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Role of reactive oxygen species in antibiotic action and resistance

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The alarming spread of bacterial strains exhibiting resistance to current antibiotic therapies necessitates that we elucidate the specific genetic and biochemical responses underlying drug-mediated cell killing, so as to increase the efficacy of available treatments and develop new antibacterials. Recent research aimed at identifying such cellular contributions has revealed that antibiotics induce changes in metabolism that promote the formation of reactive oxygen species, which play a role in cell death. Here we discuss the relationship between drug-induced oxidative stress, the SOS response and their potential combined contribution to resistance development. Additionally, we describe ways in which these responses are being taken advantage to combat bacterial infections and arrest the rise of resistant strains.

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Introduction

The emergence of bacteria that have developed resistance to nearly all single and combinatorial antibiotic therapies used in the treatment of infection is a worldwide clinical threat. We are faced with an expanding list of microbial species that can actively escape, with mechanistic heterogeneity, the killing action of structurally and functionally diverse drug classes. It is clear that novel antibacterial tactics, including systematic exploration and exploitation of microbial drug stress response and defense systems, are required to combat antibiotic resistance [1] (Figure 1).

Bacteria are capable of resisting the action of antibiotics as a result of genetic alterations, including the physical exchange of genetic material with another organism (via plasmid conjugation, phage-based transduction, or horizontal transformation), the activation of latent mobile genetic elements (transposons or cryptic genes), and the mutagenesis of its own DNA [2]. The last of these mechanisms, chromosomal mutagenesis, may arise directly from interaction between the chromosome and the antibacterial agent or antibiotic-induced oxidative stress, or indirectly from the bacterium's error-prone DNA polymerases during the repair of a broad spectrum of DNA lesions. The efficacy of inhibiting essential bacterial processes by antibiotics, and thus their capacity to prevent infection, is diminished following any of the aforementioned resistance-conferring events. This is due to the microbe's new-found ability to modify or destroy the structure of a given drug, reduce access to the drug target by an alteration in permeability/active transport, or abolish stable interactions between the drug molecule and its target [2,3].

Clearly, the encounter between a thriving bacterial population and antibacterial molecules that threaten the existence of this population presents an enormous stress to each microbe in the population. The critical effect of this antibiotic-induced stress is the generation of an intracellular environment that is highly conducive to genetic evolution, owed to a tremendous degree of selective pressure and the physiological responses of the microbe [4,5]. Core responses include the SOS DNA stress response (first described by Radman and Witkin [6,7]), the heat-shock protein stress response (recently reviewed in [8]), and the oxidative stress response [9,10•]. Any bacterium surviving the initial wave of antibacterial attack, due to any or all of these defensive responses, may therefore serve as 'patient zero' in the rise of populations resistant to single drugs or multiple drugs. In this same manner, just a few 'patient zeros' may contribute to the burgeoning phenomenon of heteroresistance, where isolates from a given resistant population exhibit heterogeneous levels of resistance to an antibiotic.

In this commentary, we discuss the mechanism by which cellular-generated oxidative stress is induced by antibiotic treatment, and the role of reactive oxygen species (ROS) in drug-mediated bacterial cell death. Further, we consider the relationship between the SOS response and antibiotic-stimulated ROS, as well as the mutagenic potential of these reactions, and describe current efforts to exploit cellular responses in fighting drug-resistant strains.

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ROS formation

The formation of ROS in living organisms is an unfortunate by-product of respiration in an oxygen-rich environment [9]. There is an extensive literature providing evidence for the role of endogenous ROS as a causative agent in mutagenesis and as a significant contributor to the mutational burden experienced by microbes during periods of oxidative stress (e.g. [11–13]). This notion is supported by the existence of several overlapping enzymatic mechanisms employed by bacteria to combat ROS toxicity [14•].

ROS are generated intracellularly during aerobiosis-fueled oxygen metabolism via successive single-electron reductions, thereby producing superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and highly destructive hydroxyl radicals ($OH^{\bullet}$). Environmental molecular oxygen (O_2) can readily diffuse into microbes and interact with a host of cellular biomolecules. Of particular importance to ROS formation are those interactions between O_2 and biomolecules like respiratory flavoenzymes, which have accessible catalytic redox cofactors within their active sites and readily participate in electron transfer reactions with O_2. While $O_2^{\bullet-}$ and H_2O_2 can both be generated in this manner, recent evidence has shown that the respiratory chain is responsible for the production of biologically relevant levels of $O_2^{\bullet-}$ [15], but not H_2O_2 [16]; the specific cytoplasmic mechanism for the generation of H_2O_2 in appreciable quantities during steady state is not well understood.

