Structural basis of *Streptomyces* transcription activation by zinc uptake regulator

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### ABSTRACT

*Streptomyces coelicolor* (*Sc*) is a model organism of actinobacteria to study morphological differentiation and production of bioactive metabolites. *Sc* zinc uptake regulator (*Zur*) affects both processes by controlling zinc homeostasis. It activates transcription by binding to palindromic Zur-box sequences upstream of −35 elements. Here we deciphered the molecular mechanism by which *Sc* Zur interacts with promoter DNA and *Sc* RNA polymerase (RNAP) by cryo-EM structures and biochemical assays. The *Sc* Zur-DNA structures reveal a sequential and cooperative binding of three *Sc* Zur dimers surrounding a Zur-box spaced 8 nt upstream from a −35 element. The *Sc* RNAPσ²⁷₀-*Zur*-DNA structures define protein-protein and protein-DNA interactions involved in the principal housekeeping (σ²⁷₀) dependent transcription initiation from a noncanonical promoter with a −10 element lacking the critical adenine residue at position −11 and a TTGCCC −35 element deviating from the canonical TTGACA motif. *Sc* Zur interacts with the C-terminal domain of *Sc* RNAP α subunit (α-CTD) in a complex structure trapped in an active conformation. Key *Sc* Zur-α-CTD interfacial residues accounting for *Sc* Zur-dependent transcription activation were confirmed by mutational studies. Together, our structural and biochemical results provide a comprehensive model for transcription activation of Zur family regulators.

### INTRODUCTION

*Streptomyces* is the largest genus within the *Actinobacteria*, producing about half of all clinically used antibiotics and other important pharmaceuticals of natural origin (1). *Streptomyces* bacteria undergo a complex life cycle of morphological differentiation that is closely associated with the bioactive metabolite production. Both processes are subject to strict regulation at the transcriptional level. Transcription initiation steps catalysed by RNA polymerase (RNAP) are the most common targets for regulation. As observed in other bacteria, a single RNAP core enzyme (α₂ββ’ω) is used by streptomycetes to transcribe all genes, whereas most streptomycetes contain one primary σ factor and multiple alternative σ factors to coordinate expression of genes by recognizing alternative promoters (2,3). *Streptomyces coelicolor* is a model organism of actinobacteria. Among 64 σ factors encoded in *S. coelicolor* genome, σ²⁷₀ is the principal housekeeping σ factor and analogous to σ₇₀ of *Escherichia coli*. The presence of σ²⁷₀ is essential across streptomycetes. ChIP-seq analysis of σ²⁷₀ identifies a total of 2137 protein-coding genes in *S. coelicolor* (4). Due to its importance in transcription regulation, streptomycete RNAP has been genetically and biochemically characterized (5–7), whereas no structural information is available to date.

Transcription factors bind to specific sequences overlapping or near RNAP binding sites of promoters to repress or activate the expression of genes (8). The catabolite activator protein (CAP; also known as the cAMP receptor protein, CRP) is one of the most studied transcription factors (9). In the presence of the allosteric effector cAMP, it activates transcription at >100 promoters by binding to a 22-base pair (bp) palindromic cis element in *Escherichia coli*, via either class I or class II mechanism. One of the best characterized class I CAP-dependent promoters is the *lac* promoter, which has a CAP binding site at position −61.5 (10). Transcription activation at the class I CAP-dependent promoter involves protein-protein interactions between CAP and the carboxyl-terminal domain of the α subunit (α-CTD) of RNAP, which enhance RNAP binding to the promoter by a recruitment mechanism. At class II CAP-dependent promoters, CAP binds at position −41.5 and forms protein-protein interactions with multiple subunits of RNAP (11,12). Both recruitment and isomerization mechanisms are involved in transcription activation at...
class II CAP-dependent promoters. A few bacterial transcription factors, such as members of MerR family, bind between the −10 and −35 elements at target promoters and activate transcription by shortening the length of non-optimal −35/−10 spacer (13,14). Although most transcription activators binding to specific DNA sequences of promoters, actinobacteria have unique transcription factors, such as CarD and RbpA, that active transcription regardless of promoter sequences (15,16).

Zinc (Zn) uptake regulator (Zur) is the most prevalent regulator of Zn uptake genes in bacteria, regulating its regulon genes in response to the fluctuations of the intracellular Zn levels (17,18). It belongs to the ferric uptake regulator (Fur) family, which is a family of bacterial proteins that regulate metal ion uptake to maintain metal homeostasis. Zur is first discovered in *E. coli* as a repressor of *znuABC* genes encoding high-affinity Zn importer and further in other bacteria including *S. coelicolor*, *Mycobacterium tuberculosis*, *Xanthomonas campestris* etc. (19–22). In the presence of sufficient Zn, the Zn-bound form of Zur acts predominantly as a transcriptional repressor by blocking RNAP binding sites. In Zn deficiency, the Zn-free form of Zur has a low DNA-binding affinity, leading to the transcription of Zn regulon genes. Available structures reveal that Zur proteins act as a homodimer, of which each monomer contains an N-terminal DNA binding domain (DBD), a C-terminal dimerization domain (DD), and a hinge region linking the two domains (19–22). The Zur may contain two or three Zn sites per monomer. According to structural and biochemical analyses of *S. coelicolor* Zur (*ScZur*), the C-site with four conserved cysteines for Zn coordination is a common structural site, while the other two sites are involved in regulatory functions (16). Zn binding in regulatory sites affects the orientation of the two DBD domains and regulates DNA binding. In contrast to the well-studied Zur binding in several Zur proteins, Zur-DNA interaction has only been structurally characterized for *E. coli* Zur (*EcZur*), which is crystallized in complex with its target DNA, a conserved 30-bp AT-rich sequence called a Zur-box (19). The *EcZur* binds to the Zur-box as a dimer of dimers, which is stabilized by a pair of salt bridges between neighbouring monomers. Each monomer of *EcZur* recognizes the DNA sequence of the Zur-box by both base readout and shape readout.

Although Zur proteins usually act as repressors, they may also activate transcription of genes, at least in *S. coelicolor* and *X. campestris*, where Zur proteins activate putative Zn exporters of cation diffusion facilitator (CDF) superfamily (23,24). In *X. campestris*, footprinting identifies a 59-bp GC-rich sequence with a 20-bp inverted repeat in the upstream region of a CDF gene. Intriguingly, the 59-bp GC-rich sequence shows no significant similarity with the Zur-box of *X. campestris*. In *S. coelicolor*, the promoter region of *zitB* gene encoding a CDF exporter contains a 15-bp Zur-box, that is spaced 8 nt upstream from the −35 element (Supplementary Figure S1). However, in the presence of enough Zn, the Zur protects both the Zur-box motif and the further upstream region up to position −138 relative to the transcription start site (TSS) of *zitB* (24). The size of the protected regions in both cases suggests that oligomeric Zur binding is required for transcription activation. The effectiveness of cis-regulatory elements in Zn-dependent transcription activation is tested *in vivo*. The regulatory region up to position −60 only allows partial activation of *zitB*, whereas the regulatory region up to position −228 enables full gene activation.

