Oxidation of the Guanine Nucleotide Pool Underlies Cell Death by Bactericidal Antibiotics

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A detailed understanding of the mechanisms that underlie antibiotic killing is important for the derivation of new classes of antibiotics and clinically useful adjuvants for current antimicrobial therapies. Our efforts to understand why DinB (DNA polymerase IV) overproduction is cytotoxic to *Escherichia coli* led to the unexpected insight that oxidation of guanine to 8-oxo-guanine in the nucleotide pool underlies much of the cell death caused by both DinB overproduction and bactericidal antibiotics. We propose a model in which the cytotoxicity of beta-lactams and quinolones predominantly results from lethal double-strand DNA breaks caused by incomplete repair of closely spaced 8-oxo-deoxyguanosine lesions, whereas the cytotoxicity of aminoglycosides might additionally result from mistranslation due to the incorporation of 8-oxo-guanine into newly synthesized RNAs.

Elevated levels of reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals (OH$^{\bullet}$), within prokaryotic cells potentiate cell death. For example, three different classes of bactericidal antibiotics (β-lactams, quinolones, and aminoglycosides), regardless of macromolecular target, ultimately result in cell death in both Gram-negative and Gram-positive bacteria via a common mechanism that produces OH$^{\bullet}$ (*1*). Because elevated intracellular levels of OH$^{\bullet}$ can damage DNA, lipids, and proteins, generalized oxidation catastrophe could result in cell death; however, our current work suggests that cell death is predominantly elicited by specific oxidation of the guanine nucleotide pool and its subsequent use in nucleic acid transactions.

DinB overproduction lethality due to 8-oxo-deoxyguanosine (8-oxo-dG) incorporation. The ribonucleotide reductase inhibitor hydroxyurea, although best known for stalling DNA replication forks and eliciting double-strand DNA breaks (DSBs), also causes bacterial cell death through the production of OH$^{\bullet}$ (*2*). This stimulated us to test whether the cytotoxicity associated with overproduction of the *E. coli* translesion DNA polymerase DinB (DNA Pol IV) might result from an OH$^{\bullet}$-dependent process, because, like hydroxyurea, it slows the speed of replication forks and results in bacterial cell death presumably because of DSBs (*3*, *4*). To test whether OH$^{\bullet}$ radicals underlie cell death resulting from DinB overproduction, we measured cell survival in the presence of thiourea (OH$^{\bullet}$ scavenger) and 2,2′-dipyridyl (an iron chelator that prevents the Fenton reaction required for OH$^{\bullet}$ production) (*5*–*8*). Thiourea and 2,2′-dipyridyl, which do not strongly affect cell growth (*1*), completely prevent cell death caused by DinB overproduction (Fig. 1A). In addition, DinB overproduction is not toxic in an anaerobic environment (Fig. 1B), which is consistent with OH$^{\bullet}$'s mediating DinB-induced cell death. However, in contrast to hydroxyurea, which significantly increases intracellular OH$^{\bullet}$ levels (~10-fold) (*2*), DinB overproduction has a more modest effect (~1.6-fold) (Fig. 1D). Thus, it seemed most likely that the elevated levels of DinB were lethal because of the increased use of oxidized deoxynucleotides, rather than because of the induction of high levels of OH$^{\bullet}$.

The nucleotide pool is an important target of ROS, and guanine is particularly susceptible to oxidation because of its low redox potential (*9*, *10*). One of the most intensively studied major products of guanine oxidation is 7,8-dihydro-8-oxoguanine (8-oxo-guanine) (*10*). Its deoxyribonucleotide, 8-oxo-dG, is potentially mutagenic because of its ability to form base pairs with both cytosine and adenine (fig. S1). DinB, like its human ortholog DNA Pol κ (*11*), is a translesion DNA polymerase that can use 8-oxo-deoxyguanosine triphosphate (8-oxo-dGTP) as the incoming nucleotide, pairing it with either deoxycytidine or deoxyadenosine (dC or dA), with a preference for dA (Fig. 1C). Mutation of DinB's steric gate (F13V, in which Phe^{13} is replaced by Val) severely reduces its ability to use 8-oxo-dGTP as an incoming nucleotide (Fig. 1C), as does mutation of the corresponding steric gate of Pol κ (Y112A, in which Tyr^{112} is replaced by Ala) (*11*). Therefore, to test the hypothesis that DinB overproduction causes cell death by incorporating more 8-oxo-dG than the cell can tolerate, we overproduced DinB F13V and, as predicted, observed that this variant is not cytotoxic (Fig. 1A).

