Hypochlorous acid (HOCI) is ubiquitously produced in higher eukaryotes, including humans and Drosophila (1-3). HOCI is also commonly used to kill microorganisms in food industries, hospitals, and households due to its high reactivity toward macromolecules that cause oxidation of proteins and extensive damage to DNA (4-6). HOCI directly oxidizes amino acids containing amines and sulfurs by electrophilic reaction (6, 7). In particular, methionine is oxidized to methionine sulfoxide by HOCI at physiological pH via the chlorinated forms at the reaction rates approaching the diffusion limit (4-6, 8).

Many transcription factors that respond to different oxidative stresses have been identified (9). Among these, OxyR has been well characterized as a major H$_2$O$_2$-specific transcription factor in most Gram-negative bacteria (10-12). OxyR is a typical LysR-type transcriptional regulator (LTR). These usually function in a tetrameric assembly that involves an N-terminal DNA-binding domain (DBD) and a C-terminal regulatory domain (RD). The crystal structure of Pseudomonas aeruginosa OxyR revealed that OxyR specifically recognizes H$_2$O$_2$ in a small ligand-binding site located in an interface between RD-I and RD-II, the two subdomains of RD (13). OxyR has two redox states for two invariant cysteine residues in RD-II. In the absence of H$_2$O$_2$, the cysteine residues remain as free thiol forms in the conformation representing the inactive state. In the presence of H$_2$O$_2$, the cysteine residues are oxidized to a disulfide, leading to a conformational change to the active state (14).

In this study, we identified an ortholog of E. coli HypT in Salmonella enterica serovar Typhimurium (S. Typhimurium) as a crucial factor in survival within macrophages. We determined the crystal structures of HypT from hypochlorous acid | LysR-type transcription regulator | HypT | methionine oxidation | repression mechanism

Hypochlorous acid (HOCI) is generated in the immune system to kill microorganisms. In Escherichia coli, a hypochlorite-specific transcription regulator, HypT, has been characterized. HypT belongs to the LysR-type transcriptional regulator (LTR) family that contains a DNA-binding domain (DBD) and a regulatory domain (RD). Here, we identified a hypT gene from Salmonella enterica serovar Typhimurium and determined crystal structures of the full-length HypT protein and the RD. The full-length structure reveals a type of tetrameric assembly in the LTR family. Based on HOCI-bound and oxidation-mimicking structures, we identified a HOCI-driven methionine oxidation mechanism, in which the bound HOCI oxidizes a conserved methionine residue lining the putative ligand-binding site in the RD. Furthermore, we proposed a molecular model for the oxidized HypT, where methionine oxidation by HOCI results in a conformational change of the RD, inducing a counter rotation of the DBD dimers. Target genes that are regulated by HypT and their roles in Salmonella were also investigated. DNase I footprinting experiments revealed a DNA segment containing two pseudopalindromic motifs that are separated by $\sim$100 bp, suggesting that only the oxidized structure makes a concomitant binding, forming a DNA loop. An understanding of the HypT-mediated mechanism would be helpful for controlling many pathogenic bacteria by counteracting bacterial HOCI defense mechanisms.

Significance

Hypochlorous acid (HOCI) is a powerful oxidant that kills microorganisms. HypT has been identified as a HOCI-sensing transcription factor regulating several genes to enhance survival during HOCI stress in Escherichia coli. However, the structure and a detailed action mechanism of HypT have not yet been reported. In this study, we identified Salmonella Typhimurium HypT as a crucial factor in survival within macrophages and presented structures of HypT. The full-length structure shows interesting features describing a type of tetrameric assembly for the LysR family transcription regulator. The regulatory domain structures at various states give important clues to understanding the HOCI-sensing mechanism. Combining these results, we provided a molecular mechanism for HypT that explains its HOCI-sensing ability and structural changes.