Unlike $O_2^{\bullet-}$ and H_2O_2, which can be enzymatically eradicated by the activity of superoxide dismutases ($2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$) and catalases/peroxidases ($2H_2O_2 \rightarrow 2H_2O + O_2$), respectively, there exists no known enzyme that catalyzes the cellular detoxification of $OH^{\bullet}$. Instead, $OH^{\bullet}$ is capable of indiscrimining oxidative attack on proteins, lipids, and DNA in a manner that may be cytotoxic or mutagenic [17]. $OH^{\bullet}$ is generated *in vivo* via the Fenton reaction [18], during which cytoplasmic, solvent-accessible ferrous iron (Fe^{2+}) is oxidized by H_2O_2 to yield $OH^{\bullet}$ ($H_2O_2 + Fe^{2+} \rightarrow OH^{\bullet} + OH^- + Fe^{3+}$). The Fenton reaction is interdependent on the Haber–Weiss reaction, during which ferric iron (Fe^{3+}) is reduced by $O_2^{\bullet-}$ to yield Fe^{2+}. It is important to note that $O_2^{\bullet-}$ may interact with 'free', unincorporated Fe^{3+}, or it may reductively attack iron–sulfur cluster-bearing enzymes, thereby destabilizing and/or releasing Fenton-ready Fe^{2+} [19,20].

As such, during periods of oxidative stress, $O_2^{\bullet-}$ is produced at the membrane by the respiratory chain and is dismutated by superoxide dismutases to H_2O_2 and reduces Fe^{3+} by Haber–Weiss chemistry. H_2O_2 can then oxidize Fe^{2+} by Fenton chemistry to yield $OH^{\bullet}$ and Fe^{3+}, therefore potentially establishing a vicious redox cycle of ROS attack and generation. Because Fe^{2+} is capable of localizing to DNA, proteins, and lipids, generation of $OH^{\bullet}$ may occur in the immediate vicinity of these biomolecules and thus focus its deleterious effects. Along these lines, it has been shown that Fe^{2+} exhibits a sequence-specific preference when binding DNA and participating in the Fenton reaction [21,22]. Interestingly, this sequence can be found within the operator sites that enable binding of the iron regulatory transcription factor, Fur, to iron homeostasis-related gene promoters [23].

Antibiotic-induced ROS formation

As noted earlier, a better understanding of the specific sequences of events leading to cell death from the wide range of bactericidal antibiotics is needed for the development of more effective antibacterial therapies. One promising approach involves the identification of bacterial response network targets that can be exploited to combat the rise of resistant microbes [24].

Along these lines, we recently employed a systems biology approach to identify novel mechanisms that contribute to bacterial cell death upon DNA gyrase inhibition by the widely used fluoroquinolone antibiotic, norfloxacin [25•]. It is well known that fluoroquinolone drugs achieve their deadly effect through direct binding with DNA gyrase (topoisomerase II, product of *gyrA* and *gyrB*) and/or topoisomerase IV (product of *parC* and *parE*), trapping the topoisomerase(s) between the DNA strand breakage and rejoining steps that take place during modulation of DNA supercoiling [26]. This interaction results in the formation of double-stranded DNA breaks and ultimately replication arrest by blocking replication forks.

