How Microbes Defend Themselves From Incoming Hydrogen Peroxide

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Microbes rely upon iron as a cofactor for many enzymes in their central metabolic processes. The reactive oxygen species (ROS) superoxide and hydrogen peroxide react rapidly with iron, and inside cells they can generate both enzyme and DNA damage. ROS are formed in some bacterial habitats by abiotic processes. The vulnerability of bacteria to ROS is also apparently exploited by ROS-generating host defense systems and bacterial competitors. Phagocyte-derived $O_2^-$ can toxify captured bacteria by damaging unidentified biomolecules on the cell surface; it is unclear whether phagocytic H$_2$O$_2$, which can penetrate into the cell interior, also plays a role in suppressing bacterial invasion. Both pathogenic and free-living microbes activate defensive strategies to defend themselves against incoming H$_2$O$_2$. Most bacteria sense the H$_2$O$_2$ via OxyR or PerR transcription factors, whereas yeast uses the Grx3/Yap1 system. In general these regulators induce enzymes that reduce cytoplasmic H$_2$O$_2$ concentrations, decrease the intracellular iron pools, and repair the H$_2$O$_2$-mediated damage. However, individual organisms have tailored these transcription factors and their regulons to suit their particular environmental niches. Some bacteria even contain both OxyR and PerR, raising the question as to why they need both systems. In lab experiments these regulators can also respond to nitric oxide and disulfide stress, although it is unclear whether the responses are physiologically relevant. The next step is to extend these studies to natural environments, so that we can better understand the circumstances in which these systems act. In particular, it is important to probe the role they may play in enabling host infection by microbial pathogens.

Keywords: reactive oxygen species, OxyR regulator, peroxide sensing repressor (PerR), Yap1p, nitric oxide

THE THREATPOSED BY OXYGEN

Life evolved 3.8 billion years ago in an anoxic world. The biochemical pathways of these primordial organisms were based upon iron-cofactored enzymes, as this transition metal is adept at both redox and ligand-exchange processes. One billion years later, the appearance of photosystem II began the release of diatomic oxygen into the atmosphere. The oxygen levels in the atmosphere remained relatively low for another billion years, because photosynthetically generated O$_2$ was quickly consumed through its chemical reduction by environmental ferrous iron and sulfide. Only later, once these reductants had been largely titrated, did oxygen accumulate to higher concentrations (1). However, when it did, O$_2$ created an environment that was—and remains—incompatible with...
extant organisms, which have inherited their iron-centric metabolic plans from their anoxic forbears.

In part the problem is that molecular oxygen oxidizes ferrous iron to insoluble ferric hydroxide precipitates, making it difficult for cells to acquire enough iron to charge their enzymes. That problem has been substantially ameliorated by the evolution of a variety of iron-import tactics (2). However, in addition, oxygen is kinetically active as a univalent oxidant (3). It disrupts the metabolism of anaerobic organisms by oxidizing their low-potential metal cofactors, thereby inactivating key enzymes, and by adding the radical-based enzymes that play specialized roles in metabolism. Contemporary anaerobes have not solved this problem: In order to optimize their anaerobic growth, they continue to rely upon enzymes that are directly disrupted by oxygen, and so these microbes are constrained to anoxic niches (4).

In contrast, organisms that committed to life in oxic environments were able to dispense with low-potential catalytic strategies, and they employ enzymes that molecular oxygen does not damage at an important rate. Yet aerobes have residual problems with oxygen. Molecular oxygen is a passable univalent oxidant, and inside cells it adventitiously steals electrons from the cofactors of redox-active enzymes (5, 6). The transfer of a single electron results in the formation of a more-potent univalent oxidant, which is superoxide (Figure 1A); the transfer of two electrons results in the formation of hydrogen peroxide. Both species are potent oxidants that can directly oxidize all cellular biomolecules and by adducting the radical-based enzymes that play specialized roles in metabolism. Contemporary aerobes have not solved this problem: In order to optimize their anaerobic growth, they continue to rely upon enzymes that are directly disrupted by oxygen, and so these microbes are constrained to anoxic niches (4).

The transfer of two electrons results in the formation of hydrogen peroxide. Both species can oxidize the solvent-exposed iron centers of enzymes that are found throughout metabolism. Further, a secondary reaction between hydrogen peroxide and cellular iron pools creates hydroxyl radicals (Figure 1), which are extremely potent species that can directly oxidize all cellular biomolecules (7). This vulnerability to partially reduced oxygen species (ROS) is universal among contemporary organisms. This review specifically aims to describe the strategies that are used by organisms to defray the toxicity of hydrogen peroxide—and to highlight the circumstances in which these defenses may not be adequate.

### REACTIVE OXYGEN SPECIES ARE CONTINUOUSLY FORMED INSIDE OXIC CELLS

The significance of hydrogen peroxide (H$_2$O$_2$) was first suggested by the discovery in 1900 of an enzyme devoted to degrading it: catalase (8). The discoverer, Oscar Loew, noted that it is found in virtually all tissues, and he made the inference that H$_2$O$_2$ was likely a by-product of metabolism that, if not removed, must be toxic to cells. Seventy years later Joe McCord and Irwin Fridovich chanced upon an enzyme that degrades superoxide (O$_2$) (9). Subsequent work has extended the cohort of scavenging enzymes to include peroxidases and superoxide reductases, and it has confirmed that virtually no organism lacks the ability to degrade H$_2$O$_2$ and O$_2$.

The model bacterium *Escherichia coli* contains two superoxide dismutases in its cytoplasm and one in its periplasm. Its cytoplasm also features both an NADH peroxidase (AhpCF) and two catalases (10) (Figure 2). Interestingly, some of these enzymes take advantage of the fact that iron can react with O$_2$ and H$_2$O$_2$: The original superoxide dismutase was likely an iron-dependent SOD, and most catalases use heme to degrade H$_2$O$_2$. Looking more broadly, similar scavenging systems are distributed throughout all biological kingdoms and in most cellular compartments, including the mitochondria, peroxisomes, and cytoplasm of eukarya.

The importance of these enzymes was revealed by genetic studies of *E. coli*: Mutants that lack cytoplasmic SODs, or that lack Ahp and catalases, were found to be unable to grow under oxic conditions in a standard glucose medium (10, 11). The observation confirmed Loew’s postulate, proving both that these species are generated internally and that if not scavenged they will cripple cellular metabolism. Direct observation of O$_2$ production is not possible, but mutants that cannot degrade H$_2$O$_2$ release it into growth media at a rate that connotes an internal production rate of 10 μM/sec (12). Measurements show that as little as 0.5 μM intracellular H$_2$O$_2$ is sufficient to poison select biosynthetic pathways (13–15). The titers and kinetics of the scavenging enzymes are sufficient to suppress internal concentrations to about 50 nM—low enough to enable metabolism to operate without bottlenecks (12).

