Peroxisome Responsive Regulator PerR of group A Streptococcus Is Required for the Expression of Phage-Associated DNase Sda1 under Oxidative Stress

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Abstract

The peroxide regulator (PerR) is a ferric uptake repressor-like protein, which is involved in adaptation to oxidative stress and iron homeostasis in group A streptococcus. A perR mutant is attenuated in surviving in human blood, colonization of the pharynx, and resistance to phagocytic clearance, indicating that the PerR regulon affects both host environment adaptation and immune escape. Sda1 is a phage-associated DNase which promotes M1T1 group A streptococcus escaping from phagocytic cells by degrading DNA-based neutrophil extracellular traps. In the present study, we found that the expression of sda1 is up-regulated under oxidative conditions in the wild-type strain but not in the perR mutant. A gel mobility shift assay showed that the recombinant PerR protein binds the sda1 promoter. In addition, mutation of the conserved histidine residue in the metal binding site of PerR abolished sda1 expression under hydrogen peroxide treatment conditions, suggesting that PerR is directly responsible for the sda1 expression under oxidative stress. Our results reveal PerR-dependent sda1 expression under oxidative stress, which may aid innate immune escape of group A streptococcus.

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Introduction

Streptococcus pyogenes (group A streptococcus, GAS) is a facultative Gram-positive human pathogen which causes mild to severe infectious diseases including pharyngitis, cellulitis, necrotizing fasciitis, and toxic shock syndrome [1,2]. Although it lacks catalase, GAS has developed other defense mechanisms against oxidative stress, including NADH oxidase, superoxide dismutase, peroxidases, and Dps-like peroxide resistance protein [3,4]. In addition, a peroxide operon regulator, PerR, has been identified and characterized as the peroxide responsive regulator in GAS [3,5,6].

PerR is a ferric uptake repressor (Fur)-like protein, which regulates genes involved in oxidative stress responses and iron homeostasis in GAS [3,6-8]. PerR protein has been shown to bind to zinc and iron, and the metal binding of PerR is required for optimal responses to peroxide [5,9]. The perR mutant is attenuated in virulence in murine air sac and baboon pharyngitis infection models [3,10]. In addition, the perR mutant is more susceptible to phagocytic cell clearance [10], indicating that the PerR regulon also contributes to immune escape. Transcriptome analysis further showed that PerR not only regulates peroxide detoxifying enzyme expression, but also coordinates DNA and protein metabolisms and DNA repair system activity, which may contribute to GAS virulence and adaptation to the host environment [5,11].

DNase is one of the important virulence factors of GAS [12,13]. Until now, DNases Sda1, Spd, Mf3, and SpnA have...
Our results suggest that PerR contributes to GAS immune escape through regulating sda1 expression under oxidative conditions.

Materials and Methods

Bacterial strains and culture media

GAS strain A20 (emm1/ST28), SW612 (the perR isogenic mutant), and SW665 (perR complementation strain) were described in the previous study [21]. GAS strains were cultured in tryptic soy broth (Becton, Dickinson and Company, Sparks, MD) supplemented with 0.5% yeast extract (TSBY) at 37°C. GAS strains were cultured in TSBY for 5 h) were used for 5' rapid amplification of cDNA ends (RACE) analysis 5’ RACE analysis was performed according to methods described in the manual (5’ RACE System for Rapid Amplification of cDNA Ends, Version 2.0, Life Technologies Cooperation).

Plasmid Constructions

Plasmid pMW508 used for mutared the perR gene in wild-type strain A20 was described previously [21]. Briefly, the 1.7 kb of perR region was amplified by PCR and cloned in to the isogenic mutant was confirmed by Southern hybridization with the Cm probe [21]. For complementation of the perR mutant, the 1.5 kb of DNA containing the perR gene and the promoter region were amplified by PCR and cloned in to the shuttle vector pDL278 (copy number is around 20 to 30) [24]. For measurement of the sda1 promoter activity, the sda1 promoter (400 bp) was amplified and fused with a promoter-less cat gene on plasmid pMW398 [25] by using the BamHI restriction enzyme site to construct.