Author contributions: I.J., D.K., T.N., S.R., and N.-C.H. designed research; I.J., D.K., and T.N. performed research; I.J., D.K., T.N., S.H., J.A., S.R., and N.-C.H. analyzed data; and I.J., D.K., T.N., S.R., and N.-C.H. wrote the paper.

The authors declare no conflict of interest.

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Data deposition: The atomic coordinates and structure factors have been deposited in the Protein Data Bank, www.pdb.org (PDB ID codes SYDV, SYDG, SYDV, SYER, and SYED).

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S. Typhimurium at various states, which account for its molecular mechanisms of action and regulation.

Results

Identification of HypT in S. Typhimurium. For a HypT ortholog candidate, we found an uncharacterized LysR-type transcription regulator gene (WP_000383509) in the S. Typhimurium genome that showed high sequence identity (82%) to E. coli HypT. The gene contained three methionine residues (Met123, Met206, and Met230) that were previously characterized as important HOCl-sensing residues in E. coli HypT (19). However, two cysteine residues (Cys4 and Cys150) in E. coli HypT are replaced like other HypT homologs (19). Our results indicate that the tetramer is the functional unit of StHypT, an intrinsic asymmetric feature. One subunit exhibited the so-called “extended conformation,” in which the DBD is extended away from the RD, and there is no direct interaction between them (22–25) (SI Appendix, Figs. S5B, Left, yellow). The other subunits exhibited the “compact conformation,” in which a folded-in DBD

Fig. 1. Functional importance of hypT (WP_000383509) from S. Typhimurium. (A) Viabilities of the ΔrclR strain and ΔhypT (WP_000383509)/ΔrcrR strain depending on the concentration of HOCl. Error bars represent the SD (N.D., not detected). (B) Survival of Salmonella strains in RAW264.7 murine macrophage cell line. Wild-type strain (wt), the hypT-deleted strain (ΔhypT), and the hypT-deleted strain harboring pH2E21::hypT (∆hypT+pHypT) were added to monolayer of RAW264.7 cells at a multiplicity of infection of 10. After 30 min, 0, 8, or 24 h after infection, intracellular bacteria were plated for enumeration. Each strain was tested in triplicate, and the average cfu ml−1 was calculated. Asterisks indicate statistical significance (*P < 0.05; **P < 0.01; ***P < 0.001) by unpaired t test comparing the ΔhypT to the wild-type strain.

A Configuration in the StHypT Tetramer. We determined the structure of the full-length StHypT protein (StHypT-FL) at 3.3-Å resolution using crystals grown under reducing conditions. The asymmetric unit contained two StHypT molecules, and each molecule consisted of the N-terminal DBD (residues 1–93) and the RD (residues 100–302), which are linked by a flexible hinge (residues 94–99) (Fig. 2A). A globular homotetramer was built by combining two neighboring asymmetric units, which is consistent with the measured molecular mass using the size exclusion chromatography results (SI Appendix, Fig. S3). These observations strongly suggest that the tetramer is the functional unit of StHypT, as in most LTRR family proteins. The StHypT tetramer can be described as having dimer-of-dimers symmetry, formed by two dimeric interfaces (the DBD-to-DBD in SI Appendix, Fig. S4A and the RD-to-RD in SI Appendix, Fig. S4B) (Fig. 2B). The structure of the DBD dimer is very similar to that of the typical LTRRs (21), and the dimeric interface between the DBDs is also largely stabilized by hydrophobic interactions in the linker helix (α4), as in most LTRRs (SI Appendix, Fig. S4A) (13). Likewise, the overall structure of the RD dimer of StHypT is similar to that of typical LTRRs. The RD dimer of StHypT is stabilized by β/β packing interactions between the β4-β8-β3-β1-β2 sheets of one protomer and the β6-β5-β7-β4-β8 sheets of the other protomer. The angle between each protomer is ∼60° (SI Appendix, Fig. S4B).

