The molecular mechanisms and physiological consequences of oxidative stress: lessons from a model bacterium

James A. Imlay

Abstract | Oxic environments are hazardous. Molecular oxygen adventitiously abstracts electrons from many redox enzymes, continuously forming intracellular superoxide and hydrogen peroxide. These species can destroy the activities of metalloenzymes and the integrity of DNA, forcing organisms to protect themselves with scavenging enzymes and repair systems. Nevertheless, elevated levels of oxidants quickly poison bacteria, and both microbial competitors and hostile eukaryotic hosts exploit this vulnerability by assaulting these bacteria with peroxides or superoxide-forming antibiotics. In response, bacteria activate elegant adaptive strategies. In this Review, I summarize our current knowledge of oxidative stress in *Escherichia coli*, the model organism for which our understanding of damage and defence is most well developed.

Reduced
With a lower oxidation state, typically as a result of acceptance of an electron from another molecule or atom.

Department of Microbiology, University of Illinois at Urbana-Champaign, Urbana, Illinois USA.
e-mail: jimlay@illinois.edu.
doi:10.1038/nrmicro3032
Published online 28 May 2013

Microbial life first evolved in a world devoid of oxygen and rich with reduced iron[1]. By three billion years ago, the extant microbial life forms shared basic biochemical mechanisms and a common metabolic plan, which persist today. The subsequent oxygenation of the atmosphere by photosynthetic organisms created a crisis: oxygen is a reactive chemical, and organisms had to devise strategies to defend themselves against it.

Molecular oxygen (O_2) is small and nonpolar, and it diffuses across typical biological membranes as quickly as through water[2]. Consequently, even the most active cells cannot respire quickly enough to lower the intracellular O_2 concentration substantially below the concentration immediately outside the cell. Some microorganisms escape oxidative stress by residing in anaerobic microhabitats; all others must contend with intracellular O_2. Cursory examination shows that the ability to do so varies widely: obligate anaerobes cannot tolerate oxygen at all, microaerophiles require a low-micromolar O_2 concentration, and aerobes thrive in air-saturated fluids. However, virtually all of these microorganisms suffer poor growth, elevated mutagenesis or even death when they are exposed to O_2 levels that exceed those of their native habitats. This also holds true for the facultative anaerobe *Escherichia coli*[3].

The nature of the underlying injuries is not self-evident, as amino acids, carbohydrates, lipids and nucleic acids — the structural molecules from which organisms are made — are essentially unreactive with O_2. In the 1950s, Gerschman *et al.* suggested that oxygen toxicity derives from the same events that underlie the toxicity of ionizing radiation: the formation of partially reduced reactive oxygen species (ROS)[4]. The four-electron reduction series of O_2 is depicted in FIG. 1a and shows that the addition of consecutive electrons generates superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$). The lethal effects of ionizing radiation had recently been shown to derive from $HO^{\bullet}$ radicals, so Gerschman's idea seemed plausible. It was not immediately obvious how intracellular O_2 might obtain the three electrons that could reduce it to $HO^{\bullet}$, but catalases and peroxidases (enzymes that degrade H_2O_2) had long been recognized to be ubiquitous among aerobic organisms[5] (FIG. 1b). The existence of these enzymes implied that organisms must somehow routinely encounter H_2O_2 and that, were it not scavenged, H_2O_2 would harm the cell. In 1969, McCord and Fridovich reported the existence of an enzyme that dismuted $O_2^{\bullet-}$, and so a similar inference was drawn for this molecule[6] (FIG. 1b). These ideas were finally confirmed when mutants of *E. coli* were generated[7]. Strains that lack both cytoplasmic superoxide dismutases (SODs) were found to grow as well as their wild-type parents in anaerobic cultures, but to grow poorly in aerobic media. Analogous results were later observed for strains that lack the primary catalases and peroxidases[8]. These studies set the stage for three fundamental questions

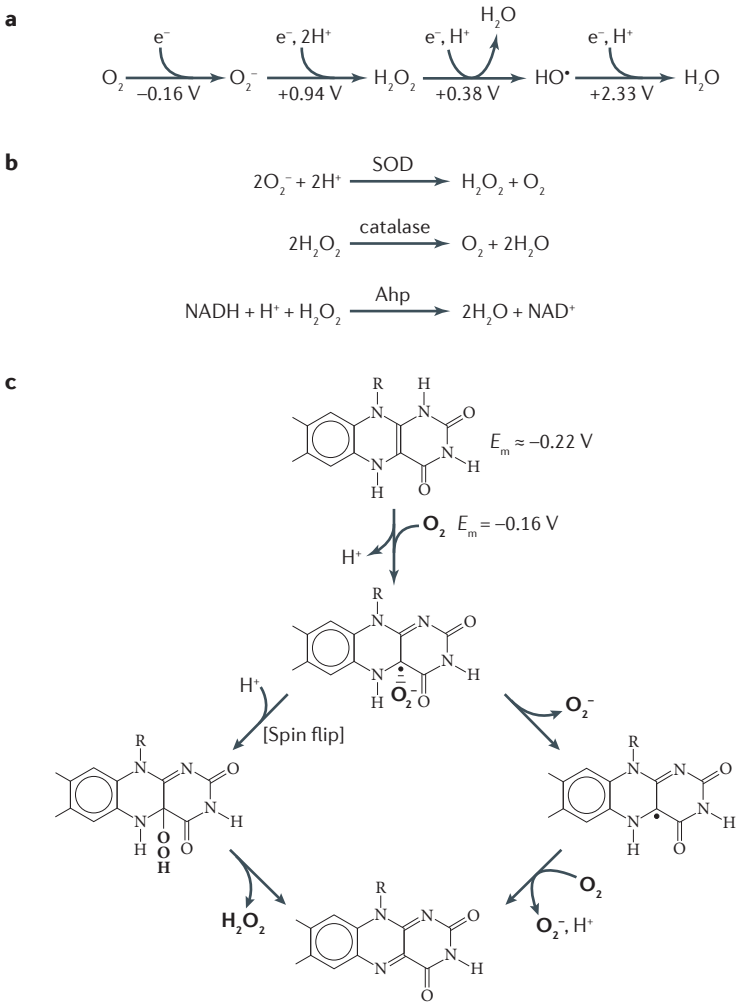

Figure 1 | **The generation of reactive oxygen species and the enzymes used for scavenging. a** | The univalent reduction series of oxygen. The standard reduction potentials (pH 7) of molecular oxygen (O_2), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO•) indicate that, with the exception of O_2, these compounds are potentially potent univalent oxidants. The standard concentration of O_2 is regarded as 1 M. **b** | The *Escherichia coli* enzymes that are responsible for degrading reactive oxygen species (ROS). O_2^- is dismuted by superoxide dismutase (SOD). H_2O_2 is targeted by catalases (catalase E and catalase G), as well as by alkyl hydroperoxide reductase (Ahp). **c** | Two pathways of adventitious $FADH_2$ oxidation on flavoproteins. Flavin autoxidation is possible because enzymatic flavins commonly have univalent reduction potentials as low as that of O_2. The pathway to the left requires an electron spin flip by either flavosemiquinone or O_2^-, allowing adduction and, ultimately, H_2O_2 release[141]. The pathway on the right releases two consecutive molecules of O_2^- to the bulk solution. The left pathway predominates in most enzymes studied to date. ROS and O_2 are shown in bold. E_m, redox potential.

Spin-aligned electrons
Electrons that are in separate orbitals and have the same spin quantum number. Two electrons must have opposite spins to reside in the same orbital.

Reduction potential
The measure of the thermodynamic affinity of a compound for an electron.

that continue to frame the field. First, how do O_2^- and H_2O_2 arise inside aerobic cells? Second, what types of damage do these species create? And finally, what strategies do cells use to defend themselves against these compounds? This Review highlights the damage caused by O_2^-, H_2O_2 and HO•, as these are the oxidants that are most relevant in aerobic environments. That said, other oxidizing species do arise and cause discrete types of cellular injuries (BOX 1). In this Review, I discuss the endogenous and exogenous sources of H_2O_2 and O_2^- and describe the damage that they can cause in the *E. coli* cell. Furthermore, I consider the defence strategies and repair programmes that *E. coli* has evolved to survive exposure to these toxic species.

Sources of intracellular O_2^- and H_2O_2

O_2 has an even number of electrons, but the final two reside in discrete orbitals as unpaired, spin-aligned electrons. This arrangement constrains O_2 to accept electrons one at a time[9]. Because the univalent reduction potential of O_2 is slightly negative (–0.16 V; FIG. 1a), its affinity for that first electron is low, so O_2 can take electrons only from good univalent electron donors, such as metal centres, flavins and respiratory quinones. Such cofactors are prominent electron carriers in respiratory chains, and indeed both O_2^- and H_2O_2 have been detected as trace products when submitochondrial particles or inverted bacterial membranes respire *in vitro*[10,11]. The flavins of dehydrogenases were subsequently identified as the primary sources of O_2^- and H_2O_2 (REFS 12,13). However, the rate at which cells produce H_2O_2 is not substantially diminished for mutants lacking respiratory enzymes, which led to the conclusion that both O_2^- and H_2O_2 are primarily produced by the accidental autoxidation of non-respiratory flavoproteins[14,15]. Indeed, such flavoproteins are found throughout metabolism, and a wide variety of them release ROS *in vitro*, including glutathione reductase, lipoamide dehydrogenase and glutamate synthase[16–18].

Flavoprotein autoxidation occurs when O_2 adventitiously collides with the dihydroflavin of the reduced enzyme (FIG. 1c). Resultant electron transfer generates O_2^- and a flavosemiquinone species. Sometimes the O_2^- immediately diffuses away, but most of the time a second electron transfer occurs before O_2^- escapes the active site, and H_2O_2 is the species that enters the bulk solution. Thus, such enzymes are probably responsible for both O_2^- and H_2O_2 production. The autoxidation rates of flavoproteins vary, as these rates depend on the degree of flavin exposure, the flavin midpoint potential and the residence time of electrons on it[19]. Therefore, it is plausible that the degree of ROS stress that an organism experiences depends on the titres of the most autoxidizable enzymes in the cell. For example, fumarate reductase is an abundant anaerobic respiratory enzyme that reacts unusually rapidly with oxygen, and it is the predominant source of O_2^- when facultative and obligately anaerobic bacteria enter aerobic habitats[15,20]. Because the rate of enzyme autoxidation also depends on collision frequency, the rate of ROS formation is greater when oxygen concentration is high[14] — a fact that explains the toxicity of hyperoxia. In *E. coli*, a minor fraction of ROS formation derives from the autoxidation of menaquinone, the low-potential electron carrier in the respiratory chain[21]. Like redox enzymes, it autoxidizes in proportion to the oxygen concentration.

Only 0.1–1% of the electron flux through any particular enzyme is likely to be intercepted by O_2. However, measurements of H_2O_2 efflux from scavenger-deficient cells indicate that the aggregate rate of H_2O_2 formation inside aerobic *E. coli* is 10–15 μM per second (REF. 14). O_2^- formation is estimated to be about 5 μM per second,

Box 1 | Confused about oxidative stress and antioxidants?

The term oxidative stress is unfortunately broad. This Review focuses on the damage created by superoxide (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (HO•), which are the oxidants most likely to plague aerobic organisms. However, other oxidizing species can cause injuries that are both important and chemically distinct. Molecular oxygen (O_2) itself can directly inactivate specialized free-radical and low-potential enzymes that are required for some forms of anaerobic metabolism[131,132]. For example, in *Escherichia coli*, O_2 rapidly poisons pyruvate:formate lyase and the anaerobic ribonucleotide reductase, and in other organisms it can directly inactivate enzymes such as pyruvate:ferredoxin oxidoreductase and nitrogenase, which use low-potential Fe–S clusters to conduct difficult chemistry. These mechanisms of toxicity restrict some bacteria and archaea to anaerobic habitats.

