DNA Distortion Mechanism for Transcriptional Activation by ZntR, a Zn(II)-responsive MerR Homologue in Escherichia coli*

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MerR-like DNA distortion mechanisms have been proposed for a variety of stress-responsive transcription factors. The Escherichia coli ZntR protein, a homologue of MerR, has recently been shown to mediate Zn(II)-responsive regulation of zntA, a gene involved in Zn(II) detoxification. To determine whether the MerR DNA distortion mechanism is conserved among MerR family members, we have purified ZntR to homogeneity and shown that it is a zinc receptor that is necessary and sufficient to stimulate Zn-responsive transcription at the zntA promoter. Biochemical, DNA footprinting, and in vitro transcription assays indicate that apo-ZntR binds in the atypical 20-base pair spacer region of the promoter and distorts the DNA in a manner that is similar to apo-MerR. The addition of Zn(II) to ZntR converts it to a transcriptional activator protein that introduces changes in the DNA conformation. These changes apparently make the promoter a better substrate for RNA polymerase. We propose that this zinc-sensing homologue of MerR restructures the target promoter in a manner similar to that of other stress-responsive transcription factors. The ZntR metalloregulatory protein is a direct Zn(II) sensor that catalyzes transcriptional activation of a zinc efflux gene, thus preventing intracellular Zn(II) from exceeding an optimal but as yet unknown concentration.

Zinc is an essential element that must be maintained at certain levels within all cells. However, like many transition elements, zinc is also harmful at elevated concentrations. Zinc starvation and zinc toxicity both lead to transcription of Zn-responsive genes while bound to the spacer region at a palindrome (2). In vivo experiments by Brocklehurst et al. (2) have revealed that ZntR is a trans-activator of zntA transcription, whereas gel shift assays have shown that ZntR binds to the zntA promoter (PzntA). A construct containing a 2-bp deletion in the spacer region of the zntA promoter displayed constitutive activity, indicating that the wild-type 20-bp spacing between the −35 and −10 sites plays a role in regulation. These experiments indicate significant similarities between ZntR and MerR function in vivo and lead us to address the question of how ZntR activates transcription. We investigated this question using tools developed to probe the regulatory mechanism of MerR.

Suboptimal spacing (19 bp) between the −35 and −10 promoter elements is a key feature of the merT promoter (Pt) regulated by MerR (30) and is at the heart of the DNA distortion mechanism. Promoters regulated by members of the MerR family typically have spacer elements longer than the consensus length of 17 bp which makes them poor substrates for RNA polymerase (RNAP) (31). MerR controls transcription of the mer genes while bound to the spacer region at a palindrome located between the −35 and −10 sites of Pt (21, 22). Several members of the MerR family are also known to bind in the spacer regions of their target promoters, including SoxR (32), TipAL (33), BmrR (34), and Mta (35).

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¶ The abbreviations used are: bp, base pair(s); PAGE, polyacrylamide gel electrophoresis; IgG, γ globulin; TPEN, N,N,N’,N’-tetraakis(2-pyridylmethyl)ethylenediamine; TBE, 100 mM Tris borate, 1 mM EDTA, pH 8.3; RNAP, RNA polymerase; IPTG, isopropyl-1-thio-β-D-galactopyranoside; DTT, dithiothreitol; MES, 4-morpholineethanesulfonic acid; ICP-AES, inductively coupled plasma atomic emission spectroscopy; PCR, polymerase chain reaction.
In the MerR DNA distortion mechanism, the transition from repression to activation involves several alterations in the local DNA structure. A series of specific distortions are proposed to make the promoter a more optimal substrate for RNAP (Fig. 1) (24, 36, 37). Evidence for these conformational changes comes from both physical studies and comparison of nuclease cleavage patterns for repressed and activated states of the MerR-DNA complex. The DNase I cleavage pattern observed for the MerR-DNA complex is very similar to that observed for the CAP-DNA complex (37). Inspection of the CAP-DNA complex crystal structure reveals that sites of cleavage are immediately 3' to the DNA distortion sites. These distortion sites are two 40° kinks in the DNA which widen the exposed minor groove and bend the DNA toward the CAP protein (37, 38). Addition of Hg(II) to MerR relaxes these bends at the two kink sites, resulting in a loss of the DNase I hypersensitive bands. This model was corroborated by phase bend studies that confirmed bending of the DNA toward the protein for apo-MerR and a relaxation of these bends by Hg-MerR (37).

