            | 23.5/27.0  | 82.9/96.7     |
| Bond length deviation (Å)           | 0.007      | 0.008         |
| Bond angle deviation (%)            | 1.07       | 1.22          |
| Average B factor ($Å^2$)             | 65.4       | 60.2          |
| PDB accession code                  | 1R8D       | 1R8E          |

Derivative data collection and phasing statistics

| tT25A                          | tT25ab                         | tT25ab                          | T25Ai                          | tT25ai                         |
|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| Data collection                | 51.7-2.60                       | 35.7-2.85                       | 57.7-2.70                       | 73.9-2.85                       | 52.0-2.70                       |
| Resolution (Å)                | 2.60-2.74                       | 2.70-2.85                       | 2.70-2.85                       | 2.85-3.0                       | 2.70-2.85                       |
| Unique reflections             | 82,391                          | 105,157                         | 78,328                          | 98,843                          | 106,708                         |
| Compleness (%) (outer shell)   | 99.5 (100)                      | 99.8 (100)                      | 100 (100)                       | 100 (100)                       | 95.7 (95.7)                     |
| $I/\sigma(I)$ (outer shell)    | 8.3 (1.4)                       | 6.8 (1.9)                       | 6.8 (1.5)                       | 6.6 (1.5)                       | 7.3 (1.6)                       |
| $R_{merge}$ (%)                | 6.6 (52.2)                      | 7.2 (39.1)                      | 8.0 (50.1)                      | 9.1 (50.0)                      | 7.4 (46.4)                       |
| MIR phasing                    | 16.9                            | 12.7                            | 12.5                            | 12.0                            | 13.4                            |
| Number of sites found by SOLVE | 4                               | 2                               | 2                               | 2                               | 2                               |
| SOLVE Z-score                  | 19.4                            |                                 |                                 |                                 |                                 |
| Phasing power                  | 0.54                            | 0.42                            | 0.66                            |                                 |                                 |

- $R_{merge} = \sum I_{ijkl} - I_{ijkl}/\sum I_{ijkl}$, where $I_{ijkl}$ is observed intensity and $I_{ijkl}$ is the final average value of intensity.
- $R_{merge} = \sum |F_{calc} - |F_{obs}|/\sum |F_{calc}|$, where $|F_{calc}|$ is the protein structure factor amplitude and $|F_{obs}|$ is the heavy atom derivative structure factor amplitude.
- $\phi$ is the figure of merit (FOM)/FOM after solvent flipping.

Fig. 1. Stereoview of the simulated annealing composite omit map around the central base pairs of the mta promoter contoured at 1σ. The electron density is shown as green mesh, and the DNA is shown as a stick model with atom colors as follows: gray, carbon; orange, phosphorus; red, oxygen; blue, nitrogen.
in a cryoprotecting solution containing 0.7 M imidazole, pH 6.5, and 30% DMSO. Each addition of protein, the samples were incubated for 30 s to reach equilibrium before a measurement was taken. Different concentrations of fluorescein-labeled DNA were used in these experiments to ensure that we were measuring binding for each oligonucleotide under equilibrium binding conditions. 1 nM fluorescein-labeled DNA was used in the binding experiments for MtaN, whereas either 0.1 or 0.2 nM fluorescein-labeled DNA was used for the BmrR binding experiments. However, we report only those BmrR-DNA binding constants that were generated with the latter concentration. To measure DNA binding and activation for BmrR, the protein was presaturated with 1 mM TFP− and titrated into the binding buffer containing 1 mM TFP− and 0.2 nM fluorescein-labeled DNA. Each binding isotherm was fit to Equation 1 using Kaleidagraph.

\[
P = \frac{(P_{\text{bound}} - P_{\text{free}}) [\text{protein}]/K_d + [\text{protein}]) + P_{\text{free}}}{P_{\text{free}} + P_{\text{bound}}} \quad (\text{Eq. 1})
\]

where \(P\) is the polarization measured at a given total protein concentration, \(P_{\text{free}}\) is the initial polarization of free fluorescein-labeled DNA, and \(P_{\text{bound}}\) is the maximum polarization of specifically bound DNA. [Protein]\text{tot} = [\text{Protein}]_{\text{free}} + [\text{Protein}]_{\text{bound}} is assumed because the concentration of free fluorescein-labeled DNA used for each individual binding isotherms was at least 10-fold below the \(K_d\). Nonlinear least squares analysis was used to determine \(P_{\text{free}}, P_{\text{bound}}, K_d\).