Other compounds create stresses that similarly fall within the broader notion of oxidative stress. Singlet oxygen is a potent divalent oxidant that can be formed by excited chromophores in photosynthetic microorganisms and plants[133]. It can covalently damage both enzymes and lipids; carotenoids, which quench singlet oxygen, are crucial for protection. Disulphide stress is often created in laboratory organisms by the addition of diamide, a synthetic electrophile that oxidizes thiols without the involvement of oxygen species[134]. Diamide was initially thought to mimic the action of H_2O_2, but at this point there is uncertainty about whether physiological H_2O_2 oxidizes many thiols; natural sources of errant disulphide bonds await identification. Hypochlorous acid (bleach) is a potent oxidant that is generated by the myeloperoxidases of neutrophils. It reacts especially rapidly with sulphurous amino acids, and it seems that *E. coli* possesses both a transcriptional regulator[135] and a chaperone[136] that respond to it. Finally, even the redox-cycling compounds that stimulate O_2^- formation exert their own direct effects by abstracting electrons from enzymatic flavins and Fe–S clusters[52]. In all these cases, the particular chemistry of the oxidant results in distinct physiological effects, and cells deploy different strategies to defend themselves. Thus, it is important that the stresses induced by different compounds not be equated.

So what is an antioxidant? Exogenous 'scavenging' agents have virtually no chance of substantially diminishing the intracellular level of either O_2^- or H_2O_2 because the cell is already loaded with high titres of very efficient enzymes that do the same thing. Similarly, it seems improbable that exogenous agents can have much impact on intracellular HO• lifetimes, as cells are already crowded with biomolecules that react with this radical at nearly diffusion-limited rates. The upshot is that actual antioxidants must work through other mechanisms. Manganese can protect cells by substituting for iron in mononuclear enzymes (see main text) and possibly by quenching nascent ferryl radicals before HO• is released[137]. Cell-penetrating iron chelators, such as dipyridyl and *o*-phenanthroline, bind unincorporated intracellular iron; they thereby block its participation in Fenton chemistry and prevent oxidative DNA damage. Supplements of thiols (such as acetylcysteine and thiourea) and reductants (such as ascorbate) have been widely used in experiments to diagnose oxidative stress, but it is much less clear how they might help, as these species are not especially effective at scavenging ROS. One possibility is that extremely large doses of thiols will penetrate cells and chelate iron.

on the basis of *in vitro* studies[22]. Because O_2^- and H_2O_2 react rapidly with vulnerable targets (see below), these rates are high enough to require the synthesis of ample scavenger enzymes (BOX 2).

Scavenging enzymes

E. coli contains three SODs: cytoplasmic iron- and manganese-cofactored enzymes (Fe SOD and Mn SOD) and a periplasmic copper–zinc-cofactored enzyme (Cu–Zn SOD). O_2^- cannot cross membranes, so scavenging enzymes must be located within the cellular compartment that they are intended to protect. Periplasmic O_2^- formation has been detected during exponential growth[21], but apparently it is not harmful enough to warrant periplasmic SOD synthesis during this growth phase, as Cu–Zn SOD is synthesized only when cells enter stationary phase[23]. To date, no phenotype has been reported for *E. coli* mutants that lack Cu–Zn SOD [24].

The cytoplasmic SODs are abundant enough to keep steady-state O_2^- at a subnanomolar concentration[22] (BOX 2). Titration studies have shown that this high SOD activity is necessary to avoid enzyme damage and to ensure vigorous growth[25]. The two isozymes Fe SOD and Mn SOD are coordinately regulated in response to iron levels. The iron-loaded repressor Fur blocks Mn SOD synthesis when iron levels are high; when iron levels are low, the deactivation of Fur stimulates both Mn SOD synthesis and transcription of the small RNA (sRNA) RyhB, which triggers degradation of the Fe SOD-encoding mRNA[26,27]. Control by Fur also ensures that the manganese importer MntH is induced, thereby enabling activation of Mn SOD[28]. Together, this arrangement ensures that Fe SOD is the default isozyme and that Mn SOD is synthesized when Fe SOD cannot be activated. Mn SOD synthesis is also stimulated whenever O_2^--generating antibiotics are present, with the control being exerted by the SoxRS system (described below)[29,30]. SoxRS is not active during normal aerobiosis, in the absence of these exogenous O_2^- sources.

The picture of H_2O_2 scavenging is more complex. Over the years, researchers have identified a handful of *E. coli* enzymes that can degrade H_2O_2 *in vitro*. Of these, only three have important roles *in vivo* under standard culture conditions: alkyl hydroperoxide reductase (Ahp), catalase G (KatG) and catalase E (KatE)[8]. Ahp is a two-component (AhpC–AhpF) thiol-based peroxidase that transfers electrons from NADH to H_2O_2, thereby reducing H_2O_2 to water. This peroxidase is the primary scavenging enzyme under routine growth conditions, as evidenced by the fact that *ahpCF*-null mutants accumulate enough H_2O_2 to activate the OxyR H_2O_2-stress response. KatG belongs to the catalase–peroxidase family and is only weakly expressed in exponential cells. However, OxyR strongly induces both *ahpCF* and *katG* when cells are stressed by exogenous H_2O_2 (REF. 31). KatE is strongly expressed in stationary phase cells only, as it is induced by the RpoS system[32].

Why are so many enzymes used to scavenge H_2O_2? Catalases are generally more problematic than peroxidases, as when H_2O_2 concentrations are low, the two-step catalytic cycle of catalases can stall with the haem in its intermediate ferryl/radical form. This ferryl/radical species is a potent oxidant and, if left unresolved, can abstract electrons from the surrounding polypeptide[33]. In fact, to minimize this problem, KatE-type catalases bind NAD(P)H as a rescuing reductant, whereas KatG-type catalases feature a channel that enables a range of metabolites to approach and reduce the ferryl/radical form[34]. By contrast, Ahp does not form a dangerous oxidizing species and is therefore the more efficacious scavenger during low-level H_2O_2 stress. However, Ahp can degrade H_2O_2 only as quickly as metabolism provides NADH as the stoichiometric reductant, so Ahp becomes saturated when intracellular H_2O_2 exceeds 20 μM or when catabolic substrates are scarce. Under these conditions, catalases, which do not require stoichiometric reductants, can turn over much more quickly than Ahp. Taken together, these constraints have prompted organisms to rely on Ahp when H_2O_2 levels are low, and

Metal centres
Metal atoms that confer structure and/or catalytic function on a protein. Redox enzymes commonly use the transition metals iron, copper, manganese, molybdenum, nickel and selenium for electron transfer reactions.

Flavins
Organic cofactors that bind to redox enzymes in the form of FAD or flavin mononucleotide (FMN). These cofactors are commonly used to mediate electron exchange between divalent electron donors and univalent acceptors.

Box 2 | **Reactive oxygen species by numbers: teetering on the brink**

Does hydrogen peroxide (H_2O_2) or superoxide (O_2^-) cause any damage in healthy aerobic *Escherichia coli*, or has the evolution of scavenging enzymes fully solved the problem? Direct measurements indicate that intracellular H_2O_2 is formed at a rate of 10 μM s^{-1} (REF. 14). Given the titre (~5 μM)[138] and rate constant ($k_{cat}/K_m = 4 \times 10^7$ M^{-1} s^{-1})[139] of alkyl hydroperoxide reductase C (AhpC), the primary scavenger, the steady-state H_2O_2 concentration must be ~50 nM. This seems extremely low. However, the rate constants for reactions between H_2O_2 and the dehydratases and mononuclear enzymes are typically in the range of 10^3 to 10^4 M^{-1} s^{-1} (REFS 76,92), which means that the half-time for enzyme inactivation by H_2O_2 must be as short as 20 minutes. Because the half-time for repair of dehydratase clusters is about 5 minutes[140], at any moment during aerobic growth a significant minority of the enzyme population is inactive. Any additional H_2O_2 stress will exacerbate the situation. Indeed, the OxyR system is calibrated to be activated when intracellular H_2O_2 reaches ~200 nM, and growth defects become evident when levels rise to 400 nM (REFS 38,96).

The rate of endogenous O_2^- formation is probably slightly lower (~5 μM s^{-1})[22]. The titre (20 μM) and rate constant (10^9 M^{-1} s^{-1}) of superoxide dismutases (SODs) mean that steady-state O_2^- levels are ~0.2 nM, or fewer than one molecule per cell. But it is necessary for O_2^- to be this scarce: the rate constants for inactivation of dehydratases and mononuclear enzymes by O_2^- exceed 10^6 M^{-1} s^{-1} (REF. 82), so this steady-state level of O_2^- should also cause an inactivation half-time of as little as 20 minutes. Indeed, experiments confirm that a modest decrease in SOD titre elicits enzymatic and growth defects[25]. Thus, there is nothing excessive about the high titres of scavenging enzymes: the cell needs everything it has got. By the same token, any additional stress, as a result of H_2O_2 influx from the environment or O_2^- formation by redox drugs, easily pushes the cell over the edge. This is why the OxyR and SoxRS systems are necessary.

on catalases when H_2O_2 levels are high or when cells are starved.

Many oxygen-tolerant bacteria have additional enzymes that exhibit peroxidase activity *in vitro*. *E. coli* contains thiol peroxidase[35], bacterioferritin comigratory protein (Bcp)[36] and a glutathione peroxidase homologue[37]. However, although these three enzymes are synthesized by *E. coli* under laboratory conditions, they seem to lack H_2O_2-scavenging ability *in vivo*, as *ahpCF katG katE* mutants degrade little H_2O_2 (REF. 8).On the basis of these observations, it is possible that the true physiological activities of these enzymes have not yet been correctly identified.

Exogenous oxidative stress

The basal scavenging systems described above are just barely sufficient to protect *E. coli* from endogenous O_2^- and H_2O_2 (BOX 2). However, environmental circumstances can elevate the rates at which these oxidants enter or are formed in the cell, and under such conditions the basal defences become inadequate.

The OxyR system. Environmental H_2O_2 arises from various sources: the chemical oxidation of sulphur and reduced metals at anoxic–oxic interfaces, the photochemical formation of oxidants by flavins and other chromophores, the excretion of H_2O_2 by lactic acid bacteria that contain lactate and pyruvate oxidases, and the deliberate action of the antimicrobial NADPH oxidases of macrophages, amoebae and plants. Because H_2O_2 is small and uncharged, it crosses membranes at a moderate efficiency that is similar to that of water. In fact, whenever the extracellular H_2O_2 concentration exceeds 200 nM, the rate of influx into *E. coli* exceeds the rate of endogenous H_2O_2 formation[38]. Therefore, when bacteria enter H_2O_2-containing environments, oxidative stress is likely to occur.

This threat is sensed by OxyR, a transcription factor containing an active-site Cys residue that reacts rapidly with H_2O_2 (REFS 39,40) (FIG. 2a). OxyR is normally inactive during routine aerobiosis, when the intracellular H_2O_2 concentration is ~50 nM. However, an intracellular concentration of ~200 nM is sufficient to drive OxyR into a disulphide-bonded form that actively promotes the transcription of a dozen operons around the chromosome. Because basal Ahp activity can establish a fivefold outside-to-inside gradient, an extracellular H_2O_2 concentration of 1 μM is required to activate the regulon[38]. Members of the regulon have been identified[41] (FIG. 2a). OxyR induces the synthesis of KatG and Ahp more than tenfold in an effort to drive the H_2O_2 concentration back down to innocuous levels. Other members of the regulon are best understood in the context of the damage that H_2O_2 causes, and their roles in H_2O_2 resistance are discussed below. Interestingly, an alternative mechanism of sensing and responding to H_2O_2 exists in many Gram-positive bacteria. In these organisms, peroxide-responsive repressor (PerR) is inactivated when H_2O_2 oxidizes the prosthetic iron atom in this enzyme[42]. Strikingly, this induces homologues of many of the enzymes that OxyR controls. It is currently unclear why two distinct mechanisms of H_2O_2 sensing have arisen.