Studies of E. coli RNAP with MerR and the mer operator further corroborate this mechanism. RNAP is able to bind to the –35 site of Pznt with apo-MerR bound, however the –10 site remains inaccessible (22). RNAP binds adjacent to apo-MerR in this closed complex. In the DNA distortion mechanism, Hg(II) binding to MerR leads to underwinding of the DNA (36), resulting in a Cu-OP hypersensitive site at the center of the operator (24). In vivo methylation and permanganate footprinting are also consistent with a change in the conformation of the RNAP-MerR complex (39). One effect of this underwinding, which is different from unbending, is to bring the –35 and –10 sites into proper alignment for RNAP binding to both simultaneously so that transcription can proceed (Fig. 1B). The underwinding may also facilitate the energetics of strand separation, a requisite step in open complex formation (37). Thus, underwinding and the relaxation of bending act together to remodel the promoter, making it a better substrate for RNAP and dramatically increasing the transcription initiation rate (28).

In order to delineate which mechanistic aspects are conserved among MerR family members (40–45), we have examined the mechanism that ZntR employs in transcriptional regulation of zntA. Protein/DNA footprinting and in vivo transcriptional assays reveal that, like MerR, ZntR functions primarily as a transcriptional activator, but it only weakly, if at all, represses expression of the zntA gene. Our results are consistent with a ZntR mechanism in which a series of MerR-like DNA unbending and underwinding steps restructure the promoter, allowing efficient transcription of zntA.
The promoter, pUC19Zntfoot, was digested with EcoRI and BamHI and inserted into pUC19 (New England Biolabs) digested with the same enzymes, creating pUC19Znt. Two more primers (Zntfoot-1, 5′-GTC GGC CAA CTT GGA AGG GC-3′ and Zntfoot-2, 5′-GAG AGA GTT GGC GCC CCG GAA CAT CGG C-3′) were then used for PCR with pUC19Znt to clone out a smaller portion of the promoter. The 486-bp PCR fragment was digested with EcoRI and AvaI and inserted into pUC19 digested with the same enzymes. The resulting plasmid, pUC19Zntfoot, was used for labeling in the footprinting assays.

**Promoter Extension**—Total RNA was isolated from exponentially dividing cells of strain DH5α using the Qiagen RNeasy RNA isolation kit. Cells induced with zinc, were exposed to 1 mM ZnSO4 for 1 h, lysed, and RNA isolation. Primer ZntA-PF1 (5′-GTC TCT TGG CCG TGA TTG TCA GG-3′), labeled with [γ-32P]ATP by T4 polynucleotide kinase (New England Biolabs), was used with 10 µg of total RNA for promoter extension analysis. Primer and RNA were heated to 65 °C for 5 min, quenched on ice, added to a reaction mixture of M-MulV reverse transcriptase (New England Biolabs), and placed at 42 °C for 1 h. Sequencing of pUC19Znt was carried out using labeled primer ZntA-PF1 as directed in the CircumVent Thermal Cycle Dideoxy DNA Sequencing Kit (New England Biolabs). Primer extension and sequencing samples were run together on an 8% polyacrylamide, 8M urea, 1.2× TBE sequencing gel.

**P_zntA Labeling for Footprinting**—To label the non-template strand of the promoter with EcoRI, labeled with [α-32P]ATP using the Klenow fragment of DNA polymerase (New England Biolabs), and then digested with EcoRI. To label the template strand, the fragment was digested first with EcoRI, labeled, and then digested second with BamHI. In each case, the labeled fragments were purified by gel electrophoresis.