Table 1. Primers used in this study.

| Target gene (focus tag) | Use | Forward primer | Reverse primer |
|------------------------|-----|----------------|----------------|
| mf3 (M5005_Spy_1169)   | Real-time PCR | Ggactgagacagccaggaga | ttgctgacagccagggag |
| spd (M5005_Spy_1738)   | Real-time PCR | Cgcagcagctctggaactcta | cggtaccagccagcagcagc |
| sda1 (M5005_Spy_1415)  | Real-time PCR | Tgctgacagccagccaggaga | cggtaccagccagcagcagc |
| spnA (M5005_Spy_0671)  | Real-time PCR | Tgtggctaaagcagtgacca | gcctactaattgtcttcccat |
| gyrA (M5005_Spy_0874)  | Real-time PCR | Cgctgacagctctggaactcta | gcctactaattgtcttcccat |
| Psda f                  | Construction | cggggtgctaggttagt | ggggtgctaggttagt |
| perR gene with His99Ala mutation | Construction | atgagccatcaagcagcag | aacgaaatgtcgctagccg |

been identified in GAS [12,14-16]. Mf3 and Sda1 are encoded by prophages, but Spd and SpnA are suggested to be chromosomally encoded [17,18]. SpnA is the only cell-wall-anchored DNase found in GAS [15]. Sumby et al. (2005) showed that extracellular DNase activity is required for normal progression of GAS infection in mouse and non-human primate infection model. Among them, Sda1 is the major DNase that contributes to the virulence of the clinical relevant M1T1 clone [17,19]. Buchanan et al. (2006) showed that Sda1 is both necessary and sufficient to promote GAS virulence in a murine model of necrotizing fasciitis. Compared to the wild-type strain, the sda1 mutant is impaired in its ability to degrade neutrophil extracellular traps (NETs), resulting in efficient clearance by neutrophils [12,19]. Furthermore, a recent study further showed that GAS employs Sda1 to prevent Toll-like receptor-9 recognition of degraded bacterial DNA [20]. These results indicate that Sda1 has important roles in GAS escaping immune clearance.

In the present study, we show that the increased expression of sda1 under oxidative stress is PerR-dependent. In addition, mutation of the metal-binding site of PerR abolished its positive regulation of sda1, suggesting that the peroxide responsive activity of PerR is crucial for regulating sda1 expression. These results suggest that PerR contributes to GAS immune escape through regulating sda1 expression under oxidative conditions.

PerR and Sda1 Expression

DNA and RNA manipulation

GAS genomic DNA extraction, RNA extraction, and reverse transcription were described previously [23]. Real-time PCR reactions were performed in a 10 µl mixture containing 2 µl of cDNA, 0.3 µl of primers (10 µM), and 5 µl of KAPA SYBR® Fast qPCR pre-mixture (KAPA Biosystems, Woburn, MA). The mixtures were incubated at 95°C for 10 min, followed by 45 cycles of 95°C (20 sec), 60°C (1 min), and 72°C (15 sec). Real-time quantitation was analyzed by LightCycler software (version 3.0, Roche Diagnostics, Indianapolis, IN). Expression level of each target gene was normalized to gyrA (Table 1) and analyzed using the ∆∆Ct method. Biological replicate experiments were performed from at least three independent RNA preparations in duplicate. Primers used for real-time PCR analysis were designed by Primer3 (v. 0.4.0, http://frodo.wi.mit.edu) according to the MGAS5005 chromosomal DNA sequence (NCBI reference sequence: NC_007297.1) and are described in Table 1. RNAs extracted from A20 (cultured in TSBY for 5 h) were used for 5’ rapid amplification of cDNA ends (RACE) analysis 5’ RACE analysis was performed according to methods described in the manual (5’ RACE System for Rapid Amplification of cDNA Ends, Version 2.0, Life Technologies Cooperation).
perR gene was mutated by the mutagenesis method, encoding for histidine (residue 99) was changed to alanine by site-directed mutagenesis with primers described in Table 1. The mutated perR gene with its native promoter was ligated to pDL278 by BamHI and EcoRI to construct plasmid pMW680.

