Structural basis of copper-efflux-regulator-dependent transcription activation

Highlights
- Cryo-EM structures of intact CueR-TAC complex without and with RNA synthesis
- CueR dimer activates transcription through bending promoter DNA
- The structures reveal the interactions between σNCR and CueR
- The σNCR/CueR interactions play an auxiliary role in CueR-dependent transcription

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Structural basis of copper-efflux-regulator-dependent transcription activation

Wei Shi,1,9 Baoyue Zhang,2,3,9 Yanan Jiang,1,4,9 Chang Liu,5,9 Wei Zhou,2,3 Ming Chen,2,3 Yang Yang,6,7,8,* Yangbo Hu,2,* and Bin Liu1,10,*

SUMMARY
The copper efflux regulator (CueR), a representative member of mercury resistance regulator (MerR) family metalloregulators, controls expression of copper homeostasis-regulating genes in bacteria. The mechanism of transcription activation by CueR and other MerR family regulators is bending the spacer domain of promoter DNA. Here, we report the cryo-EM structures of the intact CueR-dependent transcription activation complexes. The structures show that CueR dimer bends the 19-bp promoter spacer to realign the −35 and −10 elements for recognition by σ70 RNA polymerase holoenzyme and reveal a previously unreported interaction between the DNA-binding domain (DBD) from one CueR subunit and the σ70 nonconserved region (σNCR). Functional studies have shown that the CueR-σNCR interaction plays an auxiliary role in CueR-dependent transcription, assisting the activation mechanism of bending promoter DNA by CueR dimer. Because DBDs are highly conserved in sequence and structure, this transcription-activating mechanism could be generally used by MerR family regulators.

INTRODUCTION
Copper is an essential and precisely regulated trace element for biological function in all living organisms. However, it can be highly toxic at high concentrations, and thus, the maintenance of copper homeostasis is vital to the survival of cells (Baksh and Zamble, 2019; Finney and O’Halloran, 2003; Lutsenko, 2010; Robinson and Winge, 2010; Waldron et al., 2009). As a member of the mercury resistance regulator (MerR) family regulators, which respond to environmental stimuli, such as metal-ion overload, oxidative stress, or xenobiotics (Brown et al., 2003), the copper efflux regulator (CueR) is sensitive to +1 transition-metal ions including Cu⁺, Ag⁺, and Au⁺ ions (Brown et al., 2003; Changela et al., 2003; Stoyanov and Brown, 2003). Other metal-responsive metalloregulators in the family include MerR, HmrR, PmtR, ZmrR, CadR, PbrR, SctR, and CoaR, all of which contribute to homeostasis of heavy metal ions in bacteria via regulating the transcription of heavy-metal-resistance genes (Brown et al., 2003). CueR controls the transcription of two copper-homeostasis genes, copA encoding a Cu⁺-transporting P-type ATPase pump and cueO encoding a copper oxidase for detoxification (Outten et al., 2000; Petersen and Moller, 2000; Stoyanov et al., 2001; Yamamoto and Ishihama, 2005). Because CueR shows an extremely high affinity ($K_{d} = 2 \times 10^{-21} \text{M}$) to Cu⁺, it is often found in the activator form (Changela et al., 2003; Philips et al., 2015). Like many other MerR family regulators (Bachas et al., 2011; Heldwein and Brennan, 2001; Newberry et al., 2008; Watanabe et al., 2008), CueR acts on the DNA promoter with a 19-bp spacer, which exceeds the 17-bp optimal length for recognition by σ70 factor (Brown et al., 2003; Philips et al., 2015; Typas and Hengge, 2006).

Previous structural studies of CueR have explained its metal-ion selectivity and revealed the general activation mode by modulating local DNA conformations within the promoters (Changela et al., 2003; Joshi et al., 2012; Philips et al., 2015; Sameach et al., 2017). However, the complete mechanism of transcription activation, such as whether and how the regulators directly interact with RNA polymerase (RNAP), necessitates the structure of the transcription activation complex (TAC) of RNA polymerase with MerR family regulators.