**DNase I Footprinting**—DNase I footprinting reactions were carried out in 50 µl of foot-printing buffer (10 mM Tris, pH 8.0, 2 mM MgCl2, 1 mM CaCl2, 100 mM K-glutamate, 1 mM DTT, 100 µg/ml bovine serum albumin, 2.5 µg/ml sonicated salmon sperm DNA, 1 µM TPEN, 5% glycerol). Labeled DNA, ZntR, ZnSO4, and RNAP were added to the reaction mixture in sequential order with a 5-min incubation at 37 °C after each addition. The mixture was then incubated for 30 min at 37 °C before DNase I digestion. The dNTPs were removed by phenol-chloroform extraction. The samples were loaded onto the same type of sequencing gel used in the footprinting assays, and the levels in each lane were determined relative to the lanes with RNAP minus salmon sperm DNA. The TPEN concentration was 10 mM rather than 1 mM as used in the footprinting assays. Procedures were the same as described previously (22) with a few modifications. Two nanomolar labeled DNA was used in the reaction rather than 4–6 nM, 5 µCi of [α-32P]UTP was used rather than 10 µCi, and the reaction was terminated with 200 µl of stop buffer (10 mM EDTA, pH 8.0, 100 µg/ml RNA) prior to phenol-chloroform extraction. The samples were loaded onto the same type of sequencing gel used in the footprinting assays, and the signals were digitized in the same manner as the footprinting gels. RNA levels in each lane were determined relative to the lanes with RNAP alone, whereas the labeled DNA was used to normalize the DNA loading for each lane. The Zn titration transcription results were fitted to a sigmoidal function using the Igor Pro Version 2.04 software program (Wavemetrics, Inc., Lake Oswego, OR).

RESULTS

**ZntR Purification and Metal Content**—Fig. 2 shows an SDS-PAGE gel of different steps in the ZntR purification process. A 9 liter prep typically yielded ~180 mg of pure protein. The purified protein was found by ICP-AES to contain less than 0.05 Zn/monomer and less than 0.05 Cu/monomer. Zn-ZntR was easily prepared by adding excess Zn(II) to ZntR in aerobic buffer containing DTT. The protein was found to bind 0.9 ± 0.3 Zn/monomer. In aerobie buffer without DTT present, the complex was unstable and precipitated. In anaerobic conditions with 2 equivalents of Zn added but no DTT present, the protein bound 2.1 ± 0.4 Zn/monomer.

**Transcription Start Site**—To map the start of transcription and determine the approximate location of essential promoter elements, primer extension analysis of the zntA transcript was carried out (Fig. 3). While Brocklehurst et al. (2) have recently reported a primer extension analysis of zntA, in our hands, the start of transcription appears to occur one base 5′ to the previously reported result. We find that the zntA start of transcription mapped to a T rather than an A (see Fig. 3 and Ref. 2). The previous primer extension results were presumably ob-
tained using a temperature of 37 °C for the reverse transcription reaction (44) while our reverse transcription was carried out at 42 °C to reduce possible secondary structure that can abort termination of the cDNA before reaching the 5' end of the transcript. Possibly, this temperature difference results in a slightly truncated primer extension product at 37 °C as com-

Fig. 4. ZntR footprinting of PzntA. A, template strand; B, non-template strand. For DNase I and KMnO₄ footprinting, the concentrations of ZntR, Zn(II), and RNAP used were 75 nM, 25 μM, and 100 nM, respectively. In lanes 5, 7, 14, and 16, 100 μM UpA and 10 μM ATP, GTP, and UTP were added to the reactions. In the phenyl-phenanthroline-Cu footprinting experiments, the concentration of Zn(II) was 35 μM while the ZntR and RNAP concentrations were the same as the other footprinting techniques. All buffers contained 1 μM TPEN. Lanes G are guanine-specific sequence ladders.
pared with 42 °C. Alternatively, there may be two different transcripts formed upon induction of zntA. We did observe a slightly shorter primer extension product in a faint band below the primary product (Fig. 3 and other data not shown). Resolution of these competing hypotheses awaits further experimentation. A transcription start site at the T shown in Fig. 3 is consistently observed in our experiments.