The SoxRS system. O_2^- differs from H_2O_2 in that it is a charged species at physiological pH ($pK_a = 4.8$), so it cannot penetrate membranes[43,44]. This means that cytoplasmic O_2^- must be formed inside the cell. However, hostile plants and bacteria manage to induce the production of O_2^- in the cytoplasm of target bacteria by excreting redox-cycling organic compounds — typically phenazines or quinones — that can passively enter the interior of the target cell[45,46]. There, these compounds abstract electrons from low-potential metal centres, respiratory quinones and flavins. The compounds can then transfer the electrons to O_2, thereby generating O_2^-. In phenazine- or plumbagin-treated *E. coli*, the rate of O_2^- formation can increase by ~100-fold[47].

Early investigators discovered that under these conditions Mn SOD titres increase by more than tenfold[46]. This response was subsequently shown to be driven by the SoxRS system, which is inactive during regular growth but becomes highly active when cells are exposed to a wide variety of redox-cycling compounds[29,30]. SoxR is a homodimeric regulatory protein containing one sensory [2Fe–2S] cluster per subunit. During exposure to redox-active drugs, the cluster becomes oxidized. Both reduced and oxidized SoxR bind upstream of the *soxS* gene, but only the oxidized form stimulates transcription (FIG. 2b). The SoxS protein then acts as a secondary transcription factor, enhancing the expression of many genes scattered around the chromosome, including *sodA*, the gene encoding Mn SOD (see REF. 48 for a full list). When the inducing compounds are removed, SoxR returns to its reduced state, largely owing to electron transfer from the Rsx complex[49]. The extant SoxS is quickly degraded

Respiratory quinones
Lipid-soluble organic molecules that carry electrons between membrane-bound redox enzymes.

Autoxidation
Electron transfer from a reduced enzyme or cofactor to molecular oxygen.

Hyperoxia
Molecular oxygen concentrations above that of air (22%).

Thiol-based peroxidase
An enzyme that uses a redox-active Cys residue to reduce hydrogen peroxide to water.

RpoS system
The regulon that is governed by RNA polymerase σ–factor RpoS. RpoS is activated in stationary phase and under many stress conditions that suppress growth.

Chromophores
Light-absorbing compounds.

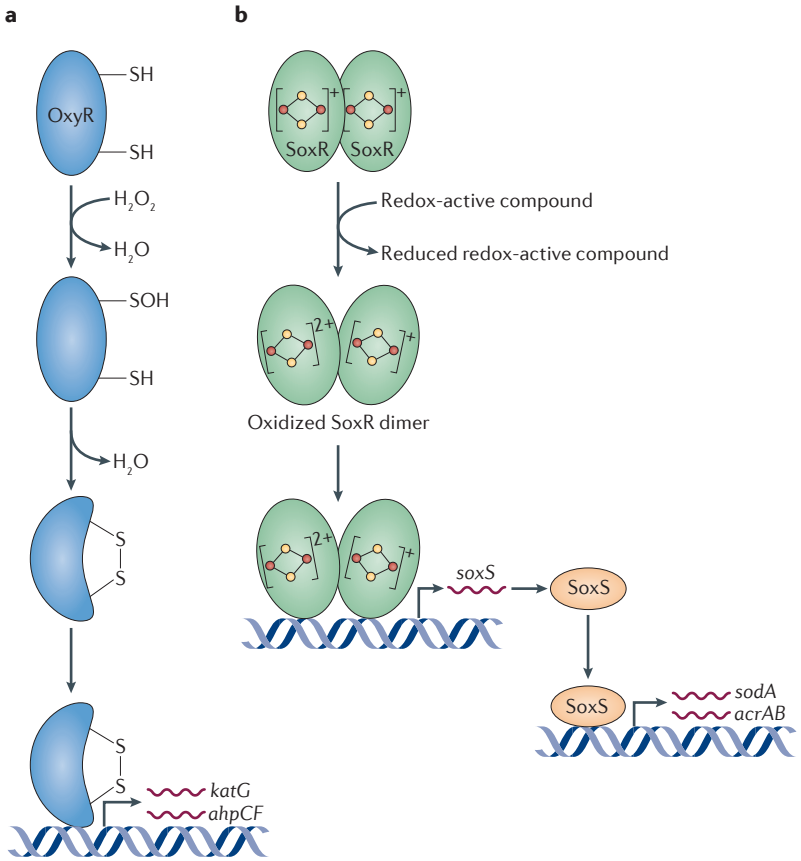

Figure 2 | **Activation of redox-sensitive transcriptional regulators in *Escherichia coli*.** *Escherichia coli* has two defence systems that are induced under conditions of oxidative stress: the OxyR system, which responds to hydrogen peroxide (H_2O_2), and the SoxRS system, which responds to redox-active compounds. **a** | Activation of the OxyR system occurs when a sensory Cys residue in OxyR reacts rapidly with H_2O_2, forming a sulphenic acid moiety that then condenses with a resolving Cys[40]. The resultant disulphide bond locks OxyR into a conformation that enables it to act as a positive transcription factor for OxyR regulon members, such as *katG* (encoding catalase G) and *ahpCF* (encoding alkyl hydroperoxide reductase), among other genes. **b** | SoxR is a homodimeric transcription factor, and each monomer contains a [2Fe–2S] cluster. The dimer becomes activated through the direct oxidation of these clusters by redox-active compounds[52], typically phenazines or quinones, which are produced by plants and bacterial competitors. Oxidized SoxR stimulates transcription of *soxS*, and the SoxS protein acts as a secondary transcription factor that goes on to activate expression of SoxRS regulon members, including *sodA* (encoding Mn-cofactored superoxide dismutase) and *acrAB* (encoding a multidrug efflux pump), among a large array of other genes.

because the amino-terminal tail is targeted by the Clp protease system, leading to an end to the response[50].

Initially, it was expected that O_2^- would be the oxidant that activates SoxR, but *in vivo* experiments strongly indicate that the redox-cycling compounds themselves are the primary physiological activators[51–54] and that O_2^- is a relatively ineffective activator. (There is also disagreement in the literature about whether O_2^- can oxidize SoxR *in vitro*[52,55].) One rationale for SoxR being activated by the redox-cycling compounds rather than by O_2^- is that redox-cycling compounds are toxic to cells even under anoxic conditions (when O_2^- cannot be generated) because of their ability to oxidatively destabilize enzymes and to act as Michael acceptors.

Michael acceptors
Unsaturated carbonyl compounds that are vulnerable to addition reactions by nucleophiles.

Thus, SoxRS needs to sense the threat even when O_2^- is absent.

Many of the SoxRS-induced proteins function to exclude redox-cycling compounds from the cytoplasm. This can be achieved by slowing their entry through modifications of the charge and porin content of the cell envelope (via WaaZY and the sRNA MicF), by actively pumping them back out of the cell (via AcrAB and TolC) or by chemically modifying them (via NfsA and YgfZ)[56–61]. A few induced enzymes — glucose-6-phosphate dehydrogenase and NADPH:ferredoxin oxidoreductase — help replenish the NADPH pools that are depleted when the drugs oxidize NADPH-reduced enzymes[62]. Other components of the SoxRS regulon serve to mitigate specific types of cell damage (discussed below). The aggregate SoxRS response has a strong impact on the ability of *E. coli* to tolerate redox-cycling compounds.

SoxRS-type systems exist in many bacteria, albeit sometimes in a modified form. SoxR often activates regulon members directly, without the intermediacy of SoxS[63]. Furthermore, SoxR seems to control the synthesis and export of phenazines in many bacteria that excrete these compounds; in such organisms, SoxR does not control SOD synthesis[51,64,65]. The benefits of phenazine excretion for the producing organisms are being actively investigated. In addition to the suppression of competitors, phenazines can solubilize iron[66], deliver electrons to insoluble oxidants[67] and act as signalling molecules[63]. The picture that has emerged is that these redox compounds are widely used by plants and microorganisms and are widespread enough that many bacteria carry a SoxR-type system to defend themselves against them. O_2^- formation is one element of the stress that these compounds impose, which explains why targeted organisms include SOD induction as part of their response.

It has also been suggested that traditional clinical antibiotics trigger the endogenous formation of ROS and thereby contribute to bacterial death[68], but this idea has since been challenged by several studies[69–71].

Damage caused by O_2^- and H_2O_2

The idea that O_2^- might be toxic to cells was initially contentious, and in fact early studies failed to identify biomolecules that this species could easily damage[72–75]. This problem was ultimately solved by investigations into the growth defects of O_2^--stressed cells. In 1976, Brown reported that hyperbaric oxygen imposed several amino acid auxotrophies on wild-type *E. coli*[3]. Ten years later, Carlioz and Touati reported that SOD-deficient mutants exhibited similar phenotypes: they were unable to grow unless their medium was supplemented with branched-chain (Leu, Ile, Val), aromatic (Tyr, Trp, Phe) and sulphurous (Met, Cys) amino acids[7]. These observations implied that hyperoxia might exert its effects by accelerating O_2^- formation and that O_2^- specifically disrupts these amino acid biosynthesis pathways. More recently, an analogous approach has revealed defects that arise when H_2O_2 accumulates in cells lacking catalase and peroxidase[76].

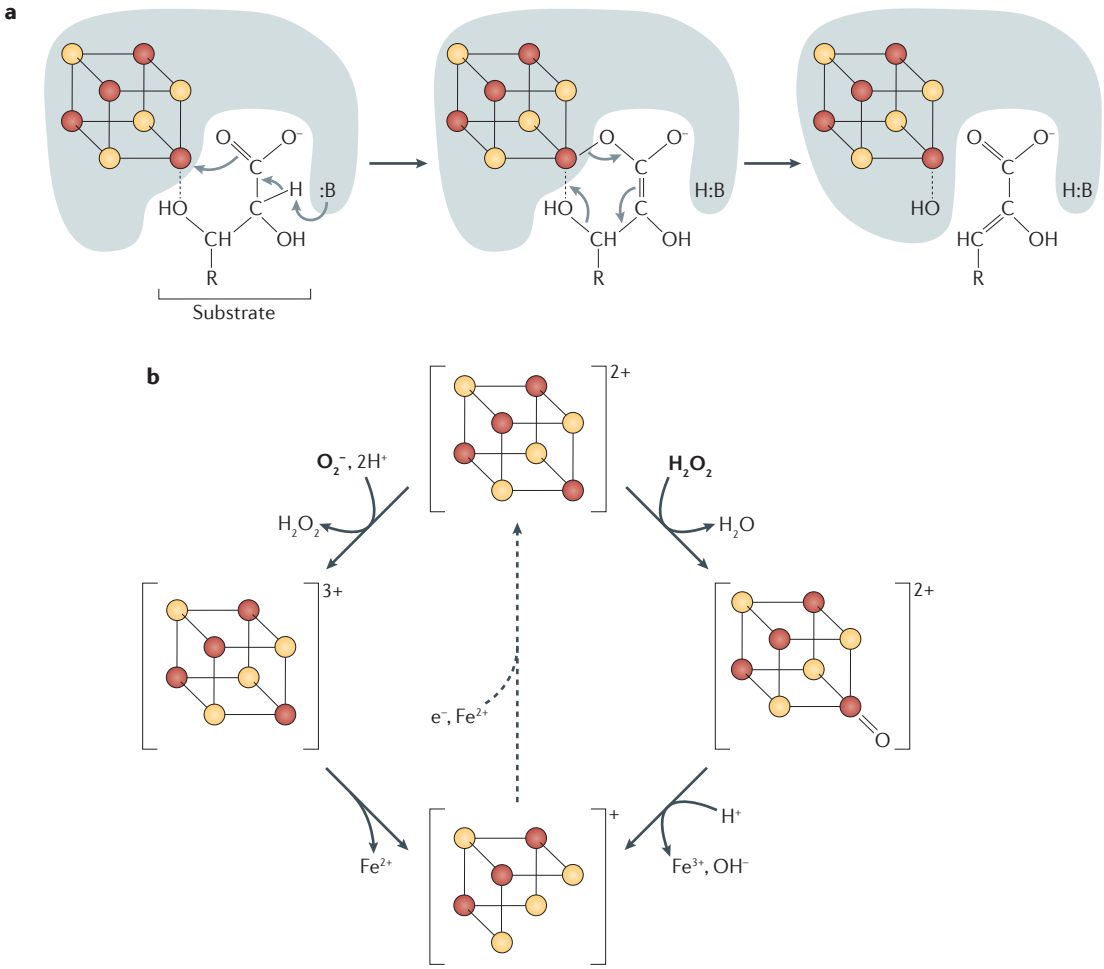

Figure 3 | **The role and oxidative vulnerability of dehydratase [4Fe–4S] clusters. a** | Cellular dehydratases reversibly dehydrate α,β-dihydroxy acids, releasing enol products (shown) that subsequently tautomerize to α-ketoacid products (not shown). The cluster coordinates substrates through their β-hydroxyl and carboxylate groups. Deprotonation by a nearby base (B:) triggers hydroxide abstraction by the catalytic iron atom, comprising a net dehydration. Light grey arrows denote electron shifts towards new bonding partners. **b** | The left pathway shows the exposed cluster being oxidized by superoxide (O_2^-), resulting in the formation of hydrogen peroxide (H_2O_2) and conversion of the cluster to an unstable $[4Fe–4S]^{3+}$ species, which then releases Fe^{2+} (REF. 82). The loss of the catalytic iron atom destroys enzyme activity. The right pathway shows oxidation of the cluster by H_2O_2, which presumably creates a transient ferryl species that abstracts a second electron from the cluster; Fe^{3+} dissociates[92]. After damage by either oxidant, the resultant $[3Fe–4S]^+$ cluster can be reactivated *in vitro* and *in vivo* by reduction and remetallation (dashed line). Reactive oxygen species (ROS) are shown in bold.