In this study, we reported the structures of the intact CueR-dependent TAC (hereinafter referred to as CueR-TAC). In addition to elucidating the role of CueR in modulating promoter DNA in TAC, the structures also reveal a previously unreported interaction between CueR and σ70, the auxiliary role of which is further corroborated by in vitro and in vivo functional assays. This transcription-activating mechanism could be generally used by other members in the MerR family.
RESULTS AND DISCUSSION

Overall structures of CueR-TACs

The cryo-EM structures of the intact *Escherichia coli* CueR-TAC comprising a CueR dimer, σ70-RNAP holoenzyme, and a complete CueR-specific promoter (P*copA*) without or with a de novo synthesized RNA transcript were determined at overall resolutions of 3.9 Å and 4.1 Å, respectively (Figures S1–S3, Table S1). The CueR-TACs were reconstituted on a synthetic DNA scaffold that corresponds to positions −40 to +14 of P*copA* and contains the −35 element, −10 element, and the inverted repeat sequence (IRS) for CueR binding (Figure 1A). The cryo-EM maps show well-defined density for all major components of the complexes and support reliable model building (Figures 1B, S4, and S5A). The two CueR-TAC structures are readily superimposable with an RMSD of 0.52 Å over their Cα atoms (Figure S5B), indicating that transitioning from open complex to initial transcribing complex does not require further conformational changes in CueR or promoter spacer DNA. The following structural analyses will focus on the CueR-TAC containing a de novo synthesized RNA transcript.

Promoter DNA modulation by CueR in TAC

In the complex, the CueR dimer sits on the top of the promoter spacer region with two N-terminal DNA-binding domains (DBDs) “gripping” the DNA backbone of the IRS like a clamp (Figure 2A). Although the IRS recognized by CueR has 3 bp overlap with the −35 element, CueR and RNAP holoenzyme bind to the opposite faces of spacer DNA to avoid possible steric clashes or interference with the −10 and −35 elements recognition (Figures 1B and 2A). The conformations of CueR dimer and its bound DNA in CueR-TAC are highly similar to those in the activator CueR/DNA complex (Figure 2A) (Philips et al., 2015), except that the bending of promoter spacer DNA in CueR-TAC is slightly reduced by ~3° compared with that in the activator CueR/DNA complex (Figure 2B), possibly owing to the insertion of α helix-turn-helix (HTH) motif into the major groove from the opposite side. Using the canonical promoter with a 17-bp
the trajectory of the helical axis is altered by ~25° for the promoter spacer DNA in CueR-TAC (Figure 2C). In comparison with the repressor CueR/DNA complex structure in which the Cu⁺-binding sites of CueR were deleted (Philips et al., 2015), the spacer DNA in CueR-TAC undergoes a significant directional change of ~37° (Figure 2D).

Like many other MerR family metalloregulators, CueR acts on the promoter with a 19-bp spacer, which is 2 bp longer than the optimal spacer length of 17 bp in σ70-TIC. The additional 2 bp extends the distance between the –10 and –35 elements and hampers the proper recognition of –35 or –10 element, once one of the elements is fixed by σ70-holoenzyme. The CueR dimer significantly bends DNA promoter, reducing the distance between the –35 and –10 elements to 55.4 Å, which is close to the 55.0 Å distance in the canonical promoter of σ70-TIC (Figure S6). In addition, as observed in the activator CueR-DNA complex structure (Philips et al., 2015), this bending also under-twists the promoter, compensating for the phase angle change between the –35 and –10 elements caused by the extra 2 bp in the promoter spacer region. As a result, the –35 and –10 elements are properly recognized by σ70 in CueR-TAC as they are in σ70-TIC (Figures S6B and S6C). In vitro promoter binding assays showed that P_copA was poorly bound by σ70-RNAP holoenzyme in the absence of CueR protein and addition of CueR significantly increased the interaction between P_copA and σ70-RNAP holoenzyme (Figures S6D and S6E).