**RESULTS AND DISCUSSION**

**Overall Structure of the MtaN-nta Promoter Complex**—The structure of the MtaN-DNA complex was determined to 2.70-Å resolution by multiple isomorphous replacement using 5-iodouracil-containing oligodeoxynucleotides as heavy atom derivatives. The asymmetric unit contains one dimer of MtaN bound to a 26-base pair oligodeoxynucleotide that contains the 19-bp spacer between the −35 and −10 regions of the nta promoter as well as the entire −35 hexamer and 1 bp of the −10 hexamer. Each subunit of the MtaN dimer contains an N-terminal DNA binding domain (residues 2–74) followed by an eight-turn helix \(a5\) (residues 75–107) (Fig. 2a). The MtaN dimer is stabilized by an antiparallel coiled coil formed by the \(a5\)-\(a’5\) helices (where a prime indicates the other subunit). The independent MtaN subunits are structurally identical with a root mean squared deviation (r.m.s.d.) of 0.3 Å between all \(C_{\alpha}\) atoms. The DNA labeling domain belongs to the winged helix-turn-helix family, which consists of a four-helix bundle and a three-stranded antiparallel β sheet (18, 19). Each MtaN monomer has the topology of β1-α1-α2-β2-β3-α3-α4-α5 that is identical to that of the previously solved apoMtaN structure (9).

Superposition of \(a1\) to \(a4\) of DNA-free MtaN and DNA-bound MtaN shows an r.m.s.d. of only 0.60 Å, and a superposition of \(a5\) from each structure gives an r.m.s.d. of only 0.30 Å. However, upon binding, DNA-bound MtaN undergoes an 11° rotation and a 6 Å inward translation of each of its DNA binding domains, resulting in a shortening of the center-to-center distance between the two DNA recognition helices (\(a2\) to \(a’2\)) from 33.2 to 29.5 Å (Fig. 2b). Conformational changes were anticipated for the DNA-free MtaN for the dimer to be able to bind to successive major grooves in the shortened DNA structure seen in the BmrR−TPP−−DNA complex (7, 9). However, a difference distance matrix calculated between the DNA-free and DNA-bound conformers of MtaN reveals that the positions of the coiled coils (\(a5\), \(a’5\)), which were expected to drive the movement of the DNA binding domains, do not change with respect to each other but rather the rotation and translation are propagated through a hinge movement of the DNA binding domains toward each other (Fig. 2b). The center of this hinge is located N-terminal to \(a5\) and spans residues 71–75. These residues display the largest differences in their \(\phi/\psi\) angles between the DNA-free and DNA-bound conformers of MtaN. The \(\phi/\psi\) differences of these residues range from 30 to 100°, which is significantly well above the average \(\phi/\psi\) differences (13° and 16°(\(\phi\)) calculated for all other MtaN residues. An analogous

**Fig. 2. Overview of MtaN structures.** a, structure of the MtaN-DNA complex. The secondary structural elements of one subunit are colored blue, and the other are colored as follows: green, \(\beta\)1 (residues 2–4) and \(\alpha1\) (residues 5–12); yellow, \(\alpha2\) (residues 16–24); orange, \(\beta\)2 (residues 33–34) and \(\beta\)3 (residues 40–42); magenta, \(\alpha3\) (residues 44–59); cyan, \(\alpha4\) (residues 63–70); red, \(\alpha5\) (residues 77–109). The wings of the winged helix DNA binding motif are labeled W1 and W2. The sequence of the nta oligodeoxynucleotide used for crystallization is shown below the structure with 5-iodouracil sites shown in red, a superimposition of the \(a5\) helices of DNA-bound MtaN and apoMtaN. DNA binding requires rotation (11°) and translation (6.0 Å) of the DNA binding domains. Green, ApoMtaN; blue, MtaN bound to DNA. An arrow points to the conformational hinge.