In our study, we performed phenotypic and microarray analyses on *Escherichia coli* treated with norfloxacin to identify additional contributors to cell death resulting from gyrase poisoning [25•]. In the course of this work, we discovered and characterized a novel oxidative damage cell death pathway which involves ROS formation, due in part to a breakdown in iron regulatory dynamics following norfloxacin-induced DNA damage. More specifically, we showed that gyrase-inhibiting antibiotic treatment resulted in the activation of the SoxR-regulated $O_2^{\bullet-}$ stress response [27], the IscR-regulated *iscRUSA* operon for repair/synthesis of heavily degraded iron–sulfur clusters [28], Fur-regulated iron import and utilization [29], as well as the SOS DNA stress response [30]. We demonstrated *in vivo* that these events promote the Fenton reaction-catalyzed generation of $OH^{\bullet}$, and that these highly destructive molecules play a critical role in the bactericidal efficacy of norfloxacin [25•]. Key contributors to $OH^{\bullet}$ production and cell killing were *atpC* (a structural and proton-translocating component of ATP synthase) and *iscS* (a component of the aforementioned IscR-regulated iron–sulfur cluster synthesis machinery). Importantly, to prove that the observed generation of ROS was not due to redox-cycling of the antibiotic, we treated quinolone-resistant *E. coli* strains harboring

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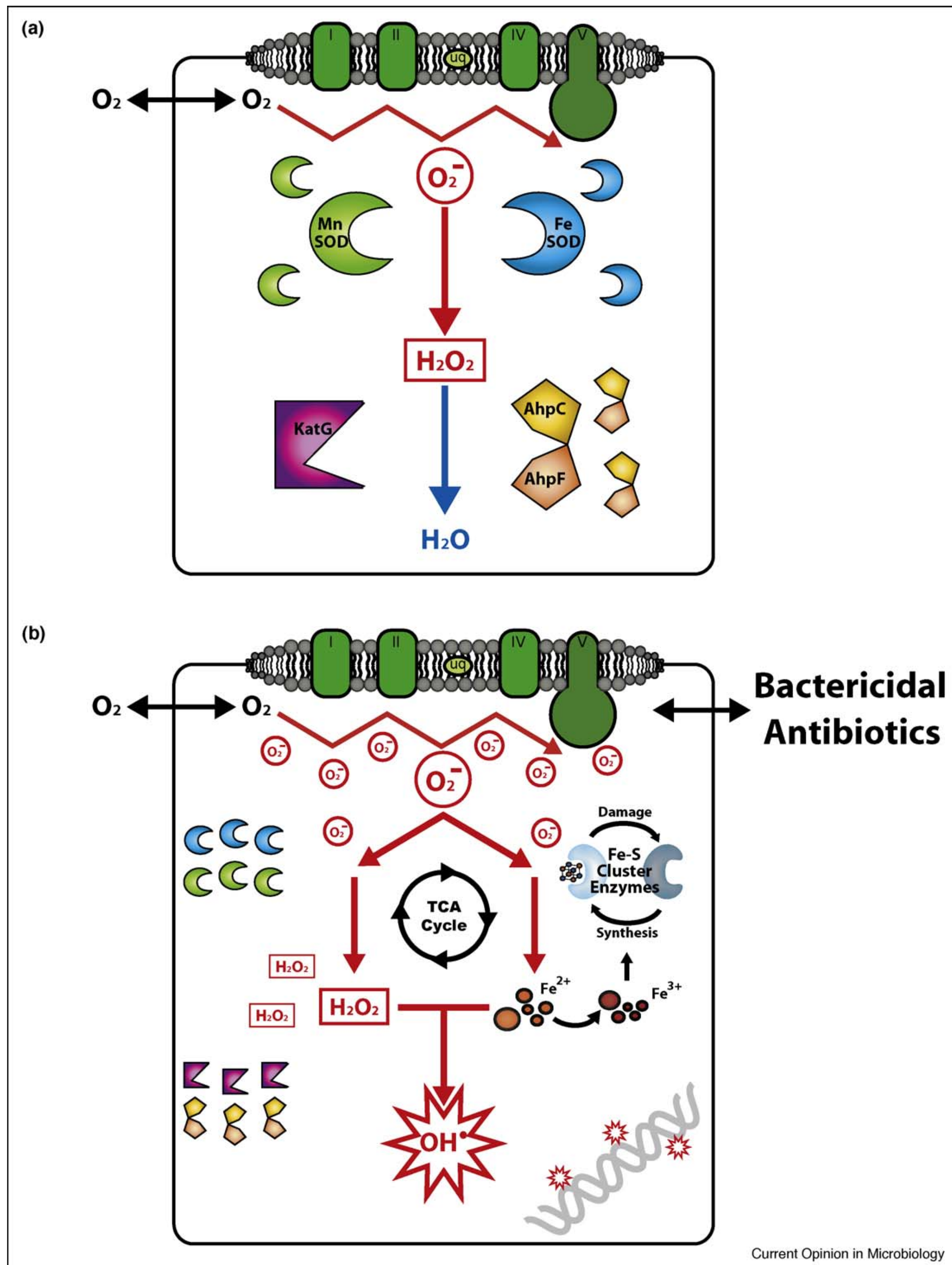
Figure 1