**DNA/Protein Interactions**—The footprinting results for both strands of P zntA are shown in Fig. 4. On the template strand (Fig. 4A), ZntR alone protects bases −23 to −12 from DNase I cleavage, whereas bases −11 and −30 show hypersensitivity to cleavage (lane 2). On the non-template strand (Fig. 4B), bases −35 to −10 are protected from cleavage, whereas bases −17 and −42 are hypersensitive to DNase I cleavage (lane 2). Upon addition of Zn(II), the length of the protected region on both strands does not change (Fig. 4, lanes 3). However, the hypersensitive sites within the footprinted region decrease (−30 on template and −17 on non-template), whereas the protection of −27 on the template strand and −20 on the non-template strand is diminished. The other hypersensitive bands outside the footprint do not change (−11 on template and −42 on non-template). When RNAP is added to ZntR without Zn(II) present (lanes 4), there is little change in the footprint from ZntR alone (lanes 2). A similar result is observed with the addition of nucleotide triphosphates ATP, UTP, and GTP and the dinucleotide initiator UpA (lanes 5) to ZntR and RNAP. As shown in lanes 6, the addition of ZntR, Zn(II), and RNAP to the reaction mixture results in an extended footprint covering −55 to −52 and −46 to +21 on the template strand and −44 to +25 on the non-template strand. Weaker protection is observed at +22 to +25 on the template and at −55 to −50 and +26 to +28 on the non-template strand. Hypersensitive bands are apparent at −60, −39, and −38 on the template strand and at −58, −47, and −46 on the non-template strand.
The addition of ATP, UTP, GTP and UpA allows the production of a 13-base RNA transcript of zntA before the incorporation of the first cytosine residue. The resulting halted transcription complex cannot proceed any further without the addition of CTP. Fig. 4, lanes 7 shows the footprint of this complex that is formed with the addition of ZntR, Zn(II), RNAP, UpA, and the three NTPs to the reaction mixture. On the template strand, −36, −27, and −15 are less protected, whereas +22 to +32 are more protected. Similarly on the non-template strand, the regions between −45 to −32 and −17 to −12 are less protected, whereas +26 to +35 are more protected. The hypersensitive bands seen in lanes 6 (ZntR, Zn(II), and RNAP) are also diminished, while new hypersensitivities appear at −34 to −36 on the non-template strand. These results are consistent with a 3′ movement of RNAP after formation of the initiated, open complex.

Reagents that delineate the melted-out region also indicate that the addition of Zn(II) to ZntR causes the transcription bubble to move in the 3′ direction. KMnO₄ footprinting indicates the position of unpaired thymines (and sometimes other bases) in regions of non-base-paired DNA. Fig. 4, lanes 10–15 indicate that KMnO₄ reactivity is greatly enhanced with ZntR, Zn(II), and RNAP in the reaction mixture. Without the addition of UpA and NTPs, unpaired bases are found at −10, −11, −12 on the template strand and −9, −5, −1, +1 and +4 on the non-template strand (lanes 15). As shown in lanes 16, adding UpA, ATP, UTP, and GTP shifts the reactive bases downstream (−10 to −12, +1 to +3, and +11 to +13 on the template and −1, +1, +4 on the non-template). Since it is unlikely that the transcription bubble extends from −12 to +13, the footprinting data may indicate a mixture of the RNAP open complex and halted complex.

More subtle distortions of DNA structure have been detected with copper-phenanthroline complexes (24). 5-Phenyl-1,10-phenanthroline-Cu cleaves DNA by binding to the minor groove and forming a copper-oxygen species, therefore it is sensitive to changes in minor groove geometry (47). Hypersensitivity to 5-phenyl-OP-Cu cleavage was visible only with ZntR, Zn(II), and RNAP together (Fig. 4, lanes 22). The hypersensitive areas were found in the center of the palindromic site between ZntR (−22 to −24) and near the transcription start site (−2 to −4). Similarly on the non-template strand and −1 to −4 and +5 to +7 on the non-template). These hypersensitivities were all diminished upon 5′ movement of RNAP with the addition of UpA and the three NTPs (data not shown). The footprinting data for ZntR are summarized in Fig. 5.

**Transcription Assays—In vitro** run-off transcription assays using the P₁₇₂₆₄₆ template are shown in Fig. 6. RNAP alone (Fig. 6A, lanes 1 and 9) produces very low levels of the zntA transcript. When ZntR is titrated with excess TPEN present (10 μM) but no added Zn(II), zntA transcript levels increase very slightly up to 20 nM ZntR, and by 500 nM ZntR, they drop off to a value that is ~20% lower than RNAP alone (lanes 2–8 in panel A and graph in panel B). When this titration is repeated with 10 μM Zn(II) present, transcriptional activation steadily increases and is enhanced by a factor of 6–8 once ZntR levels reach 50–100 nM (lanes 10–16 in panel A and graph in panel B). Transcription levels then drop off slightly at 500 nM ZntR. Similarly, with constant [ZntR] and increasing [Zn(II)], half-maximal transcriptional activation is achieved at 5.9 ± 0.2 μM Zn(II) in the presence of 10 μM TPEN (lanes 17–24 in panel A and graph in panel C). Similar transcription results were obtained using ApA rather than UpA as the dinucleotide initiator (data not shown). Without TPEN in the transcription buffer, ZntR did not require added Zn(II) for activation (data not shown), suggesting that the buffer still contains sufficient levels of Zn(II) to activate ZntR despite chelating overnight. Any zinc present in the buffer was too low to detect by ICP-AES analysis (detection limit for Zn = 200 nM).