When two protomers in the asymmetric unit were superposed using the RD as a reference, we observed a translational variation (3–5 Å) in the subdomain RD-II of the RD and a rotational variation (∼10°) of the DBD (Fig. 2C). The two variations are structurally related mainly through the interaction between Arg93 of the DBD and the α8 in RD-II of the other subunit in the dimer (Fig. 2B and D). The interdomain hinge and the intersubunit interaction between the DBDs and the RDs suggest that the structural changes in the RD result in the structural change of the DBD.

The domain arrangement of StHypT is remarkably different from any other LTRR structure (22–26). To analyze the conformational difference between StHypT and the typical LTRRs, the dimeric units of StHypT and OxyR from P. aeruginosa were taken from the tetramers based on the RD-RD dimeric interface (SI Appendix, Figs. S5 and S6). OxyR, a typical LTRR, displayed an intrinsic asymmetric feature. One subunit exhibited the so-called “extended conformation,” in which the DBD is extended away from the RD, and there is no direct interaction between them (22–25) (SI Appendix, Fig. S5B, Left, yellow). The other subunits exhibited the “compact conformation,” in which a folded-in DBD
makes close contact with the RD of the same subunit (SI Appendix, Fig. SSB, Left; tan). In contrast, all subunits in the StHypT tetramer were in a largely single conformation, distinguished from both the extended and the compact conformations (SI Appendix, Fig. SSB, Right). In StHypT, the DBD of one subunit in the dimer was near the RD of the other subunit, with close proximity (albeit no direct interaction) between the linker helix (α4) and the RD-II (SI Appendix, Fig. SSB, Right).

These conformational differences between the subunits of StHypT and OxyR resulted in large differences in the DNA-binding modes. The two DBD dimers in the OxyR tetramer face the same direction (SI Appendix, Fig. SSB, Left), and the OxyR tetramer binds to two adjacent sites of a slightly curved DNA molecule (SI Appendix, Fig. SCC). In contrast, each of the DBD dimer in the StHypT tetramer face opposite sides with a parallel arrangement (Fig. 2B).

HOCl-Binding Site in the RD. To reveal more detailed features of the StHypT RD, we determined its crystal structure (StHypT-RD) at a higher resolution (2.0 Å; SI Appendix, Table S1). The wild-type StHypT-RD showed a very similar structure as the RD region of HypT-FL (rmsd 0.628 Å for 188 C atoms). The typical ligand-binding site cavity of LTTR was well conserved in StHypT-RD, located between RD-I and RD-II (Fig. 3A) (22). Interestingly, Met206, one of three important methionine residues in E. coli HypT (19), was placed near the ligand (or solvent)-accessible cavity (Fig. 3B). The Met206 residue was in the α8 helix of RD-II, and its side chain was inserted into a small hydrophobic pocket composed of Leu174, Tyr200, Tyr205, Leu209, Ile210, Pro248, Tyr250, and Ile273 residues (Fig. 3B). Regarding the other two methionine residues, Met123 was surrounded by the hydrophobic residues in RD-I, and Met230 was close to the twofold axis of the RD dimer (Fig. 3A and SI Appendix, Fig. S6 A and B).

In the apo-structure of StHypT-RD, two water molecules were bound in the cavity around the Met206 residue (Fig. 3C). To better define the HOCl-binding site, we briefly soaked the HypT-RD crystals in HOCl and determined the structure at 1.75-Å resolution (SI Appendix, Table S1). Remarkably, a peanut-shaped electron density map was found in the cavity (consistent with the typical ligand-binding site of LTTRs), and the conserved Met206 residue was placed near the peanut-shaped electron density map (SI Appendix, Fig. S7A). Difference Fourier maps excluded the possibility that the electron density represented two water molecules. Furthermore, we examined a bromine ion in the place of the electron density map in a crystal structure grown in the presence of NaBr (SI Appendix, Fig. S7B). Collectively, we concluded that the electron density map represents an HOCl (or ClO \textsuperscript{-}) molecule. The local environment surrounding the electron density map also likely holds an HOCl molecule (Fig. 3D). The backbone -NH group of Ser110, Tyr205, and Met206 and the side chain hydroxyl group of Ser110 are together involved in its binding. In summary, HypT-RD captures an HOCl molecule near the Met206 residue, which might be important for the selectivity and specificity of StHypT.