The Zur-box in the *zitB* promoter of *S. coelicolor* is spaced 8 nt upstream from the −35 element (Supplementary Figure S1A), suggesting that the *ScZur* may activate transcription via the class II activation mechanism (11,12,24). However, the precise mechanism by which ScZur interacts with *zitB* promoter DNA and ScRNAP to activate transcription remains obscure. In the present study, we determined cryo-EM structures of *ScZur*-DNA and ScRNAP<sub>H<sup>rdB</sup></sub>-Zur-DNA complexes. The ScZur-DNA structures unveil sequential trimerization of ScZur surrounding the Zur-box while the ScRNAP<sub>H<sup>rdB</sup></sub>-Zur-DNA structures demonstrate that ScZur activates ScRNAP<sub>H<sup>rdB</sup></sub> by contacting αCTD. These results from structural analysis are supported by the Mango-based *in vitro* transcription assays. Together, these findings provide a comprehensive model for transcription activation of Zur family regulators.

**MATERIALS AND METHODS**

**Plasmids**

All primers used for amplification by PCR are listed in Supplementary Table S1. Genomic DNA of *S. coelicolor* M145 was used as a template. The *rpoA*, *rpoB*, *rpoC* and *rpoZ* encoding RNAP core enzyme (α<sub>5</sub>ββ′ω) were cloned into a single plasmid pET28a to get a final vector, pET28a-RNAPcore. Restriction sites *Xba*I, *Nde*I, *Bam*HI, *Eco*RI and *Hind*III were used for inserting *rpoA*, *rpoB*, *rpoC* and *rpoZ* genes. In the resulting vector, β and β′ were fused in frame by inserting a 9 amino-acid polylinker between the C-terminal of β and the N-terminal of β′. An 8xHis tag was engineered on the C-terminal of *rpoB* and *rpoC*. An 8xHis tag was engineered on the 3′ end of the connected β and β′ gene. Structures of reported bacterial RNAP complexes show that the two terminals are close to each other and the strategy of fusing β and β′ has been used in a heterogeneous expression of *Mycobacterium smegmatis* RNAP core (25). The dissociable σ<sup>H<sub>rdB</sub></sup> that directs RNAP to promoters of housekeeping genes and the Zur monitoring in *S. coelicolor* Zn were inserted into the pET28a vector via *Nde*I and *Eco*RI restriction sites to obtain pET28a-*hdB* and pET28a-*zur* respectively.

**ScRNAP core purification**

The vector pET28a-RNAPcore was transformed into *E. coli* strain BL21(DE3) for expression. Cultures were incubated at 37°C, transferred to 16°C when reaching an optical density at 600 nm (OD<sub>600</sub>) of 0.6, and allowed the cultures to cool for 20 minutes before inducing with 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 16°C for 16 h. Cells were harvested, washed, and resuspended in lysis buffer (50 mM Tris (pH 8.0), 500 mM NaCl, 10 mM MgCl<sub>2</sub>, 10% glycerol (v/v), 1 mM PMSF, 1 mM β-mercaptoethanol, 0.1 mg/ml sodium deoxycholate and 5 mM imidazole). Following sonication on ice, cell debris was removed by centrifugation at 15 000g for 40 min at 4°C. The resulting supernatant was loaded onto nickel-NTA resin equilibrated
with lysis buffer, followed by washing with lysis buffer containing 20 mM imidazole (pH 8.0) and eluting with elution buffer (20 mM Tris (pH 8.0), 200 mM NaCl, 5 mM MgCl₂, 10% glycerol (v/v) and 200 mM imidazole). The eluted fractions were pooled and loaded onto a HiTrap Heparin HP affinity column pre-balanced in buffer A (20 mM Tris (pH 8.0), 5 mM MgCl₂, 10% glycerol (v/v)). The RNAP core was eluted by buffer B (20 mM Tris (pH 8.0), 1 M NaCl, 5 mM MgCl₂, 10% glycerol) that was increased from 0 to 100% in 20 ml. The fractions were pooled, concentrate and polished by a Superose 6 gel filtration column equilibrated with size exclusion chromatography (SEC) buffer (20 mM Tris (pH 8.0), 150 mM NaCl, 2 mM MgCl₂, 3 mM DTT and 10% glycerol). The resulting protein was concentrated to 3 mg/ml, and then stored at −80°C (Supplementary Figure S2). The RNAP core carrying truncated or mutated α subunit was prepared by same protocol.

**σ^{HrdB} purification**

*Escherichia coli* strain BL21(DE3) transformed with pET28a-hrdB was incubated at 37°C until the OD₆₀₀ reached ~0.6. Expression was induced with 0.3 mM IPTG at 16°C for 16 h. Cells were harvested, washed, and resuspended in the lysis buffer without MgCl₂ and sodium deoxycholate. Cells were lysed by using sonication. Supernatant obtained by centrifugation was applied to nickel-NTA resin equilibrated with lysis buffer, washed with lysis buffer containing 20 mM imidazole (pH 8.0) and eluting with elution buffer. The eluted fractions were pooled, loaded onto a HiTrap Heparin HP affinity column pre-balanced in buffer A and eluted by buffer B that was increased from 0 to 100% in 20 ml. The fractions were pooled, concentrate and polished with a Superdex 200 gel filtration column equilibrated with SEC buffer. The resulting protein was concentrated to 6 mg/ml and stored at −80°C (Supplementary Figure S2).

**ScZur purification**

*Escherichia coli* strain BL21(DE3) transformed with pET28a-zur was cultured, harvested and lysed as described for σ^{HrdB}. The purification protocol was also similar to σ^{HrdB} except that Zur was eluted with lysis buffer containing 250 mM imidazole (pH 8.0) and polished with a Superdex 75 gel filtration column equilibrated with SEC buffer with 1 μM ZnSO₄. According to Roe et al. (21), the Kₐ of ScZur for Zn is 0.45–0.78 fM. The resulting holo-Zur (∼1.5 mg/ml) was stored at −80°C. To obtain apo-Zur without Zn, the elution fractions from nickel-NTA column was dialyzed into buffer C (20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 5% glycerol and 5 mM EDTA) to remove imidazole and nickel. The EDTA was removed by dialyzing against buffer D (20 mM Tris–HCl, pH 8.0, 50 mM NaCl, 10% glycerol and 1 mM DTT), concentrated to 1 mg/ml, and stored at −80°C (Supplementary Figure S2). The Zur mutants were prepared by same protocol.

**ScRNAP^{HrdB} holoenzyme**

*S. coelicolor* RNAP core enzyme and σ^{HrdB} were incubated in a 1:2 ratio for 1 h on ice. The reaction mixture was loaded onto Superose 6 gel filtration column equilibrated with SEC buffer and eluted with the same buffer. Fractions containing *S. coelicolor* RNAP-σ^{HrdB} holoenzyme were pooled and stored at −80°C (Supplementary Figure S2).