**DISCUSSION**

The in vitro results outlined here have shown that the purified ZntR protein is a Zn(II) receptor that is both necessary and sufficient to directly mediate zinc-responsive activation at P₁₇₂₆₄₆. At the heart of the transcriptional activation mechanism employed by ZntR are DNA distortions that are proposed to make the promoter a more optimal substrate for RNAP. This DNA distortion mechanism is apparently a widespread attribute of MerR family members which generally mediate responses to a large variety of physical and chemical stresses.

A transcriptionally active, heparin-resistant open RNAP complex at P₁₇₂₆₄₆ is only formed when ZntR, Zn(II), and RNAP are present in the reaction mixture. The in vitro activation and the sequence of metal-dependent changes in the ZntR footprinting data show many similarities with those obtained for MerR (22, 24, 25, 37) and SoxR, another MerR homologue in E. coli that responds to oxidative stress (40). SoxR contains a reoxosear Fe-S cluster that activates transcription in the oxidized form but not in the reduced form (48). As shown in Fig. 7, MerR, SoxR, and ZntR all bind to a palindromic sequence located between the −35 and −10 regions of their target promoters. The spacing between these promoter elements is suboptimal in each case (19 bp for merT and soxS, and 20 bp for zntA). Without the activating metal, all three proteins produce DNase I hypersensitive areas within the footprinted region. With metal bound (or in the oxidized state of the Fe-S cluster in the case of SoxR), some of these hypersensitivities are significantly decreased (37, 49). Ansari et al. (37) proposed that two of the DNase I hypersensitive sites in MerR footprints (marked...
with a asterisk in Fig. 7) arise from protein-induced bending that creates kinks in the DNA structure much like those observed in the CAP-DNA complex. Phasing and topoisomerase assays indicate that, upon Hg(II) binding, the protein-induced bends are relaxed and the operator is untwisted (36, 37). These changes realign the promoter elements in a manner that leads to more effective RNAP binding. The footprinting data for ZntR indicates similar, but not identical, DNA distortions occurring upon Zn(II) binding. Furthermore, like Hg-MerR, Zn-ZntR greatly enhances RNAP binding and open complex formation at the target promoter. While promoter remodeling by sensor proteins may be one of several general mechanisms for transcriptional activation, a comparison of the detailed conformational changes reveals a variety of subtle differences in DNA structure from system to system.

Analysis of the crystal structure of DNase I-DNA complexes provides an understanding of how protein-induced bending affects DNase I cleavage. DNase I widens the minor groove while bending the DNA away from itself toward the major groove (50). Therefore, distortions in DNA structure that create a widened minor groove result in excellent substrates for DNase I hypersensitivity within a footprint suggests that the DNA in the nucleoprotein complex is bent toward the major groove or that the DNA is highly flexible (52). Like MerR, SoxR, and ZntR, the CAP protein also exhibits these DNase I hypersensitivities within the footprinted region (54). The crystal structure of the CAP-DNA complex indicates the exact position of the protein-induced kinks (38), and the DNase I hypersensitivities are found immediately 3’ to these sites (37). Therefore, the footprinting similarities seen between the MerR family and CAP coupled to the crystallographic data available for the CAP-DNA complex strongly support the bending aspect of the DNA distortion mechanism proposed for MerR and ZntR.

Support for the localized DNA unwinding in the MerR-like DNA distortion mechanism was obtained from chemical nuclease probes. The footprints of ZntR, MerR, and SoxR show hypersensitivity to 5-phenyl-OP-Cu cleavage at the center of the palindromic binding site with the activating metal bound (24, 55). For Zn-ZntR and the oxidized Fe$_3$S$_2$ form of SoxR, this nuclease hypersensitivity is apparent only in the ternary open RNAP complexes, whereas Hg-MerR displays this feature both with and without RNAP. This hypersensitivity is proposed to be caused by a protein-induced underwinding of the DNA helix. Additional 5-phenyl-OP-Cu hypersensitive sites are also found around the transcription start site for each metal-activated protein when RNAP is added to the reaction. This cleavage by Cu-OP at positions −3 to −7 on the template strand and +4 to +5 on both strands correlates well with the presence of an open, transcriptionally active complex at other promoters (56, 57).