Although the observation that DinB F13V is not cytotoxic is consistent with our hypothesis that DinB overproduction is incorporating more 8-oxo-dG than the cell can handle, it does not exclude the possibility that the ability of DinB to copy over N^2-dG adducts (*12*) is contributing to cell death. Therefore, we cooverproduced MutT, a nucleotide sanitizer of the GO system that, together with MutM and MutY, minimizes the deleterious effects of oxidized guanine (*13*). MutT, which hydrolyzes 8-oxo-dGTP to 8-oxo-deoxyguanosine monophosphate (dGMP) (*13*), eliminates the cytotoxicity of DinB when cooverproduced (Fig. 1A). DinB overproduction in a Δ*mutT* strain is as cytotoxic as it is in wild-type (fig. S2), which suggests that DinB levels are rate-limiting for death, even though levels of 8-oxo-dGTP are extremely low in a Δ*mutT* mutant (*14*), a conclusion that is consistent with trace amounts of 8-oxo-dGTP usage during replication potentially having important biological consequences (*15*).

Our results are consistent with the hypothesis that DinB is incorporating more 8-oxo-dG than the cell can tolerate, but two potential mechanisms of intolerance are possible: the accumulation of lethal mutations or the formation of lethal DSBs. It is unlikely that ~50% of the cells have obtained a lethal mutation within 90 min (Fig. 1A), as DinB moves as slowly as 1 base pair (bp) per s and thus has only copied ~5400 bp per replication fork (*4*). Instead, it seems more likely that cells are dying from DSBs as previously suggested (*3*), a possibility consistent with our observation that DinB overproduction results in the up-regulation of the SOS response as measured by microarray analysis (tables S1 and S2) and cell filamentation as measured by flow cytometry (4.76-fold increase) (Fig. 1D).

Although the absolute levels of 8-oxo-dG present in DNA in an unstressed Δ*mutT* strain are too low to cause chromosomal fragmentation (*16*), a change in the levels of 8-oxo-dGTP and/or a change in the ratios of the particular DNA polymerases operating in a cell (i.e., DinB overproduction in this case) could result in closely spaced 8-oxo-dG nucleotides. Closely spaced DNA lesions are potentially problematic, because the proximity of individual DNA lesions can alter the cell's ability to repair damage (*17*) and subsequently result in DSBs (*18*). A single DSB in an *E. coli* cell has been known for decades to be potentially lethal (*19*). Thus, in principle, incomplete base excision repair by MutM and MutY glycosylases acting at closely spaced dC:8-oxo-dG and dA:8-oxo-dG pairs, respectively, could result in the generation of a lethal DSB (fig. S3). A DNA polymerase prone to using 8-oxo-dGTP as a substrate, such as DinB, would increase the likelihood of two 8-oxo-dG lesions being incorporated in close proximity and, thus, the potential for a DSB. Direct incorporation of an 8-oxo-dG by a DNA polymerase close to an existing 8-oxo-dG could also lead to a lethal DSB event via the action of GO system glycosylases (fig. S3). DSB formation by either of these mechanisms could be

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suppressed by MutT overproduction. In principle, the occurrence of a closely spaced dC:8-oxo-dG and 8-oxo-dG:C pair as a consequence of direct oxidation of DNA could also result in DSBs (fig. S3), but these would not be suppressible by overproduction of MutT.

