H$_2$O$_2$ is an unavoidable cytotoxic by-product of aerobic life. Dpr, a recently discovered member of the Dps protein family, provides a means for catalase-negative bacteria to tolerate H$_2$O$_2$. Potentially, Dpr could bind free intracellular iron and thus inhibit the Fenton chemistry-catalyzed formation of toxic hydroxyl radicals (H$_2$O$_2$ + Fe$^{3+}$ → OH + ·OH + Fe$^{2+}$). We explored the in vivo function of Dpr in the catalase- and NADH peroxidase-negative pig and human pathogen Streptococcus suis. We show that: (i) a Dpr allelic exchange knockout mutant was hypersensitive (~10$^6$-fold) to H$_2$O$_2$, (ii) Dpr incorporated iron in vivo, (iii) a putative ferroxidase center was present in Dpr, (iv) single amino acid substitutions D74A or E78A to the putative ferroxidase center was present in Dpr, and (v) the H$_2$O$_2$ hypersensitive phenotype was complemented by wild-type Dpr or by a membrane-permeating iron chelator, but not by the site-mutated forms of Dpr. These results demonstrate that the putative ferroxidase center of Dpr is functionally active in iron incorporation and that the H$_2$O$_2$ resistance is mediated by Dpr in vivo by its iron binding activity.

Aerobic metabolism is essential for many living organisms for the production of energy. However, partially reduced forms of oxygen superoxide anion radical (O$_2$·$^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (·OH) are formed and able to cause serious damage to cellular macromolecules (1–3). Despite the lack of oxidative phosphorylation, H$_2$O$_2$ is produced by streptococci (4–7). In addition, H$_2$O$_2$ is encountered as a part of host defenses (8), and under certain conditions streptococci seem to utilize it as their own virulence factor (4, 9, 10). However, streptococci lack the H$_2$O$_2$-degrading enzyme catalase, which in many organisms is responsible for the elimination of H$_2$O$_2$. Identification and characterization of the components mediating H$_2$O$_2$ resistance in streptococci may eventually contribute to the development of means to prevent and treat streptococcal diseases.

Dpr (Dps-like peroxide resistance protein) is a recently discovered aerotolerance and H$_2$O$_2$ resistance factor of Streptococcus mutans (11). Although Dpr is widely conserved in other Gram-positive bacteria, including the important human pathogens Streptococcus pyogenes and Streptococcus pneumoniae (11), relatively little is known of the molecular mechanisms of its action. The primary amino acid sequence shares similarity with Escherichia coli Dps (DNA-binding protein from starved cells) (12, 13), a prototype for a large group of similar oligomeric proteins (14), which is abundantly expressed in starved cells and involved in DNA protection by DNA-Dps biocrystal formation (15, 16). Studies on Dps family members also indicate that the protective function of Dpr against H$_2$O$_2$ might be mediated by H$_2$O$_2$ degradation due to a catalase-like activity (14) or by chelation of free intracellular iron (17).

It is known that toxicity of H$_2$O$_2$ is relatively weak (3), although it easily diffuses across biological membranes (18, 19) and oxidizes thiols (3). However, if reduced transition metal ions, especially iron, are present, H$_2$O$_2$ is nonenzymatically cleaved into highly toxic hydroxyl radicals by Fenton chemistry (H$_2$O$_2$ + Fe$^{3+}$ → OH + ·OH + Fe$^{2+}$) (20, 21). Dps family members share a conserved amino acid motif, similar to mammalian ferritin 1-subunit iron nucleation center, in the N-terminal halves of the proteins (14, 22–24). Because several members, including the streptococcal Dpr (11, 25), are known to bind iron (17, 22, 26, 27), the conserved motif is believed to serve a functional role. It has been suggested that the motif catalyzes iron oxidation by its putative ferroxidase activity and also directs the formation of an iron core into the inner cavity of the oligomer (17, 22, 28, 29). However, there is no direct experimental evidence for any of the Dps family members to support the functionality of the putative ferroxidase center in iron incorporation in vivo. Furthermore, the biological significance of iron incorporation is not well established.

Streptococcus suis is an important pig pathogen that causes severe infections such as sepsis and meningitis, and it occasionally causes life-threatening disease also in humans (30). We have previously identified in S. suis a galactose-specific adhesion activity (31–33). One of the proteins identified as a candidate adhesin displaying binding activity to glycoproteins had a 64% primary amino acid sequence identity with S. mutans Dpr. S. suis seemed an ideal model organism to study the in vivo function of Dpr in H$_2$O$_2$ resistance because it not only lacks catalase but also lacks NADH peroxidase (34). The results of the present study demonstrate that the putative ferroxidase center of Dpr is involved in iron incorporation and that the H$_2$O$_2$ resistance mediated by Dpr depends on its iron incorporation activity in vivo.
Molecular Basis of Dpr-mediated H₂O₂ Resistance

**Table I**

| Strains and plasmids | Description, antibiotic resistance (reference or source; otherwise this study) |
|----------------------|--------------------------------------------------------------------------------|
| **S. suis**          |                                                                                |
| 628                  | Wild-type strain of serotype 2, isolated from human brain (31)                  |
| D282                 | Wild-type strain of serotype 2, isolated from a pig with clinical signs of meningitis (35) |
| D282Δdpr             | Allelic replacement dpr knockout mutant; spc                                  |
| D282LA                | D282Δdpr plus pLZ12-L4A; sac, kan                                             |
| D282P3               | D282Δdpr plus pLZ12-P3; sac, kan                                              |
| D282LA-74            | D282Δdpr plus pLZ12-L4A-74; sac, kan                                         |
| D282LA-78            | D282Δdpr plus pLZ12-L4A-78; sac, kan                                         |
| **E. coli**          |                                                                                |
| DH5α                 | General-purpose host strain for cloning (laboratory stock)                     |
| Plasmids             |                                                                                |
| pBR322               | E. coli low copy cloning vector; amp, tet (New England Biolabs)               |
| pLZ12                | Streptococcal suicide vector; cat (36)                                         |
| pKUN19-Spc           | Streptococcal suicide vector; amp, sac (37)                                    |
| plZ12-Km             | E. coli/Streptococcus sp. shuttle vector; kan (Michael Caparon)               |
| plZ12-Km/Spc         | pLZ12-Km with sac cassette from pKUN19-Spc; sac, kan                          |
| pBR6500              | 6.5 kb dpr locus of strain 628 cloned into pBR322, amp                        |
| pDPR1                | 2.6 kb dpr containing fragment of strain 628 cloned into pD700; cat           |
| pDPR2                | pDPR1 with dpr replaced by sac from pKUN19-Spc; cat, sac                     |
| plZ12-L4A            | dpr cloned into Km site of sac in pLZ12-Km/Spc (see Fig. 2); kan              |
| plZ12-P3             | dpr cloned into Km site of sac in pLZ12-Km/Sac (see Fig. 2); kan              |
| plZ12-L4A-74         | pLZ12-L4A with dpr codon GAT encoding Asp-74 mutated to GCA (Ala); kan       |
| plZ12-L4A-78         | pLZ12-L4A with dpr codon GAG encoding Glu-78 mutated to GCA (Ala); kan        |