**In vitro transcription**

The transcription activities of ScRNAP^{HrdB} in the absence or presence of ScZur are evaluated by measuring the fluorescence intensities of Mango III (26,27). A 296 bp DNA fragment consisting of the zitB promoter (~228 to +15) and the mango III coding sequence were synthesized and cloned into pUC19 via restriction sites EcoRI and HindIII (Supplementary Figure S1). The resulting plasmid was used as a template to amplify the DNA scaffold carrying the zitB regulatory region up to ~68 (TP-68), ~100 (TP-100) or ~140 (TP-140), and the mango III by using primers listed in Supplementary Table S1. Mango III transcription assays were performed in black 96-well plates. 0 or 1 μM Sc-Zur was mixed with 10 nM nucleic acid scaffold in 100 μl transcription buffer (20 mM Tris–HCl, pH 8.0, 0.1 M KCl, 5 mM MgCl₂, 20 μM ZnSO₄, 2 mM DTT, 4 U RNaseIn, 1 μM TO1–PEG–biotin, and 5% glycerol) at room temperature for 10 min, followed by supplementation of 0.1 μM ScRNAP^{HrdB} and 1 mM NTPs. The reactions were incubated for 10 min at 30°C and stopped by 0.5 ng heparin. Corresponding reactions without NTP were used as blanks. Fluorescence emission intensities were measured using SpectraMax Id5 Multi-Mode Microplate Reader (Molecular Devices) with excitation wavelength of 510 nm and emission wavelength of 535 nm.

**Electrophoretic mobility shift assay (EMSA) for ScZur–DNA binding**

The dsDNA containing a 5’-conjugated FAM was prepared by annealing complementary oligonucleotides (10 μM each, Supplementary Table S1) in 20 mM Tris pH 8.0 by heating the reaction to 95°C for 10 min and allowing it to cool to room temperature slowly. For Zn-dependent EMSA reactions, 1 ng of labelled dsDNA and 100 nM purified apo-ScZur or mutants were mixed in 10 μl binding buffer (20 mM Tris–HCl (pH 8.0), 100 mM KCl, 2 mM DTT, 0.1 mg/ml BSA, 5% glycerol, 0.1 mg of poly(dI-dC) and 0–40 μM ZnSO₄) and incubated at room temperature for 20 min. For Zur-dependent EMSA reactions, 0–800 nM purified ScZur or mutants and 1 ng of labelled dsDNA were mixed in 10 μl binding buffer (20 mM Tris–HCl (pH 8.0), 100 mM KCl, 2 mM DTT, 0.1 mg/ml BSA, 5% glycerol, 0.1 mg of poly(dI-dC) and 20 μM ZnSO₄) and incubated at room temperature for 20 min. The binding mixture was then subjected to electrophoresis at 4°C on a 5% polyacrylamide gel at 130 V in TB (89 mM Trizma base, 89 mM boric acid) buffer, followed by a visualization using Amerham Typhoon RGB Biomolecular Imager with Cy2 filters. Signals from all shifted bands were combined to obtain the bound fraction, and the signal from non-shifted band was used for non-bound fraction. Data from three independent experiments were fitted to a Hill equation to obtain the nₘ and the Kₐ,ave.
Assembly of ScRNAP$_{\text{HdB}}$–Zur–DNA complex

The $\text{zitB}$ promoter DNA carrying 13-bp ($\text{zitB}_{13}$) or 6-bp ($\text{zitB}_{6}$) non-complementary transcription bubble were prepared by annealing nontemplate (nt) strand (28 $\mu$M final) and template (t) strand (30 $\mu$M) in annealing buffer (10 mM Tris (pH 8.0), 50 mM NaCl, 10 mM MgCl$_2$). RNA (5'-GUAGG-3'; 42 $\mu$M) was supplemented for preparing nucleic-acid scaffold carrying 13-bp non-complementary transcription bubble. The reactions were heated to 95°C for 10 minutes and cooled to room temperature slowly. To assemble ScRNAP$_{\text{HdB}}$–Zur-DNA, 270 $\mu$l core enzyme of 11 $\mu$M and 20 $\mu$l a$_{\text{HdB}}$ of 243 $\mu$M were added into 700 $\mu$l assembling buffer (20 mM HEPES pH 8.0, 50 mM KCl, 5 mM MgCl$_2$, 20 $\mu$M ZnSO$_4$, 3 mM DTT) and incubated for 10 min at room temperature, followed by supplementation of 100 $\mu$l promoter DNA of 28 $\mu$M and incubation of another 10 minutes. The complexes were loaded onto a Supernos 6/10/300 GL column (GE Healthcare), eluted by assembling buffer, concentrated to 7 mg/ml and stored at −80°C (Supplementary Figure S2).

Cryo-EM data acquisition

Two batches of data (5550 movies in total) were collected for ScRNAP$_{\text{HdB}}$–Zur–$\text{zitB}_{13}$. 3 $\mu$l ScRNAP$_{\text{HdB}}$–Zur–$\text{zitB}_{13}$ of 0.8 mg/ml were applied to Quantifoil R1.2/1.3 Au 300 mesh grids freshly glow-discharged at 25 mA for 60 s. Grids were blotted for 2 s at 16°C with 100% chamber humidity and then plunge-frozen in liquid ethane in a Vitrobot (FEI, Inc.). 3523 movies were collected by using SerialEM on Titan Krios 300 kV microscopes with Gantan K2 detector operated in counting mode with a pixel size of 1.1 Å/pixel, a defocus ranging from −1.0 to −2.0 $\mu$m, and a total dose of 40 e$^-$/Å$^2$ fractionated into 32 frames. 2027 movies were collected by Gantan K3 detector using EPU with the same pixel size, defocus ranging and dose fractionation.

For data acquisition of ScRNAP$_{\text{HdB}}$–Zur–$\text{zitB}_{6}$, 3 $\mu$l samples of 1.0 mg/ml were used for preparing the grids as described above. 15232 movies were collected by using SerialEM on Titan Krios 300 kV microscopes with Gantan K2 detectors operated in counting mode. Exposures of a total dose of 40 e$^-$/Å$^2$ were fractionated into 32 frames with a pixel size of 1.05 Å/pixel and a defocus ranging from −1.0 to −2.0 $\mu$m.

Cryo-EM data processing

Datasets of ScRNAP$_{\text{HdB}}$–Zur–$\text{zitB}_{13}$ acquired with Gantan K2 detector were subjected to motion correction using RELION’s own implementation of the UCSF MotionCor2 program in RELION-3.1 (28). The aligned images were imported into CryoSPARC suite v3.3.1 (29) and subjected to patch CTF estimation, manually curate exposures, and template picker with good 2D classes from blob picker as templates, followed by two rounds of 2D classification of picked particles. Datasets acquired with Gantan K3 detector were processed by the same protocol. Particles of good 2D classes of the two datasets were combined and subjected to the third round of the 2D classification, followed by ab-Initio reconstruction, homogeneous refinement, non-uniform refinement and 3D variability analysis. Good 3D classes were selected, iteratively refined with C1 symmetry, and subjected to local resolution estimation and local filtering to produce final maps. The final particle stacks were subjected to particle subtraction to keep only the signal of ScZur region, followed by masked local refinements to improve the map quality and interpretability (Supplementary Figure S3).