Gel mobility shift assay

Purification of recombinant His<sub>6</sub>-PerR protein (rPerR) and the gel mobility shift assay were described previously [21]. A DNA probe of the sda1 promoter region containing the PerR-binding sequence was amplified by primer sdaD2 promoter-1-SacI (CGAGC TCGCA ACACT TCTTC CACTT TTT) and sdaD2 promoter-2-KpnI (GGGCT ACCCT ATTTA TGTCC TCTCT TTGT). The control DNA probe (Pdpn) and a non-specific promoter DNA of SPy1840 were amplified by primers described previously [21]. rPerR and 50 ng of DNA probes were incubated in binding buffer (20 mM of Tris-HCl pH 8.0, 5% glycerol, 50 µg/ml of bovine serum albumin, and 50 mM of KCl) at room temperature for 15 min. The reaction mixtures were analyzed with 6% native polyacrylamide gel and DNA-protein complexes were visualized by staining with ethidium bromide. Competition assays were performed according to the manual (DIG Gel Shift Kit, Roche, Indianapolis, IN). Unlabeled DNA probes (cold probes) were amplified by primers described in Table 1.

Statistical analysis

Statistical analysis was performed by using Prism software, version 4 (GraphPad, San Diego, CA). A P value of student's t test < 0.05 was taken as significant.

Results

Expression of DNase genes in the perR mutant

A perR mutant is more resistant to oxidative stresses in vitro, but more susceptible to phagocytic cell clearance when compared to the wild-type strain [3,10]. DNases of GAS have been reported to have important roles in escaping immune clearance by degrading the DNA of the neutrophil extracellular traps [12,13]. The roles of PerR in regulating expression of DNase genes were therefore analyzed. The wild-type (A20), perR mutant (SW612), and complementation (SW665) strains were cultured in TSBY broth. No significant difference was found in the growth of these strains (Figure S1). The expression of m3f, spd, spnA, and sda1 in A20, SW612, and SW665 was analyzed by real-time RT-PCR. Results showed that the expression of m3f and spd are significantly increased in SW612 when compared to A20 and SW665 (Figure 1A and 1B). However, the expression of sda1 and spnA showed no difference among these strains (Figure 1C and 1D).

Expression of the DNase sda1 under Oxidative Stress Is PerR-Dependent

PerR is a peroxide responsive regulator in GAS [5,6,9,27]. The expression of DNase genes in the wild-type (A20), perR mutant (SW612), and complementation (SW665) strains were therefore analyzed under oxidative conditions. SW612 expresses more m3f under normal conditions and 0.1 mM hydrogen peroxide treatment when compared to A20 and SW665 (Figure 2A). However, the expression of m3f in A20 and SW612 were both significantly decreased after 0.5 mM hydrogen peroxide treatment (Figure 2A). The spd expression of A20 and SW665 was decreased in the presence of 0.5 mM of hydrogen peroxide, but showed no significant changes in SW612 (Figure 2B). Furthermore, the expression of sda1 in A20 and SW665, but not SW612, was significantly increased after hydrogen peroxide treatment (Figure 2C). The spnA expression of A20 and SW612 showed no significant difference in the presence of or without hydrogen peroxide (Figure 2D).

Determination of the transcriptional start site and putative Per box

The observation that PerR regulates the sda1 expression under oxidative stress conditions suggests that PerR may regulate sda1 through the putative PerR-binding sequence (Per box) [7]. The transcriptional start site of sda1 was determined by 5′-RACE assay (Figure 3A, indicates by arrow), and a putative Per box was found near the -35 region (Figure 3A). To further verify that the sda1 transcription is respond to hydrogen peroxide treatments, the activity of the sda1 promoter in the wild-type strain and perR mutant in the presence or absence of H₂O₂ were analyzed. Results showed that, in consistent with the sda1 RNA expression (Figure 2C), the promoter activity of sda1, which was evaluated by the expression of the reporter gene cat (described in Materials and Methods), was only increased in the wild-type (A20) but not in the perR mutant (SW612) after hydrogen peroxide treatment (Figure 3B).