**DNA Techniques**

Genomic DNA of *S. suis* was isolated as described previously (38). Standard protocols were used for PCR, DNA modification, cloning, *E. coli* transformation, and Southern and colony hybridization as described by Sambrook and Russel (39). DNA-modifying enzymes were purchased from Promega and Fermentas, and Vent DNA polymerase was purchased from New England Biolabs. DNA molecular mass markers were from Promega. Plasmids amplified in *E. coli* DH5α were isolated using QIAprep Spin Miniprep Kit (Qiagen) as described by the manufacturer. DNA fragments were purified from agarose gels using QIAquick Gel Extraction Kit or from PCR and other enzymatic reactions using QIAquick PCR Purification Kit as described by the manufacturer (Qiagen). DNA sequences were determined by ABI Prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit (PerkinElmer Life Sciences) with AmpliTaq DNA Polymerase FS (Roche Molecular Biochemicals). Sequencing primers were purchased from Interactive Biotechnologie GmbH. For Southern, Northern, and colony hybridization, radioactive DNA probes were labeled with [α-32P]CTP (Amersham Biosciences) using Prime-a-Gene Labeling System (Promega) according to the instructions of the manufacturer. Templates for labeling were generated by PCR using primers DPR-5'-IN and DPR-3'-IN specific for dpr and 168-5' and 168-3' specific for 16S rRNA genes and gel-isolated before labeling. The hybridized membranes were analyzed with Fuji-film BA-2500 Phosphor Imaging Plate System (Fuji Photo Film Co.) according to the instructions of the manufacturer.

**EXPERIMENTAL PROCEDURES**

**Bacterial Strains, Plasmids, and Primers**

The bacterial strains, plasmids, and primers used in this study are listed in Tables I and II. *S. suis* was cultured aerobically or under 6% CO₂ at 37 °C with or without agitation in Todd Hewitt Broth medium (DIFCO) supplemented with 0.5% (v/v) yeast extract (Biokar Diagnostics) (THY). *E. coli* was grown aerobically at 37 °C with agitation in Luria-Bertani medium. When needed, media were solidified by 1.5% agar. All bacteria were stored at −70 °C in growth media containing 15% (v/v) glycerol. Antibiotics (Sigma) were used at the following concentrations unless otherwise indicated: (i) *E. coli*, 100 μg/ml ampicillin, 30 μg/ml kanamycin, 100 μg/ml spectinomycin, and 30 μg/ml chloramphenicol; and (ii) *S. suis*, 20 μg/ml ampicillin, 500 μg/ml kanamycin, 1000 μg/ml spectinomycin, and 10 μg/ml chloramphenicol.

**Cloning and Sequence Analysis of the dpr Locus**

Southern hybridization was used to identify a 6.5-kb ClaI-EcoRI genomic fragment that seemed to contain the entire dpr from *S. suis* serotype 2 strain 628 (data not shown). To clone the 6.5-kb fragment, we ligated gel-isolated, ClaI- and EcoRI-digested DNA fragments ranging from 6 to 7 kb into EcoRI-NarI-digested pBR322, electrotornformed the digestion mixture into *E. coli* DH5α cells, and screened the transformants by colony hybridization. One hybridizing clone, designated pDPR6500, was isolated and sequenced. Sequencing data were assembled, and the consensus sequence was edited using the MAC DNAAsis software (Hitachi). The web-based program ORF Finder (www.ncbi.nlm.nih.gov/orf) was used to predict the coding regions. The BLAST software package at the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) was used to search for protein sequences homologous to the deduced amino acid sequences. Promoter sequence features were searched using WWW Signal Scan (bimas. dcrth.nih.gov/molbio/signal).

**Electrotornformation of *S. suis***

Overnight *S. suis* cultures were diluted 100-fold into 50 ml of fresh THY supplemented with 30 mm glycine. Cultures were incubated at 37 °C with slight agitation to an *A* of 0.2. Cells were harvested by centrifugation (2000 × g, 20 min, 4 °C) and washed twice with 10 ml of ice-cold 0.5 M sucrose (2000 × g, 20 min, 4 °C) and once with 10 ml of ice-cold 0.5 M sucrose supplemented with 15% (v/v) glycerol (2000 × g, 20 min, 4 °C). The cells were resuspended in 50 μl of ice-cold 0.5 M sucrose supplemented with 15% glycerol (v/v) and were either used directly or stored at −70 °C. Electrotormformations were done using the Gene Pulser II Electroporation System (Bio-Rad). 1 μg of suicide vector or 100 ng of shuttle vector was mixed with 50 μl of the electrocompetent cells on ice. The mixtures were transferred into prechilled sterile Gene Pulser cuvettes (inter electrode distance, 0.1 cm; Bio-Rad) and pulsed with a setting of 70 microfarads, 1.8 kV, and 200 ohms. After the electric pulse, the cells were diluted in 1 ml of THY supplemented with 0.3 M sucrose and incubated for 2 h at 37 °C under 6% CO₂. The cells were then plated on THY agar containing the appropriate antibiotics. The cells routinely yielded 10⁶ transformants/μg shuttle vector.

**Isolation of Total RNA and Northern Hybridization**

For extraction of total RNA, overnight *S. suis* cultures were diluted 100-fold into fresh THY and grown at 37 °C with vigorous shaking. At various levels of turbidity, 1-ml aliquots were taken from the cultures. The cells were harvested by centrifugation (15,000 × g, 2 min, 4 °C), immediately snap-frozen in liquid N₂, and stored at −70 °C. The cells were later thawed, and total RNA was extracted using the RNeasy Mini kit (Qiagen) according to instructions of the manufacturer. 20 μg of RNA was electrophoresed on a 1% agarose gel containing 2.2 M form-
aldehyde. The RNA Ladder (New England Biolabs) served as a molecular mass marker. After electrophoresis, the RNA was transferred to a Protran Nitrocellulose Transfer Membrane (Schleicher & Schuell) using the 2117 Multiphor II Electrophoresis Unit (LKB). The membranes were saturated with 3% (w/v) BSA and 0.1% (v/v) Tween 20 in P/NaCl at room temperature for 1 h. The anti-Dpr polyclonal antibodies were diluted 1:10,000 in 1% (w/v) BSA and 0.05% (v/v) Tween 20 in P/NaCl, and the membranes were incubated at room temperature for 30 min. After washing with P/NaCl, the membranes were incubated at room temperature for 30 min in 1% BSA (w/v) and 0.05% (v/v) Tween 20 in P/NaCl containing a 1:10,000 dilution of peroxidase-conjugated goat anti-rabbit immunoglobulins (Dako). After washing with P/NaCl, the ECL chemiluminescence detection kit (Amersham Biosciences) was used to detect the binding according to instructions of the manufacturer.