\(b = 107.3 \text{ Å}, c = 145.7 \text{ Å}, \alpha = \beta = \gamma = 90°\). Crystals were soaked for 30 s in a cryoprotecting solution containing 0.7 M imidazole, pH 6.5, and 30% glycerol. X-ray intensity data were collected on beamline 11-1 at the Stanford Synchrotron Radiation Laboratory (Table I). Molecular replacement using Evolutionary Programming for Molecular Replacement (16) was used to place the 3.0-Å resolution BmrR−TPP−−DNA model into the unit cell of the frozen crystals. The structure was refined against the higher resolution data using the crystallography and NMR system (12). Side chains were placed for an additional 44 residues previously modeled as alanine, and 135 water, 11 imidazole, and 6 glycerol molecules were added to the structure. The final model was verified by inspection of simulated annealing-composite omit maps and contains residues 2–277 of the BmrR monomer plus 12 base pairs of the DNA half-site. The final \(R_{\text{free}}\) was 22.8% and \(R_{\text{free}}\) was 26.7%. The stereochemistry of the final structure was assessed with PROCHECK (17) and showed 94.1% of all \(\phi/\psi\) angles in the most favored regions of the Ramachandran plot and none in generously allowed or disallowed regions.

**Measurement of DNA Binding Affinity—Fluorescence polarization binding experiments were done using a PanVera Beacon Fluorescence Polarization System (PanVera Corp.). One strand of each DNA duplex contained a 5’-fluorescein label, and the increase in polarization of the fluorophore upon protein binding was measured. Each binding experiment contained 0.1, 0.2, or 1 nM fluorescein-labeled DNA in a binding buffer of 150 mK Cl, 50 mK Tris, pH 7.6, 5 μg of poly(dI-dC). For each set of experiments either BmrR or MtaN was titrated into the DNA. After each addition of protein, the samples were incubated for 30 s to reach equilibrium before a measurement was taken. Different concentrations of fluorescein-labeled DNA were used in these experiments to ensure that we were measuring binding for each oligonucleotide under equilibrium binding conditions. 1 nM fluorescein-labeled DNA was used in the binding experiments for MtaN, whereas either 0.1 or 0.2 nM fluorescein-labeled DNA was used for the BmrR binding experiments. However, we report only those BmrR-DNA binding constants that were generated with the latter concentration. To measure DNA binding and activation for BmrR, the protein was presaturated with 1 mM TFP− and titrated into the binding buffer containing 1 mM TFP− and 0.2 nM fluorescein-labeled DNA. Each binding isotherm was fit to Equation 1 using Kaleidagraph.

\[
P = \frac{(P_{\text{bound}} - P_{\text{free}}) [\text{protein}]/K_d + [\text{protein}]) + P_{\text{free}}}{P_{\text{free}} + P_{\text{bound}}} \quad (\text{Eq. 1})
\]

where \(P\) is the polarization measured at a given total protein concentration, \(P_{\text{free}}\) is the initial polarization of free fluorescein-labeled DNA, and \(P_{\text{bound}}\) is the maximum polarization of specifically bound DNA. [Protein]\text{tot} = [\text{Protein}]_{\text{free}} + [\text{Protein}]_{\text{bound}} is assumed because the concentration of free fluorescein-labeled DNA used for each individual binding isotherms was at least 10-fold below the \(K_d\). Nonlinear least squares analysis was used to determine \(P_{\text{free}}, P_{\text{bound}}, K_d\).
conformational hinge is suggested for other MerR family members by the recent crystal structures of MerR family members CueR and ZntR, which reveal that this hinge region sits adjacent to the metal binding sites of CueR and ZntR (20). Although the mechanism of transcription activation by these and other MerR family members is unknown in the absence of relevant protein-DNA complex structures, it is plausible that the coactivator binding signal is transduced through similar hinges.