To investigate the role of Met206 in the bacteria, we added a plasmid that overexpressed mutant hypT (M206Q or M206L) to the hypT/rnr-disrupted Salmonella strain. Glutamine was chosen to mimic methionine sulfoxide, an oxidized methionine (27, 28), and leucine was chosen to represent the oxidation-free state because leucine is rarely oxidized. The transcriptional impact of the HypT M206Q mutant was similar to the wild-type HypT, while that of the HypT M206L mutant was significantly reduced (SI Appendix, Fig. S2B). We did not see better activity for hypT M206Q, possibly because methionine sulfoxide is bulkier than glutamine, and thus glutamine seems not to fully mimic the oxidized methionine. However, substitution with the leucine residue failed to activate HypT, indicating the importance of the oxidation of Met206 for the activity of HypT. Taken together, our results further confirmed the importance of Met206 in sensing HOCl.

The Roles of Methionine Residues in the Oxidized Conformation. We determined the structure of a HypT RD M206Q mutant protein at 2.6-Å resolution to understand the HOCl sensing mechanism of HypT. The asymmetric unit of the crystal contained two RD dimers in different conformations (SI Appendix, Fig. S8). Of these, one dimer (chain A:B) showed essentially the same structure as the wild-type RD structure (rmsd 0.802 Å for 367 C atoms). The Gln206 in the mutant structure (chain A:B) was in the reduced conformation, since the side chain was inserted into the small hydrophobic pocket (as is Met206 in the wild-type structure) (Figs. 3B and 4A). However, the other dimer (chain C:D) showed substantial structural differences from the RD structure in the reduced conformation (rmsd 2.401 Å for 308 C atoms), especially in the active site near the Gln206 residue and in the intersubdomain orientation in the RD (Fig. 4A).

The Gln206 residue was moved away from the small hydrophobic pocket consisting of α5 in RD-I, resulting in the oxidized conformation (Fig. 4A). This oxidized conformation might result from the polar side chain of glutamine, which is not as suitable for the hydrophobic pocket as the side chain of methionine. Interestingly, this conformational change in Gln206 induced asymmetric changes between the protomers (chain C:D) in the oxidized conformation. In chain C, the entire RD-I subdomain rotated around RD-II by ∼30° within the RD, as the α5, β1, and β2 in RD-I were moved because the small hydrophobic pocket was collapsed (Fig. 4B). In chain D, RD-II was rotated in the dimer instead of RD-I. The β6-β5 sheet in RD-II of chain D together with the α1 and α2 of RD-I in chain C (SI Appendix, Fig. S4B). The rotation of RD-I in chain C caused the rotation of RD-II in chain D. As a result, the RD-II of chain D is rotated by ∼30°, with movement of α6 in the RD-II by 9 Å with respect to RD-I (Fig. 4C). These observations suggest that the oxidation of Met206 leads to a global positional shift of the RD dimers and the DBDs in the full-length HypT tetramer.
We built a structural model of the full-length HypT in the oxidized state. The RD structures of the M206Q mutant in the oxidized conformation was superposed onto the full-length structure of the HypT tetramer in the reduced conformation. Given that the molecular contact between α4 of the DBD and α8 in the RD governed the angle of the DBD with respect to the RD (Fig. 2D), the rotation of the DBD dimers was expected in response to the HOCl-driven oxidation in the RD. The movement of β1 and α8 of the other subunit resulted in the clockwise rotation of the DBD dimers by ∼20° and a total ∼40° rotation between the DBD dimers in the tetrameric assembly (SI Appendix, Fig. S9).