Preparation of Cellular Protein Extracts of S. suis

Overnight S. suis cultures were diluted 100-fold into fresh THY and grown at 37 °C with vigorous shaking. At various levels of turbidity, cells were harvested by centrifugation (2000 × g, 20 min, 4 °C), washed with P/NaCl (10 mM sodium phosphate buffer and 0.15 mM NaCl (pH 7.4)) (2000 × g, 20 min, 4 °C), and resuspended in 750 μL of P/NaCl. The cells were disrupted by sonication five times (20 s each time), with a chilling interval of 1 min between the sonications. EDTA-free Protease Inhibitor Mixture Tablets (Roche Molecular Biochemicals) were added to the sonicates according to instructions of the manufacturer. After unbroken cells and cell debris were removed by centrifugation (15,000 × g, 30 min, 4 °C), the cleared lysate was collected and stored at 4 °C. Protein concentrations were determined at least in triplicate using the Bio-Rad Protein Assay, based on the Bradford dye binding procedure (40), using bovine serum albumin (BSA) (Sigma) as a standard.

Preparation of Polyclonal Antibodies Specific for Dpr

Expression and purification of recombinant Dpr have been described previously (41). Two rabbits were immunized subcutaneously with 1.0 ml of a mixture of the recombinant Dpr protein and Freund's complete adjuvant (1:1, v/v). Booster injections with the same protein mixture in Freund's incomplete adjuvant were given at 28 and 56 days, and sera were collected 14 days after each immunization. The sera collected 14 days after the last booster had the highest activity against Dpr and were used for subsequent work.

Western Analysis of Dpr Expression

For Western blotting, the proteins were resolved under denaturing conditions in a 12% polyacrylamide gel or under nondenaturing conditions in a 4–15% gradient Tris-HCl Ready Gel (Bio-Rad) using SDS-PAGE Low Range (Bio-Rad) or equine spleen type I ferritin (Sigma) and BSA as the molecular mass markers, respectively. Proteins were subsequently transferred to a Protran Nitrocellulose Transfer Membrane (Schleicher & Schuell) using the 2117 Multiphor II Electrophoresis Unit (LKB). The membranes were saturated with 3% (w/v) BSA and 0.1% (v/v) Tween 20 in P/NaCl at room temperature for 1 h. The anti-Dpr polyclonal antibodies were diluted 1:10,000 in 1% (w/v) BSA and 0.05% (v/v) Tween 20 in P/NaCl, and the membranes were incubated at room temperature for 30 min. After washing with P/NaCl, the membranes were incubated at room temperature for 30 min in 1% BSA (w/v) and 0.05% (v/v) Tween 20 in P/NaCl containing a 1:10,000 dilution of peroxidase-conjugated goat anti-rabbit immunoglobulins (Dako). After washing with P/NaCl, the ECL chemiluminescence detection kit (Amersham Biosciences) was used to detect the binding according to instructions of the manufacturer.

Inactivation of dpr in S. suis

We generated a genetically stable deletion mutant (D282Δdpr) of the dpr by adopting the double cross-over method.

Construction of the Suicide Vector pDPDR2—A 2.8-kb fragment around the dpr was amplified by PCR using primers TOT-5′ and TOT-3′. The resulting PCR product was digested with KpnI and KpnI, and the resulting 2.6-kb fragment was cloned into the KpnI-BamHI site of pID700 to generate pDPDR1. The dpr was deleted from the pDPDR1 by PCR with primers DEL-5′ and DEL-3′ and replaced by a spectinomycin resistance gene (spc) devoid of terminator generated by PCR with primers SPSC and SPFA using pKUN19-Spc as the template. The resulting plasmid μl was analyzed by restriction enzyme digestions and PCR to contain the spc in the same direction of transcription as the dpr.

Generation of the Mutant—Because strain 628 has been poorly transformable in our hands, we used D282 cells of same serotype and with identical dpr sequence as strain 628, and colonies with the single cross-over genotype (ChlR, SpcR) were selected. One ChlR and SpcR colony was chosen, and the double cross-over genotype (Spca) was selected as follows. Single cross-over mutant was first grown overnight with spectinomycin (1000 μg/ml) allowing the vector to excise out of the genome, leaving the spc in the place of the dpr. Resulting double cross-over mutants were enriched by using the bacteriostatic activity of chloramphenicol (10 μg/ml) and at the same time killing the dividing bacteria (still having the vector insertion) by using ampicillin (20 μg/ml). The enriched bacteria were plated on THY with spectinomycin, and the double cross-over genotype (Spca) was verified by replica plating. The replacement of dpr by spc was further verified by PCR with primers Dpr-5′-OUT and Dpr-3′-OUT annealing upstream and downstream of dpr, respectively, and by Western analysis.

Ectopic Expression of Dpr in D282Δdpr

The dpr mutation was complemented by introducing wild-type dpr into D282Δdpr in an E. coli/Streptococcus sp. shuttle vector pLZ12-Km/Sp, pLZ12-KmSp was generated by ligating the spc generated by PCR with primers SPSC and SPFA using pKUN19-Spc as the template to Ncol/EcoRI-digested plasmid pLZ12-Km. Two dpr-containing DNA fragments, LaA and P3, were amplified by PCR using primers 5′-DCOMP and 3′-COMP or 5′-UPCOMP and 3′-COMP, respectively. These DNA fragments were digested with KpnI and ligated into KpnI-digested and dephosphorylated plasmid pLZ12-Km/Sp to generate the plasmid constructs pLZ12-LaA and pLZ12-P3, respectively. In these constructs, dpr transcription is under the control of spc promoter or the possible promoter activity of dpr upstream sequence. The constructs were sequenced to ensure that no mistakes were introduced into dpr during the amplification and introduced into D282Δdpr, and complementation genotype (Spca, KanR) was selected on agar plates. The expression of Dpr was verified by Western analysis.