2D class averages corresponding to ScZur-DNA unexpectedly obtained in the 2D classification of picked particles from ScRNAP$_{\text{HdB}}$–Zur–$\text{zitB}_{13}$ sample were used and were the templates to pick ScZur-DNA particles from the two datasets respectively in CryoSPARC suite v3.3.1 (29). The picked particles were subjected to two rounds of 2D classification respectively before combination. The combined particles were subjected to the third round of the 2D classification. Particles in good 2D classes were selected and subjected to ab-initio reconstruction, followed by iterative refinement, local resolution estimation, and local filtering to produce final maps (Supplementary Figure S4).

For the datasets of ScRNAP$_{\text{HdB}}$–Zur–$\text{zitB}_{6}$, dose-fractionated movies were subjected to patch motion correction, patch CTF estimation, manually curate exposures, and template picker using good 2D classes from ScRNAP$_{\text{HdB}}$–Zur–$\text{zitB}_{13}$ as templates, followed by three rounds of 2D classification of picked particles and ab-Initio reconstruction in CryoSPARC suite v3.3.1 (29). Particles in good 2D classes were selected and imported into RELION-3.1 using esparc2star.py module for re-extraction, 3D auto-refinement with the model from ab-Initio reconstruction as a starting reference, CTF refinement and Bayesian polishing (28). The polished particles were imported back into CryoSPARC suite v3.3.1 again for non-uniform refinement, 3D variability analysis and 3D classification. Good 3D classes were selected, iteratively refined with C1 symmetry, and subjected to local resolution estimation and local filtering to produce final maps. The ScZur region was subjected to masked local refinement with subtracted particle to improve the map quality and interpretability (Supplementary Figure S5). The charge density distribution map of a local refinement map was prepared as described by Wang et al. (30).

Cryo-EM model building and refinement

The initial atomic models of ScRNAP$_{\text{HdB}}$ subunits were generated by SWISS-MODEL (31). The crystal structure of Zn-bound ScZur (21) and the homology model of ScRNAP$_{\text{HdB}}$ were fitted into the cryo-EM density maps using ChimeraX (32). The model of the promoter DNA and the RNA oligomer, were built manually in Coot (33). The coordinates were refined in Phenix with secondary structure restraints, rotamer restraints and Ramachandran restraints (34). The final coordinates were validated using MolProbity (35). The Map versus Model FSCs of five cryo-EM maps and their corresponding atomic models of this study were generated by Phenix (Supplementary Figure S6). The statistics of cryo-EM refinement were summarized in Supplementary Table S2.
RESULTS

Zur activates zitB via Zur-box and further upstream regions

We purified the RNAP core enzyme, and housekeeping σHdb of S. coelicolor for in vitro run-off transcription reactions. Although the zitB promoter lacks the critical adenine residue at position −11 (Supplementary Figure S1A), it can be recognized by σHdb in previous in vitro transcription reactions (24). ScZur protects a region of 98 bp (−138 to −40) in a footprint on zitB promoter. In addition to the 7−1−7 Zur-box (−45 to −59), inspection of the 98 bp DNA sequence identified another two palindromic (−67 to −93 nt, and −117 to −133), of which both show similarity with the Zur box (Supplementary Figure S1B). Previous in vivo assays demonstrate that the zitB regulatory region with the Zur-box motif alone can only allow marginal gene activation (~1.3-fold), whereas the upstream sequence up to −228 nt enables full gene activation (~6.5-fold) (24). The DNA templates used for the in vitro run-off transcription reactions contain the zitB region from −140, −100 or −68 to + 15 respectively, followed by sequence encoding Mango III (36,37), which is a fluorogenic RNA aptamer used for direct readout of transcription (Figure 1, Supplementary Figure S1A). As expected, the ScRNAP-σHdb holoenzyme can initiate basal transcription of all three DNA templates. In the presence of the SCzur, transcription was enhanced ~1.6-fold for the DNA template containing the zitB region from −68 to +15 (TP−68), whereas the level of enhancement observed in transcription reactions of the other two templates (up to −100 or −140, TP−100 or TP−140) was more than 4-fold.

A 33 bp zitB Zur-box DNA probe (~68 to ~36) has been proposed to binding two ScZur dimers according to the estimated molecular weight of the retarded band in previous EMSA assays (18). We observed three retarded bands in the EMSA assay at 20 μM ZnSO4 with ScZur from 0 to 800 nM (Supplementary Figure S7). An average dissociation constant $K_{D-ave}$ of 34.0 ± 0.7 nM and a Hill coefficient $n_H$ of 2.3 ± 0.1 were obtained from a Hill Plot. We examined ScZur binding to DNA sequences containing palindromes 1 or 2, using DNA probes from −141 to −109 or from −101 to −69. Oligomeric ScZur binding was observed on both DNA probes at higher concentration (200 nM) compared to the Zur-box DNA probe (6.25 nM) (Supplementary Figure S8). The palindromic 7−1−7 Zur-box contains highly conserved GC base pairs at positions 2, 8 and 14 (Supplementary Figure S1B), which are the signatures of the Zur-box. The modified zitB Zur-box DNA probe (M1) with the three GC base pairs replaced by AT base pairs showed a $K_{D-ave}$ of 97.0 ± 7.7 nM and an $n_H$ of 1.6 ± 0.2 (Supplementary Figure S7B). Replacing the corresponding second G by C in EcZur-box results in ~4 times increase of the $K_{D-ave}$ (from 2.9 ± 0.8 to 11.9 ± 0.4 nM), while replacing the 14th C by G results in ~13 times increase of the $K_{D-ave}$ (from 2.9 ± 0.8 to 39.1 ± 1.3 nM) (19). Consequently, a zitB Zur-box M2 probe was constructed by introducing C, T and G at positions 2, 8 and 14, showing a $K_{D-ave}$ of 223.1 ± 30.5 nM and an $n_H$ of 0.9 ± 0.1 (Supplementary Figure S7C). Introducing T and A at positions 3 and 13 into the M2 probe further decreased the binding of ScZur (Supplementary Figure S7D). No binding of ScZur was observed on the DNA probe downstream of the Zur-box (~29 to +50) (24). We replaced the sequence from −100 to −69 of the TP−100 by the sequence from +18 to +49 of zitB to obtain a hybrid template TP−100H (Supplementary Figure S8C and D). In the presence of ScZur, transcription was enhanced ~1.7-fold, indicating that Zur binding to the upstream regions is necessary for full activation.