The orbital structure of O$_2$ restricts it to accepting a single electron at a time, and its reduction potential (−0.16 V) is too
modest for it to directly oxidize most biomolecules (3). However, O2 can accept electrons from electron donors such as metal centers, flavins, and quinones. These are all prominent electron carriers in the E. coli respiratory chain, yet the rate at which cells produce endogenous H2O2 did not substantially diminish in mutants that lacked the respiratory enzymes, suggesting that in this bacterium O−2 and H2O2 are primarily produced by the accidental autooxidation of non-respiratory flavoproteins (5, 6) (Figure 1). These proteins are found throughout metabolism, and many, including glutathione reductase, lipoamide dehydrogenase, and glutamate synthase, have been shown to release ROS in vitro (16–18). The small size of O2 prevents its exclusion from most active sites, and its collision with reduced flavins triggers the consecutive transfer of one or two electrons, generating O−2 or H2O2, respectively (19). The rate is naturally proportionate to collision frequency, meaning that ROS formation is more rapid in highly oxic environments. This math presumably underlies the observation that most organisms cannot tolerate oxygen levels that substantially exceed those of their natural habitat.

THE CLASSES OF DAMAGE CAUSED BY SUPEROXIDE AND HYDROGEN PEROXIDE

The phenotypes of SOD mutants and of catalase/peroxidase mutants enabled investigators to track down the specific injuries that these ROS create. The mutants can grow at wild-type rates under anoxic conditions, but they require supplementation with aromatic and branched-chain amino acids if they are to grow in oxic media (11, 13–15, 20). They are also unable to use any carbon source, such as acetate, that required a fully functional TCA cycle (21, 22). Further scrutiny identified particular enzymes whose damage resulted in these defects.

The aromatic biosynthesis defect derives from the ability of H2O2 to oxidize the Fe(II) cofactor of the first enzyme of the pathway, DAHP synthase (15). The resulting Fe(III) atom dissociates. The role of the iron atom is both to bind substrate and to stabilize an oxyanion intermediate in the catalytic cycle; therefore, the resultant apoenzyme is completely inactive and the pathway fails. Other mononuclear Fe(II) enzymes such as ribulose-5-phosphate 3-epimerase, peptide deformylase, threonine dehydrogenase, and cytosine deaminase are similarly damaged (13, 14). Notably, the reaction between Fe(II) and H2O2 also generates a hydroxyl radical. If the catalytic Fe(II) atom is coordinated by a cysteine side chain, the hydroxyl radical reacts immediately with this sacrificial residue, creating a sulfenic acid (Figure 3) (13). Cellular thioredoxins and glutaredoxins can reduce this moiety back to a native cysteine residue, thereby allowing Fe(II) to bind and the activity to be restored (13). In contrast, when H2O2 oxidizes the Fe(II) of enzymes that lack such a residue, the nascent hydroxyl radical oxidizes other active-site ligands, creating lesions that are irreversible. These reactions are one source of the protein carbonylation that can be detected in H2O2-stressed cells (13).
The branched-chain biosynthetic defects as well as the TCA-cycle defects are caused by the oxidation of the \([4\text{Fe}-4\text{S}]\) clusters of dehydratase enzymes \((20, 22–26)\). A solvent-exposed iron atom of these clusters binds substrate directly, activating it for deprotonation and subsequently completing the dehydration by abstracting a hydroxide anion. But the same exposed iron atom can be oxidized by hydrogen peroxide to an unstable \([4\text{Fe}-4\text{S}]^{3+}\) state \((\text{Figure 4})\); this valence is unstable, and the cluster quickly disintegrates into a \([3\text{Fe}-4\text{S}]^+\) form that lacks the catalytic, solvent-exposed iron atom \((24)\). Interestingly, the hydroxyl radical that is formed during this process pulls a second electron from the iron-sulfur cluster; thus, a hydroxide anion, rather than a hydroxyl radical, is released into the active site, and polypeptide oxidation is avoided. Cells continuously repair these damaged clusters, so that the steady-state activity of these enzymes reflects the balance between the oxidation and repair rates \((21, 27, 28)\).

In addition to the metabolic defects described above, \(\text{H}_2\text{O}_2\) can also react with the cytoplasmic pool of loose iron that is used to metallate nascent iron-dependent enzymes \((\text{Figure 1B})\). Iron is sticky, and this pool is thought to adhere to a wide variety of biomolecules, including the surface of nucleic acids \((29)\). DNA thereby acts as a locus of hydroxyl radical production, and so DNA damage is a universal consequence of \(\text{H}_2\text{O}_2\) stress \((30–33)\). Some oxidative base lesions are mutagenic; others comprise replication blocks. All organisms therefore wield enzymes devoted to the excision or recombinational repair of oxidative lesions. Even though the level of endogenous \(\text{H}_2\text{O}_2\) is well-controlled in scavenger-proficient \(E.\ coli\), the rate of DNA oxidation remains high enough that mutants lacking these repair pathways cannot grow in oxic environments \((34, 35)\).

Thus, organisms that dwell in oxic habitats can do so only because they have acquired an array of both scavenging and repair functions. The level of these enzymes is high and their synthesis is costly; accordingly, their titers have been calibrated to barely withstand the amount of stress commensurate with the oxygen level of the native environment \((36, 37)\). This arrangement is successful under routine growth conditions. However, we shall see that it becomes inadequate if special circumstances elevate the production of ROS.

**THE PROBLEM OF EXOGENOUS OXIDATIVE STRESS**

**Exogenous Sources of Superoxide**
Both environmental photochemistry and chemical redox reactions generate \(\text{O}_2^-\) \((38)\), but the steady-state level of \(\text{O}_2^-\) formed in this way is unlikely to be high enough to pose a risk for cells. Notably, the known targets of \(\text{O}_2^-\) are iron enzymes that are cytoplasmic, and \(\text{O}_2^-\) is a charged species that cannot cross membranes to get at them \((39, 40)\). However, both microbes and higher organisms have evolved mechanisms by which they can use \(\text{O}_2^-\) to poison unwanted competitors.