**CueR-σNCR interaction plays an auxiliary role on transcription activation**

In addition to CueR-DNA interaction, CueR also makes protein-protein interaction with σ70 (Figure 3A). The DBDs of both CueR subunits interact with promoter spacer DNA by inserting the characteristic HTH motifs into DNA major grooves (Figures 3A and 3B), whereas only the DBD of CueR-I subunit makes contact with the σ70 nonconserved region (σNCR). The loop wing of CueR-I DBD interacts with the A153-S159 loop (loop L1) between σNCR helices H2 and H3, likely through the hydrogen bond interactions between S159 in σNCR and S32/T38 in CueR-I and between σNCR R157 and main chain oxygen atom of R31 in CueR-I, as well as a possible salt bridge interaction between σNCR K264 and CueR-I E33 (Figure 3C). Consistent
with the aforementioned structural observations, mutations of S32, E33, and T38 of CueR to alanines (referred to as CueR-SETm) undermined the CueR’s abilities in facilitating the binding of RNAP holoenzyme to $P_{copA}$ and activating transcription on the promoter (Figures 3D, 3E, and S7A). Similarly, alanine substitutions of R157, S159, and K264 of $\sigma^{70}$ (referred to as $\sigma^{70}$-RSKm) also decreased the transcription activation on $P_{copA}$ by CueR (Figures 3D and S7A) and reduced the CueR-dependent enhancement of $P_{copA}$ binding by RNAP holoenzyme, whereas the basal promoter binding activity of the $\sigma^{70}$-RSKm holoenzyme without CueR was not weakened (Figures 3E and S7B). Sequence alignments show that the residues involved in CueR-$\sigma^{NCR}$ interactions are highly conserved (Figure S8). Therefore, the CueR-$\sigma^{NCR}$ interaction plays an auxiliary role in CueR-dependent transcription, assisting the activation mechanism of bending promoter DNA by CueR dimer.

It should be noted that during the preparation of our manuscript, Fang et al also reported the cryo-EM structures of *E. coli* CueR transcription activation complex (Fang et al., 2020). All of their and our structures show that CueR activates transcription through bending the promoter DNA, supporting the DNA distortion
paradigm of allosteric transcriptional control. They found that mutation of residue E33 alone does not alter CueR activity, while simultaneous mutations of S32, E33, and T38 of CueR to alanines in our study slightly impaired CueR activity, indicating an auxiliary role of CueR-sNCR interaction in CueR-dependent transcription.

The overall structures and promoter recognition patterns are highly similar between CueR-TAC and σ70-TIC (PDB 4YLN) (Zuo and Steitz, 2015) (Figures 4A–4D and S6A–C), but the sNCR conformations are noticeably different in the two structures, likely owing to the CueR-sNCR interaction (Figures 4A and 4B). The loop wing of CueR-I DBD inserts into the gap between sNCR loop L1 and promoter DNA and pushes the loop L1 and two neighboring helices (H2 and H3) 7.6 Å away from their original positions (Figures 4B and S9A). Other sNCR helices also exhibit distance shifts to various extents (Figures 4B and S9A).

The residue R157 of sNCR has been suggested to interact with promoter template strand DNA at −16/−17 position. Disruption of the interaction by mutating the arginine to either alanine or glutamate impeded promoter DNA opening and greatly decreased transcription activity (Narayanan et al., 2018). In our CueR-TAC structure, R157 has established an interaction with CueR-I DBD instead (Figure 3C) and lost the proposed interaction with DNA. The DNA region that was contacted by sNCR in RNAP-σ70 open complex (PDB 6CA0) is now interacted by CueR-I DBD (Figures S9B and S9C). Therefore, in CueR-TAC, R157 adopts a role of stabilizing the CueR binding, while its original function in interacting with spacer DNA is substituted by the loop wing of CueR-I DBD, which is sandwiched between sNCR and spacer DNA (Figures 3C, S9B, and S9C).
The positional effects of IRS on CueR-\(\alpha\)NCR interaction