Inactivation of Fe–S-dependent dehydratases. The origin of the branched-chain auxotrophy of SOD-deficient *E. coli* was determined when Kuo and Fridovich showed that O_2^- destroys the catalytic [4Fe–4S] cluster of dihydroxy-acid dehydratase (DHAD)[77]. This cluster serves to bind α,β-dihydroxy acid substrates and, acting as a Lewis acid, directly participates in the dehydration reaction (FIG. 3a). The problem is that O_2^- is electrostatically driven to bind the solvent-exposed cluster. Following protonation, O_2^- becomes a strong univalent oxidant (FIG. 1a), and it abstracts a single electron from the cluster[78] (FIG. 3b). The oxidized cluster is unstable, and the substrate-coordinating iron atom dissociates from it, rendering the enzyme inactive. DHAD is just one member of a family of Fe–S cluster-cofactored dehydratases, and other members of the family were subsequently found to be equally sensitive to O_2^-. These include aconitase A, aconitase B, fumarase A and fumarase B of the tricarboxylic acid (TCA) cycle, a fact that explains the inability of SOD mutants to grow on TCA cycle substrates such as succinate and acetate[79–82].

Importantly, work in yeast demonstrated that SOD-deficient *Saccharomyces cerevisiae* suffers the same type of injuries. *S. cerevisiae* cytoplasmic Cu–Zn SOD gene (*sod1*) deletion mutants are defective in the activities of two [4Fe–4S] dehydratases (isopropyl malate isomerase (Leu1) and homoaconitase (Lys4)), which leads to disrupted synthesis of branched-chain amino acids and Lys[83,84]. Deletion of the mitochondrial Mn SOD gene (*sod2*) also impairs aconitase activity and thereby growth on glycerol, which requires robust TCA cycle function[85,86]. These observations were gratifying, as they

Lewis acid
A molecular moiety that can share an electron pair provided by a donor compound.

confirmed that the nature of oxidative damage in *E. coli* is likely to be predictive of the types of damage that oxidants induce in other organisms.

The rate constant for Fe–S cluster damage by O_2^- (up to $5 \times 10^6\ M^{-1}\ s^{-1}$ (REF. 82)) is so high that even in wild-type cells, in which abundant SODs keep O_2^- at a miniscule 10^{-10} M concentration, the half-time for enzyme damage is ~20 minutes (BOX 2). This time frame is short compared with the usual doubling time of cells in natural habitats, and this situation is tolerable only because cells continuously repair the damaged clusters by reduction and remetallation. It is not clear whether dedicated proteins assist the repair process. The protein YggX — a member of the SoxRS regulon — can help[52,87], although the mechanism by which it does so is unclear. A di-iron protein, YtfE, can assist repair when clusters are nitrosylated during nitric oxide stress, but this protein seems to be minimally expressed under other conditions[88].

Interestingly, two members of the SoxRS regulon encode O_2^--resistant dehydratases that can functionally replace the sensitive enzymes. Fumarase C, a non-Fe–S enzyme, is strongly induced when SoxRS detects redox-cycling compounds; fumarase C expression restores flux through the TCA cycle[25,81]. Similarly, aconitase A — which has an Fe–S cluster but seems to be resistant to inactivation *in vivo* — replaces the highly sensitive housekeeping enzyme aconitase B[89,90].

H_2O_2 also attacks Fe–S dehydratases, although the chemistry of this attack differs from that of O_2^- attack (FIG. 3b). The ability of H_2O_2 to oxidize Fe^{2+} has long been recognized:

$$Fe^{2+} + H_2O_2 \rightarrow [FeO]^{2+} + H_2O$$
$$[FeO]^{2+} + H^+ \rightarrow Fe^{3+} + HO^{\bullet}$$

The overall reaction is dubbed the Fenton reaction[91]. $HO^{\bullet}$ is an extremely powerful oxidant (FIG. 1a) that reacts at nearly diffusion-limited rates with virtually all organic molecules. Similarly to O_2^-, H_2O_2 can directly ligand and oxidize the catalytic iron atom of dehydratase clusters, precipitating Fe^{3+} loss and enzyme inactivation. If $HO^{\bullet}$ were generated during this process, one might expect it to fatally damage the local active-site residues. Therefore, it was surprising to learn that when H_2O_2 inactivates these enzymes *in vitro*, it is possible to restore full activity by supplying iron and a reductant[92]. The reason is that before the ferryl radical ($[FeO]^{2+}$) can decay into $HO^{\bullet}$, it abstracts a second electron from the cluster, converting the radical into an innocuous hydroxide anion (OH^-) (FIG. 3b). The end result is that although the enzyme is temporarily disabled by the release of Fe^{3+}, a repairable $[3Fe–4S]^+$ species is left behind.

This damage mechanism pertains only to the small set of [4Fe–4S] enzymes that are dehydratases. In most Fe–S proteins, including those involved in electron transfer reactions, the clusters are fully coordinated by polypeptide and are thereby protected from these oxidants, which must directly contact the cluster to oxidize it. However, experiments have shown that when cells are exposed to H_2O_2 for several generations, even non-dehydratase cluster enzymes are at risk[93]. This is because H_2O_2 poisons the Isc system (Fe–S cluster synthesis system), which is responsible for the transfer of Fe–S clusters to newly synthesized apoenzymes[94]. The mechanism of poisoning is unclear, although it seems plausible that H_2O_2 attacks the nascent clusters on the scaffold protein IscU, which mediates the assembly of new clusters. In any case, *E. coli* compensates for this problem by using OxyR to induce the Suf system[95]. This alternative machinery also builds clusters, but somehow it assembles them and transfers them to client proteins without interference by H_2O_2.

Inactivation of mononuclear iron proteins. O_2^- and H_2O_2 also disable a family of enzymes that use a single iron atom as a prosthetic group[76,96] (FIG. 4). As is the case in dehydratases, these iron atoms directly bind substrate and, by stabilizing oxyanionic intermediates, catalyse a wide range of reactions. This enzyme family includes epimerases, dehydrogenases, deformylases and deaminases. Inactivation by H_2O_2 involves a Fenton reaction between H_2O_2 and the iron atom, with release of Fe^{3+}. The inactivation rate constants resemble those for the dehydratases, and as little as 0.5 μM intracellular H_2O_2 disables them. In the case of ribulose-5-phosphate 3-epimerase, after enzyme damage by a single bolus of H_2O_2, the oxidized enzyme can be largely reactivated by the addition of Fe^{2+}, but a minor fraction is irreversibly inactivated owing to polypeptide damage by the $HO^{\bullet}$ that is produced[96]. Cycles of repair and re-oxidation progressively cause the loss of all activity. Other enzymes, such as threonine dehydrogenase and peptide deformylase, use a Cys residue as one of the iron-coordinating ligands. $[FeO]^{2+}$ apparently oxidizes this residue to a sulphenic acid, effectively quenching $HO^{\bullet}$ before it can be released. Sulphenates are easily reduced by cellular reductants, and remetallation allows the damaged enzyme to be fully repaired.

O_2^- also oxidizes and releases the iron atoms of mononuclear enzymes[97] (FIG. 4). Because this reaction does not form a strong oxidant such as $[FeO]^{2+}$ or $HO^{\bullet}$ radicals, the polypeptide is wholly undamaged. However, inside cells, repeated cycles of this process ultimately lead to mismetallation of the enzyme with zinc, an alternative metal that is reasonably abundant *in vivo*. Zinc is not as catalytically efficient as iron in these enzymes[76,96], so there is a progressive decline in enzyme function.

E. coli has a clever strategy to maintain activity of these mononuclear enzymes during H_2O_2 stress: it replaces the iron atom with manganese[98]. The OxyR regulon triggers the strong induction of Dps (an iron-sequestering ferritin) and MntH (a manganese importer). Thus, when the cell is under oxidative stress, the intracellular level of manganese increases. When manganese is bound in the mononuclear site, the metal serves as a H_2O_2-resistant cofactor that is almost as catalytically active as iron itself.

DNA damage. In some circumstances, the most consequential impact of oxidative stress is mutagenesis. Neither H_2O_2 nor O_2^- can damage DNA directly, but both SOD and catalase/peroxidase mutants exhibit high mutation rates[99,100]. In the case of H_2O_2, the reason is obvious: by

Half-time
In an exponential decay process, the time needed for conversion of half of the reactant to product.

Isc system
(Fe–S cluster synthesis system). A multiprotein complex that assembles Fe–S clusters on a scaffold protein and then transfers them to client proteins.

Suf system
A protein complex that assembles and transfers Fe–S clusters to recipient proteins. The Suf system comprises different proteins to the Fe–S cluster synthesis (Isc) system, and the activity of the Suf system is more resistant than that of the Isc system to chemical stress and iron deficiency.