Mammals, plants, and amoebae have all weaponized an NADPH oxidase to kill bacteria \((41, 42)\). Mammalian phagocytes engulf microbial invaders and spray them with...
superoxide that is formed by an inducible NADPH oxidase (Figure 5). The importance of this enzyme is reflected by the observation that humans and mice that lack it are vulnerable to infections (43, 44). However, it is still unclear how the phagocytic ROS production inhibits microbial growth. The acidic environment inside the phagosome can partially protonate $\text{O}_2^-$, resulting in a neutral species that in principle can penetrate captive bacteria; however, *Salmonella enterica* mutants lacking the periplasmic superoxide dismutase are hypersensitive, suggesting that $\text{O}_2^-$ does not gain access into the cytosol and instead acts on a target on the cell surface or in the periplasm (45, 46). Because protonated HO$_2^+$ is a better oxidant than $\text{O}_2^-$, it is possible that the acidity of the phagosome expands the range of biomolecules that superoxide can damage. The target has not yet been identified. A key difficulty is that *in vitro* systems have been unable to match the micromolar doses of superoxide (46, 47) that are sustained in the phagosome.

Bacteria also have found a way to impose $\text{O}_2^-$ stress upon competitors—and in this case the $\text{O}_2^-$ is aimed at the cytoplasm. A wide range of bacteria (and plants) secrete redox-cycling antibiotics (48–51). These are primarily soluble quinones and phenazines; they penetrate target cells, oxidize their redox enzymes, and transfer the electrons to oxygen. Enteric bacteria protect themselves from these compounds by activating the SoxRS regulon (52, 53). Its components elevate the titer of cytoplasmic SOD, pump out the drugs, and modify the cell envelope to diminish their entry (54).

Over the past dozen years microbiologists have examined the possibility that other bacterial stresses might also owe their potency, in part, to oxidative stress. The clinical antibiotics ampicillin, kanamycin, norfloxacin and trimethoprim have been particular foci of these studies, but similar hypotheses have been ventured for metal overload, nanoparticles, solvent stress, toxin/antitoxin systems, and many others (55). Key observations have been that the stressed cells accumulate oxidized forms of cell-penetrating dyes, which are thought to be oxidized by hydroxyl radicals; that cell death is slowed by the administration of thiol compounds, which might scavenge ROS, and by iron chelators that would block hydroxyl-radical formation; and that toxicity is diminished in mutants whose TCA cycle is blocked, ostensibly diminishing the rate of
respiration and any associated ROS formation (56). However, this interpretation has been challenged (55, 57–59). Most of these protectants also have the capacity to slow metabolism, and it is well known that the efficacy of antibiotic action depends upon a robust growth rate. Moreover, tests of antibiotic action did not detect oxidative damage to ROS-sensitive enzymes or to DNA; the rate of H2O2 formation (measured in scavenging mutants) was not accelerated; and the E. coli response to H2O2 stress (below) was not triggered. Finally, no clear model has emerged to explain how such diverse stresses could create toxic levels of ROS. More work must be done to resolve these contradictory observations.

**Exogenous Sources of Hydrogen Peroxide**

H2O2 is chemically formed in habitats though abiotic reactions between sulfur and oxygen at oxic/anoxic surfaces and the photochemical reduction of oxygen by chromophores (60–63). Levels can reach 1 µM in the ocean (64, 65). It can also be produced as part of the plant wound response, during the inflammatory response of mammalian hosts—and, notably, as a primary metabolic by-product of many lactic acid bacteria (66–70). The latter organisms often lack respiratory chains and use the two-electron reduction of molecular oxygen to recycle reduced NADH, thereby indirectly improving the ATP yield of what is otherwise a fermentative process. It seems likely that the excreted H2O2 may suppress the growth of competitors. The ability of lactic acid bacteria to tolerate their own H2O2 is impressive: They can achieve high densities in lab cultures in which millimolar H2O2 has accumulated. This tolerance appears to be due to the absence of oxidant-sensitive dehydratases and mononuclear Fe(II) enzymes. This adaptation is not without a price: These bacteria are unable to synthesize many amino acids, and they lack a TCA cycle and the improved energy yield that comes with it.

Unlike superoxide, H2O2 is an uncharged, albeit polar, molecule that can cross cell membranes. Because this process is relatively slow, when bacteria venture into environments containing extracellular H2O2, the high activities of intracellular catalases and peroxidases succeed at lowering internal H2O2 concentrations below that of the external environment (12). The transmembrane gradient for E. coli has been estimated to be 5- to 10-fold between the external world and the cytoplasm. Indeed, although 0.5 µM internal H2O2 is sufficient to impair the growth of this bacterium in lab cultures, external concentrations of up to 5 µM seem to be tolerated without an overt growth defect (60). Thus the limited permeability of membranes to H2O2 is essential to the efficacy of scavenging enzymes and to the ability of bacteria to grow in many habitats.

Of great interest to biologists is the role that phagocyte-derived H2O2 may play in suppressing microbial infection (Figure 5). The O2 that is produced by host NADPH oxidase will dismutate, either spontaneously or via enzymatic catalysis, to generate H2O2. The ability of H2O2 to cross membranes likely enables it to enter phagocytosed bacteria—but it also allows it to diffuse across the phagosomal membrane, into the producing cell, and potentially out into extracellular environments. Modeling suggests that this diffusion sharply limits the amount of H2O2 inside the macrophage phagosome, despite the rapid rate at which it is formed. Estimates are that the steady-state level falls well below 10 µM (46). Such doses may be enough to induce stress responses in the captive bacteria, but they are unlikely to be lethal. The major caveat to this analysis is that it presumes that the environment acts as a one-way sink for the H2O2. However, if H2O2 accumulates within inflamed tissue, the H2O2 flow is bidirectional, and the level that accumulates may in principle be far higher. Clearly, this question cries out for direct measurements of H2O2 in vivo.

The rate of H2O2 production in neutrophils is substantially higher than that in macrophages—but the fact that H2O2 is a substrate of myeloperoxidase has once again been projected to cap the level at which it can accumulate (47). In sum, although at first blush it seems a no-brainer that H2O2 would contribute mightily to the killing actions of these cells, that point is not yet resolved.

**THE ROLE OF OxyR DURING HYDROGEN PEROXIDE STRESS**

When external levels of H2O2 exceed a few micromolar, its flux into microbes threatens to elevate its internal concentration to toxic levels, despite the action of scavenging enzymes. To cope, virtually all microbes possess inducible stress responses that are focused upon H2O2. The paradigmatic system is the OxyR response of E. coli (71). OxyR is a H2O2-activated transcription factor. It is not activated merely by movement of E. coli into oxic environments, and oxyR mutants are capable of normal aerobic growth. However, OxyR is activated when exogenous H2O2 accumulates to ~0.2 µM in the cytoplasm, which is achieved by about 3 µM external H2O2 (60, 72). Null mutants cannot grow at these levels of environmental H2O2.