Recombinant PerR protein binds to the sda1 promoter region

To further clarify whether PerR bound to Psda1, the recombinant PerR protein (rPerR) was incubated with partial Psda1 and the DNA binding activity of rPerR was analyzed by a gel retardation assay. Results showed that rPerR bound to the dpr promoter region (containing a PerR-binding box) but not to the SPy1840 promoter (PSpy1840, without a PerR-binding box, Figure 4A). In addition, rPerR caused a band-shift of Psda1 in a dose dependent manner (Figure 4A). To further investigate the specificity of binding, five hundred to one thousand-fold of unlabeled cold-specific probes (Psda1 and Pdpr DNA probes) and cold-non-specific probe (PSpy1840) were used to compete against the protein-DNA interaction. Results showed that unlabeled Psda1 probe can compete with the rPerR-Psda1 interaction in a dose-dependent manner (Figure 4B). In addition, the cold-specific probe (Pdpr probe) but not a cold-non-specific probe (PSpy1840) significantly competes with the rPerR-Psda1 interaction (Figure 4B).

Metal binding site of PerR is required for regulating sda1 under oxidative conditions

Structural analysis of B. subtilis PerR (BsPerR) showed that BsPerR binds to a ferrous ion by the regulatory site, which is coordinated by three histidine (H37, H91, H93) and two
aspartate (D85, D104) residues (Figure 5A) [26,28]. Under oxidative conditions, residues H37 and H91 of BsPerR are oxidized, resulting in altering PerR protein structure and its DNA-binding activity [26,27]. A recent study showed that mutation of histidine residues (H6, H19, and H99) of the regulatory site in GAS PerR reduces the metal occupancy but not affect DNA-binding ability of the PerR protein [9]. Peroxide sensing by PerR in GAS also requires regulatory metal [9]. To further clarify that whether the peroxide sensing ability of PerR of GAS is required for regulating sda1 under oxidative conditions, the histidine residue (H99) of regulatory site in GAS PerR was mutated to alanine (PerR<sub>H99A</sub>, Figure 5A), and the expression of sda1 in the perR mutant (SW612), and SW612 complemented with the wild-type PerR or PerR<sub>H99A</sub> under normal and hydrogen peroxide treatment conditions were analyzed by real-time RT-PCR. Results showed that the expression of sda1 in both SW612 and the PerR<sub>H99A</sub> complemented strain were not responsive to the hydrogen peroxide treatment (Figure 5B). However, the increased expression of sda1 under oxidative conditions was found in the wild-type PerR complementation strain (Comp_PerR, Figure 5B).

**Discussion**

The peroxide response regulator (PerR) of GAS is important for bacterial survival in human blood, colonization of the pharynx, and resistance to phagocytic clearance, indicating that the PerR regulon is crucial for GAS to establish successful infections [3,10,11]. In the present study, we showed that PerR is required for the expression of phage-associated DNase sda1 under oxidative conditions. Sda1 is a DNase of GAS and has
roles in GAS escaping from immune recognition and clearance. The interaction between PerR and Sda1 may have important roles in GAS pathogenesis.

PerR is a metal-binding protein [27]. In Bacillus subtilis, a ferrous ion in the regulatory site of the PerR protein is involved in PerR protein conformation changes and DNA-binding activity under oxidative conditions [26,29]. In GAS, previous studies showed that PerR protein could bind to iron or manganese, and the DNA-binding activity of PerR is regulated by oxidative stress in vitro [5,9,21,30]. In the present study, EMSA analysis showed that rPerR binds to the Psda1 promoter region, suggesting PerR may directly interact with the putative Per box of the sda1 promoter (Figure 3). In addition, mutation of the histidine residue (H99) in the putative PerR regulatory site abolishes sda1 expression under oxidative conditions (Figure 5), suggesting that oxidative stress-sensing ability of PerR is crucial for sda1 expression. PerR generally is a repressor through directly binding to a target genes’ promoter [31,32]. Under oxidative conditions, the oxidized cations trigger the conformational changes in PerR, which involves decreasing PerR DNA-binding activity and derepression of target gene expression [27]. Although our results showed that PerR positively regulates sda1 expression under oxidative conditions in GAS, the molecular mechanism of this positive regulation is still unknown. In B. subtilis, PerR positively regulates srfA gene (encodes for surfactin) and is required for its expression [32]. Although the mechanism by which PerR activates srfA remains unclear, Hayashi et al (2005) suggested that PerR may interact with other regulator to induce the full activation of srfA expression [32]. In GAS, according to the transcriptome