MtaN and BmrR Reconfigure Promoter DNA in a Similar Manner—During the course of this study, we were able to

Fig. 3. MtaN-\(mta\) and BmrR-\(bmr\) promoter binding. a, a view of the contacts between MtaN and the \(mta\) promoter (left) and BmrR and the \(bmr\) promoter (right). DNA and protein atoms are colored as follows: gray, carbon; orange, phosphorus; red, oxygen; blue, nitrogen. Hydrogen bonds are depicted by small black spheres. Only one half-site is shown as the contacts of the other half-site are identical. b, schematic diagram of MtaN-DNA contacts (left) and BmrR-DNA contacts (right) shown for one DNA half-site. Blue arrows, side chain-DNA hydrogen bonds; green arrows, backbone amide-DNA contacts; yellow arrows, van der Waals contacts. DNA contacting residues at MtaN and BmrR equivalent positions are: Ser-15 (MtaN) = Ser-18 (BmrR), Arg-17 = Lys-20, His-21 = Tyr-24, Tyr-22 = Tyr-25, Tyr-38 = Tyr-42, Arg-39 = Arg-43, Lys-56 = Lys-60, Leu-62 = Leu-66.
extend the resolution of the structure of a BmrR-TPP\(^{-}\)-DNA complex to 2.4-Å resolution, allowing a detailed comparison between the DNA contacts and activation mechanism of this MerR family member to those of MtaN (Fig. 3). MtaN recognizes the mta promoter via its helix-turn-helix motif and wings, W1 and W2, in a fashion nearly identical to that of BmrR (Fig. 3, a and b). Superposition of a1 through a4 of the DNA binding domains of the MtaN-DNA and BmrR-TPP\(^{-}\)-DNA structures reveals an r.m.s.d. of only 1.02 Å (Fig. 4a). Strikingly, and despite their different 19-bp spacer sequences (8/19 identities) and overall lengths (22 versus 26), the structure of the MtaN-bound mta DNA nearly is identical to the structure of the bmr DNA when bound to BmrR-TPP\(^{+}\). The r.m.s.d. is only 1.38 Å between the atoms of their phosphate backbones (Fig. 4, b and c). Although the DNA structures and DNA binding domains of each complex are nearly structurally identical, superposition of the DNA binding domains of one subunit of each complex reveals significant differences in their dimeric structures, whereby the relative positions of the helices of the coiled coils of each complex are different. Specifically, the second helix of the coiled coil of BmrR is rotated 6.2° and translated 6.9 Å with respect to the location of the corresponding helix of the MtaN-DNA complex. As a result, the DNA binding domain of the second promoter of BmrR sits higher in the major groove (Fig. 4a). Yet the DNA structures and DNA contacts nearly are identical in each complex (Figs. 3b and 4, b and c).

Analysis of the MtaN-DNA complex reveals that the DNA is bent by 47°. The bend is centered at the central TpT base pair step where the bases of the Thy1'-Ade1 base pair slide and twist away from each other, resulting in very poor Watson-Crick hydrogen bonds. Base pair steps involving Thy1 and Thy2' of the mta promoter are distorted with respect to B-form DNA with rise, roll, and twist values of 5.3 Å, 23.5°, and 17.0°, respectively (21) (Fig. 4b). The overall end-to-end length of the DNA helix is shortened by 5.9 Å when compared with a canonical B-DNA structure (from 75.0 to 69.1 Å). Despite differences in sequence, such distortion of the central (TpT) base pair step and end-to-end shortening of the DNA nearly are identical to those observed in the BmrR-TPP\(^{+}\)-DNA complex. The rise, roll, and twist values of the central base pair step of the bmr promoter that involve Thy1 and Ade2 are 5.1 Å, 25.3°, and 26.4°, respectively, and the end-to-end length of the DNA helix is shortened by 5.0 Å. The near identical properties of these DNA pieces strongly suggests that all MerR family members with 19-bp spacers activate transcription via the same structural mechanism, which involves localized distortion and reconfiguration of the DNA to reorient the −10 and −35 promoter elements to allow open complex formation by RNA polymerase (1, 3).

**Fig. 4. Structural mechanism of transcription activation by the MerR family.** *a*, superimposition of the MtaN-DNA and BmrR-TPP\(^{-}\)-DNA complexes. Helices 1 to 4 of one subunit from each dimer, but not W1, were overlaid. *Yellow*, BmrR; *blue*, MtaN. *b*, overlay of the MtaN-mta and BmrR-bmr promoter structures using all equivalent phosphate backbone groups. *Blue*, mta DNA; *yellow*, bmr DNA. *c*, close up of the superimposed mta and bmr promoters near base pair 1. Notice their nearly identical structures at the site of DNA distortion. *Blue* sticks, mta promoter DNA; *yellow* sticks, bmr promoter DNA.