To further understand the role of CueR-\(\alpha\)NCR interaction in CueR-activated transcription, we designed two promoter mutants by moving the CueR core binding sequence (CCTTCC-\(\gamma\)-GGAAGG) (Yamamoto and Ishihama, 2005) 1 bp upstream or downstream in the \(P_{\text{copA}}\) promoter (referred to as \(P_{\text{copA}}^{\pm\text{1bp}}\) and \(P_{\text{copA}}^{\pm\text{1bp}}\), respectively) in which the length of the spacer DNA was kept to be 19 bp as the wild-type \(P_{\text{copA}}\) (Figure S10A). For the two promoter mutants, the \(P_{\text{copA}}^{\pm\text{-1bp}}\) is predicted to weaken the CueR-\(\alpha\)NCR interaction in CueR-TAC complex owing to a longer distance between position of CueR-binding sequence and the \(-10\) element (Figure 4E). By contrast, the \(P_{\text{copA}}^{\pm\text{+1bp}}\) is predicted to cause a significant steric clash between CueR and \(\alpha\)NCR owing to the closer position of CueR-binding sequence to the \(-10\) element (Figure 4E). Both promoter mutants showed unaffected binding to CueR in the absence of RNAP (Figure S10B). However, the \(P_{\text{copA}}^{\pm\text{-1bp}}\) caused a moderate decrease in CueR-dependent promoter binding by RNAP and activation of transcription, and both the CueR-dependent promoter binding and transcription activation were almost abolished on \(P_{\text{copA}}^{\pm\text{+1bp}}\) (Figures 4F, S10C, and S10D). These data further indicate that CueR-\(\alpha\)NCR interaction could stabilize the CueR-RNAP-promoter complex in activating transcription. In addition, the position of IRS on the \(P_{\text{copA}}\) is likely evolutionarily optimized for proper CueR-\(\alpha\)NCR interaction and maximum activation of bacterial transcription by CueR.

Concluding remarks

Taken together, our data show that CueR bends the promoter DNA to facilitate the \(-10\) and \(-35\) elements recognition by RNAP and its interaction with \(\alpha\)NCR stabilizes the CueR-RNAP-promoter complex. Our findings support that RNAP and CueR work synergistically in regulating transcription and increase each other’s affinity to \(P_{\text{copA}}\) (Martell et al., 2015). This study advances the understanding of the transcription activation process regulated by bacterial metallosensor proteins and suggests that other MerR family factors likely also adopt a similar mode of action to bend promoter DNA and make contacts with \(\alpha\)NCR domain during transcription activation, given that their N-terminal DNA-binding domains are highly conserved in the family (Brown et al., 2003; Philips et al., 2015).

Limitations of the study

In this study, we have captured the structures of CueR-TAC without RNA or with 3-nt RNA, showing the key activation states. However, the complete transcription activation process by CueR requires the elucidation of more states with RNA transcripts of different lengths.

STAR Methods

Detailed methods are provided in the online version of this paper and include the following:

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Supplemental Information

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102449.
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AUTHOR CONTRIBUTIONS

B.L. and Y.H. initiated and designed the experiments. W.S. and Y.J. performed protein sample preparations and assembly of the complexes used in the structure determination. W.S., Y.J. and B.L. performed cryo-EM grid preparation, screening, and optimization. B.L. conducted high throughput data collection on Titan Krios, Y.Y., C.L., W.S. and B.L. performed image processing, map reconstruction, and model building and refinement. B.Z., W.Z. and M.C. constructed mutations and purified proteins. B.Z., W.Z., M.C. and Y.H. performed in vitro biochemical and in vivo promoter activity tests. W.S., B.Z., Y.J., C.L., Y.Y., Y.H. and B.L. analyzed data. W.S., C.L., Y.Y., Y.H. and B.L. wrote the manuscript with the contributions from all the authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Bacterial and virus strains** | | |
| Ec-parent | Hu et al., 2016 | N/A |
| Ec-CueR-SETm | This study | N/A |
| Ec-a235-RSKm | This study | N/A |
| *E. coli* BL21(DE3) | Novagen | Cat#69450-3 |
| *E. coli* DH5α | Shenzhen KT Life | Cat#KTSM101L |
| **Chemicals, peptides, and recombinant proteins** | | |
| rNTP | Promega | P1221 |
| CuSO4 | SinoPharm | 10008218 |
| AgNO3 | Aladdin | S116264 |
| \(\alpha-32P\)GTP | PerkinElmer | BLU006H500UC |
| **Deposited data** | | |
| Combined cryo-EM map of CueR-TAC complex with clearer nCR density | This study | EMD-22289 |
| The atomic coordinates for the model of CueR-TAC complex without RNA transcript | This study | 6XH7 |
| The atomic coordinates for the model of CueR-TAC complex with RNA transcript | This study | 6XH8 |
| Cryo-EM map of CueR-TAC complex without RNA transcript | This study | EMD-22184 |
| Cryo-EM map of CueR-TAC complex with RNA transcript | This study | EMD-22185 |
| **Oligonucleotides** | | |
| | See Table S2 for oligonucleotides and sequences. |
| **Recombinant DNA** | | |
| pET21a-a70-RSKm | This study | N/A |
| pET21a-CueR | This study | N/A |
| pET21a-CueR-SETm | This study | N/A |
| pCas | Jiang et al., 2015 | N/A |
| pTargetF | Jiang et al., 2015 | N/A |
| pTargetF-EcoroD | This study | N/A |
| pTargetF-EccueR | This study | N/A |
| pZT100 | Li et al., 2014 | N/A |
| pZT-PcopA | This study | N/A |
| pZT-PcopA\_1 bp | This study | N/A |
| pZT-PcopA\_1 bp | This study | N/A |
| pVS10-RNAP | Belogurov et al., 2007 | N/A |
| pET21a-a70 | This study | N/A |
| **Software and algorithms** | | |
| ResMap | Kucukelbir et al., 2014 | N/A |
| UCSF Chimera 1.14 | Pettersen et al., 2004 | https://www.cgl.ucsf.edu/chimera/ |