Site-directed PCR Mutagenesis

Cods encoding Asp-74 (GAT) and Glu-78 (GAG) were independently mutated into GCA (Ala) in the pLZ12-LaA, resulting in plasmids

| Primer | Sequence 5′→3′ with KpnI restriction sites underlined and mutagenic sites in bold | Target gene or locus (NCBI Nucleotide Database accession number) and annealing position |
|--------|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|
| 16S-5′ | GCATAAACGTCGAGCCGACAG                                                          | 16S rRNA (X59021) 409→427                                                        |
| 16S-3′ | TCAGCAGAGGTCGACGAG                                                             | 16S rRNA (X59021) 982→964                                                        |
| DPR-5′-IN | CAGCAGAATTTGCGCTTTC          | dpr (AY154459) 2969→2979                                                       |
| DPR-3′-IN | TTAGTTGGTCAAGACTAAGTCA          | dpr (AY154459) 5430→5451                                                        |
| TOT-5′ | TCAAGTATGGAGCGGTTGGAGAG          | dpr locus (AY154459) 1712→1730                                                  |
| TOT-3′ | TTTTGTGACCCCACTCTGATGCCAAG         | dpr locus (AY154459) 4530→4551                                                  |
| DEL-5′ | ATATGCCCTTCTTTTTCATTATTA          | dpr locus (AY154459) 2931→2951                                                  |
| DEL-3′ | ATAGATGGAGGTCGACACAG              | dpr locus (AY154459) 3451→3469                                                  |
| SPCS | TCAGTTATGCTCTGGTAA             | spc (M69221) 2→19                                                               |
| SPFA | TTATAAATTTTTTTTTAATC          | spc (M69221) 1038→1021                                                          |
| SPCB | CATATGCAAGGTTTATTATTCA          | spc (M69221) 1158→1170                                                          |
| DPR-5′-OUT | CAGAGGAGGGAAGATTTATCC          | dpr locus (AY154459) 2856→2875                                                  |
| DPR-3′-OUT | GAAGAGATCTCAGGTATTAC          | dpr locus (AY154459) 3561→3540                                                  |
| 5′-UPCOMP | TTTTGTGACCCCACTCTGATGCCAAG         | dpr locus (AY154459) 2843→2863                                                  |
| 5′-DCOMP | TTTTGTGACCCCACTCTGATGCCAAG       | dpr locus (AY154459) 2913→2933                                                  |
| 3′-COMP | TTTTGTGACCCCACTCTGATGCCAAG       | dpr locus (AY154459) 3563→3543                                                  |
| DPR-AspA | TATTAGGAGGTCGACGAG              | dpr locus (AY154459) 3145→3171                                                  |
| DPR-NAT-3′A | ACCATATTTTCTTTAATGATTAC         | dpr (AY154459) 3144→3118                                                       |
| DPR-GluB | AGTCAACGTTAATACCTTATTAG         | dpr (AY154459) 3160→3183                                                       |
| DPR-NAT-3′B | CATATGCAAGGTTTATTATTCA          | dpr (AY154459) 3158→3133                                                       |

Table II

Oligonucleotide primers
Molecular Basis of Dpr-mediated H₂O₂ Resistance

RESULTS

General Features of S. suis dpr Locus—We have previously identified in S. suis a galactose-specific adhesion activity (31–33) and purified a candidate adhesin (42). Recently, the corresponding gene was cloned, based on peptide sequence data of the purified protein and by genome walking.1 The gene turned out to encode a protein with 64% identity and 82% similarity to S. mutans Dpr (NCBI Protein Database accession number BA964742) at the level of primary amino acid sequence. In the present study, we identified by Southern hybridization and subsequently cloned and sequenced a 6.5-kb Clal-EcoRI fragment containing the entire dpr. As shown in Fig. 1A, we identified eight possible ORFs in addition to dpr, some of which were in the same direction of transcription. This indicated that dpr might be transcribed in a multicistronic mRNA. To study this possibility, we first analyzed the upstream region of dpr for sequence features commonly associated with bacterial promoters. As shown in Fig. 2, we found a possible 5′-factor −10 recognition sequence TATAAT and a −35 recognition sequence TTGACA between ORFI and dpr. A putative Shine-Dalgarno box was found a few nucleotides upstream of the initiation codon. Northern hybridization demonstrated that dpr is transcribed monocistronically with an approximate mRNA size of 550 bp (Fig. 1B). Thus, there seems to be a functional promoter between ORFI and dpr possibly including the sequence features found in this study (Fig. 2). However, further work including primer extension analysis is needed to characterize the putative dpr promoter.

Inactivation and Complementation of dpr in S. suis—The sequence information of the 6.5-kb Clal-EcoRI fragment allowed us to construct a suicide plasmid pDPR2 and subsequently generate a dpr knockout mutant (D282Δdpr) using allelic replacement (Fig. 3A). PCR analysis with primers DPR-5′-OUT and DPR-3′-OUT annealing upstream and downstream of dpr, respectively, confirmed the replacement of dpr by spc by homologous recombination (Fig. 3B). This was further verified by Western analysis (Fig. 3C). The dpr mutation was complemented by introducing wild-type dpr into D282Δdpr in an E. coli/Streptococcus sp. shuttle vector pLZ12-Km/Spc (Fig. 2). In pLZ12-L4A, dpr was inserted into spc containing only its putative Shine-Dalgarno box, leading to a Dpr expression level comparable with that of the wild-type strain D282 at the early-stationary phase (Fig. 3C). In pLZ12-P3, dpr was inserted into spc with its own putative promoter and Shine-Dalgarno box, leading to overexpression of Dpr at the early-stationary phase (Fig. 3C). The overexpression might have resulted from better stability of pLZ12-P3 or from inclusion of the putative dpr promoter. In any case, the complementation strains constructed allowed us to analyze the function of Dpr with two cellular levels of the protein. Both of the constructed plasmids pLZ12-L4A and pLZ12-P3 induced a constant Dpr expression level in early-stationary phase as analyzed by Western blotting of several independent protein extracts.

Role of Dpr in Aerotolerance of S. suis—Dpr is an important aerotolerance factor for S. mutans (11). To study whether Dpr is involved in aerotolerance of S. suis, we analyzed the growth of our strains in solid and liquid THY incubated under normal or 6% CO₂ atmosphere. At the same time, we quantified dpr mRNA and Dpr levels at different time points of the growth phase. We first grew D282 and D282Δdpr to early-stationary phase in THY at 37 °C under 6% CO₂. The cultures were then diluted 100-fold into fresh THY and incubated at 37 °C with vigorous shaking, which causes an extensive aeration of the...
culture medium. The growth was monitored by measuring A_{600} (Fig. 4A). As shown in Fig. 4B, Dpr expression was transcriptionally induced just after the culture entered the actively growing state. The transcriptional activity sharply decreased when the bacterial population reached the stationary phase. The protein levels, on the other hand, reached the maximum in the actively growing state and remained relatively unchanged at the stationary phase (Fig. 4C). Thus, wild-type D282 cells clearly expressed Dpr during the growth period assayed. Yet, as shown in Fig. 4A, there was no significant growth retardation of D282Δdpr as compared with D282. As analyzed by plating of early-stationary phase cultures, there were no significant differences between the colony forming abilities of D282Δdpr and D282, regardless of whether the THY plates were incubated aerobically or under 6% CO_{2} (data not shown). Taken together, the results indicate that Dpr is not an essential aerotolerance factor for S. suis.