ROS generation and proposed contribution to resistance following treatment of bacteria with antibiotics. (A) Generation of ROS during steady-state conditions. Molecular oxygen (O_2) can diffuse without impediment into bacteria and interact with a range of cellular biomolecules, including respiratory flavoenzymes which catalyze the redox reactions that fuel aerobic electron transport chain activity. O_2 can readily abstract electrons from respiratory enzymes due to its reduction potential, making the electron transport chain the dominant source of superoxide (O_2^-) in the cell. During homeostasis, an abundance of superoxide dismutase enzymes (manganese-bearing Mn-SOD and iron-bearing Fe-SOD) exist in the cytoplasm to appropriately defend the bacterium against superoxide-based oxidative damage. The product of superoxide dismutation is hydrogen peroxide (H_2O_2), which is also toxic and requires additional layers of ROS defense. The primary scavenger of hydrogen peroxide is alkylhydroperoxide reductase (which is composed of AhpC and

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mutations in the *gyrA* gene and found that we could not detect OH• generation.

Building upon this work with fluoroquinolones, we have further shown that all classes of bactericidal antibiotics, regardless of their specific target, promote the generation of lethal hydroxyl radicals in both Gram-negative and Gram-positive bacteria [10•]. To get at the system-level responses underlying this phenomenon, we used microarrays to collect time-course gene expression response profiles for *E. coli* exposed to representatives of the major bactericidal drug classes, including β-lactams and aminoglycosides, in addition to fluoroquinolones. We analyzed microarray data using transcriptional regulatory and biochemical pathway classifications in order to refine the list of significantly changing genes (by *z*-score) and identify coordinately responding functional groups that were common across the diverse drug classes. By taking this approach, we were able to predict and subsequently confirm experimentally that the general mechanism of lethal OH• production involves tricarboxylic acid (TCA) cycle metabolism and a transient depletion of NADH, in addition to iron–sulfur cluster destabilization and iron misregulation.

The role of ROS in drug-induced killing has been expanded upon in several recent studies. For example, Wang and Zhao, in an attempt to determine which components of cellular ROS defense systems play a role in this phenotype, showed that the combined activity of superoxide dismutases SodA and SodB (containing manganese and iron as cofactors, respectively) were critical to killing by fluorquinolones, while the peroxidase AhpC (which uses NADH as reducing equivalent when breaking down H_2O_2) was critical to killing by β-lactams and aminoglycosides [31]. Additionally, Engelberg-Kulka and colleagues have reported on a potential connection between ROS generation, the chromosomally encoded MazF toxin and the extracellular death factor (EDF) signaling peptide [32].

Moreover, three recent and separate comprehensive screens of the contribution of single-gene disruptions to increased antibiotic susceptibility have provided further support for antibiotic-induced ROS formation and the role of ROS in drug-mediated killing [33,34•,35•]. Specifically, Miller and coworkers observed that the impairment of ROS defenses in *E. coli* potentiates killing by rifampin (a rifamycin) and metronidazole (a nitroimidazole) [33], while Tavazoie and coworkers showed that the inhibition of aerobic respiration reduced the susceptibility of *E. coli* to aminoglycoside antibiotics [34•], and Hancock and colleagues observed that the mutations in TCA cycle metabolism and respiratory electron transport chain components decreased killing by tobramycin (an aminoglycoside) in *Pseudomonas aeruginosa* [35•].

Taken together, these studies have begun to establish a mechanism for ROS production in bacteria during stressful versus steady-state conditions. Along these lines, we have recently attempted to elucidate the cellular events that connect treatment of bacteria with aminoglycoside antibiotics and the oxidative stress cell death pathway [36]. Our results show that aminoglycoside-induced mistranslation and misfolding of membrane-associated proteins activate the envelope stress response and redox-responsive two-component signal systems, leading to the production of hydroxyl radicals. Moreover, we found that these two-component systems are broadly involved in bactericidal antibiotic-mediated oxidative stress and cell death, providing additional insight into the common mechanism of killing induced by bactericidal antibiotics.