(Continued on next page)
RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and materials should be directed to and will be fulfilled by the Lead Contact, Bin Liu (liu00794@umn.edu).

Material availability
Materials are available upon reasonable request.

Data and code availability
The cryo-EM density maps of our CueR-TAC complex have been deposited in the Electron Microscopy Data Bank under the accession number EMD-22184 (without RNA transcript), EMD-22185 (with RNA transcript) and EMD-22289 (combined map with clearer NCR density). The corresponding atomic coordinates for the atomic model have been deposited in the Protein Data Bank under the accession number 6XH7 (without RNA transcript) and 6XH8 (with RNA transcript).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

For clone construction, we used the Escherichia coli strain DH5α; for recombinant protein expression, we used the Escherichia coli strain BL21(DE3); for in vivo promoter activity test, we used Escherichia coli strains Ec-parent, Ec-CueR-SETm and Ec-s70-RSKm. Bacteria were grown at 37°C as described in the Method Details section.

METHOD DETAILS

Purification of RNA polymerase core enzyme and preparation of σ70-RNAP holoenzyme
To express σ70 protein, the coding fragment was cloned into pET21a plasmid between Nhe I and Hind III sites to produce pET21a-σ70. Mutations in σ70 were introduced by oligos on pET21a-σ70 plasmid following the Quickchange site-directed mutagenesis protocol (Stratagene). All oligonucleotides for constructing clones are listed in Table S2. The procedures for expression and purification of E. coli RNAP core enzyme and σ70 have followed the reported protocol (Belogurov et al., 2007; Liu et al., 2017; Zuo and Steitz, 2015). E. coli core RNAP was expressed in E. coli BL21(DE3) and purified using HisTrap HP affinity chromatography column (GE Healthcare), followed by HiTrap Heparin HP column (GE Healthcare), HiTrap Q HP anion exchange chromatography column (GE Healthcare), and HiLoad 16/600 Superdex 200 pg size-exclusion chromatography column. Wild-type and mutant σ70 were expressed in E. coli BL21(DE3) and purified using HisTrap HP affinity chromatography column (GE Healthcare), followed by HiTrap Heparin HP column (GE Healthcare) and HiLoad 16/600 Superdex 200 pg size-exclusion chromatography column. The σ70-RNAP holoenzyme was assembled by mixing the purified core enzyme with purified σ70 protein (1:3 molar ratio) at room temperature for 15 minutes followed by size exclusion chromatography to remove the extra σ70 protein (Zuo and Steitz, 2015).

Expression and purification of CueR
For construction of the plasmid pET21a-CueR, the E. coli CueR gene was PCR-amplified from E. coli genomic DNA and ligated into pET21a vector with C-terminal 6×His-tag using Nde I and Xho I restriction.
sites. Mutations in the plasmid were introduced by oligos following the Quickchange-site-directed mutagenesis protocol (Stratagene).