Figure 2. Expression of DNase genes in wild-type (A20), perR mutant (SW612), and complementation strain (SW665) under oxidative conditions. Bacteria were grown in TSBY broth for 3 h and treated with 0.1, 0.3, and 0.5 mM of H2O2 for another 2 h. The expression of DNase genes, including (A) mf3, (B) spd, (C) sda1 and (D) spnA, were analyzed by real-time RT-PCR. Biological replicate experiments were performed from at least three independent RNA preparations in duplicate. Expression level of each target gene was normalized to gyrA. *: P < 0.05 to both A20 and SW665 in each culture condition; #: P < 0.05 to A20 and SW665 without H2O2 treatment, respectively.

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Grifantini et al (2011) suggests that the oxidation of PerR may not lead to its release from bound promoters in vivo [5]. In addition, protein structure analysis showed that GAS PerR possesses distinctive structural and amino acid features when compared to other Fur family proteins [9], suggesting that the molecular mechanism of PerR in GAS may be unique. Further studies of the interaction between PerR and the sda1 promoter may provide clues for understanding the mechanism of this positive transcriptional regulation in GAS.

Transcriptome analysis showed that PerR in GAS not only involves regulating ROS-detoxifying enzymes, but also coordinates DNA and protein metabolic functions, and DNA repair system activity, which may contribute to GAS survival in the host environment [5,11,30]. Adapting to the host environment and escaping from immune clearance are required for bacteria to establish successful infections. Previous studies showed that a perR mutant is more susceptible to phagocytic cell clearance [10], indicating that the PerR regulon participates in immune escape of GAS. In the present study, we found that PerR may involve in repression of spd expression under oxidative stress (Figure 2B), but the putative Per box could not be found in the spd promoter region. Under oxidative stress, the expression of mf3 was repressed in both the wild-type strain and perR mutant (Figure 2A), suggesting that PerR may not directly participate in mf3 regulation under oxidative conditions. Unlike mf3 and spd, the expression of sda1 under oxidative stress relies on the regulatory activity of PerR. Sda1, one of the most potent DNases in GAS, has been found to help GAS to escape from immune clearance by degrading neutrophil extracellular traps (NETs) [12,19]. These results suggest that GAS up-regulates sda1 expression through sensing peroxide signals by PerR, which may help GAS to escape from the NETs.

In summary, our results showed that the peroxide responsive activity of PerR may directly contribute to GAS expressing sda1 under oxidative stress. The molecular mechanism of the positive regulation of PerR on the sda1 promoter remains for further studies.
Figure 4. **Interaction between rPerR and the sda1 promoter.** (A) DNA-binding activity of rPerR to different regions of the sda1 promoter. rPerR protein was incubated with 50 ng of DNA probes (Pdpr and PSPy1840 as positive and negative control, respectively) and the DNA-binding activity was evaluated by the mobility shift of the protein-DNA complex in a 6% polyacrylamide gel. (B) Binding specificity of rPerR to Psda1 DNA probe. DIG-labeled Psda1 DNA probe was incubated with cold-specific probe (500, 1000, and 1500-fold of unlabeled Psda1 DNA probe, and 500 and 1000-fold of Pdpr DNA probe) or cold-nonspecific probe (Pspy1840 DNA probe). The protein-DNA complex in a 6% polyacrylamide gel was transferred to a membrane for visualizing the DIG signal.