The processivity of DinB interacting with the β clamp is 300 to 400 nucleotides (*20*), and thus, in principle, it could continue to replicate long enough to introduce multiple 8-oxo-dGs into DNA and so set up the potential for a MutM- and MutY-mediated DSB. Consistent with this model, a Δ*mutM* Δ*mutY* mutant is less sensitive to the cytotoxic effects of DinB overproduction (Fig. 1E). The protective effect of Δ*mutM* Δ*mutY* may be less than that of MutT overproduction, because other base excision repair enzymes that recognize 8-oxo-dG may additionally contribute to DSB formation (*13*). In addition, it is possible that MutT is also able to sanitize another oxidized deoxynucleotide triphosphate, as can the human MutT homolog (*21*), and that its incorporation into DNA contributes to DSB formation through a MutM- and MutY-independent process. Because cells repair DSBs by homologous recombination, the increased sensitivity of a Δ*recA* strain to DinB overproduction (Fig. 1E) is consistent with this model of 8-oxo-dG–mediated DSB formation. Collectively, the above data suggest that incorporation of 8-oxo-dG into DNA during replication is cytotoxic because of DSBs generated by the incomplete action of base-excision repair systems designed to protect cells from the mutagenic effects of oxidized nucleotides, i.e., the cellular protector has become the executioner.

Bactericidal antibiotic lethality due, in part, to the oxidation of guanine nucleotides. We then wondered whether the principle revealed by these experiments—an increase in closely spaced 8-oxo-dG lesions leading to DSBs—might also underlie cell death induced by bactericidal antibiotics. Antibiotics can generally be classified as being bacteriostatic (preventing cell growth) or bactericidal (killing cells) (*22*). Bactericidal antibiotics had long been thought to kill by means of class-specific drug interactions, which usually fall into three categories: inhibitors of cell wall biosynthesis, of DNA replication, and of protein synthesis (*23*). However, it was recently shown that a common pathway that produces $OH^{\bullet}$ substantially contributes to killing induced in Gram-negative and Gram-positive bacteria by major classes of bactericidal antibiotics (*1*). Despite having different macromolecular targets, β-lactams (cell wall synthesis inhibitors), quinolones (DNA gyrase inhibitors), and aminoglycosides (protein synthesis inhibitors) generate $OH^{\bullet}$ through the tricarboxylic acid cycle, a transient depletion of the reduced form of nicotinamide adenine dinucleotide, destabilization of iron sulfur clusters, and a stimulation of the Fenton reaction (*1*). Because $OH^{\bullet}$ are the most powerful oxidizing agent in living cells and have a half-life of nanoseconds (*24*), cell death could result from the cumulative effect of oxidizing various classes of cellular molecules and macromolecules. However, from the insights we gained into the mechanistic basis of DinB overproduction cytotoxicity, we hypothesized that, instead, the oxidation of the guanine nucleotide pool is specifically responsible for much of the death caused by bactericidal antibiotics.

To test this hypothesis, we overproduced MutT in *E. coli* and treated the resulting culture with representatives of the three different classes of antibiotics that increase $OH^{\bullet}$: ampicillin (β-lactam), norfloxacin (quinolone), and kanamycin (aminoglycoside). It was striking that simply overproducing MutT was sufficient to reduce the sensitivity of *E. coli* cells to killing by all three drugs, consistent with the hypothesis that oxidation of the guanine nucleotide pool underlies much of the cytotoxicity of bactericidal antibiotics (Fig. 2A and fig. S4A). Similarly, overproduction of RibA, an alternative 8-oxo-dGTP sanitizer (*25*), was also sufficient to reduce sensitivity to killing by ampicillin and norfloxacin (Fig. 2B). The inability of RibA to reduce the sensitivity of cells to kanamycin (Fig. 2B) is probably the consequence of kanamycin's blocking of protein synthesis coupled with the inherent instability of the RibA protein, which results in a substantial reduction of RibA protein levels after addition of the drug (fig. S5). In contrast, overproduction of NudB, a nucleotide sanitizer that preferentially hydrolyzes 8-OH-deoxyadenosine triphosphate (8-OH-dATP) in vitro and has only a twofold effect on mutation rate when overproduced in a Δ*mutT* mutant (*26*, *27*), did not affect antibiotic sensitivity (Fig. 2C).