ScZur binding to Zur-box as a trimer of dimers

ScZur shows low to moderate sequence identity to Zur of E. coli (21%), X. campestris (24%) and M. tuberculosis (55%) (19,20,22). The α3 recognition helices of DBD domains are highly conserved, accounting for their Zur-box similarity (Supplementary Figure S9). The structure of Zn-bound ScZur has been reported(21), whereas the DNA-bound structure is still unavailable. The cryo-EM structures of ScZur-promoter complexes were unexpectedly obtained during an initial attempt to assemble ScRNAP-σHdb-Zur–DNA complexes. In the course of cryo-EM data processing, 2D classification revealed the presence of ScZur–DNA complexes. 3D classification of the complexes revealed two structures, which were determined at overall resolutions of 3.4 Å (a trimer of dimers, TOD) and 3.8 Å (a dimer of dimers, DOD) respectively (Supplementary Figure S4). The two structures of ScZur-promoter complexes are essentially the same except that the ScZur dimer close to the upstream terminal of the promoter DNA (DIIH) is missing in the DOD structure (Figure 2, Supplementary Figure S10). The
three ScZur dimers in TOD complex protect the DNA sequence from −68 to −36, which comprises the 7−1−7 Zur-box (−59 to −45). The ScZur dimer in the middle (DII) sits in the Zur-box and interact with the palindrome by both monomers. In contrast, the other two ScZur dimers (DI and DIII) make contacts with the Zur-box by using only one monomer (Figure 3). Interactions with the Zur-box are indispensable since ScZur binding to DNA downstream of the Zur-box is not observed. The three retarded bands observed in EMSA assays of the zitB Zur-box DNA probe and the TOD and DOD structures suggest a sequential binding of each ScZur dimer. Superimposition of ScZur dimer with EcZur dimer gives an rmsd of 2.4 Å (187 aligned Ca atoms). The first N-terminal helix observed in EcZur is missing in ScZur (Supplementary Figure S9A). The EcZur binds to its cognate DNA as a dimer of dimers instead of the trimer of dimer observed in the ScZur structure (14). The relative orientation between dimers is different. When the two structures are superposed according to DI of the ScZur-promoter complex, the rotation and displacement between the second dimers are 53° and 21 Å respectively (Supplementary Figure S11). Compared to the EcZur−DNA complex, the ScZur binding only results in slight bends in the target DNA. The ScZur-promoter structures reveal direct protein-protein interactions between adjacent dimers. D37 and H41 residues of each DII monomer interact with their counterparts in the neighbouring dimers (Figure 2D). In EMSA analysis, retarded bands of high molecular weights appear as Zn increased, however the enhancement of Zn on oligomeric ScZur binding is partially reduced when D37 and/or H41 are mutated to alanine. Compared to the wild type protein, all mutants display a reduction of transcription activation (Supplementary Figure S12).

Overall conformations of the two monomers of the dimers are essentially the same with an overall rmsd of 1.1 Å. Moderate differences (rmsd 1.6 Å) are revealed by superimposition of the DNA-bound ScZur dimer with the DNA-free ScZur dimer (16). DNA binding results in an increased distance (from 27 to 34 Å) between the N-terminals of the recognition helices of the two monomers (measured from the Ca atoms of L48 residues) (Figure 3A). Otherwise, the α1 N-terminal would conflict with the phosphodiester backbone of the DNA duplex. The ScZur dimer utilizes two recognition helices (Figure 3B), each from one monomer, to make contacts with the DNA in the major groove, by using residues R53, which is conserved in Zur proteins (Supplementary Figure S9). The R53A mutation compromises both the DNA binding and the transcriptional activation of ScZur (Supplementary Figure S13). The R53 residues of two DII monomers interact with the GC base pair at posi-
Figure 3. Interaction between ScZur and targeted dsDNA. (A) Superimposition of the DNA-bound ScZur dimer with the DNA-free ScZur dimer. Distance between the α1 helices of the two monomers is increased upon DNA binding. (B) The R53 residues of two DII monomers interact with the GC base pair at positions 2 (G_{-58}(nt)) and 14 (G_{-46}(nt)) of the Zur-box respectively. (C) The GC base pair at position 8 (G_{-52}(nt)) is close to the R53 residues of both DI monomer and DIII monomer. (D) Protein-nucleic acid interactions between ScZur dimers and the targeted dsDNA. The figures were prepared by Chimera X and DNAproDB.
of TP−68, whereas no correlation between transcription activities and DNA-binding abilities of the mutants could be observed. The TP−100 was used in the transcription assays of T49A and Y52A, and the transcription activities correlated with their DNA-binding abilities.

**Overall structure of ScRNAP<sup>PrHB</sup>−Zur−DNA**

Pre-melted promoter DNA fragments are often used to assemble RNAP-promoter open complex (RPo) and RNAP-promoter initial transcribing complex (RPitc) to improve the sample homogeneity and enhance protein−DNA interaction. Initially, we designed an 84-bp *zitB* promoter DNA fragment comprising a pre-melted 6-bp (−68 to −12) upstream promoter double-stranded DNA (dsDNA), a 13-bp (−11 to +2) non-complementary transcription bubble, and a 14-bp (+3 to +16) downstream promoter ds-DNA (Supplementary Figure S1A). To assemble the RPitc complex, ScZur was incubated with the 84-bp *zitB* promoter DNA carrying the 13-bp non-complementary bubble (*zitB<sub>13</sub>*) followed by supplementation of the ScRNAP<sup>PrHB</sup> holoenzyme. We added a 5-nt RNA primer to stabilize the template single-stranded DNA (ssDNA) of the unwound transcription bubble. The RPitc structures carrying one (ScRNAP<sup>PrHB</sup>−Zur<sub>d1</sub>−*zitB<sub>13</sub>* or two ScZur dimers (ScRNAP<sup>PrHB</sup>−Zur<sub>d2</sub>−*zitB<sub>13</sub>*)) were obtained at 4.1 and 3.7 Å resolution respectively (Supplementary Figure S3). The Zur regions are visible at a lower contour level compared to the RNAP regions in the density maps, suggesting their flexibility. Interactions between ScZur and ScRNAP<sup>PrHB</sup> are not observed. In vitro transcriptional assays were carried out by using a template with the *zitB<sub>13</sub>* promoter carrying a pre-melted 13-bp bubble. The results reveal that the ScZur does not enhance the transcription of the *zitB<sub>13</sub>* promoter (Figure 4), suggesting that the RPitc with a pre-melted 13-bp bubble may be not a target of ScZur activation.