All constructs were transformed into chemically competent E. coli BL21(DE3) cells. The cells were grown in LB medium with 100 μg/mL ampicillin at 37 °C to the OD600 value of 0.6, and protein expression was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) at 20 °C for 16 h. The harvested cells were suspended in lysis buffer containing 50 mM Tris-HCl, pH 8.0, 300 mM NaCl, 10 mM imidazole, 5% (v/v) glycerol and lysed via sonication. The lysate was then centrifuged at 80,000 g for 1 h. The recombinant CueR protein was purified through a 5-mL HisTrap column (GE Healthcare) and 5-mL HiTrap Heparin column (GE Healthcare) and further loaded onto a gel filtration column, 120-mL HiLoad 16/600 Superdex 200 in a buffer containing 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5 mM DTT. The final sample was aliquoted, flash-frozen in liquid nitrogen, and stored at −80 °C until use.

Assembly and purification of E. coli CueR-TAC complex

The synthesized promoter DNA used in the assembly includes the region from −10 element and −35 element of the Pcedure (Figure 1A). The promoter DNA was prepared by annealing non-template (NT) strand DNA to an equal molar amount of template (T) strand DNA (Table S2). The CueR-TAC complexes were assembled by incubating αRNAP-holoenzyme, the DNA promoter and CueR protein (1:3:6 molar ratio) in a buffer containing 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM MgCl2 and 5 mM DTT at 37 °C for 15 min without or with GTP and ATP (300 μM each). CueR shows high affinity to +1 transition-metal ions, and a majority of the purified CueR protein should already contain bound metal ion, and therefore, extra metal ion is not added in the reaction. CueR, RNAP, and DNA could form a stable CueR-TAC during the complex assembly for cryo-EM study. The reaction mixture was then purified through Superose 6 Increase 10/300 column (GE Healthcare) to remove the extra CueR protein and nucleic acids.

Cryo-EM sample preparation and data acquisition

A drop of 3.5 μL of the purified CueR-TAC at about 1 μM was applied to Quantifoil R2/2 200 mesh Cu grids (EM Sciences) glow-discharged at 15 mA for 60 s. The grid was then blotted for 3 s at 4 °C and 100% humidity and vitrified by plunging into liquid ethane using a Vitrobot Mark IV (FEI).

Cryo-EM data were acquired using a 300-kV Titan Krios microscope (FEI) equipped with a Falcon III direct electron detector. Automated data acquisition was carried out using EPU software (FEI) in counting mode with a pixel size of 0.89 Å and a defocus range from −1.0 to −2.4 μm. Each micrograph contains 30 dose-framed fractions and was recorded with a dose rate of 0.8 e/μm²/sec (1 e/Å²/sec). Each fraction was exposed for 1 s, resulting in a total exposure time of 30 s and the total dose of 30 e/Å².

Image processing

Cryo-EM data were processed using cryoSPARC v2.15 (Punjani et al., 2017), and the procedure is outlined in Figures S1 and S2. A total of 4,611 (CueR-TAC without RNA) or 4,498 (CueR-TAC with RNA) movies were collected. Beam-induced motion and mechanical drift were corrected with dose-weighting using the Patch motion correction (Rubinstein and Brubaker, 2015). The contrast transfer functions (CTFs) of the summed micrographs were determined using Patch CTF estimation (Rohou and Grigorieff, 2015). Particles were then automatically picked using Blob picker with the parameters: minimum particle diameter (110 Å) and maximum particle diameter (210 Å). In total, 1,421,781 and 1,299,989 particles were picked with a pixel size of 0.89 Å and a defocus range from −1.0 to −2.4 μm. Each micrograph contains 30 dose-framed fractions and was recorded with a dose rate of 0.8 e/μm²/sec (1 e/Å²/sec). Each fraction was exposed for 1 s, resulting in a total exposure time of 30 s and the total dose of 30 e/Å².