The OxyR protein contains a sensory cysteine residue (C199) that is directly oxidized by H2O2, generating a sulfenic acid (SOH) (73). As a result, the residue moves from the hydrophobic pocket in which it is buried and swings toward the C208 residue, which then condenses to form a disulfide bond (Figure 6). This bond locks OxyR into its activated conformation, and its DNA binding ability differs from that of the reduced protein. In E. coli the reduced form has little transcriptional impact upon most genes, but the oxidized form recruits RNA polymerase and thereby activates the expression of genes that possess an OxyR binding site. In many other bacteria, reduced OxyR acts as a repressor, and upon its oxidation it releases the DNA, stimulating gene expression (74, 75). In still other bacteria gene expression is both repressed by the reduced form and activated by the oxidized form. The conformational change is manifested by an alteration of its DNA footprint (76).

Free cysteine reacts very slowly with H2O2 (2 M−1 s−1), and typical cysteine residues of proteins do, too (77). Yet in OxyR the sensing cysteine residue reacts with a rate constant of 106 allowing it to detect micromolar H2O2 in seconds (72, 78). In that respect the hyperreactive cysteine of OxyR resembles the catalytic cysteine residue of thiol-based peroxidases, including
that deal with the damage that \( \text{H}_2\text{O}_2 \) produces (Table 1). The peroxidase \( \text{AhpCF} \) and the catalase \( \text{KatG} \) are each induced more than 10-fold in order to scavenge \( \text{H}_2\text{O}_2 \) (Figure 2). Why two enzymes? \( \text{AhpCF} \) is an efficient scavenger when the \( \text{H}_2\text{O}_2 \) concentration is less than 10 \( \mu \text{M} \) and the cell is well-fed (10). However, \( \text{AhpCF} \) requires \( \text{NADH} \) as a reductant, and so its activity becomes limited when catabolic substrates are scarce. In contrast, catalases do not require reductants, and they can degrade \( \text{H}_2\text{O}_2 \) faster than \( \text{Ahp} \). However, catalases are problematic when the \( \text{H}_2\text{O}_2 \) concentrations are low, since their two-step catalytic cycle can stall with the heme in its intermediate ferryl radical form. This species is a potent oxidant, and unless it is quenched by a reductant, it can abstract electrons from the surrounding polypeptide and inactivate the enzyme (81). The KatG catalase of \( \text{E. coli} \)—and of many other bacteria—has a channel that apparently enables small-molecule reductants to approach the active site and quench the high-valence heme (84); for this reason the enzyme is denoted a catalase/peroxidase, though the peroxidase activity by itself is too slow to comprise an efficient scavenging mechanism (85).

When \( \text{H}_2\text{O}_2 \) levels are high, the existential threat to bacteria is that DNA oxidation will prove lethal. This damage is driven by the intracellular iron pool (86), and so the OxyR system employs several mechanisms to diminish it (Figure 7). Dps, a dodecameric mini-ferritin, is induced to sequester unincorporated iron in its hollow core (87–89). This action requires that loose \( \text{Fe(II)} \) be oxidized to \( \text{Fe(III)} \), and for this reaction Dps apparently uses \( \text{H}_2\text{O}_2 \) as a co-substrate. One benefit is that Dps will stop storing iron when \( \text{H}_2\text{O}_2 \) concentrations drop. The iron-uptake repressor Fur is also induced (90). In unstressed cells Fur:Fe(II) complexes signal that the cell has sufficient iron, and this form occludes the promoters of genes that encode iron import systems (2). When \( \text{H}_2\text{O}_2 \) is present,

AhpC of \( \text{E. coli} \). In the latter enzyme an adjacent cationic residue facilitates the deprotonation of cysteine, which provides an order-of-magnitude improvement in its reactivity with \( \text{H}_2\text{O}_2 \) (79). A plausible explanation for the remaining enhancement is that the nucleophilic cysteine is arranged in a large hydrophobic cleft. Surrounding hydrogen bonds could polarize the dioxygen bond, making it vulnerable to attack, and one of the residues can protonate the hydroxide leaving group, pulling the reaction forward. As a result, thiol-based peroxidases have a rate constant of \( 10^7 \text{M}^{-1} \text{s}^{-1} \), which is appropriate for their physiological role (80, 81). A similar physical arrangement may explain the high rate constant of OxyR as well.

It follows that the activation of OxyR is an excellent marker of \( \text{H}_2\text{O}_2 \) stress. This effect can be tracked by monitoring the expression of OxyR-controlled genes—or by visualizing the intrinsic fluorescence of HyPer, an engineered chimera of OxyR and yellow-fluorescent protein (82). HyPer is as responsive to \( \text{H}_2\text{O}_2 \) as is OxyR itself, and its oxidation status can be visualized either by microscopy or flow cytometry. Importantly, two-wavelength analysis can correct for variable HyPer content in different samples, thereby avoiding loading artifacts that can arise when redox-active dyes are employed as ROS sensors.

THE DEFENSES THAT OxyR TURNS ON

When \( \text{E. coli} \) is stressed by an influx of \( \text{H}_2\text{O}_2 \), activated OxyR stimulates the transcription of over two dozen genes (83). These mainly fall into three categories: proteins that reduce the \( \text{H}_2\text{O}_2 \) concentration, proteins that shrink the iron pool, and proteins that deal with the damage that \( \text{H}_2\text{O}_2 \) produces (Table 1). The peroxidase \( \text{AhpCF} \) and the catalase \( \text{KatG} \) are each induced more than 10-fold in order to scavenge \( \text{H}_2\text{O}_2 \) (Figure 2). Why two enzymes? \( \text{AhpCF} \) is an efficient scavenger when the \( \text{H}_2\text{O}_2 \) concentration is less than 10 \( \mu \text{M} \) and the cell is well-fed (10). However, \( \text{AhpCF} \) requires \( \text{NADH} \) as a reductant, and so its activity becomes limited when catabolic substrates are scarce. In contrast, catalases do not require reductants, and they can degrade \( \text{H}_2\text{O}_2 \) faster than \( \text{Ahp} \). However, catalases are problematic when the \( \text{H}_2\text{O}_2 \) concentrations are low, since their two-step catalytic cycle can stall with the heme in its intermediate ferryl radical form. This species is a potent oxidant, and unless it is quenched by a reductant, it can abstract electrons from the surrounding polypeptide and inactivate the enzyme (81). The KatG catalase of \( \text{E. coli} \)—and of many other bacteria—has a channel that apparently enables small-molecule reductants to approach the active site and quench the high-valence heme (84); for this reason the enzyme is denoted a catalase/peroxidase, though the peroxidase activity by itself is too slow to comprise an efficient scavenging mechanism (85).