Next, we synthesized a similar 84-bp *zitB* promoter DNA fragment comprising a pre-melted 6-bp (−6 to −1) bubble (*zitB<sub>6</sub>* (Figure 4A)). The transcription of the *zitB<sub>6</sub>* promoter was enhanced by the ScZur in the Mango-based *in vitro* assays (Figure 4D) and was used to assemble the RPo complexes. 127 088 single particles were obtained from 2D classification and used to reconstruct a map of 3.3−5 Å resolution at the ScRNAP<sup>PrHB</sup> and 8−12 Å resolution at the ScZur. The low resolution of the ScZur region compared to the ScRNAP<sup>PrHB</sup> region in the density map indicates that the ScZur region is flexible as observed in the ScRNAP<sup>PrHB</sup>−Zur−*zitB<sub>13</sub>*. The density of the ScZur dimer binding at the upstream terminal of the promoter DNA is visible at a lower contour level compared to the rest portions in the density map. We observed extra density between ScZur and ScRNAP at a lower contour level. The density is close to ScRNAP αNTD, suggesting that it likely belongs to ScRNAP αCTD. We used 3D variability analysis (3DVA), an algorithm that fits a linear subspace model of conformational change to cryo-EM data (41), to visualize the detailed molecular motions of ScRNAP<sup>PrHB</sup>−Zur−*zitB<sub>6</sub>* (Movie 1) and to cluster the particles into 10 classes (Supplementary Figure S5D). The ninth class carrying one ScZur dimer (ScRNAP<sup>PrHB</sup>−Zur<sub>d1</sub>−*zitB<sub>6</sub>*) is refined to a resolution of 3.8−7 Å at ScRNAP<sup>PrHB</sup> and 10−15 Å at ScZur (Figure 4B, Supplementary Figure S5D). Local refinement focused on the ScZur<sub>d1</sub> region generated a 11-Å-resolution map. A charge density distribution map tends to be better resolved than the corresponding EM map (30). We then used the 11-Å-resolution map to generate a charge density distribution map of ~7 Å using Laplacian (30), which allowed more confident docking of ScZur and ScRNAP αCTD components (Figure 4C) and demonstrated direct contact between ScZur and ScRNAP αCTD. Particles of another seven classes were combined and subjected to 3D classification focused on ScZur region, generating density maps of RPo complexes carrying DOD (ScRNAP<sup>PrHB</sup>−Zur<sub>d2</sub>−*zitB<sub>6</sub>*) or TOD (ScRNAP<sup>PrHB</sup>−Zur<sub>d3</sub>−*zitB<sub>6</sub>*) of ScZur (Supplementary Figure S5D). The densities for the ScRNAP αCTD domains are visible at a lower contour level in both maps. ScRNAP αCTD domains docked in both maps are distant from the ScZur dimers (Figure 5, Supplementary Figure S15), whereas the flexibility of this regions suggests the possibility of ScZur to directly contact ScRNAP αCTD.

**ScRNAP<sup>PrHB</sup>−promoter DNA interactions**

Among the three ScZur-bound RPo complexes, map quality was highest for ScRNAP<sup>PrHB</sup>−Zur<sub>d1</sub>−*zitB<sub>6</sub>* and therefore subsequent analysis focused on this structure. The final cryo-EM map was reconstructed using a total of 54 799 single particles and refined to an overall 3.45 Å resolution. The resolution at the ScZur region was ~ 10 Å and increased to 5.37 Å by local refinement (Supplementary Figure S5D). The 84-bp promoter DNA (−68 to +16), four major regions of the α<sup>PrHB</sup> subunit (α<sub>1.2</sub>, α<sub>2</sub>, α<sub>3</sub> and α<sub>4</sub>), five subunits of ScRNAP core (α<sub>α</sub>Bβ′α), three ScZur dimers, and αCTD were docked into the map (Figure 5A). The overall structure of ScRNAP closely resembles the reported actinobacteria RNAP structures of *M. tuberculosis* RPo (PDB ID: 6VVY) and *M. smegmatis* RPitc (PDB ID: 5V1S) with rmsd of 1.079 Å (2759 C<sub>α</sub> aligned) and 1.047 Å (2636 C<sub>α</sub> aligned) respectively (Supplementary Figure S16). The α<sub>2</sub> and α<sub>4</sub> of α<sup>PrHB</sup> occupy the same positions and make the same interactions with RNAP (35−37). Actinobacteria RNAPs contain a lineage-specific insertion of ~90 residues that folds into two long anti-parallel α-helices at the N-terminal of the β′ subunit (44). In the ScRNAP, the lineage-specific insertion (β′1l) roughly spans residues 141−228. A large non-conserved region (NCR) is inserted between regions 1.2 and 2.1 in housekeeping σ factors, such as *E. coli* σ<sup>70</sup>. As observed in σ<sup>70</sup> factors of *M. tuberculosis* and *M. smegmatis*, the NCR region of *S. coelicolor* α<sup>PrHB</sup> is significantly shorter compared to that of *E. coli* σ<sup>70</sup> (Supplementary Figure S16C and D) (45).

Only 20% of streptomycese promoters contain similar sequences to those recognized by *E. coli* σ<sup>70</sup> (46). The −10 element of *zitB* promoter (−12 to −7, TTGACT) lacks the critical adenine residue at position −11, while the −35 element (−36 to −31, TTGCCC) deviates from the canonical TTGACA motif (24). The RPo structures reveal numerous interactions between ScRNAP<sup>PrHB</sup> and the *zitB* promoter (Figure 5). ScRNAP<sup>PrHB</sup> recognizes the promoter DNA by interactions between the σ<sup>PrHB</sup> and the conserved −10 and −35 DNA elements (Figure 5B). The σ<sub>4</sub> binds to the
Figure 4. The overall structure of ScRNAP$_{H9268}$-Zur$_{41}$-zitB$_6$. (A) DNA scaffold with a 6-bp pre-melted bubble used in structure determination. The zur-box, −35 element, and −10 element are highlighted in red. (B) The cryo-EM density map and structure model of ScRNAP$_{H9268}$-Zur$_{41}$-zitB$_6$. The density map and cartoon representation structure are colored as indicated in the color key. NT, non-template-strand promoter DNA; T, template-strand promoter DNA. The map was generated by merging the consensus map of the full ScRNAP$_{H9268}$-Zur$_{41}$-zitB$_6$ complex and the focused map of the Zur$_{41}$-DNA subcomplex from the complex (Supplementary Figure S5) in Chimera X. (C) Two views of the charge density distribution map generated from the cryo-EM map of local 3D refinement focused on the ScZur$_{41}$ region. (D) ScZur activates transcription from zitB$_6$ promoter with a 6-bp pre-melted bubble instead of zitB$_{13}$ with a 13-bp pre-melted bubble. Figures of density maps and structures are prepared by Chimera X.

−35 element in its major groove. R483 and E484 extend into the major groove and likely make base-specific polar interactions with nucleotides of −35 element. T482 and R485 make interactions with the phosphate backbone of the non-template strand while R461, A471, L472, D473 and R487 contact the phosphate backbone of template strand. Duplex DNA between the −35 and −10 elements contributes to RNAP-promoter interaction through the phosphodiester backbone of the non-template strand. The −17/−18 ‘Z-element’ interacts with Y36 and R37 of β′ zipper, V353 and H354 of σ3.0, and R350 of σ2.4 (38). S. coelicolor promoters lacking the conserved −35 element often have a conserved GG motif (−13 and −14) (39), of which G-13(nt) contacts side chains of Q336 and R340 of H9268$_{2.4}$. The invariant W-dyad (W332/W333, corresponding to W433/W434 in E. coli or W256/W257 in T. aquaticus) maintains the ds/ss DNA junction at the upstream edge of the transcription bubble, where W332 forms a stack with the base of T$_{-12}$(nt). Bases of both T$_{-11}$(nt) (corresponding to the conserved A$_{-11}$(nt) of a canonical −10 element) and T$_{-7}$(nt) are flipped out and captured by a pocket on σ2 formed by K322, Y324 and Y329, and a pocket made of L227 of σ1.2, and Asn282, R284, Leu285, Phe326 and Ser327 of σ2 respectively (Figure 5B). The ‘discriminator’ (DSR) region of non-template strand (−6 to −4) interacts with residues of σ1.2. The ‘core recognition element’ (CRE) (−3 to +2) interacts with residues of β-subunit, of which R169, I356, R362, L449 and V458 form a pocket to accommodate G$_{+2}$(nt). The template strand of −10 element is bent by ∼90° at position −11, wraps around the σ3 globular domain, and passes through a cleft between σ2 and σ3 to place the downstream template strand ssDNA into the main primary channel. However, the
Figure 5. Interactions between $\text{ScRNAP}^{\text{HdB}}$ and promoter DNA. (A) Two views of the density map and structure of $\text{ScRNAP}^{\text{HdB}}$–HrdB–Zurd3–zitB. The density map shown as transparent surface was generated by merging the consensus map of the full $\text{ScRNAP}^{\text{HdB}}$–Zurd3–zitB complex and the focused map of the Zurd3–DNA subcomplex from the complex (Supplementary Figure S5) in Chimera X. The map and cartoon representation structure are colored as indicated in the color key. (B) Interactions between promoter DNA and $\text{ScRNAP}^{\text{HdB}}$. The density map of DNA is shown as transparent surface. The residues involved in the interactions are shown as sticks. The figures were prepared by Chimera X.
density of the template ssDNA in the transcription bubble is not well-defined.