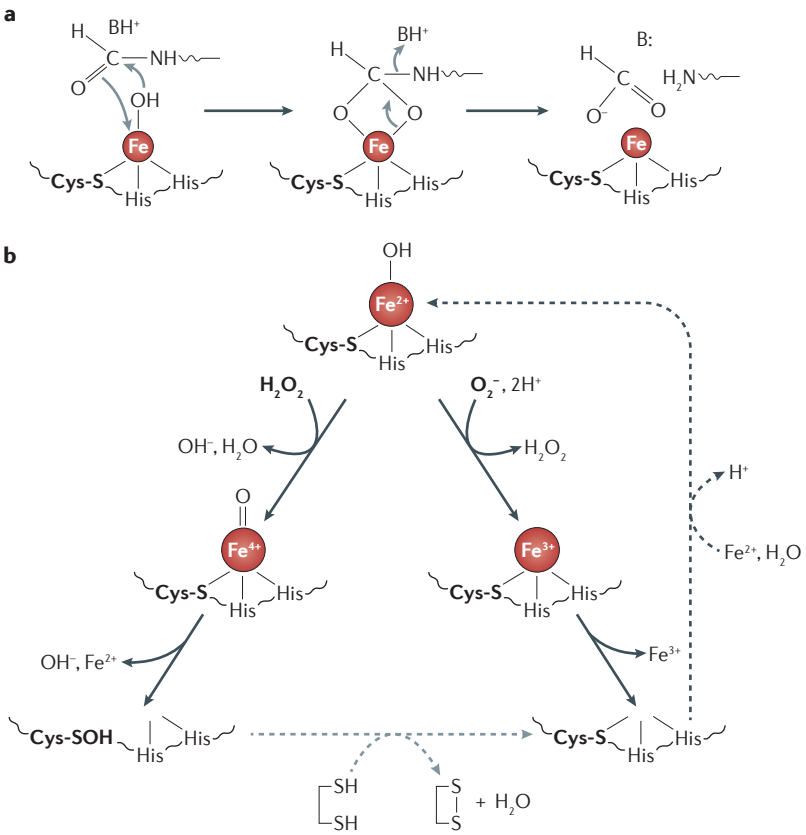

Figure 4 | **The role and oxidative vulnerability of mononuclear iron enzymes.** Hydrogen peroxide (H_2O_2) and superoxide (O_2^-) diminish the activity of mononuclear iron enzymes, which use single iron atoms as prosthetic groups. **a** | Peptide deformylase is presented as an example of this enzyme class. The cationic iron atom of peptide deformylase both activates a water molecule to provide a strong hydroxyl nucleophile (left) and stabilizes the negatively charged oxygen atom of the reaction intermediate (centre)[142]. BH^+ represents the enzymatic proton donor that ultimately cleaves the carbon–nitrogen bond in the substrate. Light grey arrows denote electron shifts towards new bonding partners. **b** | In the left pathway, oxidation of the mononuclear iron enzyme by H_2O_2 generates a transient ferryl species (Fe^{4+}=O) that is then quenched by a coordinating Cys residue. As a sulphenic species (–SOH) is the ultimate product[76], it seems likely that a thiyl radical electron is transferred to the departing iron atom. The right pathway shows oxidation by O_2^- to generate Fe^{3+}, which dissociates. The activity of the O_2^--generated apoprotein can be restored by simple remetallation (dashed black arrows), although mismetallation of these enzymes by zinc can progressively diminish activity. Reactivation of the H_2O_2-damaged enzyme requires sulphenic reduction before remetallation (dashed grey arrows). Reactive oxygen species (ROS) and the oxidizable Cys are shown in bold.

reacting with the cellular pool of unincorporated iron, some of which adventitiously associates with DNA[101], H_2O_2 produces HO•, which can oxidize both base and ribose moieties of the DNA, giving rise to a wide variety of lesions[102,103]. Guanine is disproportionately damaged because its lower reduction potential allows its electrons to hop to electron holes in nearby oxidized base radicals[104]. For example, although an adenine residue might be the initial site of HO• attack, immediate electron movement from a neighbouring guanine 'repairs' the adenine radical, and a lesion ultimately resides on the guanine. One common product is 8-hydroxyguanine, which is highly mutagenic owing to its ability to base pair with adenine in a way that eludes the intrinsic mispair detection system of DNA polymerases[105]. By contrast, thymine oxidation produces lesions that are more likely to be non-coding; such lesions block polymerase progression and tend to be lethal rather than mutagenic[106]. Similarly, the oxidation of ribose moieties generates polymerase-blocking single-strand breaks with 3′ glycolate residues 5′ to the break. These residues cannot be used as primers and must be removed before repair (see below).

Because iron is the co-reactant in the Fenton reaction, the rate of DNA damage is elevated when iron levels are high. For this reason, O_2^--stressed cells and *fur* mutants that oversynthesize iron importers exhibit high levels of DNA damage[107,108]. Conversely, during periods of H_2O_2 stress, OxyR induces Dps[109–111], a ferritin-class protein that strongly suppresses the amount of DNA damage by sequestering the unincorporated iron[100]. YaaA, another OxyR-induced protein, also has a role in controlling iron levels, although the mechanism involved is unknown[112]. Interestingly, because Fur is itself a mononuclear iron protein, it tends to lose activity during both O_2^- and H_2O_2 stress, and this could potentially lead to derepression of iron acquisition systems and the disastrous import of more iron[113]. To avoid this situation, both the SoxRS and OxyR systems stimulate Fur synthesis and thereby curb the synthesis of iron importers[114].

Repair of damaged DNA. When DNA damage occurs, repair is essential. Formamidopyrimidine DNA glycosylase (also known as MutM and Fpg), endonuclease IV and endonuclease VIII initiate the excision of oxidized bases[115–117]. These enzymes are not highly specific for particular lesions; instead, they scan for helical distortions, thereby enabling removal of the many disparate adducted bases that oxidation can produce. Exonuclease III and endonuclease IV excise fractured ribose moieties and restore a 3′ primer for DNA polymerase I-driven repair synthesis[106]. When excision systems fail to recognize lesions or when replication forks overtake adducts before they can be repaired, post-replication recombination is the back-up strategy. Strains that lack recombination (*rec*) genes are hypersensitive to exogenous H_2O_2, and under aerobic conditions *recA* mutations are synthetically lethal with either catalase/peroxidase or SOD deficiency[100,107]. In fact, strains that are deficient in both recombination and excision repair strategies, such as *recA xth* mutants (deficient in RecA and exonuclease III) and *polA recB* mutants (deficient in DNA polymerase I (Pol I) and RecB), are fully viable only in anaerobic media. Thus, routine aerobiosis creates enough oxidative DNA lesions that repair is an essential function.

RecA also controls expression of the SOS system. This regulon includes *sfiA* (also known as *sulA*), which encodes a protein that suspends cell septation until replication resumes and daughter chromosomes can be formed. Error-prone lesion by-pass is a final option to cope with DNA damage, allowing replication to proceed past lesions that have not been repaired. It is likely that this process is facilitated by one or more of

SOS system
The global response to DNA damage that is exhibited by many bacteria.

the translesion polymerases[118]. Two of these — Pol IV and PolV, encoded by *dinB* and *umuDC*, respectively — are regulated by the SOS system. Expression of these proteins is induced later than the other repair systems, presumably because mutagenesis is a collateral outcome that is appropriate only when excision and recombination efforts have failed.

Interestingly, no DNA repair activity is controlled by OxyR, and only endonuclease IV (encoded by *nfo*) is regulated by SoxRS[30]. Why is this? DNA repair processes are distinguished from other defensive functions in that DNA repair needs to continue after the oxidative stress itself has abated. Therefore, the SOS system continues to induce repair proteins as long as replication blocks persist. When *E. coli* is exposed to a high concentration of H_2O_2, the initial effect is a suspension of growth and metabolism owing to enzyme inactivation. Only after the H_2O_2 is removed by extant peroxidase and catalase activities can metabolism resume, and it is at this point that the SOS system is activated, its member proteins are synthesized and DNA damage is addressed.

Oxidation of Cys residues or lipids. Biochemists long ago recognized that dissolved O_2 in cell extracts can promote the oxidation of protein Cys and Met residues and the peroxidation of lipids. Consequently, at the outset of investigations into bacterial oxidative stress, the expectation was that similar injuries would lie at the heart of cell dysfunction. However, to date none of the phenotypes of SOD- or catalase/peroxidase-deficient cells have been linked to such events. In fact, most Cys residues react very sluggishly with O_2^- and H_2O_2 — certainly orders of magnitude more slowly than iron[119]. Indeed, experiments that have detected general protein disulphide formation inside H_2O_2-stressed *E. coli* cells have typically resorted to millimolar concentrations of H_2O_2 (REFS 120,121). (The active-site thiols of OxyR and Ahp are unusually reactive, for reasons that are unclear, and their oxidation inside cells can be detected after the application of low-micromolar concentrations of H_2O_2 (REF. 39).) Met is even less reactive than Cys. Thus, most proteins are not targeted by physiological doses of these oxidants.

Why, then, do glutaredoxin 1 (Grx1), thioredoxin 2 (Trx2) and DsbG, redoxins that excel in reducing disulphide bonds, belong to the OxyR regulon? Grx1 helps deactivate OxyR by reducing the disulphide bond when H_2O_2 stress has passed[122], but this does not explain why OxyR induces the other proteins. One possibility is that enzymes with hypersensitive Cys residues do exist, but researchers have not yet chanced upon them. A second possibility, given that bicarbonate accelerates H_2O_2-driven thiol oxidation[123,124], is that disulphide formation is relevant in CO_2-rich environments. A third possibility is that these redoxins help restore the coordinating Cys ligand that can be oxidized by $[FeO]^{2+}$ in the active sites of mononuclear iron enzymes (FIG. 4). But the most intriguing answer might be that OxyR moonlights as a

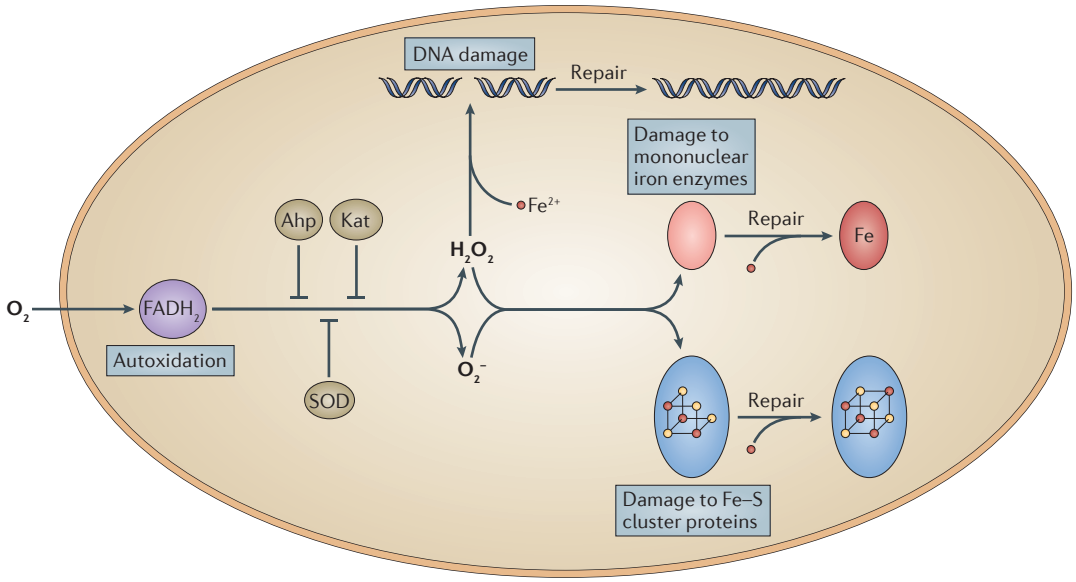

Figure 5 | **Overview of damage caused by reactive oxygen species in *Escherichia coli*.** The autoxidation of redox enzymes (such as the oxidation of $FADH_2$ on flavoproteins) leads to continuous hydrogen peroxide (H_2O_2) and superoxide (O_2^-) formation. Catalases (Kats), peroxidases (such as alkyl hydroperoxide reductase (Ahp)) and superoxide dismutases (SODs) minimize the accumulation of these two oxidants. Nevertheless, both species damage [4Fe–4S] dehydratases and mononuclear iron enzymes. The disabled enzymes are continuously repaired, so their steady-state activities represent the balance between damage and repair processes. H_2O_2 also reacts directly with the pool of unincorporated Fe^{2+}, which loosely associates with biomolecules, including DNA. The resultant hydroxyl radicals damage DNA, requiring the action of repair enzymes. The basal defences of the cell keep the rates of these injuries low enough that growth and viability are not noticeably affected. However, when O_2^--generating redox compounds and/or H_2O_2 enter the cell, the intracellular levels of these oxidants rise; consequently, the vulnerable enzymes become predominantly disabled, and metabolic pathways fail. Under these conditions, the induction of OxyR- and SoxRS-directed defence regulons is essential for cell recovery.

Peroxidation
Lipid damage in which peroxyl groups are added to unsaturated bonds, thereby disrupting lipid packing in the membrane.

Redoxins
Proteins that use their Cys residues to deliver electrons to oxidants. Thioredoxins and glutaredoxins reduce disulphide bonds in cellular proteins.

sensor of other stresses that modify protein thiols more effectively than H_2O_2 does. Recent work has shown that during anaerobic growth, some nitric oxide (NO) escapes nitrate and/or nitrite reductases and then nitrosylates protein thiols, including the sensory Cys residue of OxyR[125]. The induction of the OxyR regulon protects *E. coli* from this stress, perhaps by providing redoxins that reductively release the NO. Thus, it may be wrong to assume that every member of the OxyR regulon provides an important defence against H_2O_2.