Recent results indicate that RNAP open complex formation in general involves wrapping of the DNA strand around RNAP to form a left-handed superhelix (58–61). The DNase I footprinting data for the RNAP/P$_{zntA}$Zn-ZntR complex are consistent with a similar model in which a series of upstream bends lead to spooling of DNA onto the polymerase. A model for the interaction of Zn-ZntR with the wrapped RNAP-DNA open complex is given in Fig. 8. A MerR-like DNA distortion mechanism conforms nicely with this model for RNAP/DNA interaction. In the repressor conformation, apo-MerR may bend DNA away from RNAP, preventing the wrapping of DNA around the RNAP protein core. Relaxation of these bends by Hg-MerR coupled with unwinding and changes in writhe may stimulate transcription by facilitating the wrapping characteristic of a productive open complex. Apo- and Zn-ZntR would follow this same mechanism, although the extent of bending and underwinding remains to be seen in this system.

Another unusual aspect of the MerR regulation mechanism is the ability of RNAP to form closed complexes at promoters bound to apo-MerR (Fig. 1B) (24). DNase I footprinting analyses indicated that with apo-MerR bound to the DNA, RNAP binds upstream of the MerR binding site, only accessing the −35 site (24, 37). In this state, apo-MerR has also been shown to weakly repress transcription at merT (24). ZntR-P$_{zntA}$ did not readily form a closed RNAP complex in this manner at 37 °C (data not shown). While the transcription assays indicate strong activation of zntA transcription with Zn-ZntR, apo-ZntR only weakly represses at concentrations exceeding 100 nM. The slight increase in transcription at lower apo-ZntR concentrations may be attributed to ZntR scavenging any exogenous zinc from the buffer. As more apo-ZntR is added, Zn-ZntR is out-competed by the apo protein for the DNA binding site.

Without a strong zinc chelator like TPEN (log K = 18.0) (62), which can sequester contaminating Zn(II), ZntR shows activation with no additional metal added (data not shown). The fact that TPEN is required to see the lowest levels of transcript in the absence of Zn(II) suggests that ZntR is very sensitive to free Zn(II) concentrations in the buffer. As Zn(II) is titrated into the assay buffer, the onset of transcriptional activation is observed just before Zn(II) levels exceed the TPEN concentration, indicating that ZntR can compete to a limited extent with TPEN for Zn(II). This provides further evidence that ZntR has a strong affinity for Zn(II). While MerR binds 1 mol of Hg per MerR dimer (22, 63), ZntR can bind 1–2 mol of Zn(II) per monomer depending on the buffer conditions. However, the minimal metal occupancy required for function remains to be established.

In vivo transcriptional data for ZntR (2) reveals another similarity to MerR—the cooperative or ultrasensitive transcriptional response to metal ions (25). Brocklehurst et al. (2) have shown that in vivo expression of zntA exhibits a sigmoidal response to changes in Zn(II) concentrations in the media. This phenomenon has been observed for both in vivo (7, 64) and in vitro (25) activation of P$_z$ by the MerR protein. The Zn(II) induction curve from our transcription results, however, should not be interpreted as ultrasensitivity because of the presence of TPEN in the assay. TPEN is a strong Zn(II) chelator and thus buffers the Zn(II) concentration. Once the buffer capacity is exceeded, Zn(II) binding to ZntR will be stoichiometric and thus will not reflect metal effects in a simple manner. The interesting ultrasensitivity feature of both the MerR and ZntR regulation systems is another important similarity of these stress response systems, however the molecular basis of this cooperative response phenomenon remains elusive.

The transcription and footprinting data presented here provide strong evidence that the stress-responsive transcriptional activation of P$_{zntA}$ by ZntR involves a MerR-like DNA distortion mechanism. ZntR acts as a genetic switch that becomes a
strong activator upon Zn(II) binding. The metal selectivity and Zn(II) affinity of ZntR are being evaluated and may shed light on the molecular basis of metal ion recognition by this MerR family member.

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