We next compared the possible DNA-binding modes between the reduced and the oxidized structures of the HypT tetramer. The reduced form of the StHypT tetramer could bind to two parallel DNA segments that are curved by interaction with the DBD dimer (Fig. 5A, Left and Center). The RD surfaces of StHypT between the two DBD dimers are highly basic, indicating that the side surface can bind a DNA segment (25–30 nt) (SI Appendix, Fig. SSD) and, thus, contact the two curved bound DNA segments (Fig. 5A, Left). This DNA-winding mode is comparable to the histone octamer that scaffolds the DNA in eukaryotic cells (29).

In contrast to two parallel DNA segments bound to the reduced HypT, two DNA segments adopting a crossing angle of ∼40° could be docked onto the DBD dimers of the oxidized HypT tetramer model (Fig. 5B). The crossing angle between the bound DNA segments did not allow the wrapping around the HypT tetramer in the oxidized conformation. Instead, the oxidized HypT could be bound to a negative-supercoiled DNA loop (Fig. 5B).

**Structural Modeling of the Oxidized HypT.** We built a structural model of the full-length HypT in the oxidized state. The RD structures of the M206Q mutant in the oxidized conformation was superposed onto the full-length structure of the HypT tetramer in the reduced conformation. Given that the molecular contact between α4 of the DBD and α8 in the RD governed the angle of the DBD with respect to the RD (Fig. 2D), the rotation of the DBD dimers was expected in response to the HOCl-driven oxidation in the RD. The movement of β1 and α8 of the other subunit resulted in the clockwise rotation of the DBD dimers by ∼20° and a total ∼40° rotation between the DBD dimers in the tetrameric assembly (SI Appendix, Fig. S9).

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**StHypT Target Genes and StHypT Binding DNA Segments.** We compared the transcription levels of putative target genes (feoA, feoB, fluA, fluC, sitC, sitD, metN, metB, and cysH) in *S. Typhimurium* SL1344 cultured in the M9 minimal media. In the absence of HOCl, the transcription levels of the putative target genes were not influenced by a deletion of hypT (SI Appendix, Fig. S10A). In the presence of HOCl stress, however, the hypT-deletion strain showed a twofold higher transcription level of iron acquisition-related genes (feoA, feoB, fluA, fluC, sitC, and sitD) than the wild-type strain. The increased transcription levels in the hypT-deleted mutant returned to the levels of the wild type by adding a hypT-expressing plasmid (SI Appendix, Fig. S10B). Consistently, intracellular iron level of the hypT-deleted strain cultured in the LB medium in the presence of HOCl stress were substantially lower than that of the wild-type strain (SI Appendix, Fig. S10C). However, genes related to the biosynthesis of cysteine and methionine (metN, metB, and cysH) in the hypT-deleted *S. Typhimurium* showed no significant differences in their transcript levels, unlike *E. coli* cultured in the LB medium (15) (SI Appendix, Fig. S10 A and B). These discrepancies might result from the different growth media and lower levels of HOCl treatment than for HypT of *E. coli*.

When the wild-type strain was exposed to HOCl, the transcription levels of iron acquisition-related genes were decreased (SI Appendix, Fig. S10D) without changing the mRNA levels of hypT itself (SI Appendix, Fig. S10E). Furthermore, the electrophoretic mobility shift assay (EMSA) with the sit, feo, and flu operons revealed that the purified HypT protein directly bound to their possible promoter regions (SI Appendix, Fig. S11). Based on these results, we concluded that in *Salmonella*, HypT directly represses these genes in response to HOCl stress. It is interesting that the iron acquisition-related genes are repressed even in an iron-limited condition. The M9 media used in this study contained a very limited concentration of iron, where the master
iron regulator Fur is in the inactive apo-forms and thus cannot repress the iron acquisition genes (30, 31) (SI Appendix, Fig. S10F). We further observed the recovery of the bacterial viability at a lethal dose of HOCl by addition of the membrane permeable the iron chelator dipyridyl (SI Appendix, Fig. S12), indicating that the toxicity of HOCl is mainly mediated by iron in *Salmonella*.