Role of Dpr in H\textsubscript{2}O\textsubscript{2}, Resistance of S. suis—Dpr is involved in H\textsubscript{2}O\textsubscript{2} resistance of S. mutans (11). Sensitivities of our S. suis strains to H\textsubscript{2}O\textsubscript{2} were tested by exposing early-stationary phase cultures to different concentrations of H\textsubscript{2}O\textsubscript{2} and counting viable cells after plating onto THY. Fig. 5 shows that over the whole range of H\textsubscript{2}O\textsubscript{2} concentrations used, there was only a slight loss of viability of the wild-type strain D282. In contrast, D282Δdpr was highly sensitive toward H\textsubscript{2}O\textsubscript{2} with a ∼10^{6}-fold reduced viability after exposure to 5.0 mM H\textsubscript{2}O\textsubscript{2}. To examine the possibility that the H\textsubscript{2}O\textsubscript{2} hypersensitivity of D282Δdpr was not due to its Dpr deficiency but rather was caused by polar effects of the spc insertion, genetic complementation analyses were done. These experiments were important because D282 was used for mutant construction due to poor transformability of 628, which served as the initial source of sequence information (Fig. 1A). Also, ORFV with a predicted gene product of 16.2 kDa (Fig. 1A; Table III) was in the complementary strand to Dpr and was also deleted in D282Δdpr. Fig. 5 shows that over the whole range of H\textsubscript{2}O\textsubscript{2} concentrations used, both of the complementation strains were resistant to H\textsubscript{2}O\textsubscript{2}. This confirmed that Dpr was responsible for the detected H\textsubscript{2}O\textsubscript{2} resistance in S. suis and ruled out the participation of ORFV and other downstream effects. Overexpression of Dpr in D282P3 (Fig. 3C) did not significantly increase the ability of bacteria to tolerate H\textsubscript{2}O\textsubscript{2} (Fig. 5).

Iron Chelator Complementation of the H\textsubscript{2}O\textsubscript{2} Hypersensitivity of D282Δdpr—Free intracellular Fe^{2+} nonenzymatically cleaves H\textsubscript{2}O\textsubscript{2} into hydroxyl radicals in Fenton chemistry fashion and is important for the actual toxicity of H\textsubscript{2}O\textsubscript{2} (20, 21). We analyzed how iron chelators with different membrane permeabilities altered the H\textsubscript{2}O\textsubscript{2} hypersensitivity of D282Δdpr. The hydrophilic iron chelator DFOM, a siderophore produced by Streptomyces pilosus, has been used in several studies to interfere with the intracellular iron pool of both prokaryotic and eukaryotic cells (43–46). DTPA, on the other hand, has been used as an extracellular iron chelator (46). The effects of these compounds on the H\textsubscript{2}O\textsubscript{2} hypersensitivity of D282Δdpr were assayed by exposing early-stationary phase cultures to 5.0 mM H\textsubscript{2}O\textsubscript{2} after preincubating the bacteria with different concentrations of the chelators. As shown in Fig. 6 the H\textsubscript{2}O\textsubscript{2} hypersensitivity of D282Δdpr was complemented by a preincubation with 100 μM DFOM. In contrast, DTPA did not complement the H\textsubscript{2}O\textsubscript{2} hypersensitivity at any of the concentrations studied.
The results indicate that the intracellular but not the extracellular iron pool is involved in H\textsubscript{2}O\textsubscript{2} sensitivity of the bacteria and that the absence of functional Dpr can be complemented by a molecule capable of iron chelation.

**Site-directed Mutagenesis of the Putative Ferroxidase Center of Dpr**—Amino acid sequence alignment of Dps family members with known crystal structures revealed that *S. suis* Dpr contained a putative ferroxidase center in its N-terminal half (Fig. 7). It has been suggested that this amino acid motif is involved in iron incorporation in a fashion similar to that of classical ferritins (22, 27, 28, 47), but there is no direct experimental evidence to support this proposal in vivo. When we analyzed cellular protein extracts from early-stationary phase cultures grown in the presence of \textsuperscript{55}FeCl\textsubscript{3}, Dpr had clearly incorporated iron (Fig. 8). To investigate the possible relationship of the in vivo iron incorporation activity with the putative ferroxidase center, we utilized site-directed mutagenesis. The negatively charged residues Asp-74 and Glu-78 of the putative ferroxidase center (Fig. 7) were independently substituted with Ala. As shown in Fig. 8A, the mutations had no apparent effects on the level of expression or solubility of Dpr. The mutations also seemed to have no effects on the oligomeric stability of Dpr as indicated by similar mobility to wild-type Dpr in nondenaturing polyacrylamide gels (Fig. 8B). In contrast, the mutations independently caused a complete inactivation of the iron incorporation activity in vivo (Fig. 8C). Taken together, *S. suis* Dpr was able to incorporate iron in vivo, and this process was dependent on Asp-74 and Glu-78, the amino acids conserved in the putative ferroxidase centers of several Dps family members (Fig. 7) (14, 22, 27, 28, 47).

**H\textsubscript{2}O\textsubscript{2} Sensitivities of D282\textsuperscript{Δdpr} Expressing Wild-type or Site-mutated Forms of Dpr**—The H\textsubscript{2}O\textsubscript{2} sensitivities of D282\textsuperscript{Δdpr} expressing wild-type or site-mutated forms of Dpr were assayed by exposing early-stationary phase cultures to different concentrations of H\textsubscript{2}O\textsubscript{2} and counting viable cells after plating onto THY. As shown in Fig. 9 bacteria expressing wild-type Dpr were fully resistant to H\textsubscript{2}O\textsubscript{2}. In contrast, bacteria expressing the site-mutated and iron incorporation-negative forms of Dpr were hypersensitive to H\textsubscript{2}O\textsubscript{2} with a comparable phenotype to dpr knockout. Thus, the detected H\textsubscript{2}O\textsubscript{2} resistance of *S. suis* was not only critically dependent on Dpr but specifically on its iron incorporation activity.
Molecular Basis of Dpr-mediated H₂O₂ Resistance