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the oxidation of Fe(II) by H$_2$O$_2$ can deactivate Fur, potentially leading to the disastrous import of more iron. The induction by OxyR of higher levels of Fur seems to partially correct this problem (91). Finally, YaaA is a protein whose biochemical action is not understood but which demonstrably shrinks the iron pool (92). Mutants that lack any of these proteins—Dps, Fur, or YaaA—exhibit high levels of intracellular iron and suffer rapid DNA damage during protracted H$_2$O$_2$ stress.

While the drop in iron pools helps protect DNA, it creates a problem for the synthesis or repair of iron-cofactored enzymes. Iron cofactors comprise three main types—iron-sulfur clusters, mononuclear Fe(II) groups, and heme—and the OxyR system turns on adaptations to sustain the function of enzymes that use each. *E. coli* typically uses Isc-based machinery to build iron-sulfur clusters (93, 94), but during H$_2$O$_2$ stress the secondary Suf system is induced (95, 96) (Figure 4). For unknown reasons, this system works well when iron levels are low (97), making it better than the house-keeping Isc system, which is poisoned by H$_2$O$_2$ (21). The induction of a manganese importer (MntH) (98) enables mononuclear enzymes to become metallated by Mn(II) rather than Fe(II) (Figure 3). Manganese is not as efficient a catalyst as iron, but it remains available even as Dps sequesters iron, and it is unreactive with H$_2$O$_2$ and thereby enables the mononuclear enzymes to remain functional (13). An alternative is that these sulfur reducing systems are useful if OxyR moonlights as a sensor of other thiol-derivatizing stresses.

In *E. coli* both the reduced and oxidized forms of OxyR repress oxyR itself by binding over its promoter; this action ensures that the titer of OxyR are controlled and do not change during H$_2$O$_2$ stress (76). Transcriptomic data suggest that oxidized OxyR may repress several additional genes, including those that encode the periplasmic disulfide bond chaperone DsbG, the ferric iron reductase FhuF, the inner membrane protein of unknown function YbjC, and the NADPH nitroreductase NfsA (83, 99). The significance of this regulation remains unknown.

Most members of the regulon have functions that either prevent injuries or allow the cell to tolerate them. The regulon is very successful at this: Whereas one micromolar of H$_2$O$_2$ in the

![FIGURE 7](Image)
environment fully blocks the growth of an OxyR-deficient strain, a wild-type strain is able to adapt and grow in 10 micromolar or more (60). Indeed, a final member of the OxyR regulon, cytochrome c peroxidase, is a periplasm-facing membrane-bound enzyme that allows E. coli to actually exploit environmental H$_2$O$_2$ as a respiratory oxidant when oxygen and nitrate are unavailable (100) (Figure 2). It is likely that the enzyme plays a role atoxic/anoxic interfaces near the intestinal epithelium, where H$_2$O$_2$ may be formed and may diffuse into anoxic zones.

**OxyR IS MODIFIED DEPENDING ON THE ORGANISM**

The OxyR system has been most fully studied in E. coli, where it is presumably adapted for the enteric environment. Similarly, other bacteria seem to have adapted the OxyR regulon to suit their particular niches; they exhibit differences in terms of the regulation mechanism, the number of OxyR homologs, and the identity of the genes in the regulon. *Porphyromonas gingivalis*, for example, encounters two types of environments: the oral cavity where the oxygen tension is high and the hemin concentrations are low, and the periodontal pockets, which contain mixed microbial communities that lower the oxygen levels and are bathed with proteins such as hemoglobin that serve as a source of hemin. Perhaps unsurprisingly, the OxyR protein in *P. gingivalis* senses both signals, as evidenced by the further activation of OxyR-regulated genes in a hemin-limited environment under anaerobic conditions (101). Interestingly, measurements of the OxyR regulon genes indicate that they are constitutively expressed. This observation is consistent with the idea that the OxyR protein has mutated into a locked-on form, and the residual reduced enzyme may block the action of a subpopulation of oxidized protein. In organisms that are exposed to low, continuous levels of H$_2$O$_2$ stress, AhpCF may suffice to protect the cell from H$_2$O$_2$, but when H$_2$O$_2$ levels become high, a full commitment to catalase synthesis may be called for. Conversely, in other organisms it may be more beneficial to preemptively synthesize basal levels of catalase to guard against a sudden deluge of oxidative stress. Under those conditions, the basal expression can help with the initial stress, and the activation of OxyR can further increase the scavenging enzyme titers.

> **TABLE 2** | Mechanism of OxyR control in different bacteria.

| Bacteria                     | Oxygen tolerance | OxyR control                  |
|------------------------------|------------------|--------------------------------|
| Acinetobacter baumannii      | Aerobe           | Activator and repressor (109) |
| Caulobacter crescentus       | Aerobe           | Activator (110)               |
| Burkholderia pseudomallei    | Aerobe           | Activator and repressor (104) |
| Corynebacterium diptheriae   | Aerobe           | Repressor (107, 111, 112)     |
| Corynebacterium glutamicum   | Aerobe           | Activator and repressor (113, 114) |
| Deinococcus radiodurans      | Aerobe           | Activator and repressor (102, 108, 115) |
| Pseudomonas aeruginosa       | Aerobe           | Activator and repressor (102) |
| Neisseria gonorrhoeae        | Aerobe           | Repressor (106)               |
| Neisseria meningitidis       | Aerobe           | Activator and repressor (102) |
| Streptomyces coelicolor      | Aerobe           | Activator (116)               |
| E. coli                      | Facultative anaerobe | Activator and repressor (83) |
| Haemophilus influenzae       | Facultative anaerobe | Activator (117, 118)         |
| Klebsiella pneumoniae        | Facultative anaerobe | Activator (119)              |
| Rhodobacter sphaeroides      | Facultative anaerobe | Activator (123)             |
| Magnetospirillum                | Facultative anaerobe | Activator (121)             |
| graphiwalidense               | Facultative anaerobe | Activator (123)             |
| Salmonella typhimurium        | Facultative anaerobe | Activator (71)              |
| Serratia marcescens          | Facultative anaerobe | Activator (122)          |
| Shewanella oneidensis        | Facultative anaerobe | Activator and repressor (106) |
| Vibrio cholerae              | Facultative anaerobe | Activator (123–125)         |
| Vibrio vulnificus            | Facultative anaerobe | Activator (123–125)         |
| Bacteroides fragilis         | Anaerobe         | Activator (126–128)           |
| Bacteroides thetaiotaomicron | Anaerobe         | Activator (126–128)           |
| Porphyromonas gingivalis     | Anaerobe         | Activator (101)               |
| Tannerella forsythia         | Anaerobe         | Activator (129)               |