To investigate the HypT-DNA interaction, the *fhu* operon was selected as a model promoter. Since *S. Typhimurium* and *E. coli* have nearly identical nucleotide sequences in the promoter region of the *fhu* operon, the transcription start site (TSS) and σ^70^ binding sites (−10 and −35 boxes) in the *fhuA* promoter were adopted from *E. coli* (32) (Fig. 6A). Multiple DNase I footprinting experiments revealed two HypT-binding sites: TTAGTTATAGTCA, +7 to +21 from the TSS and AACCCTTCTTTTACA, −111 to −97 from the TSS (SI Appendix, Fig. S13). The DNA region between the two binding sites contains the −10 box and −35 box, the TSS, and Fur binding site (Fur box). The HypT-binding sites exhibit pseudopalindromic patterns, as observed in other LTTRs (33, 34) (Fig. 6A). However, the spacing (102 nt) between the two binding sites in *StHypT* are far different from those of typical LTTRs (7 nt in the case of OxyR; ref. 33). The EMSA, using the various DNA fragments, confirmed the results from DNase I footprinting experiments and further suggested that the reduced and the oxidized HypT tetramers share the binding sites in the *fhu* promoter (SI Appendix, Fig. S13C).

**Discussion**

Given the high sequence similarity between *E. coli* HypT and StHypT, the structure in the functional states and the regulation mechanisms would be shared. However, previous studies on *E. coli* HypT have presented a ring-like dodecameric assembly of the *fhu* operon, the transcription start site (TSS) and σ^70^ binding sites (−10 and −35 boxes) in the *fhuA* promoter were adopted from *E. coli* (32) (Fig. 6A). Multiple DNase I footprinting experiments revealed two HypT-binding sites: TTAGTTATAGTCA, +7 to +21 from the TSS and AACCCTTCTTTTACA, −111 to −97 from the TSS (SI Appendix, Fig. S13). The DNA region between the two binding sites contains the −10 box and −35 box, the TSS, and Fur binding site (Fur box). The HypT-binding sites exhibit pseudopalindromic patterns, as observed in other LTTRs (33, 34) (Fig. 6A). However, the spacing (102 nt) between the two binding sites in *StHypT* are far different from those of typical LTTRs (7 nt in the case of OxyR; ref. 33). The EMSA, using the various DNA fragments, confirmed the results from DNase I footprinting experiments and further suggested that the reduced and the oxidized HypT tetramers share the binding sites in the *fhu* promoter (SI Appendix, Fig. S13C).

Several homologs of HypT based on the sequence similarity have been found in many Gram-negative bacteria (15). However, to date, most of these genes have not been functionally characterized, except for *E. coli* HypT and StHypT. Although further functional studies are required, Met206 is the hallmark for the HypT orthologs, since the methionine residue is directly involved in HOCl sensing, according to our results. A recent study on a HypT homolog (VV2_1132) from *Vibrio vulnificus*, in which Met206 is replaced with leucine, confirmed that it did not function as a HOCl-responsive factor (36, 37). Two other methionine residues (Met123 and Met230) were characterized in *E. coli* HypT (19).

Both methionine residues are in the moving parts and have less hydrophobic interactions in the oxidized conformation (SI Appendix, Fig. S6 C and D). The greater polarity of the oxidized methionine, the oxidized methionine residues appear to be more stabilized in the oxidized conformation. Thus, our findings suggest that oxidation of the two methionine residues (Met123 and Met230) render the oxidized conformation of HypT to be more favorable. Why was HOCl recognized by a methionine residue in HypT? This question could be simply explained by the preferential reactivity of methionine with HOCl compared to H_2O_2, as discussed in ref. 19. Methionine reacts with H_2O_2 much more slowly than with HOCl. Therefore, HypT has more selectivity for HOCl by adopting methionine residues for sensing oxidative stress, unlike cysteine-based sensors.