Table III

| ORF | Nucleotide position<sup>a</sup> | No. of amino acids/kDa | Protein name/NCBI Protein Database accession number | Organism | % Identity/similarity (length)<sup>b</sup> | Function(s) |
|-----|-------------------------------|------------------------|----------------------------------------------------|----------|----------------------------------------|-------------|
| I   | 2465–2767                     | 100/10.8               | No significant similarity found                    | S. suis  | 100/100 (172)                          | Putative H₂O₂ resistance factor (34) |
| II  | 2932–3450                     | 172/19.6               | Dpr/AAG33871                                       | S. suis  | 59/74 (289)                            | ATPase component of a putative ABC transporter |
| III | 4686–5417                     | 243/27.2               | ABC-NBD/AAAL00620                                  | S. pneumoniae | 41/65 (239)                        | Membrane-associated component of a putative ABC transporter |
| IV  | 5456–6289                     | 277/31.9               | AAL00618                                           | S. pneumoniae | 34/54 (248)                        | Sensor histidine kinase of a putative two-component system |
| V   | 3594–3178                     | 138/16.2               | No significant similarity found                    | S. suis  | 65/85 (139)                            | Hypothetical protein |
| VI  | 2423–1914                     | 169/19.7               | AAR76093                                           | S. pneumoniae | 46/66 (119)                        | Putative phosphotyrosine protein phosphatase |
| VII | 1782–1360                     | 140/15.6               | AAM75641                                           | S. pyogenes | 45/60 (396)                         | Hypothetical protein |
| VIII| 1370–138                     | 410/48.1               | AAM98960                                           | S. agalactiae | 136/140 (15.6)                     | Putative acyltransferase |

<sup>a</sup> By sequence identity at amino level according to BLAST searches on Sept. 8, 2002.

<sup>b</sup> In accordance to the coding direction.

<sup>c</sup> The length of the alignment in amino acids.

**Figure 6.** Effects of iron chelators on the H₂O₂ hypersensitivity of D282Δdpr. D282Δdpr, grown to early-stationary phase in THY, was stressed with or without 5.0 mM H₂O₂ for 2 h at 37 °C after preincubating the bacteria for 30 min at 37 °C in the presence of different concentrations of the chelators as indicated. The cell viabilities were counted by plating to THY. DFOM/H11001, DTPA/H11001, DTPA/H11001, DTPA/H11001, DTPA/H11001, DTPA/H11001, DTPA/H11001, DTPA/H11001.

**Discussion**

In this paper, we provide for the first time direct in vivo evidence on how streptococcal Dpr mediates its protective function against H₂O₂. H₂O₂ resistance is a crucial property for streptococci because several species produce it as a part of their metabolism. In addition, streptococci seem to utilize it as their own virulence factor (4, 9, 10). Thus, in contrast to normal cellular physiology, Dpr may also have a role in the pathogenesis of streptococcal infections. S. suis seemed to be an ideal model organism to study the in vivo function of Dpr because it not only lacks catalase, like other streptococci, but also lacks NADH peroxidase (34). This enzyme is capable, to some extent, of substituting for the absence of catalase (48, 49).

It is known that defects in the regulation of intracellular iron homeostasis may lead to enhanced oxidative stress (46, 50, 51). Iron in its reduced form nonenzymatically cleaves H₂O₂ into hydroxyl radicals, the most deleterious forms of reactive oxygen intermediates, by Fenton chemistry (H₂O₂ + Fe²⁺ → ·OH + ·OH + Fe³⁺) (20, 21, 52). Based on a primary amino acid sequence comparison, Dpr has been reported to be a member of the Dps protein family (11), in which one of the functional features is iron binding activity (17, 22, 26, 27). The family members seem to be able to oxidize Fe²⁺ and store it inside the oligomeric protein shell as Fe³⁺ (17, 29, 53, 54), resembling classical ferritins in this respect (23). Indeed, Bozzi and co-workers (22) identified in L. innocua Flp, a member of the Dps family that is well characterized in vitro (53, 54), a homologous region to the iron nucleation center of mammalian ferritin L-subunit (24). They suggested that this region could carry out the initial steps in iron core formation and also serve as a ferroxidase center. Although a homologous region to this amino acid motif can be found in several Dps family members (Fig. 7) (14), no direct experimental evidence is available on its functionality in iron incorporation in vivo. Furthermore, the biological significance of iron incorporation is not well established. In the present study, a putative ferroxidase center was identified in the N-terminal half of S. suis Dpr, and the structure-function relationship was studied by site-directed mutagenesis.

The targets for the point mutations in the putative ferroxidase center were chosen by using the three-dimensional structure of L. innocua Flp (28). Flp shares 44% primary amino acid sequence identity with S. suis Dpr. In Flp crystals, 12 iron atoms have been directly observed occupying the putative ferroxidase centers, which are formed at the interfaces of two adjacent subunits (28). The amino acids protruding into the interface of Flp dodecamer and seemingly involved in iron incorporation in vivo. Furthermore, the biological significance of iron incorporation is not well established. In the present study, a putative ferroxidase center was identified in L. innocua Flp (28). Flp shares 44% primary amino acid sequence identity with S. suis Dpr. In Flp crystals, 12 iron atoms have been directly observed occupying the putative ferroxidase centers, which are formed at the interfaces of two adjacent subunits (28). The amino acids protruding into the interface of Flp dodecamer and seemingly involved in the actual iron coordination (His-31, His-43, Asp-47, Asp-58, and Glu-62) (28) are all conserved and similarly spaced in S. suis Dpr as His-47, His-59, Asp-63, Asp-74, and Glu-78, respectively (Fig. 7). The involvement of these amino acids in forming a classical dinuclear ferritin-like ferroxidase center has been modeled for Flp (28). In the initial step, the first ferrous iron, guided into the interface of the dodecamer through hydrophilic channels, would bind to His-31 from the N-terminal half of S. suis Dpr, and the structure-function relationship was studied by site-directed mutagenesis.

In contrast, Asp-74 and Glu-78 were crucial for the Dpr to be a member of the Dps protein family (11), in which one of the functional features is iron binding activity (17, 22, 26, 27). The family members seem to be able to oxidize Fe²⁺ and store it inside the oligomeric protein shell as Fe³⁺ (17, 29, 53, 54), resembling classical ferritins in this respect (23). Indeed, Bozzi and co-workers (22) identified in L. innocua Flp, a member of the Dps family that is well characterized in vitro (53, 54), a homologous region to the iron nucleation center of mammalian ferritin L-subunit (24). They suggested that this region could carry out the initial steps in iron core formation and also serve as a ferroxidase center. Although a homologous region to this amino acid motif can be found in several Dps family members (Fig. 7) (14), no direct experimental evidence is available on its functionality in iron incorporation in vivo. Furthermore, the biological significance of iron incorporation is not well established. In the present study, a putative ferroxidase center was identified in the N-terminal half of S. suis Dpr, and the structure-function relationship was studied by site-directed mutagenesis.