Antibiotic-induced SOS response activation and error-prone polymerases

A great deal of recent attention has been paid to the role of the SOS stress response in the phenomenon of induced mutagenesis [37], and the potential for combating resistance by inhibiting the activity of SOS-regulated, mutagenic machinery [38•,39•,40]. Considering that the SOS response is most efficiently activated by DNA damaging agents, it is not surprising that the most convincing evidence correlating antibiotic treatment with inducible mutagenesis and acquired resistance has followed from the study of fluoroquinolone antibiotics [41–43,44•].

During times of homeostasis, the LexA repressor protein effectively represses, via steric inhibition, the expression of the genes that compose the SOS regulon [30]. Upon detection of exposed, single-stranded DNA (the result of DNA damage or stalled replication forks), the SOS coregulator, RecA, is activated. The immediate effect of this activation is the formation of nucleoprotein filaments at the site(s) of genotoxic stress. Oligomerization of activated RecA then triggers autoproteolysis of LexA, thereby inactivating LexA, alleviating LexA-mediated repression, and initializing the highly dynamic expression of SOS genes [45].

(Figure 1 Legend Continued) AhpF subunits, and uses NADH as a cofactor), which converts H_2O_2 to water. Increased concentrations of hydrogen peroxide induce the complementary activity of hydroperoxidases (including OxyR-regulated KatG), which also convert H_2O_2 to H_2O. (B) Generation of ROS during antibiotic-induced stress conditions. When treated with bactericidal antibiotics, drug-target binding and resultant common changes in metabolism have been shown to trigger the production of ROS which contribute to cell death in bacteria. Heightened respiratory activity results in the increased production of superoxide, which overwhelms superoxide dismutase defenses and leads to the oxidation of iron-sulfur clusters ($[4Fe–4S]^{2+}$) employed by abundant dehydratase enzymes. The consequence of iron-sulfur cluster oxidation is the destabilization and/or release of ferrous (Fe^{2+}) iron, which can be oxidized by superoxide dismutase-generated hydrogen peroxide to yield hydroxyl radicals (OH•) via the Fenton reaction; interestingly, the tricarboxylic acid (TCA) cycle and new iron-sulfur cluster synthesis have been shown to play critical roles in the initiation and repetition of these events. Hydroxyl radicals are highly toxic and indiscriminately reactive, therefore making this ROS a potent mutagen of DNA that likely contributes to the acquisition of resistance in bacteria.

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The majority of core SOS genes [46] have some functions in the physical repair of damaged DNA. Repair may occur via nucleotide excision, base excision, or recombination pathways, depending on the type and number of lesions. The repair process also involves the activity of specialized DNA polymerases, DNA pol II (product of *polB*/*dinA*), IV (product of *dinB*), and V (product of *umuC* and *umuD*), which catalyze error-prone DNA synthesis across lesions (translesion synthesis, or TLS) that are physically prohibitive to the normal replicative polymerase, DNA pol III [47,48]. Expression of pol V is SOS-dependent and its activity is RecA-dependent, while expression of pol II and pol IV is SOS-independent yet increased approximately 10-fold upon SOS induction [30]. While there is some degree of functional overlap, and competition between these polymerases has been observed *in vivo*, pol II and pol IV are considered more proficient at handling sterically bulky DNA adducts (i.e. benzopyrenes) [49], whereas thiamine dimers, abasic sites, and ROS-mediated oxidation products are considered better substrates for pol V [50]. The greatest difference between these specialized DNA polymerases (and when compared to pol III) is the accuracy with which they add the appropriate deoxyribonucleotide base opposite damaged or undamaged template DNA. Due, in part, to major structural differences (i.e. a more accessible active site and the absence of the so-called 'O-helix', an α-helix that plays a key role in pairing cognate deoxyribonucleotides), Y-family polymerases pol IV and pol V have been shown to synthesize DNA with significantly higher error frequencies than pol II (up to 1000-fold less fidelity), depending on the template [51•,52]. Y-family polymerases also lack the exonuclease function of polymerases like pol II, and therefore cannot correct mistakes that are made during replication.