Figures S1 and S2. A total of 4,611 (CueR-TAC without RNA) or 4,498 (CueR-TAC with RNA) movies were collected. Beam-induced motion and mechanical drift were corrected with dose-weighting using the Patch motion correction (Rubinstein and Brubaker, 2015). The contrast transfer functions (CTFs) of the summed micrographs were determined using Patch CTF estimation (Rohou and Grigorieff, 2015). Particles were then automatically picked using Blob picker with the parameters: minimum particle diameter (110 Å) and maximum particle diameter (210 Å). In total, 1,421,781 and 1,299,989 particles were picked with a pixel size of 0.89 Å and a defocus range from −1.0 to −2.4 μm. Each micrograph contains 30 dose-framed fractions and was recorded with a dose rate of 0.8 e/μm²/sec (1 e/Å²/sec). Each fraction was exposed for 1 s, resulting in a total exposure time of 30 s and the total dose of 30 e/Å².

Reconstruction of four maps. The initial models were low pass filtered to 20 Å and set as the starting references for heterogeneous refinement (3-dimensional [3D] classification) in cryoSPARC v2.15. Particles in good 3D classes were selected to perform homogeneous refinement in cryoSPARC v2.15 and then imported into RELION-3.1 (Zivanov et al., 2020) using csparc2star.py module (Asarnow et al., 2019). To improve the map quality and interpretability of the CueR part in the CueR-TACs, the final particle stacks were subjected to signal subtraction to keep only the CueR dimer and the promoter spacer region, followed by masked 3D classification in RELION-3.1. Particles in the good classes (19,894 and 25,244 for CueR-TAC without and with RNA, respectively) from the masked 3D classification were selected, reverted to the original particles, and subjected to further 3D autorefinements to generate the final 3.9 Å and 4.1 Å maps for CueR-TAC without or with a RNA transcript. Because the two CueR-TAC maps are almost identical in the regions of CueR, promoter upstream DNA, and ntNCR, the final particles stacks...
from the two data sets were combined (45,138 particles in total) and subjected to masked 3D classification focusing on σNCR and the NTD of the proximal CueR subunit with residual signal subtraction. Particles in the good class (14,935) were selected, reverted to original particles, and subjected to another round of 3D autorefinement that resulted in a 4.1-Å map with clearer density for σNCR and its interacting interface with CueR NTD. This map was used as a cross-reference during model building. Resolutions of all maps were determined by gold-standard Fourier shell correlation (FSC) at 0.143 between the two half-maps. Local resolution variation was estimated from the half-maps by ResMap (Kucukelbir et al., 2014).

**Model building and refinement**

The initial models were generated by docking the previous structures of the components in the RNAP core (PDB 6B6H) into the individual cryo-EM density maps using Chimera (Pettersen et al., 2004) and COOT (Emsley and Cowtan, 2004). The 3.9 Å and 4.1 Å cryo-EM density maps for CueR-TACs and the 4.1 Å cross-reference map allowed to dock RNAP, σ70, and the CueR dimer in Chimera and build the promoter DNA scaffold and the RNA transcript (5’-GAG-3’) in COOT. The ω subunit is visible at lower contours compared with other components of the complex likely owing to a lower occupancy. The bubble region at the template side of CueR-TAC without RNA and the C-terminal helix of ω subunit of CueR-TAC with RNA were not built owing to poor density. The intact models were then refined using Phenix (Adams et al., 2010). In the real-space refinement, minimization global, local grid search, and adp were performed with the secondary structure, rotamer, and Ramachandran restraints applied throughout the entire refinement. 3DFSC calculation shows the cryo-EM maps have sphericity values of 0.733 and 0.758 for CueR-TAC without and with RNA, respectively, suggesting the maps have sampled majority of the angular space and do not suffer from major directional resolution anisotropy issue (Tan et al., 2017). The split cryo-EM maps were generated using color zone with 1.5 Å coloring radius in volume viewer of Chimera (Pettersen et al., 2004). The final models have good stereochemistry by evaluation in Phenix. The statistics of cryo-EM data collection, 3D reconstruction and model refinement were shown in Table S1. All figures were generated using UCSF ChimeraX (version 1.0) (Goddard et al., 2018) and PyMol (Schrödinger, v.2.3.2). Sequence alignments were performed using Clustal Omega (Sievers and Higgins, 2014) and the online server ESPript 3.0 (Robert and Gouet, 2014).