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S. suis

dPpr, are marked with an asterisk. The amino acids Asp-74 and Glu-78, chosen for independent substitution with Ala in the proposed to be involved in iron coordination in Dlp-1 and Dlp-2 (27) are also shaded black. Flp, the closest Dpr homolog with a solved crystal structure, folds into a four-helix bundle, where amino acids from helixes A and B (boxed) form the intersubunit iron-binding pocket. In the dedocamer, 12 pockets are formed, each serving as a putative ferroxidase center (28). The amino acids Asp-74 and Glu-78, chosen for independent substitution with Ala in S. suis Dpr, are marked with an asterisk.

Dpr expression after resolving 2 μg of cellular protein extracts either under denaturing (A) or nondenaturing (B) conditions. Under nondenaturing conditions, equine spleen type I ferritin (Fer) and BSA were used as molecular mass markers. Recombinant Dpr (tDpr) was expressed in its N terminus (41), leading to slightly faster mobility of the Dpr oligomer. The gel was subsequently analyzed for 55Fe-containing proteins by autoradiography.

Effects of the putative ferroxidase center mutations on Dpr and its in vivo iron incorporation activity. Western analysis of Dpr expression after resolving 2 μg of cellular protein extracts from early-stationary phase cultures was loaded into each well of a 4%–15% gradient polyacrylamide gel, with equine spleen type I ferritin and BSA serving as molecular mass markers, and resolved under nondenaturing conditions. The N-terminal half of S. suis Dpr was aligned with the homologous regions of Listeria innocua ferritin Flp (CAC96173), mini-ferritins Dlp-1 (1JI5A) and Dlp-2 (1JIGA) of Bacillus anthracis, and Dps (AAA21855) of E. coli. NCBI Protein Database accession numbers in parentheses. Amino acids shared with Dpr are shaded gray. Amino acids involved in iron coordination of Flp (28) or in Pb2+ coordination of the Dps heavy atom derivative (47) are shaded black. Amino acids proposed to be involved in iron coordination in Dlp-1 and Dlp-2 (27) are also shaded black. Flp, the closest Dpr homolog with a solved crystal structure, folds into a four-helix bundle, where amino acids from helixes A and B (boxed) form the intersubunit iron-binding pocket. In the dedocamer, 12 pockets are formed, each serving as a putative ferroxidase center (28). The amino acids Asp-74 and Glu-78, chosen for independent substitution with Ala in S. suis Dpr, are marked with an asterisk.

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The dpr knockout of S. suis had a ~10^6-fold reduced viability under exposure to 6.0 mM H_2O_2 as compared with the wild-type strain. By analyzing the H_2O_2 sensitivities of dpr knockout expressing wild-type or site-mutated forms of Dpr, we determined the role played by the iron incorporation activity of Dpr in H_2O_2 resistance. Strikingly, bacteria expressing iron incorporation-negative forms of Dpr were hypersensitive to H_2O_2 with a phenotype comparable to that of dpr knockout. Thus, Dpr seemed to protect the bacteria against H_2O_2 by its iron incorporation activity. This was supported by the iron chelator DFOM, an iron chelator interfering with the intracellular iron pool (43–46), was able to rescue the viability defect of D282Δdpr. Under the same conditions, an extracellular iron chelator, DTPA (46), did not cause any effect. Yamamoto et al. (25) recently reported data indicating that S. mutans Dpr is capable of inhibiting the action of Fenton chemistry in vitro. Thus, Dpr seems to provide an indirect means for catalase-negative bacteria to tolerate H_2O_2 by inhibiting the Fe^{3+}-catalyzed cleavage of H_2O_2 into more toxic reactive oxygen intermediates.

Mechanisms other than iron incorporation have been suggested for Dps family members to explain the protection against H_2O_2, and synergy between them is possible (12, 14, 25). A convincing body of evidence indicates that E. coli Dps protects DNA by a direct association, which leads to highly oxidizing intermediates. Thus, Dps can protect DNA in a manner that is independent of its iron incorporation activity.

H_2O_2 sensitivities of D282Δdpr expressing wild-type or site-mutated forms of Dpr. Viability curves of D282 (O) and derivative strains stressed with extragenously added H_2O_2 are shown. Bacteria were grown to early-stationary phase in THY and treated for 2 h with different concentrations of H_2O_2 at 37 °C, and their viabilities were counted after plating to THY. Values are given as means of two independent experiments. Strain D282LA (C) is otherwise like D282Δdpr but expresses wild-type Dpr from the pLZ12-LAA. Strains D282LA-74 (O) and D282LA-78 (+) are otherwise like D282Δdpr but express the site-mutated and iron incorporation-negative forms of Dpr from pLZ12-LAA-74 and pLZ12-LAA-78, respectively.
ordered DNA-Dps biocrystals (12, 15, 16, 55). However, Dps also contains a putative ferroxidase center in its N-terminal half, and a dual function in \( \text{H}_2\text{O}_2 \) resistance has been proposed. Dps could directly protect the DNA by biocrystal formation but also inhibit the action of Fenton chemistry by its iron incorporation activity (17, 29, 47). Whether Dpr also binds and protects DNA remains contradictory. Yamamoto et al. (25) recently reported data indicating that S. mutans Dps has no DNA binding activity. In our own studies using hydroxyl radical DNA footprinting assays, we have not detected any DNA binding activity for S. suis Dpr. Also, some other members of the Dps family seem to lack DNA binding activity (26, 27). The possibility that Dpr could differ from Dps in lacking DNA binding activity is not unexpected, considering that the primary amino acid sequence identity shared between S. suis Dpr and E. coli Dps is only 25%. It also seems unlikely that Dpr could have a catalase-like activity as reported for the DpsA of *Synechococcus* sp. (14). S. suis Dpr shares an extremely weak homology to DpsA (undetectable in BLAST-P data base search) and does not seem to contain heme, which has been linked to the enzymatic activity of DpsA (14). Furthermore, Yamamoto et al. (25) have recently presented data indicating that *S. mutans* Dpr has no catalase-like activity. Indeed, the results of our present study indicate that it is the iron incorporation activity that is the molecular basis of Dpr-mediated \( \text{H}_2\text{O}_2 \) resistance.

The Dps family includes molecules from diverse taxonomic lineages (14) with striking similarities, even including a recent member in the archaeon *Halobacterium salinarum* (56). Proteins fold into four-helix bundles resembling the fold of mammalian ferritins and assemble into large hollow globular complexes (23, 27, 28, 47). Based on our present study, *S. suis* Dpr protected the bacteria against \( \text{H}_2\text{O}_2 \) by its iron incorporation activity. Whether this is true for the Dps family members in general is not known. However, the amino acid residues forming the putative ferroxidase centers are among the most conserved primary amino acid sequence features of the family (Fig. 7) (14). Also, all members have proven to be iron-binding proteins, if analyzed for this activity (11, 17, 22, 26, 27, 56). Thus, it is possible that in diverse bacterial species, Dps-related molecules might serve as functional ferritin-like molecules and protect bacteria against \( \text{H}_2\text{O}_2 \) as inhibitors of Fenton chemistry. However, it has become evident that, despite sharing the few highly conserved amino acid residues, the putative ferroxidase centers vary considerably (Fig. 7), which might lead to different functional properties. Indeed, Zhao et al. (17) have recently reported data on iron oxidation and incorporation properties of *E. coli* Dps that differ from those of *L. innocua* Fip in both the rates of Fe\(^{2+}\) binding and oxidation. Thus, it is obvious that more studies are needed to further elucidate the importance of iron incorporation activity for Dps family members in \( \text{H}_2\text{O}_2 \) resistance.