Like H_2O_2, HOCl generates hydroxyl radicals that are more reactive and induce damage to DNA or other essential cellular molecules in the presence of Fe^{2+} (7, 38, 39). Indeed, the reaction of Fe^{2+} with HOCl is much faster than with H_2O_2 (7). Therefore, it could be more important to reduce the cellular levels of Fe^{2+} to block the reaction in the bacterial cytosol maybe for the survival within macrophages. To better understand the HOCl-resistance mechanisms of *Salmonella*, further studies would be required on the interplay or the functional division between HypT and the HOCl-responsive cysteine-based transcriptional regulator RclR.

This study revealed that StHypT in the oxidized state is bound to a DNA segment that contains two pseudopalindromic sequences separated by ~100 bp in the promoter region of the *fhuA* promoter. A DNA docking model of the StHypT tetramer in the reduced state suggested a 25- to 30-nt spacing between two DNA motifs, as shown in Fig. 5A. Thus, the concomitant binding

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**Fig. 6.** A *fhuA* promoter and its repression mechanism by HypT. (A) The structure of the *fhuA* promoter. The *fhuA* promoter from *Salmonella* was analyzed by comparing with the *E. coli* *fhuA* promoter. The −10 and −35 boxes, recognized by σ^70^, are highlighted by gray boxes. The StrHypT binding sequences (red letters on yellow boxes) were determined by DNase I footprinting assay (SI Appendix, Fig. S13). The pseudopalindromic sites are indicated with underlines and red arrows. The transcription start site and translation initiation codon are shown in blue and green letters, respectively. Fur box is indicated by a magenta box. (B) A proposed repression mechanism of StrHypT. Red lines, yellow boxes, and gray boxes represent DNA, the StrHypT binding site, and the σ factor binding site (−35 and −10 box), respectively. The combination of purple and green illustrations presents the RNA polymerase complex (RNAP), and among them, the green shapes represent σ^70^.
of the reduced StHypT tetramer to the long fluo4 promoter DNA segment would not be allowed. Only separate binding of the StHypT tetramers on one (or both) of the two HypT-binding sites are expected. In contrast, the oxidized StHypT tetramer would remain bound to the long DNA segment by looping in a negatively supercoiling manner. From these findings, we propose a regulation mechanism for the gene expression of the fluo operon by HypT. In the absence of HOCI stress, the HypT tetramer is bound to one or two HypT-binding motifs, but it cannot repress the target gene because the σ factor is accessible to −35 and −10 boxes (Fig. 6B). When a HOCI triggers the conformational change of HypT, the concomitant binding of the two HypT-binding motifs is allowed, which ultimately form a DNA loop. Because the RNA polymerase-binding sites (−35 and −10 boxes) are in the loop, gene expression would be strongly repressed (Fig. 6B). In addition, the regulatory mechanism would be changed if the spacing between the two HypT-binding motifs were different.

We observed that the HypT-mediated defense system of Salmonella increases the lethal dose of HOCI and is important in survival of the bacteria within macrophages. An understanding of the HOCl defense mechanism at the molecular level would be helpful for controlling many pathogenic bacteria by counteracting them.

**Experimental Procedures**

Expression and Purification of HypT for Crystallization. To overexpress the full-length (1–302) and regulatory domain (RD, 97–302) of HypT (WP_000383509), the genes encoding each protein were amplified and cloned into the pProEX HTa expression vector (Invitrogen). The resulting plasmid encodes the hexa-histidine tag and TEV protease cleavage site. HTa expression vector (Invitrogen). The resulting plasmid encodes the hexa-histidine tag and TEV protease cleavage site.

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