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Figure 5. Expression of sda1 in a perR mutant (SW612) complemented with the wild-type perR (Comp_PerR) or regulatory metal-binding site mutated perR (Comp_PerR_{H99A}) under normal and H₂O₂ treatment conditions. Bacteria were grown in TSBY broth for 3 h and treated with/without 0.5 mM of H₂O₂ for another 2 h. The expression of sda1 in different strains was analyzed by real-time RT-PCR. (A) Alignment of B. subtilis and S. pyogenes PerR protein sequence. Amino acid residues involved in metal binding in B. subtilis are shown in bold. (B) Expression of sda1 in SW612, Comp_PerR, and Comp_PerR_{H99A} under normal and H₂O₂ treatment conditions. Biological replicate experiments were performed from at least three independent RNA preparations in duplicate. Expression level of each target gene was normalized to gyrA. *: P < 0.05.

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Supporting Information

Figure S1. Growth curve of the wild-type (A20), perR mutant (SW612), and complementation strain (SW665). (TIFF)

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References

1. Carapetis JR, Steer AC, Mulholland EK, Weber M (2005) The global burden of group A streptococcal diseases. Lancet Infect Dis 5: 685–694. doi:10.1016/S1473-3099(05)70267-X. PubMed: 16253886.

2. Cunningham MW (2000) Pathogenesis of group A streptococcal infections. Clin Microbiol Rev 13: 470–511. doi:10.1128/CMR.13.4.470-485.2000. PubMed: 10865988.

3. Ricci S, Janulczyk R, Björck L (2002) The regulator PerR is involved in oxidative stress response and iron homeostasis and is necessary for full virulence of Streptococcus pyogenes. Infect Immun 70: 4968–4976. doi:10.1128/IAI.70.9.4968-4976.2002. PubMed: 12183543.

4. Tsou CC, Chiang-Ni C, Lin YS, Chuang WJ, Lin MT et al. (2008) An iron-binding protein, Dpr, decreases hydrogen peroxide stress and protects Streptococcus pyogenes against multiple stresses. Infect Immun 76: 4038–4045. doi:10.1128/IAI.00477-08. PubMed: 18541682.

5. Chiang-Ni R, Toulon JG, Cola S, Lira S (2011) Peroxide stimulation and role of PerR in group A streptococcus. J Bacteriol 193: 6593–6551. doi:10.1128/JB.05924-11. PubMed: 21949080.

6. King KY, Horenstein JA, Caparon MG (2000) Aerotolerance and peroxide resistance in peroxidase and PerR mutants of Streptococcus pyogenes. Mol Microbiol 38: 5290–5299. doi:10.1046/j.1365-2958.2000.02929.x. PubMed: 10986229.

7. Benrot A, King KY, Caparon MG (2005) The PerR regulon in peroxide resistance and virulence of Streptococcus pyogenes. Mol Microbiol 55: 221–234. doi:10.1111/j.1365-2958.2004.04370.x. PubMed: 15612930.

8. Benrot A, Weston BF, Caparon MG (2007) A PerR-regulated metal transporter (PmrA) is an interface between oxidative stress and metal homeostasis in Streptococcus pyogenes. Mol Microbiol 63: 1185–1196. doi:10.1111/j.1365-2958.2006.05577.x. PubMed: 17238923.

9. Makhath N, Rastegari S, Sanson M, Ma Z, Olsen RJ et al. (2013) Crystal structure of peroxide stress regulator from Streptococcus pyogenes provides functional insights into the mechanism of oxidative stress sensing. J Biol Chem 288: 18311–18324. doi:10.1074/jbc.M113.465650. PubMed: 23645680.

10. Gryllos I, Grifantini R, Colaprico A, Gryllos I (2013) Crystal structure of the peroxide stress regulator peroxide stimulon repressor with operator. DNA - Mol Microbiol 41: 849–860. doi:10.1038/dmo.2012.162. PubMed: 23431444.

11. Le Breton Y, Mistry P, Valdes KM, Quigley J, Kumar N et al. (2013) Environmental pH changes, but not the LuxS signalling pathway, regulate SpeB expression in M1 group A streptococcus. J Med Microbiol 62: 39–49. doi:10.1099/jmm.0.05642-0. PubMed: 23849789.