The functional divergence of the Dps family is an important area of further investigation. The family members seem to be involved in aerotolerance (11), \( \text{H}_2\text{O}_2 \) resistance (11, 12, 57), cold shock adaptation (58), starvation tolerance (12), iron storage (22), neutrophil activation (59, 60), carbohydrate binding (61), and adhesion (62). The molecular determinants mediating these activities are largely unknown. Additional *in vivo* studies together with *in vitro* work using recombinant site-mutated molecules of the molecules in combination with crystal structure information (27, 28, 41, 47) are needed to reveal the molecular mechanisms behind these activities and their relationships to each other. Determining the structural basis of these biological activities may eventually contribute to the development of means to prevent and treat diseases caused by pathogenic bacteria.

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REFERENCES

1. Imlay, J. A., and Fridovich, I. (1991) *Free Radic. Res. Commun.* 12–13, 59–66
2. Imlay, J. A. (1995) *J. Biol. Chem.* 270, 19767–19777
3. Storz, G., and Imlay, J. A. (1999) *Curr. Opin. Microbiol.* 2, 188–194
4. Hiray, R. A., Sikand, K. S., and Rutman, A. (1995) *J. Biol. Chem.* 270, 15093–15098
5. Thomas, E. L., and Finne, J. (1994) *Infect. Immun.* 62, 529–535
6. Nguyen, P. T., Abrahams, J., Phan, T. N., and Marquis, R. E. (2002) *Curr. Microbiol.* 44, 262–266
7. Nathan, C., and Shliss, M. U. (2000) *Proc. Natl. Acad. Sci. U. S. A.* 97, 8841–8848
8. Jansen, T. W., Bolin, M., Balling, R., Chhatwal, G. S., and Schnabel, R. (2002) *Infect. Immun.* 70, 5202–5207
9. Spellerberg, B., Cundell, D. R., Sandros, J., Pearce, B. J., Idanpaan-Heikkila, I., Rosenow, C., and Masure, H. R. (1996) *Microb. Mol.* 19, 803–813
10. Honda, Y., Higuchi, M., Poole, L. B., and Kamio, Y. (2000) *J. Bacteriol.* 182, 3740–3747
11. Almiron, M., Link, A. J., Furlong, D., and Kolter, R. (1992) *Genes Dev.* 6, 2448–2454
12. Komovskaya, O. L., Kidwell, J. P., and Matin, A. (1994) *J. Bacteriol.* 176, 3928–3935
13. Lomovskaya, O. L., Kidwell, J. P., and Matin, A. (1994) *J. Bacteriol.* 176, 3928–3935
14. Papinutto, E., Dundon, W. G., Pitulis, N., Battistutta, R., Montecucco, C., and Zanotti, G. (1995) *Free Radic. Biol. Med.* 15, 435–445
15. Bozzi, M., Mignogna, G., Stefanini, S., Barra, D., Longhi, C., Valenti, P., and Chiancone, E. (1997) *J. Biol. Chem.* 272, 3259–3265
16. Zhoa, G., Ceci, P., Irazi, A., Giangiaco, L., Laue, T. M., Chiancone, E., and Chasteen, N. D. (2002) *J. Biol. Chem.* 277, 27689–27696
17. Cappellini, B., de Haan, L. A., and Jacobs, A. A. (1998) *Mol. Microbiol.* 29, 37619–37628
Molecular Basis of Dpr-mediated H₂O₂ Resistance

47. Grant, R. A., Filman, D. J., Finkel, S. E., Kolter, R., and Hogle, J. M. (1998) Nat. Struct. Biol. 5, 294–303
48. Crane, E. J., III, Parsonage, D., Poole, L. B., and Claiborne, A. (1995) Biochemistry 34, 14114–14124
49. Poole, L. B., and Claiborne, A. (1986) J. Biol. Chem. 261, 14525–14533
50. Touati, D., Jacques, M., Tardat, B., Bouchard, L., and Despied, S. (1995) J. Bacteriol. 177, 2305–2314
51. Morris, C. J., Earl, J. R., Trenam, C. W., and Blake, D. R. (1995) Int. J. Biochem. Cell Biol. 27, 109–122
52. Imlay, J. A., Chin, S. M., and Linn, S. (1988) Science 240, 640–642
53. Yang, X., Chiancone, E., Stefanini, S., Ilari, A., and Chasteen, N. D. (2000) Biochem. J. 349, 783–786
54. Stefanini, S., Cavallo, S., Montagnini, B., and Chiancone, E. (1999) Biochem. J. 338, 71–75
55. Wolf, S. G., Frenkuel, D., Arad, T., Finkel, S. E., Kolter, R., and Minsky, A. (1999) Nature 400, 83–85
56. Reindel, S., Anemuller, S., Sawaryn, A., and Matzanke, B. F. (2002) Biochim. Biophys. Acta 1598, 140–146
57. Chen, L., and Helmann, J. D. (1995) Mol. Microbiol. 18, 295–300
58. Hebraud, M., and Guzzo, J. (2000) FEMS Microbiol. Lett. 190, 29–34
59. Yoshida, N., Granger, D. N., Evans, D. J., Jr., Evans, D. G., Graham, D. Y., Anderson, D. C., Wolf, R. E., and Kvietys, P. R. (1995) Gastroenterology 105, 1431–1440
60. Evans, D. J., Jr., Evans, D. G., Takemura, T., Nakano, H., Lampert, H. C., Graham, D. Y., Granger, D. N., and Kvietys, P. R. (1995) Infect. Immun. 63, 2213–2220
61. Teneberg, S., Miller-Podraza, H., Lampert, H. C., Evans, D. J., Jr., Evans, D. G., Danielsson, D., and Karlsson, K. A. (1997) J. Biol. Chem. 272, 19067–19071
62. Brentjens, R. J., Ketterer, M., Apicella, M. A., and Spinola, S. M. (1996) J. Bacteriol. 178, 808–816
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DEMONSTRATION OF THE FUNCTIONAL INVOLVEMENT OF THE 
PUTATIVE FERROXIDASE CENTER BY SITE-DIRECTED MUTAGENESIS IN 
STREPTOCOCCUS SUIS 

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