Lipid peroxidation is a universal outcome of oxidative stress in eukaryotic systems, but this seems less likely in most bacteria. In the standard model of lipid peroxidation, the propagation step of the chain reaction requires that lipids be polyunsaturated. Most bacterial lipids contain only saturated and monounsaturated fatty acids[126], which are not prone to peroxidation in model systems[127]. A few studies have reported evidence that would seem to be consistent with the peroxidation of bacterial membranes, but more-specific analyses must be done to verify these data[37,128,129]. A recent study suggests that *Borrelia burgdorferi* is the exception that proves the rule: this intracellular bacterial pathogen acquires fatty acids from its eukaryotic host and can incorporate polyunsaturated ones. On examination, *B. burgdorferi* exhibited vulnerability to lipid peroxidation, whereas an *E. coli* control strain did not[130].

What's next?

For the past 40 years, studies in *E. coli* have pioneered our understanding of the oxidative-stress problem with which all organisms contend. The current overview of the damage induced by O_2^- and H_2O_2 is presented in FIG. 5. We now have basic ideas of how ROS are formed, what they damage and how *E. coli* defends itself against them. It is clear that ROS vulnerability results from the use of iron as an enzymatic cofactor. Iron is chemically versatile, facilitating both redox and surface chemistry; there is no mystery as to why ancient organisms that lived in an anaerobic, iron-rich environment recruited this metal to charge a wide diversity of enzymes. The problem is that contemporary organisms have inherited the same metabolic pathways and seek to use them in oxygen-rich habitats.

The picture is still incomplete, but as it becomes clearer, the next challenge will be to test the extent to which this paradigm applies to other microorganisms. There are reasons to think that important differences will emerge. As we learn which types of redox enzymes are most predisposed to autoxidation, we can ask whether the possession of high titres of such enzymes is what constrains some microorganisms to microaerobic or anaerobic lifestyles. Additional targets of oxidants apparently remain to be found, as SODs are found in many Gram-negative periplasms and in iron-free *B. burgdorferi*, both of which lack the classes of iron enzymes that O_2^- is known to damage. Even the ROS targets that are well established in *E. coli* might not pertain to strict aerobes; the iron-cofactored enzymes that are vulnerable to ROS in this facultative anaerobe might be routinely cofactored by other metals in aerobes, transforming what is an adaptive defence in *E. coli* into a constitutive one. Differences in environmental circumstances undoubtedly prompted different solutions to the oxidative threat, and the ongoing efforts to uncover this diversity comprise the next big step in this field.

1. Anbar, A. D. Elements and evolution. *Science* **322**, 1481–1483 (2008).
A clear overview of how metal availability has changed over geological timescales.
2. Ligeza, A., Tikhonov, A. N., Hyde, J. S. & Subczynski, W. K. Oxygen permeability of thylakoid memranes: electron paramagnetic resonance spin labeling study. *Biochim. Biophys. Acta* **1365**, 453–463 (1998).
3. Boehme, D. E., Vincent, K. & Brown, O. R. Oxygen and toxicity: inhibition of amino acid biosynthesis. *Nature* **262**, 418–420 (1976).
4. Gerschman, R., Gilbert, D. L., Nye, S. W., Dwyer, P. & Fenn, W. O. Oxygen poisoning and X-irradiation: a mechanism in common. *Science* **119**, 623–626 (1954).
The paper which initiated the notion that radicals lie at the root of oxygen toxicity.
5. Loew, O. A new enzyme of general occurrence in organismis. *Science* **11**, 701–702 (1900).
The discovery of catalase, the first known scavenging enzyme.
6. McCord, J. & Fridovich, I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **244**, 6049–6055 (1969).
A seminal paper reporting the discovery of SOD.
7. Carlioz, A. & Touati, D. Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? *EMBO J.* **5**, 623–630 (1986).
The first clear-cut demonstration that O_2^- is toxic.
8. Seaver, L. C. & Imlay, J. A. Alkyl hydroperoxide reductase is the primary scavenger of endogenous hydrogen peroxide in *Escherichia coli*. *J. Bacteriol.* **183**, 7173–7181 (2001).
9. Naqui, A. & Chance, B. Reactive oxygen intermediates in biochemistry. *Ann. Rev. Biochem.* **55**, 137–166 (1986).
10. Boveris, A. & Chance, B. The mitochondrial generation of hydrogen peroxide. *Biochem. J.* **134**, 707–716 (1973).
11. Imlay, J. A. & Fridovich, I. Superoxide production by respiring membranes of *Escherichia coli*. *Free Radic. Res. Comms.* **12–13**, 59–66 (1991).
12. Kussmaul, L. & Hirst, J. The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. *Proc. Natl Acad. Sci. USA* **103**, 7607–7612 (2006).
13. Messner, K. R. & Imlay, J. A. The identification of primary sites of superoxide and hydrogen peroxide formation in the aerobic respiratory chain and sulfite reductase complex of *Escherichia coli*. *J. Biol. Chem.* **274**, 10119–10128 (1999).
14. Seaver, L. C. & Imlay, J. A. Are respiratory enzymes the primary sources of intracellular hydrogen peroxide? *J. Biol. Chem.* **279**, 48742–48750 (2004).
15. Korshunov, S. & Imlay, J. A. Two sources of endogenous hydrogen peroxide in *Escherichia coli*. *Mol. Microbiol.* **75**, 1389–1401 (2010).
16. Massey, V. *et al.* The production of superoxide anion radicals in the reaction of reduced flavins and flavoproteins with molecular oxygen. *Biochem. Biophys. Res. Commun.* **36**, 891–897 (1969).
17. Geary, L. E. & Meister, A. On the mechanism of glutamine-dependent reductive amination of α-ketoglutarate catalyzed by glutamate synthase. *J. Biol. Chem.* **252**, 3501–3508 (1977).
18. Grinblat, L., Sreider, C. M. & Stoppani, A. O. Superoxide anion production by lipoamide dehydrogenase redox-cycling: effect of enzyme modifiers. *Biochem. Int.* **23**, 83–92 (1991).
19. Messner, K. R. & Imlay, J. A. Mechanism of superoxide and hydrogen peroxide formation by fumarate reductase, succinate dehydrogenase, and aspartate oxidase. *J. Biol. Chem.* **277**, 42563–42571 (2002).
20. Imlay, J. A. A metabolic enzyme that rapidly produces superoxide, fumarate reductase of *Escherichia coli*. *J. Biol. Chem.* **270**, 19767–19777 (1995).
21. Korshunov, S. & Imlay, J. A. Detection and quantification of superoxide formed within the periplasm of *Escherichia coli*. *J. Bacteriol.* **188**, 6326–6334 (2006).
22. Imlay, J. A. & Fridovich, I. Assay of metabolic superoxide production in *Escherichia coli*. *J. Biol. Chem.* **266**, 6957–6965 (1991).
23. Benov, L. T. & Fridovich, I. *Escherichia coli* expresses a copper- and zinc-containing superoxide dismutase. *J. Biol. Chem.* **269**, 25310–25314 (1994).
24. Gort, A. S., Ferber, D. M. & Imlay, J. A. The regulation and role of the periplasmic copper, zinc superoxide dismutase of *Escherichia coli*. *Mol. Micro.* **32**, 179–191 (1999).
25. Gort, A. S. & Imlay, J. A. Balance between endogenous superoxide stress and antioxidant defenses. *J. Bacteriol.* **180**, 1402–1410 (1998).
26. Tardat, B. & Touati, D. Two global regulators repress the anaerobic expression of MnSOD in *Escherichia coli*: Fur (ferric uptake regulation) and Arc (aerobic respiration control). *Mol. Microbiol.* **5**, 455–465 (1991).
27. Massé, E. & Gottesman, S. A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **99**, 4620–4625 (2002).
28. Kehres, D. G., Janakiraman, A., Slauch, J. M. & Maguire, M. E. Regulation of *Salmonella enterica* serovar Typhimurium *mntH* transcription by H_2O_2, Fe^{2+}, and Mn^{2+}. *J. Bacteriol.* **184**, 3151–3158 (2002).
29. Greenberg, J. T., Monach, P., Chou, J. H., Josephy, P. D. & Demple, B. Positive control of a global antioxidant defense regulon activated by superoxide-generating agents in *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **87**, 6181–6185 (1990).
30. Tsaneva, I. R. & Weiss, B. *soxR*, a locus governing a superoxide response regulon in *Escherichia coli* K-12. *J. Bacteriol.* **172**, 4197–4205 (1990).
Along with reference 29, the two independent studies that discovered SoxRS.