Map quality of the $Sc$RNAP$^{\sigma^{\text{HdB}}}$–Zur$_{d1}$–zitB$_{13}$ was better among the two RPitc complexes (Supplementary Figure S3D). The $Sc$RNAP in the $Sc$RNAP$_{d1}$–Zur$_{d2}$–zitB$_{13}$ is similar to that in the $Sc$RNAP$_{d1}$–Zur$_{d3}$–zitB$_{6}$, whereas the density of the template strand ssDNA in the transcription bubble is better resolved (Supplementary Figure S17). The 5-nt RNA base-pairing with nucleotides from $-4$ to $+1$ of template strand DNA is in a post-translocation state with the $3'$ end reaching the $\sigma3.2$ loop. The fork-loop 2 of $\beta$ subunit maintains the ds/ss DNA junction at the downstream edge of the transcription bubble by separating the dsDNA ($+3$ to $+16$) inside the DNA binding clamp between the $\beta'$ clamp, $\beta$ jaw and $\beta$ lobe.

**Zur activates transcription by contacting $\alpha$ CTD**

$Sc$RNAP$_{d1}$–Zur$_{d1}$–zitB$_{6}$ complex is trapped in an active conformation in which $Sc$Zur makes interactions with $Sc$RNAP $\alpha$ CTD, suggesting it is a $Sc$Zur-dependent transcription activation complex (TAC) (Figure 4). The $\alpha$4 helix of C-terminal dimerization domain of one $Sc$Zur monomer packs against the $\alpha1$ helix and the loop region between $\alpha3$ and $\alpha4$ helices of $Sc$RNAP $\alpha$ CTD, burying an interfacial area of $\sim200$ $\text{Å}^2$ (Figure 6). The involvement of $Sc$RNAP $\alpha$ CTD in $Sc$Zur-dependent transcription activation is supported by Mango-based transcription assay with $Sc$RNAP$^{\sigma^{\text{HdB}}}$ assemble from a truncated $\alpha$ subunit without CTD region ($\alpha_{\text{ACTD}}$) that spans residues 1–227. As shown in Figure 6D and E, the CTD is dispensable for basal transcription of zitB but is required for Zur-dependent activation. At the interface, R259, R266 and R287 of $\alpha$ CTD cluster to form a positively charged patch (Figure 6B) that is complementary to the negatively charged patch formed by E109 and E114 of the Zur (Figure 6C). Mutational studies and in vitro transcription assays confirmed the importance of these residues for $Sc$Zur-dependent transcription activation (Figure 6D and E). Intriguingly, $Sc$Zur repressed transcription of RNAP assembled from $\alpha_{\text{ACTD}}$ and $\alpha$ with a single mutant in transcription assays of TP$_{-68}$ (Figure 6D). Zur E109A mutant also repressed transcription of TP$_{-68}$. The reason of these negative effects is unclear and requires further investigation. However, negative effects are not observed in transcription assays of TP$_{-100}$ (Figure 6E). In the prototypical class II TAP-TAC (TAP, homolog of $E$. coli CAP in $T$. thermophilus, PDB ID: 512D), the $\alpha$ CTD also uses the $\alpha1$ helix and the loop region between $\alpha3$ and $\alpha4$ helices to interact with the activating region 4 (AR4) of one TAP monomer, burying a larger surface area of $\sim300$ $\text{Å}^2$ (11) (Supplementary Figure S18B). However, the distribution of surface charge in this region of $T$. thermophilus $\alpha$ CTD is mostly negative and different from the corresponding region that is positively charged in $S$. coelicolor $\alpha$ CTD.

**DISCUSSION**

Although $S$. coelicolor is a model organism for the study of antibiotic-producing and morphological development of actinobacteria, no structural information is known about the promoter recognition mechanisms employed by its RNAP. Here, we provide the first structures of $Sc$RNAP-$\sigma^{\text{HdB}}$ with a promoter. These structures show overall similarity to previously reported structures of RNAPs with $\sigma^B$ factors. $\sigma^{\text{HdB}}$ occupies the same positions, makes the same interactions with RNAP, and uses the same strategy to recognize, bind and unwind promoter DNA. The structures demonstrate protein-protein and protein–DNA interactions involved in $\sigma^{\text{HdB}}$-dependent transcription initiation in $S$. coelicolor. The actinobacteria-specific insertion $\beta1$ of $S$. coelicolor RNAP folds into two long anti-parallel $\alpha$-helices as observed in RNAP structures $M$. tuberculosis and $M$. smegmatis (42,43). The conserved structural feature of the actinobacteria $\beta1$ indicates a fundamental role requiring further investigations (Supplementary Figure S16). Housekeeping $\sigma$ factors such as $E$. coli $\sigma^{34}$ and its orthologs possess an NCR of variable length between regions 1.2 and 2.1. The NCR of housekeeping $\sigma$ factors is a target for transcription regulation (49,50). $S$. coelicolor $\sigma^{\text{HdB}}$ contains a significantly shorter NCR region compared to that of $E$. coli $\sigma^{34}$, suggesting a different regulatory strategy is adopted by $\sigma^{\text{HdB}}$.