MtaN and BmrR DNA Contacts, the Basis of Promoter Specificity—Despite the altered locations of the DNA binding domain of one subunit, the "nonspecific" DNA contacts made in the MtaN-DNA complex are very similar to those seen in the BmrR-TPP\(^{-}\)-DNA complex with residues at equivalent positions making similar DNA contacts (Fig. 3). The bent phosphate backbone near the center of the DNA element is stabilized by interactions with MtaN residues from the helix-turn-helix motif (Ser-15 and Tyr-22), as well as residues from both wings, W1 (Tyr-38) and W2 (Lys-56 and Leu-62). The N\(_2\) of Lys-56, which lies near the C-terminal end of α3, interacts with the Cyt3 phosphate. The backbone amide of residue Leu-62, which lies at the positive dipole of α4, hydrogen-bonds to the Ade2 phosphate, whereas the OH of Tyr-22 hydrogen-bonds to the Cyt3 phosphate. Residues Tyr-22 and Leu-62 are absolutely conserved among MerR family members, highlighting their functional importance in stabilizing the activated DNA conformation through backbone interactions. Another absolutely conserved residue, Arg-39, donates hydrogen bonds from its NH\(_2\) group to the phosphates of both Thy7' and Cyt8'. The position of the guanidinium group is stabilized by an interaction between its Ne atom and the O61 of Asp-23. The importance of this contact for W1-DNA interaction is underscored by the conservation of residue 23 as only an aspartate, glutamate, or glutamine. His-20, another residue of the recognition helix, hydrogen-bonds to the phosphate of Cyt8', whereas the W1 residue, Tyr-38, engages in a hydrogen bond to the N-2 of Gua10 and interacts via a water molecule with the O-2 atoms of Thy11 and Cyt10'. Additional protein-DNA contacts involve the N\(_2\) of Lys-6 and the Cyt10' phosphate and several van der Waals interactions involving residues Val-5, Arg-17, Thr-18,
To ensure equilibrium binding, 1 nM fluorescein-labeled DNA was used in this experiment. The higher resolution van der Waals contact to the N6 of Ade5/H9280 and its NH2, and the O6 of Gua4 with the latter also makes a phosphate backbone (Fig. 3).

The only promoter specifying interactions of the MtaN-DNA complex occurs between the Nε of Arg-17 and the O4 of Thy5 and its NH2, and the O6 of Gua4 with the latter also makes a van der Waals contact to the N6 of Ade5'. The higher resolution BmrR complex structure also reveals only one promoter-specific interaction per subunit, that between the Nε of Lys-20 and the O6 and N7 of Gua5. However, these interactions account for the DNA specificity of these MerR family members, as equilibrium binding experiments show a 313-fold difference for BmrR binding to the bmr (Kd = 2.4 nM) and mta (Kd = 751 nM) promoters and a 72-fold difference in MtaN binding to the mta (Kd = 96 nM) and bmr (Kd = 6900 nM) promoters (Fig. 5, Table III). Thus, MtaN appears to be a global transcription activator of multidrug efflux transporters in B. subtilis only at concentrations that are unlikely to be physiologically relevant (8).

**Importance of the Central Base Step for DNA Reconfiguration**—An alignment of promoter sequences for MerR family members that have a 19-bp spacing between the −35 and −10 promoter elements reveals that the central bases of the pseudoduplex are highly conserved as either an A or a T at positions 1 and 2' (1). We hypothesized that the conservation of A or T at these positions is needed to decrease the thermodynamic costs of bending the DNA and breaking or distorting the central base pairs relative to a three-hydrogen-bonded C-G base pair. To test this possibility, we mutated each of the central base pairs relative to a three-hydrogen-bonded C-G pair. These data revealed no difference in the binding affinity of BmrR for any of the mutants, whereas for MtaN there was only a 3-fold decrease in binding affinity when Thy2 was changed to C either singly or in the context of the first base pair change. In the absence of inducer/coactivator, e.g. TPP+, the BmrR-DNA binding experiments revealed only the energetics of equilibrium binding.