Organisms such as *Vibrio cholerae*, *Vibrio vulnificus*, and *Deinococcus radiodurans* each deploy two OxyR proteins. In *V. vulnificus*, a facultative anaerobe that occasionally encounters aeration, the two proteins are calibrated to sense different levels of H$_2$O$_2$ (123–125). The more sensitive OxyR (VvOxyR2) is activated by endogenous H$_2$O$_2$ that is formed when the cell is aerated, whereas the less sensitive OxyR (VvOxyR1) is only activated by an influx of exogenous H$_2$O$_2$ from the environment. Accordingly, VvOxyR2 induces a peroxidase (VvPrx2) that has a higher activity at lower H$_2$O$_2$ levels compared to a second peroxidase (VvPrx1) that is induced by VvOxyR1. VvPrx1 becomes necessary because high levels of...
H$_2$O$_2$ can irreversibly over-oxidize the catalytic cysteine residue of VvPpx2. It is unclear why there are two OxyR proteins in D. radiodurans. The two proteins seem to regulate different genes: OxyR1 activates katE and represses mntH and dps, and OxyR2 represses katG and the hemin transport genes (113, 114).

In some bacteria the OxyR regulon includes genes that are unrelated to iron control or H$_2$O$_2$ degradation. Superoxide dismutase is regulated by OxyR in P. gingivalis and Pseudomonas aeruginosa, seemingly implying that superoxide stress occurs concomitant with H$_2$O$_2$ stress (101, 130, 131). Perhaps the obligate anaerobe P. gingivalis, like Bacteroides thetaiotaomicron (132, 133), generates toxic doses of both superoxide and H$_2$O$_2$ whenever it enters oxic environments; thus, it may use H$_2$O$_2$ as a proxy to detect aerated habitats. In contrast, P. aeruginosa is an aerobe—but its special feature is that in competitive environments it synthesizes pyocyanin, a redox-active compound which can produce both ROS, perhaps to poison competitors (134). This behavior may necessitate the simultaneous synthesis of both superoxide and H$_2$O$_2$ defenses lest P. aeruginosa also poison itself. Meanwhile, DNA-binding assays and transcriptional analyses have shown that in Magnetospirillum gryphiswaldense OxyR induces the synthesis of genes involved in magnetosome production (121). Magnetotactic bacteria make internal magnetic particles as a way to orient themselves and dive into deeper water where there is less oxygen to impair these oxygen-sensitive bacteria; one infers that H$_2$O$_2$ is an environmental signal that triggers this defensive taxis. Together, these data indicate that OxyR has been adapted by different organisms in ways that fit their unique environmental niche.

A key question is whether OxyR plays a leading role in defending bacteria from the H$_2$O$_2$ that phagocytes produce as part of the cell-based immune response. Calculations predict phagosomal H$_2$O$_2$ levels (47, 66) that are adequate to activate OxyR, at least in E. coli (60), and local H$_2$O$_2$ levels could conceivably rise far higher in contained environments, such as abscesses. Infection data support this idea. OxyR is important in the colonizing ability of pathogens such as E. coli O1:K1:H7, Bacteroides fragilis, and Hemophilus influenzae. Mutants lacking oxyR were unable to colonize animal models or to induce abscesses in competition assays where they were mixed with wild-type cells (118, 126, 135). Biofilms can also shelter bacteria from external stressors—perhaps including H$_2$O$_2$ that is produced by an inflammatory response. The OxyR responses of Serratia marcescens, Neisseria gonorrhoeae, Klebsiella pneumoniae, and Tannerella forsythia mediate biofilm formation, a process that helps the bacteria to persist in hosts (119, 122, 129, 136). The OxyR system is required for swarming motility and the production of exotoxins by P. aeruginosa: oxyR mutants were non-motile on plates and were unable to inhibit dendritic cell proliferation (134, 137). However, while these observations demonstrate a role for OxyR in coping with host environments, they do not directly implicate the host response as the source of the H$_2$O$_2$ stress. Indeed, models of urinary tract infection demonstrated that oxyR mutants of E. coli were unsuccessful at colonization—but this phenotype persisted in a host that lacked its phagocytic NADPH oxidase (135). Presumably growth of the mutant was inhibited by H$_2$O$_2$ that was created by other environmental sources, such as competing lactic acid bacteria.

**THE THIOL-SENSING MECHANISM OF Yap1p**

The model eukaryote Saccharomyces cerevisiae also activates a defensive response when it senses hazardous H$_2$O$_2$ in its environment—but although it depends upon a thiol-based sensor to do so, the sensor is unrelated to OxyR. Yap1p is the key transcription factor (138). Its amino-terminus has a bZip DNA-binding domain, but in the absence of H$_2$O$_2$, Yap1p shuttles between the nucleus and the cytoplasm (Figure 8). However, when H$_2$O$_2$ levels rise, Yap1p is indirectly activated. The cytoplasmic Gpx3 is a glutathione peroxidase whose catalytic Cys36 residue is alternatively oxidized to a sulfenic acid by H$_2$O$_2$ and reduced to the thiol by glutathione, with the average redox state dictated by the level of H$_2$O$_2$. This sulfenic acid form can react with the C598 residue of Yap1p, which is part of the cysteine-rich domain in the N-terminus, to form an interprotein disulfide intermediate. Subsequent thiol-disulfide exchange reactions lead to the formation of an intramolecular disulfide bond between Yap1p C303 and C598, causing global conformational change. As a result, the Yap1 nuclear export signal is hidden, which blocks its interaction with the nuclear exporter Crm1 (139–141). The resultant nuclear localization of Yap1p results in the activation of several genes. Thus, unlike OxyR, the cysteine residues of Yap1p do not directly react with H$_2$O$_2$. This difference may ensure that Yap1p stays activated even after migrating into the nucleus, where the H$_2$O$_2$ may not be as high as in the cytoplasm.