Numerous positive regulators have been revealed in streptomycetes, whereas how they activate gene transcription remains obscure. Here, we show that $Sc$Zur activates zitB transcription by binding to the Zur-box positioned 8 nt upstream from the $-35$ element and contacting $Sc$RNAP $\alpha$ CTD using the $\alpha4$ helix involved in dimerization (Figure 6). The importance of interface residues is supported by Mango-based transcription assays with corresponding mutants. Sequence alignment of $Sc$Zur with the counterparts from $E$. coli, $M$. tuberculosis and $X$. campestris reveals that the E109 and E114 residues are not conserved (Supplementary Figure S9), suggesting the protein interaction observed here may be strain-specific (19,20,22). RNAP $\alpha$ CTD is a central target of transcription regulation (10,11). It is connected to $\alpha$NTD by a long, flexible linker and therefore can occupy different positions relative to the remainder of RNAP. In $E$. coli, the RNAP $\alpha$ CTD recognizes AT-rich UP elements located upstream of $-35$ elements (from $-60$ to $-40$) in some promoters to enhance transcription (51). Various transcription factors activate RNAP by interacting with $\alpha$ CTD. Structures of prototypical class I and class II TAC have been reported. Observed differences in the interfaces between $\alpha$ CTD and transcription activator were revealed by comparing our structure and the previously reported structures (Supplementary Figure S18). In the prototypical class I CAP-TAC (PDB ID: 6B6H), the $\alpha$ CTD binds both the CAP activator and the minor groove in the $-40$ to $-45$ region (10) (Supplementary Figure S18A). The 287 determinant of $\alpha3$ helix and the C-terminal loop make contact with the activation region 1 (AR1) of one CAP monomer, burying a small interface area of $\sim80$ $\text{Å}^2$. The $\alpha$ CTD also interacts with the 596 determinant of $\sigma4$ binding at the major groove in the $-35$ element mainly with its 261 determinant. In the prototypical class II TAP-TAC (TAP, homolog of CAP PDB ID: 512D), the $\alpha$ CTD uses same structural elements as observed in our structure to interact with one TAP monomer, whereas the electrostatic nature is different from that of $S$. coelicolor $\alpha$ CTD (Sup-
Figure 6. Interactions between ScZur and ScRNAP αCTD. (A) ScZur interacts with the α1 helix and the loop region between α3 and α4 helices of ScRNAP αCTD by its α4 helix of one monomer. (B) The positively charged interface of ScRNAP αCTD patch composed of R259, R266 and R287. (C) The negatively charged interface of ScZur composed of E109 and E114. (D) Transcription assays of TP−68. ScZur repressed transcription of RNAP assembled from αACTD and α with single mutant. Zur E109A mutant also repressed transcription. The reason of these negative effects requires further investigation. (E) Transcription assays of TP−100. ScZur shows no activation on ScRNAPHrdB assemble from a truncated α subunit without CTD (αACTD). Mutating interfacial residues impaired transcription activation. Figures of structures are prepared by Chimera X. Transcription data are presented as mean ± SEM from three independent assays.

We tried to assemble the ScRNAPoHrdB−Zur−DNA from both zitB6 and zitB13 promoters. The interaction between αCTD and Zur is only observed in the ScRNAPoHrdB−Zur1−zitB6 structure. Mango-based in vitro assays reveal that ScZur can activate transcription from zitB6 promoter instead of zurB6, indicating that ScZur affects steps prior to the formation of the 13-bp transcription bubble (Figure 4D). In vivo assays demonstrate that zitB gene is gradually activated by ScZur (24). The zitB regulatory region up to −60 only allows gene activation of ~1.3 folds while the upstream region up to −228 nt enables gene activation of ~6 folds. We identified two additional palindromes (~67 to ~93 nt, and −117 to −133) in the zitB promoter (Supplementary Figure S1). According to this information, three DNA templates were designed and used for the in vitro run-off transcription reactions. Consistent with the in vivo results, transcription of the template containing the zitB regulatory region up to −68 was enhanced ~1.6-fold by the Zur protein while the enhancement for the other two templates up to −100 and −140 was more than 4-fold (Figure 1B-D), indicating that full activation of zitB depends on Zur binding at palindrome II and/or I. In the case of Fur-activated genes, the Fur-box sequence is located −100 to −200 bp upstream of the TSS (53). An activator binding to a DNA region located this far distance from TSS has been proposed to activate transcription by a looping mechanism (24). An upward DNA bend toward RNAP is observed in the ScZur region in the ScRNAPoHrdB−Zur1−zitB6 structure (Supplementary Figure S20). However, it is unclear whether the DNA bending is prior to or upon binding of ScZur. We provide the first structural insight into DNA-binding characteristics of ScZur (Figure 2). Structural comparisons of apo and holo ScZur proteins reveal binding of Zn atoms in the regulatory sites drives a conformational change activating Zur proteins (22). We observed that DNA triggers
a conformational change of the DNA-binding domains. The distance between the recognition helices increased from 27 to 34 Å so that the helices are better aligned with consecutive major groove regions (Figure 3A), enabling the formation of protein-nucleic acid interactions. DNA-induced movements of the DNA-binding domains are observed in *Magnetospirillum gryphiswaldense* Fur–DNA complex structures, where the distance between the N-terminals of recognition helices decreased from 39 Å to 32 Å (measured from the Ca atoms of 152 residues) (54). In contrast, the DNA-induced conformational change is not observed in the structures of diphtheria toxin repressor (DtxR) activated by transition metal ions (55, 56). The TOD structure of ScZur-promoter complex explains the extended footprint in previous assays (24). The structure and biochemical results demonstrate that three ScZur dimers cooperatively and sequentially binding to the target DNA region with the first and third ScZur dimer docking on one side and the second ScZur dimer in the middle docking on the opposite side, surrounding the palindromic 7–1–7 Zur-box.

In summary, our structural and biochemical results demonstrate that ScZur proteins cooperatively bind to target DNA as a trimmer of dimers and activates transcriptions by contacting ScRNAP αCTD. These findings provide a mechanistic framework for understanding Zur-dependent transcription activation and may also shed light on transcription activation of other Fur-family regulators.

### DATA AVAILABILITY

Atomic coordinates of 7 structures have been deposited in PDB with accession numbers 7V09 (Zur-DNA DOD), 7V00 (Zur-DNA TOD), 7VPD (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}), 7X76 (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d2}-zitB*\textsubscript{1}), 7X75 (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}), 7VPZ (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}) and 7X74 (ScRNAP*\textsubscript{Hdb}-Zur*\textsubscript{d2}-zitB*\textsubscript{1}). 12 cryo-EM density maps have been deposited in the Electron Microscopy Data Bank with accession number EMD-32049 (Zur-DNA DOD), EMD-32048 (Zur-DNA TOD), EMDB-32063 (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}), EMDB-32033 (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d2}-zitB*\textsubscript{1}), EMDB-33032 (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d2}-zitB*\textsubscript{1}), EMDB-33031 (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}), and EMDB-33031 (ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}), EMD-33034 (Zur\textsubscript{d1}-CTD-DNA from ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}), EMD-33035 (Zur\textsubscript{d2}-DNA from ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d2}-zitB*\textsubscript{1}), EMD-33036 (Zur\textsubscript{d3}-DNA from ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d3}-zitB*\textsubscript{1}), EMD-33037 (Zur\textsubscript{d1}-DNA from ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d1}-zitB*\textsubscript{1}) and EMD-33038 (Zur\textsubscript{d1}-DNA from ScRNAP*\textsubscript{Hdb}*-Zur*\textsubscript{d2}-zitB*\textsubscript{1}).

### SUPPLEMENTARY DATA

**Supplementary Data** are available at NAR Online.

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