31. Christman, M. F., Storz, G. & Ames, B. N. OxyR, a positive regulator of hydrogen peroxide-inducible genes in *Escherichia coli* and *Salmonella typhimurium*, is homologous to a family of bacterial regulatory proteins. *Proc. Natl Acad. Sci. USA* **86**, 3484–3488 (1989).
The discovery of OxyR, the transcription factor that responds to H_2O_2.
32. Schellhorn, H. E. & Hassan, H. M. Transcriptional regulation of *katE* in *Escherichia coli* K-12. *J. Bacteriol.* **170**, 4286–4292 (1988).
33. Putnam, C. D., Arvai, A. S., Bourne, Y. & Tainer, J. A. Active and inhibited human catalase structures: ligand and NADPH binding and catalytic mechanism. *J. Mol. Biol.* **296**, 295–309 (2000).
34. Díaz, A., Loewen, P. C., Fita, I. & Carpena, X. Thirty years of heme catalases structural biology. *Arch. Biochem. Biophys.* **525**, 102–110 (2012).
35. Cha, M.-K., Kim, H.-K. & Kim, I.-H. Mutation and mutagenesis of thiol peroxidase of *Escherichia coli* and a new type of thiol peroxidase family. *J. Bacteriol.* **178**, 5610–5614 (1996).
36. Jeong, W., Cha, M.-K. & Kim, I.-H. Thioredoxin-dependent hydroperoxide peroxidase activity of bacterioferritin comigratory protein (BCP) as a new member of the thiol-specific antioxidant protein (TSA)/alkyl hydroperoxide peroxidase C (AhpC) family. *J. Biol. Chem.* **275**, 2924–2930 (2000).
37. Arenas, F. A. *et al.* The *Escherichia coli* BtuE protein functions as a resistance determinant against reactive oxygen species. *PLoS ONE* **6**, e15979 (2011).
38. Seaver, L. C. & Imlay, J. A. Hydrogen peroxide fluxes and compartmentalization inside growing *Escherichia coli*. *J. Bacteriol.* **183**, 7182–7189 (2001).
39. Aslund, F., Zheng, M., Beckwith, J. & Storz, G. Regulation of the OxyR transcriptional factor by hydrogen peroxide and the cellular thiol-disulfide status. *Proc. Natl Acad. Sci. USA* **96**, 6161–6165 (1999).
A quantification of the extreme reactivity of OxyR with H_2O_2.
40. Choi, H. *et al.* Structural basis of the redox switch in the OxyR transcription factor. *Cell* **105**, 103–113 (2001).
Work establishing the structural consequence of the reaction between OxyR and H_2O_2.
41. Zheng, M. *et al.* DNA microarray-mediated transcriptional profiling of the *Escherichia coli* response to hydrogen peroxide. *J. Bacteriol.* **183**, 4562–4570 (2001).
A transcriptome analysis that identifies members of the OxyR regulon.
42. Lee, J. W. & Helmann, J. D. The PerR transcription factor senses H_2O_2 by metal-catalyzed histidine oxidation. *Nature* **440**, 363–367 (2006).
The finding that PerR responds to H_2O_2 by undergoing a Fenton reaction that causes the oxidation of a metal-coordinating His residue.
43. Lynch, R. & Fridovich, I. Permeation of the erythrocyte stroma by superoxide radical. *J. Biol. Chem.* **253**, 4697–4699 (1978).
44. Korshunov, S. S. & Imlay, J. A. A potential role for periplasmic superoxide dismutase in blocking the penetration of external superoxide into the cytosol of phagocytosed bacteria. *Mol. Microbiol.* **43**, 95–106 (2002).
45. Hassett, D. J., Charniga, L., Bean, K., Ohman, D. E. & Cohen, M. S. Response of *Pseudomonas aeruginosa* to pyocyanin: mechanisms of resistance, antioxidant defenses, and demonstration of a manganese-cofactored superoxide dismutase. *Infect. Immun.* **60**, 328–336 (1992).
46. Hassan, H. M. & Fridovich, I. Intracellular production of superoxide radical and of hydrogen peroxide by redox active compounds. *Arch. Biochem. Biophys.* **196**, 385–395 (1979).
47. Imlay, J. & Fridovich, I. Exogenous quinones directly inhibit the respiratory NADH dehydrogenase in *Escherichia coli*. *Arch. Biochem. Biophys.* **296**, 337–346 (1992).
48. Pomposiello, P. J., Bennik, M. H. & Demple, B. Genome-wide transcriptional profiling of the *Escherichia coli* responses to superoxide stress and sodium salicylate. *J. Bacteriol.* **183**, 3890–3902 (2001).
A microarray study that identifies SoxRS-controlled genes in *E. coli*.
49. Koo, M. S. *et al.* A reducing system of the superoxide sensor SoxR in *Escherichia coli*. *EMBO J.* **22**, 2614–2622 (2003).
The discovery of the system that turns off SoxR.
50. Griffith, K. L., Shah, I. M. & Wolf, R. E. Jr. Proteolytic degradation of *Escherichia coli* transcription activators Sox and MarA as the mechanism for reversing the induction of the superoxide (SoxRS) and multiple antibiotic resistance (Mar) regulons. *Mol. Microbiol.* **51**, 1801–1816 (2004).
A satisfying explanation for how SoxS is deactivated when stress subsides.
51. Dietrich, L. E. P., Price-Whelan, A., Petersen, A., Whiteley, M. & Newman, D. K. The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*. *Mol. Microbiol.* **61**, 1308–1321 (2006).
52. Gu, M. & Imlay, J. A. The SoxRS response of *Escherichia coli* is directly activated by redox-cycling drugs rather than by superoxide. *Mol. Microbiol.* **79**, 1136–1150 (2011).
53. Krapp, A. R., Humbert, M. V. & Carrillo, N. The *soxRS* response of *Escherichia coli* can be induced in the absence of oxidative stress and oxygen by modulation of NADPH content. *Microbiology* **157**, 957–965 (2011).
54. Sheplock, R., Recinos, D. A., Mackow, N., Dietrich, L. E. & Chander, M. Species-specific residues calibrate SoxR sensitivity to redox-active molecules. *Mol. Microbiol.* **87**, 368–381 (2013).
55. Fujikawa, M., Kobayashi, K. & Kozawa, T. Direct oxidation of the [2Fe–2S] cluster in SoxR protein by superoxide: distinct differential sensitivity to superoxide-mediated signal transduction. *J. Biol. Chem.* **287**, 35702–35708 (2012).
56. Ma, D. *et al.* Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. *Mol. Microbiol.* **16**, 45–55 (1995).
57. Aiba, H., Matsuyama, S., Mizuno, T. & Mizushima, S. Function of *micF* as an antisense RNA in osmoregulatory expression of the *ompF* gene in *Escherichia coli*. *J. Bacteriol.* **169**, 3007–3012 (1987).
58. Lee, J. H., Lee, K. L., Yeo, W. S., Park, S. J. & Roe, J. H. SoxRS-mediated lipopolysaccharide modification enhances resistance against multiple drugs in *Escherichia coli*. *J. Bacteriol.* **191**, 4441–4450 (2009).
59. Liochev, S. I., Hausladen, A. & Fridovich, I. Nitroreductase A is regulated as a member of the *soxRS* regulon of *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **96**, 3537–3539 (1999).
60. Rau, J. & Stolz, A. Oxygen-insensitive nitroreductases NfsA and NfsB of *Escherichia coli* function under anaerobic conditions as lawsone-dependent Azo reductases. *Appl. Environ. Microbiol.* **69**, 3448–3455 (2003).
61. Lin, C. N. *et al.* A role of *ygfZ* in the *Escherichia coli* response to plumbagin challenge. *J. Biomed. Sci.* **17**, 84 (2010).
62. Giro, M., Carrillo, N. & Krapp, A. R. Glucose-6-phosphate dehydrogenase and ferredoxin-NADP(H) reductase contribute to damage repair during the *soxRS* response of *Escherichia coli*. *Microbiology* **152**, 1119–1128 (2006).
63. Dietrich, L. E., Teal, T. K., Price-Whelan, A. & Newman, D. K. Redox-active antibiotics control gene expression and community behavior in divergent bacteria. *Science* **321**, 1203–1206 (2008).
A paper which articulates the idea that SoxR has a variety of roles in different bacteria.
64. Kobayashi, K. & Tagawa, S. Activation of SoxR-dependent transcription in *Pseudomonas aeruginosa*. *J. Biochem.* **136**, 607–615 (2004).
65. Palma, M. *et al. Pseudomonas aeruginosa* SoxR does not conform to the archetypal paradigm for SoxR-dependent regulation of the bacterial oxidative stress adaptive response. *Infect. Immun.* **73**, 2958–2966 (2005).
66. Wang, Y. *et al.* Phenazine-1-carboxylic acid promotes bacterial biofilm development via ferrous iron acquisition. *J. Bacteriol.* **193**, 3606–3617 (2011).
67. Dietrich, L. E. P. *et al.* Bacterial community morphogenesis is intimately linked to the intracellular redox state. *J. Bacteriol.* **195**, 1371–1380 (2013).
68. Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A. & Collins, J. J. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell* **130**, 797–810 (2007).
69. Mahoney, T. F. & Silhavy, T. J. The Cpx stress response confers resistance to some, but not all bactericidal antibiotics. *J. Bacteriol.* **195**, 1869–1874 (2013).
70. Liu, Y. & Imlay, J. A. Cell death from antibiotics without the involvement of reactive oxygen species. *Science* **339**, 1210–1213 (2013).
71. Keren, I., Wu, Y., Inocencio, J., Mulcahy, L. R. & Lewis, K. Killing by bactericidal antibiotics does not depend on reactive oxygen species. *Science* **339**, 1213–1216 (2013).
72. Bielski, B. H. J. & Richter, H. W. A study of the superoxide radical chemistry by stopped-flow radiolysis and radiation induced oxygen consumption. *J. Amer. Chem. Soc.* **99**, 3019–3023 (1977).
73. Fee, J. A. Is superoxide important in oxygen poisoning? *Trends Biochem. Sci.* **7**, 84–86 (1982).
74. Fitzsimons, D. W. (ed.) *Oxygen Free Radicals in Tissue Damage* (Elsevier/North-Holland, 1979).
75. Sawyer, D. T. & Valentine, J. S. How super is superoxide? *Acc. Chem. Res.* **14**, 393–400 (1981).
76. Anjem, A. & Imlay, J. A. Mononuclear iron enzymes are primary targets of hydrogen peroxide stress. *J. Biol. Chem.* **287**, 15544–15556 (2012).
77. Kuo, C. F., Mashino, T. & Fridovich, I. α,β-dihydroxyisovalerate dehydratase: a superoxide-sensitive enzyme. *J. Biol. Chem.* **262**, 4724–4727 (1987).
The first identification of an enzyme that is rapidly inactivated by O_2^-.
78. Flint, D. H., Smyk-Randall, E., Tuminello, J. F., Draczynska-Lusiak, B. & Brown, O. R. The inactivation of dihydroxyacid dehydratase in *Escherichia coli* treated with hyperbaric oxygen occurs because of the destruction of its Fe-S cluster, but the enzyme remains in the cell in a form that can be reactivated. *J. Biol. Chem.* **268**, 25547–25552 (1993).
79. Gardner, P. R. & Fridovich, I. Superoxide sensitivity of the *Escherichia coli* 6-phosphogluconate dehydratase. *J. Biol. Chem.* **266**, 1478–1483 (1991).
80. Gardner, P. R. & Fridovich, I. Superoxide sensitivity of the *Escherichia coli* aconitase. *J. Biol. Chem.* **266**, 19328–19333 (1991).
81. Liochev, S. I. & Fridovich, I. Fumarase C, the stable fumarase of *Escherichia coli*, is controlled by the *soxRS* regulon. *Proc. Natl Acad. Sci. USA* **89**, 5892–5896 (1992).
82. Flint, D. H., Tuminello, J. F. & Emptage, M. H. The inactivation of Fe-S cluster containing hydro-lyases by superoxide. *J. Biol. Chem.* **268**, 22369–22376 (1993).
A study that defines the mechanisms and rates at which Fe–S dehydratases are poisoned by O_2^-.
83. Wallace, M. A. *et al.* Superoxide inhibits 4Fe-4S cluster enzymes involved in amino acid biosynthesis: cross-compartment protection by CuZnSOD. *J. Biol. Chem.* **279**, 32055–32062 (2004).
84. Bilinski, T., Krawiec, Z., Liczmanski, A. & Litwinska, J. Is hydoxyl radical generated by the Fenton reaction *in vivo*? *Biochem. Biophys. Res. Commun.* **130**, 533–539 (1985).
85. van Loon, A. P.G. M., Pesold-Hurt, B. & Schatz, G. A yeast mutant lacking mitochondrial manganese-superoxide dismutase is hypersensitive to oxygen. *Proc. Natl Acad. Sci. USA* **83**, 3820–3824 (1986).
86. Longo, V. D., Liou, L.-L., Valentine, J. S. & Gralla, E. B. Mitochondrial superoxide decreases yeast survival in stationary phase. *Arch. Biochem. Biophys.* **365**, 131–142 (1999).
87. Gralnick, J. A. & Downs, D. M. The YggX protein of *Salmonella enterica* is involved in Fe(II) trafficking and minimizes the DNA damage caused by hydroxyl radicals: residue CYS-7 is essential for YggX function. *J. Biol. Chem.* **278**, 20708–20715 (2003).
88. Justino, M. C., Almeida, C. C., Teixeira, M. & Saraiva, L. M. *Escherichia coli* di-iron YtfE protein is necessary for the repair of stress-damaged iron-sulfur clusters. *J. Biol. Chem.* **282**, 10352–10359 (2007).
89. Gruer, M. J. & Guest, J. R. Two genetically-distinct and differentially-regulated aconitases (AcnA and AcnB) in *Escherichia coli*. *Microbiology* **140**, 2531–2541 (1994).
90. Varghese, S. M., Tang, Y. & Imlay, J. A. Contrasting sensitivities of *Escherichia coli* aconitases A and B to oxidation and iron depletion. *J. Bacteriol.* **185**, 221–230 (2003).
91. Walling, C. Fenton's reagent revisited. *Acc. Chem. Res.* **8**, 125–131 (1975).
A foundational study of the Fenton reaction.
92. Jang, S. & Imlay, J. A. Micromolar intracellular hydrogen peroxide disrupts metabolism by damaging iron-sulfur enzymes. *J. Biol. Chem.* **282**, 929–937 (2007).
93. Nachin, L., Loiseau, L., Expert, D. & Barras, F. SufC: an unorthodox cytoplasmic ABC ATPase required for [Fe–S] biogenesis under oxidative stress. *EMBO J.* **22**, 427–437 (2003).
94. Jang, S. & Imlay, J. A. Hydrogen peroxide inactivates the *Escherichia coli* Isc iron-sulphur assembly system, and OxyR induces the Suf system to compensate. *Mol. Microbiol.* **78**, 1448–1467 (2010).