Induction of the SOS response has also been explored following treatment with antibiotics that do not directly cause DNA damage. For example, several β-lactam drugs (including ampicillin), which achieve lethality by disrupting membrane maintenance and biosynthesis or by damaging the cell wall, were shown by Miller and colleagues to trigger the SOS response via activation of the DpiAB two-component system [53•]. Additional recent studies have further explored the link between β-lactams, activation of the SOS response and SOS-related expression of error-prone polymerases [10•,54•,55•]. SOS induction has also been observed following treatment with trimethoprim (a dihydrofolate reductase inhibitor) [56], which is commonly formulated together with sulfamethoxazole (a sulfonamide) as cotrimoxazole and used to stem urinary tract infections. This latter finding has been attributed to the ability of this drug combination to exhaust intracellular pools of deoxyribonucleotides by the inhibition of ribonucleotide reductases, a mixed signal that may be perceived by the cell as a sign of overwhelming DNA stress (discussed in [57]).

A plausible explanation for the observed activation of the SOS response by a diverse set of nongenotoxic antibiotics is the antibiotic-induced cellular generation of ROS, an effect we have explored and validated experimentally [10•,25•]. It is also possible that activation of the SOS response, via direct or indirect mechanisms (e.g. two-component system signaling), acts as a catalyst for ROS production during periods of drug-based stress. ROS mutagenesis, which is addressed in the next section, may then maintain the SOS response in a chronically activated state and amplify ROS generation. A more detailed exploration of the temporal gene expression dynamics underlying interrelated antibiotic-induced SOS and oxidative stress response activation is needed to address these concepts.

DNA mutagenesis by ROS and its repair

The types of genotoxic stress induced by ROS include physical damage to the DNA base moiety and the sugar–phosphate backbone of incorporated or unincorporated (free) nucleotides, as well as single-stranded and double-stranded breaks within the double helix; in addition, DNA can be damaged by by-products of lipid peroxidation [58]. A wide variety of base adducts have been described following exposure to ROS, with the most prevalent of these being 7,8-dihydro-8-oxoguanine (8-oxoG or GO), 2,6-diamino-4-hydroxy-5-formamido-pyrimidine (FapyG), and thymine glycol (TG).

The cellular mechanisms that deal with the deleterious mutagenic effects of these stable adducts have been extensively studied. For example, because the 8-oxoG adduct can mispair with adenine nucleotides nearly as efficiently as it can pair with cognate cytosine nucleotides, this mutated base frequently results in G:C to T:A transversions when pol V-based translesion synthesis (TLS) occurs before specific cellular defenses arrive on the scene. Moreover, the 8-oxoG adduct provides a locus for further attack by ROS and reactive nitrogen species, yielding an array of DNA hyperoxidation products. A recent study performed by Neeley and colleagues examined the efficiency with which TLS polymerases pol II, pol IV, and pol V bypassed 8-oxoG and 8-oxoG hyperoxidation lesions, and monitored the frequency of 8-oxoG-related mutations *in vivo* on template DNA [59•]. They found that pol V exhibited the greatest TLS efficiency across the mutagenic spectrum tested, and that the activity of pol V was required for SOS-dependent remediation of oxidative lesions. Interestingly, the authors also concluded that the nucleotide which pol V incorporates opposite a given oxidative adduct has more to do with the lesion itself rather than the TLS abilities of the polymerase. This point may be critical to the link between acquired resistance, hypermutability and antibiotic-induced oxidative mutagenesis, for example, ROS could provide a means to rapidly diversify the breadth of mutation, which is then amplified by the activity of error-prone SOS polymerases.

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To prevent this from occurring, bacteria have evolved a three-component 8-oxoG elimination system, referred to as the 'GO system'. The GO machinery includes the MutM glycosylase which removes 8-oxoG adducts, the MutY glycosylase which removes misincorporated adenine nucleotides during the replication process, and the MutT phosphatase which sanitizes the nucleotide pool of available 8-oxoG triphosphate by hydrolyzing its conversion to 8-oxoG monophosphate; MutM also excises FapyG adducts, while the exonucleases Ndh and Nei excise TG adducts [60,61]. Glycosylases function by scanning DNA for lesions, then binding the lesion site in such a way that the base adduct is flipped outwards for *N*-glycosylic bond cleavage (between the base moiety and the sugar–phosphate backbone) within the glycosylase active site [62,63]. In this manner, the active site determines the specificity of the enzyme.