12. Buchanan JT, Simpson AJ, Quigley J, Kumar N et al. (2013) Genome-wide identification of genes required for fitness of group A streptococcus in human blood. Infect Immun 81: 2863–2871. doi:10.1128/IAI.00223-13. PubMed: 23973818.

13. Sumby P, Porcella SF, Madrigal AG, Barbian KD, Virtanena K et al. (2005) Evolutionary origin and emergence of a highly successful clone of serotype M1 group A streptococcus involved multiple horizontal gene transfer events. J Infect Dis 192: 771–782. doi:10.1086/432514. PubMed: 16353918.

14. Chiang-Ni C, Zheng PX, Ho YM, Wu HM, Chuang WJ et al. (2009) Oxidative stress and metal ions regulate a ferritin-like gene, dpr, in Streptococcus pyogenes. Int J Med Microbiol 300: 259–264. doi:10.1016/j.ijmm.2009.09.002. PubMed: 19871989.

15. Jiang-Ni C, Zheng PX, Tsai PJ, Chuang WJ, Lin MT et al. (2010) PerR confers phagocytic killing resistance and allows pharyngeal colonization by group A streptococcus. PLoS Pathog 6: e1000145. doi:10.1371/journal.ppat.1000145. PubMed: 20710271.

16. Chiang-Ni C, Tsou CC, Lin YS, Chuang WJ, Lin MT et al. (2008) The transcriptional terminal sequences downstream of the covR gene terminate covR/S operon transcription to generate covR monocistronic transcripts in Streptococcus pyogenes. Gene 427: 99–103. doi:10.1016/j.gene.2008.08.025. PubMed: 18624088.

17. Lee JW, Heilmann JD (2006) The PerR transcription factor senses H2O2 by metal-catalyzed histidine oxidation. Nature 440: 363–367. doi:10.1038/nature04537. PubMed: 16541078.

18. Dubbs JM, Mongkolsum S (2012) Peroxide-sensing transcriptional regulators in bacteria. J Bacteriol 194: 5495–5503. doi:10.1128/JB.00304-12. PubMed: 22777554.

19. Jacquemaut L, Traoré DAK, Ferrer JL, Proux O, Testemale D et al. (2009) Structural characterization of the active form of PerR: insights into the metal-induced activation of PerR and Fur proteins for DNA binding. Mol Microbiol 73: 20–31. doi:10.1111/j.1365-2958.2009.06753.x. PubMed: 19508285.

20. Herbig AF, Helmann JD (2001) Roles of metal ions and hydrogen peroxide in modulating the interaction of the Bacillus subtilis PerR peroxide regulon repressor with operator. DNA - Mol Microbiol 41: 849–859. doi:10.1046/j.1365-2958.2001.02143.x. PubMed: 11116146.

21. Toukoki C, Gryllos I (2013) PolA1, a putative DNA polymerase I, is coexpressed with PerR and contributes to peroxide stress defenses of group A streptococcus. J Bacteriol 195: 717–725. doi:10.1128/JB.01947-12. PubMed: 23204686.

Author Contributions

Conceived and designed the experiments: CCN PXZ CHW HTK. Performed the experiments: CHW HTK CCT. Analyzed the data: CCN CHW HTK. Contributed reagents/materials/analysis tools: SW PJT WJC YSL CCL. Wrote the manuscript: CCN JJW.
31. Mongkolsuk S, Helmann JD (2002) Regulation of inducible peroxide stress responses. Mol Microbiol 45: 9–15. doi:10.1046/j.1365-2958.2002.03015.x. PubMed: 12100544.

32. Hayashi K, Ohsawa T, Kobayashi K, Ogasawara N, Ogura M (2005) The H$_2$O$_2$ stress-responsive regulator PerR positively regulates srfA expression in Bacillus subtilis. J Bacteriol 187: 6659–6667. doi: 10.1128/JB.187.19.6659-6667.2005. PubMed: 16166527.