In broad outline, the membership of the Yap1p regulon overlaps with that of the OxyR regulon (Table 3). These proteins scavenge H$_2$O$_2$, change iron levels, and influence the thiol status of the cell (146, 147). Yap1p induces several peroxidases, including Ahp1, Gpx2, and Ts1, that scavenge cytosolic H$_2$O$_2$; it is currently unclear why there are three such systems. The glutathione reductase GLR1 is induced to reduce the glutathione disulfide that is formed when Gpx2 reduces H$_2$O$_2$. Additionally, Yap1p also drives synthesis of Ctt1, a cytosolic catalase. The mitochondrial iron exporter Mmt1 is also induced. It has been hypothesized that iron is exported in order to avoid H$_2$O$_2$ damage to the mitochondrial DNA (145). Alternatively, the flow of iron into the cytosol may help the repair of Fe-S clusters of H$_2$O$_2$-sensitive enzymes, such as LeuCD, that are localized there. Similar to OxyR, Yap1p is ultimately turned off by a thioredoxin system that consists of thioredoxins Trx1 and Trx2 and thioredoxin reductase Trr1 (148).

The fission yeast Schizosaccharomyces pombe features an interesting orthologue of the Yap1 system: Nuclear localization of the Pap1 transcription factor is accomplished when it receives...
A disulfide bond from thiol peroxidase [reviewed in (149)]. One intriguing feature of its regulon is that it includes not only familiar H$_2$O$_2$ defenses but also drug-resistance genes. This feature raises the possibility that H$_2$O$_2$ stress is frequently imposed upon S. pombe by natural antibiotics, much as the linkage of drug pumps and SOD to the SoxRS system of E. coli reveals that redox-cycling drugs are a natural source of superoxide stress.

**THE Fe-BASED SENSING MECHANISM OF PerR**

Some bacteria rely on a thiol-independent mechanism of H$_2$O$_2$ sensing. Unlike OxyR and Yap1p, the transcriptional repressor PerR takes advantage of the reaction between Fe(II) and H$_2$O$_2$ to detect the stress. Most extensively studied in Bacillus subtilis (150–152), PerR is a Fur homolog containing a structural zinc site and a regulatory metal binding site. It was probably easy to evolve Fur to sense H$_2$O$_2$ stress: Fur:Fe(II) already reacts with H$_2$O$_2$, and Fur and PerR are sufficiently similar in primary sequence that genomic inspection cannot reliably distinguish the two.

PerR acts as a dimeric repressor when it is bound to either Mn(II) or Fe(II). Under most growth conditions, PerR has a greater binding affinity for Fe$^{2+}$. However, in iron-limited medium that has been supplemented with manganese, PerR binds to Mn(II). The identity of the metal is functionally important, as Mn-bound PerR does not react with H$_2$O$_2$. This is probably by evolutionary design, as manganese-rich/iron-poor cells are intrinsically less vulnerable to H$_2$O$_2$. Because manganese supplants iron in mononuclear enzymes and a paucity of iron precludes much DNA damage, cells need not waste resources defending themselves against H$_2$O$_2$. In contrast, Fe-bound PerR reacts with H$_2$O$_2$ (Figure 9) with a rate constant of $10^5$ M$^{-1}$ s$^{-1}$, which approximates that of OxyR (153); the similarity in rate constants suggests that the same level of H$_2$O$_2$ may be toxic in B. subtilis as in E. coli. The reaction oxidizes two of the His ligands bound to iron, forming 2-oxo-histidine; because the oxidized ligands cannot bind metal and the oxidation cannot be reversed, the repressor is permanently inactivated (154). Intriguingly, in vivo studies have shown that the majority of PerR in Staphylococcus aureus (PerRSA) is present in the oxidized form during aerobic growth, whereas this is not true of the B. subtilis...
PerR regulon of B. subtilis are more resistant to H2O2 but have trouble growing. It is unclear why they would require both sensing systems.

Interestingly, the constitutive expression of the B. subtilis PerR regulon—in a perR mutant—causes trouble by excessively lowering the pool of intracellular iron (159). These mutants are more resistant to H2O2 but have trouble growing. It is unclear which enzyme-activity deficiency causes the poor growth. The iron deficiency was tracked to the combined repression of iron uptake by Fur, plus iron depletion due to the induction of KatA. Under these inducing conditions KatA becomes the single most abundant protein in the cell, comprising a whopping 10% of the total cell protein (159). This situation is reminiscent of OxyR-driven iron deficiency in E. coli; however, the latter is abated by induction of the Clp system (27). It seems, then, that whereas OxyR induction does not interfere with growth—and, indeed, can support it via the service of cytochrome c peroxidase—the induction of PerR is an emergency response that is incompatible with continued growth. Perhaps B. subtilis is wired to enter a period of stasis when exposed to H2O2 stress, with growth resuming only after the threat has passed, whereas OxyR allows E. coli to adjust and continue growing.

After H2O2 stress, the inactivated PerR is degraded by the protease LonA, and the repression of the PerR regulon is restored when the newly synthesized PerR binds either Mn or Fe (160).

### BACTERIA USE PerR DIFFERENTLY BASED ON THEIR NICHE

Similar to OxyR, PerR has also been adapted by bacteria to fit their particular niches. Differences have emerged in the types of PerR, what it senses, and the genes that it controls. Most bacteria have a single PerR regulator, such as Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, and Helicobacter hepaticus (161–165). On the other hand, Bacillus Licheniformis has one PerR and two PerR-like proteins, both of which can sense H2O2 by histidine oxidation (166). Other bacteria contain both OxyR and PerR, including N. gonorrhoeae, B. thetaiotaomicron, and D. radiodurans (133, 167, 168); it is not yet clear why they would require both sensing systems.

The importance of PerR in different organisms may reflect the circumstances under which these bacteria experience H2O2 stress. Low-level aeration induces the PerR regulon of the anaerobe Clostridium acetobutylicum, perhaps due to endogenous H2O2 formation (169); as oxygen levels rise, induction of the regulon is critical for cell survival. In Campylobacter jejuni, a microaerophile that lacks SoxRS and OxyR homologs, the superoxide dismutase sodB is induced in perR mutants (170). These observations indicate that PerR may not be limited to defending cells against only H2O2.