95. Lee, J. H., Yeo, W. S. & Roe, J. H. Induction of the *sufA* operon encoding Fe-S assembly proteins by superoxide generators and hydrogen peroxide: involvement of OxyR, IHF and an unidentified oxidant-responsive factor. *Mol. Microbiol.* **51**, 1745–1755 (2004).
96. Sobota, J. M. & Imlay, J. A. Iron enzyme ribulose-5-phosphate 3-epimerase in *Escherichia coli* is rapidly damaged by hydrogen peroxide but can be protected by manganese. *Proc. Natl Acad. Sci. USA* **108**, 5402–5407 (2011).
97. Gu, M. & Imlay, J. A. Superoxide poisons mononuclear iron enzymes by causing mismetallation. *Mol. Microbiol.* (in the press).
98. Anjem, A., Varghese, S. & Imlay, J. A. Manganese import is a key element of the OxyR response to hydrogen peroxide in *Escherichia coli*. *Mol. Microbiol.* **72**, 844–858 (2009).
99. Farr, S. B., D'Ari, R. & Touati, D. Oxygen-dependent mutagenesis in *Escherichia coli* lacking superoxide dismutase. *Proc. Natl Acad. Sci. USA* **83**, 8268–8272 (1986).
100. Park, S., You, X. & Imlay, J. A. Substantial DNA damage from submicromolar intracellular hydrogen peroxide detected in Hpx⁻ mutants of *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **102**, 9317–9322 (2005).
101. Henle, E. S. *et al.* Sequence-specific DNA cleavage by Fe^{2+}-mediated Fenton reactions has possible biological implications. *J. Biol. Chem.* **274**, 962–971 (1999).
102. Hutchinson, F. Chemical changes induced in DNA by ionizing radiation. *Prog. Nucl. Acid. Res.* **32**, 116–154 (1985).
103. Dizdaroglu, M., Rao, G., Halliwell, B. & Gajewski, E. Damage to the DNA bases in mammalian chromatin by hydrogen peroxide in the presence of ferric and cupric ions. *Arch. Biochem. Biophys.* **285**, 317–324 (1991).
104. Candeias, L. P. & Steenken, S. Electron transfer in di(deoxy)nucleoside phosphates in aqueous solution. Rapid migration of oxidative damage (via adenine) to guanine. *J. Am. Chem. Soc.* **115**, 2437–2440 (1993).
105. Hogg, M., Wallace, S. S. & Doublie, S. Bumps in the road: how replicative DNA polymerases see DNA damage. *Curr. Opin. Struct. Biol.* **15**, 86–93 (2005).
106. Demple, B., Johnson, A. & Fung, D. Exonuclease III and endonuclease IV remove 3′ blocks from DNA synthesis primers in H_2O_2-damaged *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **83**, 7731–7735 (1986).
A report establishing that certain DNA repair enzymes are dedicated to the repair of oxidative lesions.
107. Touati, D., Jacques, M., Tardat, B., Bouchard, L. & Despied, S. Lethal oxidative damage and mutagenesis are generated by iron in delta *fur* mutants of *Escherichia coli*: protective role of superoxide dismutase. *J. Bacteriol.* **177**, 2305–2314 (1995).
108. Keyer, K. & Imlay, J. A. Superoxide accelerates DNA damage by elevating free-iron levels. *Proc. Natl Acad. Sci. USA* **93**, 13635–13640 (1996).
109. Grant, R. A., Filman, D. J., Finkel, S. E., Kolter, R. & Hogle, J. M. The crystal structure of Dps, a ferritin homolog that binds and protects DNA. *Nature Struct. Biol.* **5**, 294–303 (1998).
110. Ilari, A., Ceci, P., Ferrari, D., Rossi, G. & Chiancone, E. Iron incorporation into *E. coli* Dps gives rise to a ferritin-like microcrystalline core. *J. Biol. Chem.* **277**, 37619–37623 (2002).
The demonstration that Dps protects cells by sequestering iron.
111. Altuvia, S., Almiron, M., Huisman, G., Kolter, R. & Storz, G. The *dps* promoter is activated by OxyR during growth and by IHF and σ^S in stationary phase. *Mol. Microbiol.* **13**, 265–272 (1994).
112. Liu, Y., Bauer, S. C. & Imlay, J. A. The YaaA protein of the *Escherichia coli* OxyR regulon lessens hydrogen peroxide toxicity by diminishing the amount of intracellular unincorporated iron. *J. Bacteriol.* **193**, 2186–2196 (2011).
113. Varghese, S., Wu, A., Park, S., Imlay, K. R. C. & Imlay, J. A. Submicromolar hydrogen peroxide disrupts the ability of Fur protein to control free-iron levels in *Escherichia coli*. *Mol. Microbiol.* **64**, 822–830 (2007).
114. Zheng, M., Doan, B., Schneider, T. D. & Storz, G. OxyR and SoxRS regulation of *fur*. *J. Bacteriol.* **181**, 4639–4643 (1999).
115. Jiang, D., Hatahet, Z., Blaisdell, J. O., Melamede, R. J. & Wallace, S. S. *Escherichia coli* endonuclease VIII: cloning, sequencing, and overexpression of the *nei* structural gene and characterization of *nei* and *nei nth* mutants. *J. Bacteriol.* **179**, 3773–3782 (1997).
116. Saito, Y. *et al.* Characterization of endonuclease III (*nth*) and endonuclease VIII (*nei*) mutants of *Escherichia coli* K-12. *J. Bacteriol.* **179**, 3783–3785 (1997).
117. Tchou, J. *et al.* 8-oxoguanine (8-hydroxyguanine) DNA glycoslyase and its substrate specificity. *Proc. Natl Acad. Sci. USA* **88**, 4690–4694 (1991).
118. Napolitano, R., Janel-Bintz, R., Wagner, J. & Fuchs, R. P. All three SOS-inducible DNA polymerases (Pol II, Pol IV and Pol V) are involved in induced mutagenesis. *EMBO J.* **19**, 6259–6265 (2000).
119. Winterbourn, C. C. & Metodiewa, D. Reactivity of biologically important thiol compounds with superoxide and hydrogen peroxide. *Free Radic. Biol. Med.* **27**, 322–328 (1999).
The demonstration that O_2^- and H_2O_2 react poorly with thiol compounds.
120. Hondorp, E. R. & Matthews, R. G. Oxidative stress inactivates cobalamin-independent methionine synthase (MetE) in *Escherichia coli*. *PLoS Biol.* **2**, e336 (2004).
121. Leichert, L. I. *et al.* Quantifying changes in the thiol redox proteome upon oxidative stress *in vivo*. *Proc. Natl Acad. Sci. USA* **105**, 8197–8202 (2008).
122. Zheng, M., Aslund, F. & Storz, G. Activation of the OxyR transcription factor by reversible disulfide bond formation. *Science* **279**, 1718–1721 (1998).
The resolution of the mechanism by which OxyR senses H_2O_2.
123. Zhou, H. *et al.* The biological buffer bicarbonate/CO_2 potentiates H_2O_2-mediated inactivation of protein tyrosine phosphatases. *J. Am. Chem. Soc.* **133**, 15803–15805 (2011).
124. Ezraty, B., Chabalier, M., Ducret, A., Maisonneuve, E. & Dukan, S. CO_2 exacerbates oxygen toxicity. *EMBO Rep.* **12**, 321–326 (2011).
125. Seth, D., Hausladen, A., Wang, Y. J. & Stamler, J. S. Endogenous protein S-nitrosylation of *E. coli*: regulation by OxyR. *Science* **336**, 470–473 (2012).
The finding that OxyR might sense nitrosative stress.
126. Nichols, D. S. & McMeekin, T. A. Biomarker techniques to screen for bacteria that produce polyunsaturated fatty acids. *J. Microbiol. Methods* **48**, 161–170 (2002).
127. Bielski, B. H. J., Arudi, R. L. & Sutherland, M. W. A study of the reactivity of HO_2/O_2^- with unsaturated fatty acids. *J. Biol. Chem.* **258**, 4759–4761 (1983).
The demonstration that monounsaturated fatty acids cannot undergo peroxidation.
128. Semchyshyn, H., Bagnyukova, T., Storey, K. & Lushhak, V. Hydrogen peroxide increases the activities of *soxRS* regulon enzymes and the levels of oxidized proteins and lipids in *Escherichia coli*. *Cell Biol. Int.* **29**, 898–902 (2005).
129. Gonzalez-Flecha, B. & Demple, B. Homeostatic regulation of intracellular hydrogen peroxide concentration in aerobically growing *Escherichia coli*. *J. Bacteriol.* **179**, 382–388 (1997).
130. Boylan, J. A., Lawrence, K. A., Downey, J. S. & Gherardini, F. C. *Borrelia burgdorferi* membranes are the primary targets of reactive oxygen species. *Mol. Microbiol.* **68**, 786–799 (2008).
131. Sawers, G. & Watson, G. A glycyl radical solution: oxygen-dependent interconversion of pyruvate formate-lyase. *Mol. Microbiol.* **29**, 945–954 (1998).
132. Pieulle, L., Magro, V. & Hatchikian, E. C. Isolation and analysis of the gene encoding the pyruvate-ferredoxin oxidoreductase of *Desulfovibrio africanus*, production of the recombinant enzyme in *Escherichia coli*, and effect of carboxy-terminal deletions on its stability. *J. Bacteriol.* **179**, 5684–5692 (1997).
Evidence that proteins might evolve to shield their oxidant-sensitive moieties from oxidants.
133. Ziegelhoffer, E. C. & Donohue, T. J. Bacterial responses to photo-oxidative stress. *Nature Rev. Microbiol.* **7**, 856–863 (2009).
134. Kosower, N. S., Kosower, E. M., Wertheim, B. & Correa, W. S. Diamide, a new reagent for the intracellular oxidation of glutathione to the disulfide. *Biochem. Biophys. Res. Commun.* **37**, 593–596 (1969).
135. Gebendorfer, K. M. *et al.* Identification of a hypochlorite-specific transcription factor from *Escherichia coli*. *J. Biol. Chem.* **287**, 6892–6903 (2012).
136. Winter, J., Ilbert, M., Graf, P. C., Ozcelik, D. & Jakob, U. Bleach activates a redox-regulated chaperone by oxidative protein unfolding. *Cell* **135**, 691–701 (2008).
137. Daly, M. J. *et al.* Small-molecule antioxidant proteome-shields in *Deinococcus radiodurans*. *PLoS ONE* **5**, e12570 (2010).
A paper that suggests a novel mechanism of antioxidant action by manganese.
138. Link, A. J., Robison, K. & Church, G. M. Comparing the predicted and observed properties of proteins encoded in the genome of *Escherichia coli* K-12. *Electrophoresis* **18**, 1259–1313 (1997).
139. Parsonage, D. *et al.* Analysis of the link between enzymatic activity and oligomeric state in AhpC, a bacterial peroxiredoxin. *Biochemistry* **44**, 10583–10592 (2005).
An excellent study of the reaction between the thiol-dependent peroxidase AhpC and H_2O_2.
140. Gardner, P. R. & Fridovich, I. Inactivation-reactivation of aconitase in *Escherichia coli*. A sensitive measure of superoxide radical. *J. Biol. Chem.* **267**, 8757–8763 (1992).
141. Massey, V. Activation of molecular oxygen by flavins and flavoproteins. *J. Biol. Chem.* **36**, 22459–22462 (1994).
A review detailing the chemistry of oxygen reduction by protein flavins, the reaction that underlies adventitious formation of O_2^- and H_2O_2.
142. Becker, A. *et al.* Iron center, substrate recognition and mechanism of peptide deformylase. *Nature Struct. Biol.* **5**, 1053–1058 (1998).

Acknowledgements
The author is grateful to past and current members of his laboratory who have contributed to many of the ideas in this Review. Work in the author's laboratory is currently supported by grants GM49640 and GM101012 from the US National Institutes of Health.

Competing interests statement
The author declares no competing financial interests.

FURTHER INFORMATION
James A. Imlay's homepage:
http://mcb.illinois.edu/faculty/profile/jimlay

ALL LINKS ARE ACTIVE IN THE ONLINE PDF