A potentially mutagenic and/or cytotoxic abasic site (lacking the base moiety) is generated following glycosylic cleavage, requiring further processing, including the activities of abasic endonucleases, DNA ligase, and DNA polymerases. Furthermore, if abasic sites on opposing DNA strands are in close proximity, double-stranded breaks may occur [64]. Pol V is among those polymerases that can efficiently synthesize DNA across abasic sites. As such, environmental conditions that promote oxidative stress, including growth in the presence of bactericidal antibiotics, provide powerful direct and indirect mechanisms for mutagenesis. There is a great deal yet to be explored in this space, including the contribution of these conditions to the development of antibiotic resistance.

Conclusions

Eighty years ago, Alexander Fleming's publication detailing his discovery of penicillin ushered in the modern era of antibacterial therapy [65]. Yet, within 15 years of his findings, Fleming presciently hypothesized that bacteria would likely attain resistance to any antibiotic treatment given the right circumstance. The continued emergence of single and multiple antibiotic-resistant bacterial strains is one of the more important societal issues today. Justifiably, the focus of antibiotic resistance research in the last half century has been on the elucidation of the mechanisms by which microbes can physically alter a drug's structure, disrupt the interaction between a drug and its cellular target, or alter the behavior and efficiency of its own transport machinery to reduce access to a drug's cellular target [2,3]. A new wave of research, however, dedicated to characterizing the physiological responses of bacteria to the presence and action of antibiotics may hold the key to thwarting the rise and spread of resistance.

With regard to the SOS response and the phenomenon of induced mutagenesis, a number of current research efforts have explored the effects of disabling the protein regulators that control expression of the SOS network of genes, including error-prone polymerases. For example, we have recently shown that a *recA* knockout strain of *E. coli* is significantly more susceptible to all classes of bactericidal antibiotics, highlighting the contributions of ROS to drug killing [10•]. Additionally, in a study by Romesberg and coworkers, it was shown that expression of an uncleavable form of LexA (thus preventing SOS activation) in drug-treated bacteria resulted in decreased survival and markedly lower mutation rates in culture (ciprofloxacin), as well as in a mouse model of infection (ciprofloxacin and rifampicin) [38•]. Along these lines, recent work by our lab has demonstrated that bacteriophage engineered to express an uncleavable LexA variant substantially increase survival in an ofloxacin-treated mouse model of systemic infection [40]; moreover, in this same study, it was shown that combinatorial treatment of ofloxacin and engineered bacteriophage can enhance the killing of fluoroquinolone-resistant bacteria by nearly four orders of magnitude compared to ofloxacin alone. Together with efforts aimed at identifying small molecule and short peptide inhibitors of RecA's ATPase and DNA filament formation abilities [66•,67•], it is clear that great potential lies in taking advantage of our current knowledge of the SOS response to combat current antibiotic resistance and prevent further development of resistant strains. These efforts may also offer the added benefit of increasing the efficacy of currently prescribed drugs, which would be particularly important given the lack of developmental efforts [1].

With regard to antibiotic-induced ROS formation and its role in bacterial resistance, a number of studies have attempted to resolve the role of ROS and the oxidative stress response in cell killing following drug treatment. In fact, it is likely that studies such as those by Demple and colleagues, which described several distinct mutations (some never before observed) in multidrug-resistant clinical isolates that increased expression of the $O_2^{\bullet-}$ response activator, SoxS [68], will become increasingly more common as the role of oxidative stress in antibiotic-mediated cell death becomes clearer. As we determine the steps between antibiotic addition and the metabolic changes that fuel ROS formation for bactericidal drug classes [10•,25•], it is vital that we compare and contrast these mechanisms with what we know about ROS generation and remediation during steady-state growth or following treatment with redox-cycling drugs [14•,17]. It may then be possible to exploit the oxidative stress response in order to enhance current antibacterial therapies, as was highlighted recently when bacteriophage engineered to overexpress SoxR significantly increased cell killing by ofloxacin [40]. Moreover, this approach may afford for the identification of novel targets within the microbe's defense systems for the development of inhibitor molecules or new antibiotics.

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