Surprisingly, it has been shown that perR mutants of S. aureus, S. pyogenes, and Group A Streptococcus have lower virulence and lower intracellular survival in infected macrophages (161, 164, 171, 172), even though the derepression of the PerR regulon might be expected to induce defenses against the oxidative stress these bacteria encounter in their hosts. It is possible that the constitutive induction of the PerR regulon causes secondary growth defects similar to what is
seen in *B. subtilis* (159). If so, it may explain why the perR mutants of these pathogens are unable to colonize their hosts. In *S. aureus* and *Staphylococcus epidermidis*, PerR represses the expression of ferritin under low-iron conditions where PerR binds to Mn, and induces it in the presence of iron, indicating that like in *B. subtilis*, PerR can regulate metal homeostasis independently of oxidative stress (161, 173).

**DO OxyR, Yap1p, AND PerR USEFULLY DETECT OTHER STRESSORS?**

The reactive sensors of OxyR and PerR—a hyperreactive thiol and Fe(II), respectively—can be modified by reactive species other than H$_2$O$_2$, and this observation raises the question of whether these transcription factors profitably respond to these other stresses. The effectors that have been examined most closely are nitric oxide (NO) and disulfide stress.

cesses (174, 175), and it is deliberately generated at toxic levels by macrophages as part of the cell-based immune response (176). It is a radical species that can pair with the unpaired d-orbital electrons of iron; as a result, NO binds heme, exposed iron-sulfur clusters, and mononuclear iron, potentially inhibiting the electrons of iron; as a result, NO binds heme, exposed iron-sulfur clusters, and mononuclear iron, potentially inhibiting the effect is merely incidental to the iron-binding activity of NO.

"Disulfide stress" is a term attached to conditions that create and disseminate disulfide bonds among cellular proteins. In many studies it is imposed by exposing microbes to diamide, a manmade reagent designed to create disulfide bonds from cellular thiols (193). Similar stresses can activate Yap1p of yeast. It is directly linked to detoxifying systems such as superoxide dismutase and catalase, and this response depended upon Yap1p (190). However, a study that used a nitric oxide donor did not detect this effect (191). The possibility exists, then, that the effects of NO and/or nitrosothiols upon OxyR and Yap1p are adventitious. Similarly, exogenous NO can react with the Fe(II) in bacterial PerR and, by inactivating the repressor, trigger induction of its regulon (192). However, members of this regulon do not provide any obvious route to remediate this stress, and so it seems likely that effect is merely incidental to the iron-binding activity of NO.

Thus far, the only condition under which disulfide stress is known to naturally occur in *E. coli* is during periods of rapid cystine import (196). This situation arises when sulfur-limited cells, which induce all forms of sulfur importers, encounter cystine. Gross over-import of cystine results, and disulfide-exchange reactions cause disulfide bonds to be transferred from the imported cystine to cytoplasmic proteins. OxyR is modified, and it induces its regulon. Both the thioredoxin and glutaredoxin systems that it induces act to minimize the disulfide stress. Disulfide stress can also be imposed by exposing cells to antimicrobial plant compounds such as diallyl thiosulfinate and diallyl polysulfanes; the induction of *ahpC*, *trxA*, and *trxC* results (197). It seems unlikely that *E. coli* naturally encounters these chemicals.

similar stresses can activate Yap1p of yeast. It is directly modified by diamide—without the mediation of Gpx3—and the H$_2$O$_2$-sensing C303 is not involved in the resultant conformational change (198). Instead, disulfide linkages are formed between C598 and C620, C620 and C629, and C598 and C629. Glutathione reductase and thioredoxin are subsequently induced. However, broadly speaking, the activation of these systems by disulfide-generating agents—and perhaps by nitrogen species—has
attracted a fair amount of attention without compelling evidence that these outcomes are not accidental.

WHAT’S NEXT?

To date, the mechanisms by which \( \text{H}_2\text{O}_2 \) poisons bacteria have been explored primarily in test tubes. From those studies we have learned that \( \text{H}_2\text{O}_2 \) stress is iron-focused, driven by reactions with enzymic iron-sulfur clusters and Fe(II) prosthetic groups and with the pool of loose iron. The high rate constants of these reactions, and the abundance of vulnerable enzymes, means that low-micromolar concentrations of \( \text{H}_2\text{O}_2 \) suffice to bring bacterial growth to a halt. The known defenses remediate the same injuries that have been discovered, which provides confidence that the overall picture has come into shape. In \( \text{E. coli} \), the most-examined microbe, it appears that the endogenous levels of \( \text{H}_2\text{O}_2 \) in the fully aerated bacterium falls just short of the threshold for \( \text{H}_2\text{O}_2 \) toxicity—and for the induction of emergency responses. Those responses, then, evidently exist to shield the cell from external \( \text{H}_2\text{O}_2 \).

The next step is to figure out how this information translates to real-world environments. The broad distribution of \( \text{H}_2\text{O}_2 \) defenses, and in particular of inducible defenses, suggests that \( \text{H}_2\text{O}_2 \) stress is a pervasive phenomenon that extends, at least episodically, to most biological habitats. Yet the actual circumstances and severity of oxidative stress remain poorly understood. Plausible sources of stress range from the redox collision that occurs at oxic/anoxic interfaces to the oxidative burst of mammalian and plant defenses. We infer that different microbes may encounter this stress in different circumstances, as the defensive reglons have been modified to suit their specific situations. The rationale for these differences is not always clear; in particular, we do not yet understand why some organisms use \( \text{OxyR} \) as a sensor, why others use \( \text{PerR} \), and why a third set employ both. Thus, if we are to fully understand oxidative stress, we will need to continue to expand these studies beyond \( \text{E. coli} \) and yeast, and we will need to evaluate the intensity of \( \text{H}_2\text{O}_2 \) stress and the role of these systems in natural habitats.

For many microbiologists, the key questions concern whether host-generated \( \text{H}_2\text{O}_2 \) plays a role in suppressing invasion by most bacteria—and, if it does so, by which strategies dedicated pathogens manage to circumvent this stress. It may seem intuitively obvious that phagocytic \( \text{H}_2\text{O}_2 \) is a potent defense, but back-of-the-envelope calculations suggest that the \( \text{H}_2\text{O}_2 \) levels may not rise as high as workers have sometimes assumed. Part of the difficulty here is that phagocytic action has typically been studied using as prey the same professional pathogens that have, by definition, developed ways to elude the toxicity. We endorse the idea of studying the process using the 99% of bacteria that phagocytes efficiently kill.

AUTHOR CONTRIBUTIONS

AS is the first author and JI is the last author. Both authors have contributed to the writing. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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