By contrast, the binding of MtaN to the mta promoter measures DNA binding and any thermodynamic cost or benefit.

**Table III**

| Protein/promoter DNA       | Kd     | Relative binding affinitya |
|----------------------------|--------|-----------------------------|
| MtaN/mtaT_x, · wild type   | 96 ± 12| 1.0                         |
| MtaN/bmr                  | 6900 ± 640 | 0.014                       |
| MtaN/mtaT_x, T_x           | 93 ± 13 | 1.0                         |
| MtaN/mtaT_x, G2            | 93 ± 13 | 0.30                        |
| MtaN/mtaT_x, C2            | 303 ± 56| 0.31                        |
| MtaN/mtaT_x, A2            | 303 ± 56| 0.31                        |
| MtaN/mtaT_x, · wild type + 1 mM TPP+ | 71 ± 5.3 | 1.4                        |
| BmrR/bmrT_x, A2            | 2.4 ± 0.25 | 1.0                        |
| BmrR/mta                  | 751 ± 75 | 0.0032                      |
| BmrR/bmrC,A2              | 3.2 ± 0.4 | 0.75                        |
| BmrR/bmrT,G2              | 4.3 ± 0.4 | 0.56                        |
| BmrR/bmrC,G2              | 4.6 ± 0.5 | 0.52                        |
| 1 mM TPP+/BmrR/bmrT/A2, · wild type | 3.3 ± 0.6 | 1.0  |
| 1 mM TPP+/BmrR/bmrT,G2    | 3.8 ± 0.7 | 0.87                        |
| 1 mM TPP+/BmrR/bmrC,G2    | 6.6 ± 1.5 | 0.50                        |
| 1 mM TPP+/BmrR/bmrC,G2    | 3.5 ± 0.6 | 0.94                        |

*a Relative Kd is the ratio of wild type promoter Kd to the mutant promoter Kd.
that is associated with the reconfiguration of the DNA into its transcriptionally active form. Therefore, the lower affinity of the mtaT1,C2 promoter might be related to the ability of MtaN to distort the DNA for activation, a phenomenon, that we would not see in the BmrR-DNA binding experiments. To address whether changes at the central base pair step of the bmr promoter affect activated BmrR DNA binding, we repeated the BmrR-bmr promoter binding experiments in the presence of saturating amounts of the coactivator TPP+. In the presence of 1 mM TPP++, the binding affinities of BmrR for the mutant bmr promoters were analogous to those observed for the MtaN mta promoter experiments i.e. there was little change, with only a 2-fold decrease in binding affinity when Ade2 was changed to G (Table III). Thus, the identity of the second of the central bases pairs as either an A or a T appears to play at best a small, perhaps context-dependent role in DNA reconfiguration. Another possibility for the conservation of these base pairs could be related to RNA polymerase rather than an MerR activation function.

As a constitutively active truncation mutant of Mta, MtaN represents a minimal structure for transcription activation by the MerR family. The lack of a coactivator binding domain in MtaN underscores the role of this structure as a “repressive” element in the absence of a signal. Only when the appropriate activation signal is sensed by this domain can the necessary conformational changes between the DNA binding domains of the dimer occur that allow reconfiguration of the DNA and subsequent transcription activation. In the absence of a regulatory domain, the MtaN dimer is free to reorient itself and reconfigure the DNA into the default state, i.e. the transcriptionally active form. Indeed, in agreement with those ideas about the evolution of MerR proteins whereby the members of this family resulted from gene fusion events between effector-binding proteins and a DNA binding coiled coil dimer (1). The MtaN-DNA structure along with the structure of the higher resolution BmrR-TPP+ -DNA complex provides a snapshot of the end point of a seemingly universal transcription activation mechanism for MerR family members. Clearly the structures of a MerR family member bound to DNA in the absence of its activation signal and to a promoter with a 20-bp spacer (the zntA promoter) (2, 22) are critical to give a complete understanding of the gene regulatory function of this large family.

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