**In vitro transcription assay**

*In vitro* transcription assays were performed using linear promoter DNA fragment as template (Shi et al., 2020) with minimal modification. First, 20 nM of 153 bp PcopA fragment (ranging from −103 to +50) was incubated with different concentration of CueR protein (ranging from 0 to 240 nM) at 37 °C for 10 min in transcription buffer (20 mM Tris-HCl, pH 7.9, 50 mM NaCl, 5 mM MgSO4, 1 mM DTT, 0.1 mM EDTA, 5% glycerol) plus 1 μM of AgNO3. To exclude the possible effects of some CueR protein without metal ion and ensure that all the purified CueR protein in the assays could be occupied by +1 transition-metal ions, we added 1 μM AgNO3 into the transcription buffer. After that, 100 nM RNAP holoenzyme, which was assembled by mixing 100 nM RNAP core and 300 nM σ70, was added and incubated at 37 °C for another 10 min. Transcription was initiated by the addition of 50 μM CTP, UTP and ATP, 5 μM GTP, and 1 μCi of [α-32P]GTP. The reactions were carried out at 37 °C for 10 min and then stopped by 1 volume of 95% formamide solution. RNA products were heated at 70 °C for 5 min and then analyzed on denaturing (7 M urea) 16% polyacrylamide gel electrophoresis (PAGE).

**DNA-binding analysis**

The electrophoretic mobility shift assays (EMSA) were performed following a reported protocol (Shi et al., 2020) with slight modification. First, 10 nM of fluorescein-labeled promoter fragments were incubated with different concentrations of CueR protein (ranging from 0 to 480 nM) and then were mixed with 25 nM σ70. RNAP holoenzyme in transcription buffer plus 1 μM of AgNO3 for 10 min at 37 °C. In this assay, we also added 1 μM AgNO3 to ensure that all the purified CueR protein in the assays could be occupied by +1 transition-metal ions. Then, 10 μg/mL of heparin was added and incubated for 5 min at 37 °C. For comparing the promoter binding of σ70-RNAP in the absence or presence of CueR protein, similar procedures were applied except that different concentrations of σ70-RNAP holoenzyme (ranging from 0 to 200 nM) were used. Afterward, samples were loaded on 6% native 0.5×TBE-PAGE. Gels were scanned by Amersham Typhoon scanner (GE Healthcare).

**E. coli mutant construction**

Mutations in *E. coli* were constructed based on a K12 MG1655 strain with deletion of lacZ gene (renamed as Ec-parent here) (Hu et al., 2016) using a CRISPR-Cas9 system (Jiang et al., 2015). Briefly, a small guide RNA
(sgRNA) targeting rpoD or cueR gene was introduced in pTargetF plasmid following the Quickchange-site-directed mutagenesis protocol (Stratagene). The Ec-parent strain carrying a Cas9 expressing plasmid pCas was cotransformed with the pTargetF constructs and a donor DNA fragment carrying aimed mutations. Mutants were selected by primer-specific PCR and confirmed by DNA sequencing.

**In vivo promoter activity test**

In vivo promoter activity was tested using a low-copy lacZ reporter fusion plasmid named as pZT100 (Li et al., 2014). A 250-bp P_<sub>copA</sub> fragment was cloned into pZT to produce the pZT-P_<sub>copA</sub> using the ClonExpress II One-Step Cloning Kit (Vazyme). Mutations in pZT-P_<sub>copA</sub> were also introduced by oligos following the Quickchange-site-directed mutagenesis protocol (Stratagene). The pZT constructs were transformed into E. coli strains. Bacterial cells were grown to exponential phase in minimal medium and then exposed to 100 µM CuSO₄ (Philips et al., 2015) for different time. The levels of β-galactosidase (Hu et al., 2009) were measured to indicate the P_<sub>copA</sub> activities.

**QUANTIFICATION AND STATISTICAL ANALYSIS**

RNAs from in vitro transcription assays and shifted DNAs in EMSA assays were quantified by ImageJ software. Data are shown as mean ± SD from three experiments. The β-galactosidase activity data were obtained from three colonies performed in duphcates for each strain and data are shown as mean ± SEM. Statistical analyses were performed using the unpaired Student’s t-test (two-tailed) between each of two groups. * p<0.05; ** p<0.01.