The amount of 8-oxo-dG incorporated into DNA is influenced by a combination of the levels of 8-oxo-dGTP in the conditions being examined and the levels and characteristics of the individual polymerases that are present. To gain insights into which of the *E. coli* polymerases might be contributing to cell death

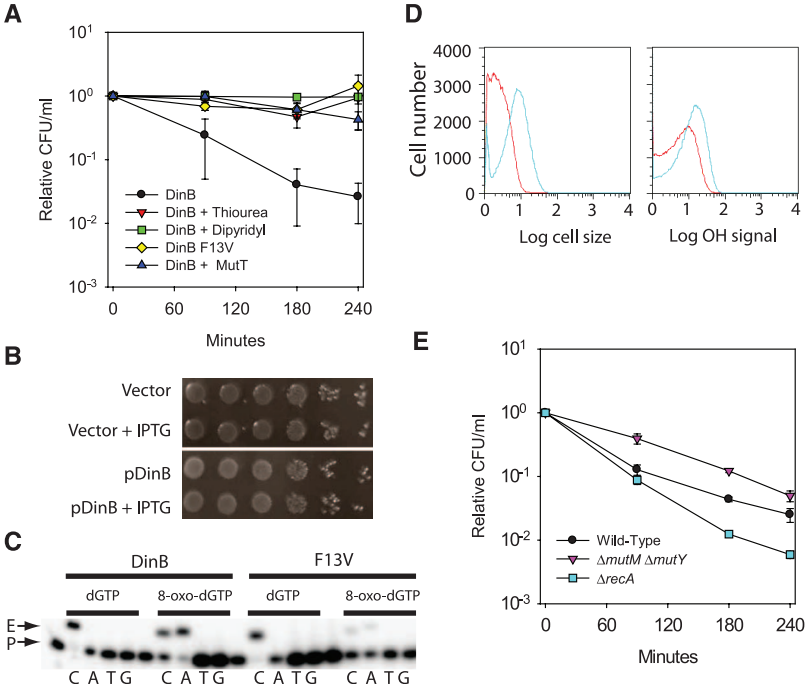

Fig. 1. DinB overproduction results in DSBs because of closely spaced 8-oxo-dG lesions. (**A**) Lethality of DinB overproduction (black), measured by colony-forming units (CFU) per ml relative to time zero, is reduced by thiourea (red), 2,2′-dipyridyl (green), and cooverproduction of MutT (blue). Overproduction of DinB F13V (yellow), which is incapable of incorporating 8-oxo-dG, is not lethal. (**B**) Under anaerobic conditions, DinB overproduction is not cytotoxic; cell viability was assayed by 10-fold serial dilutions of cells overproducing DinB with isopropyl-β-D-thiogalactopyranoside (pDinB + IPTG) and compared with noninduced (pDinB) and vector controls (vector and vector + IPTG). (**C**) DinB and DinB F13V (F13V) primer extension analysis using dGTP and 8-oxo-dGTP as the incoming nucleotide and the four different templating bases. The starting primer (P) and extended product (E) are indicated. Lanes 1, 6, 11, and 16 are unextended primer controls. (**D**) Overproduction of DinB for 3 hours (blue) results in cell filamentation but does not result in a substantial increase in intracellular $OH^{\bullet}$ levels when compared with an uninduced control (red). The forward-scatter histogram (left) of a DinB overproducing strain suggests cell elongatation (cell size, arbitrary units). (Right) Conversely, a modest increase in the 3′-(*p*-hydroxyphenyl) fluorescein fluorescence signal (1.6-fold) compared with the control is observed in a DinB-overproducing strain, which suggests that intracellular $OH^{\bullet}$ levels do not substantially increase ($OH^{\bullet}$ signal arbitrary units). (**E**) The lethality of DinB overproduction (black) is minimized in a Δ*mutM* Δ*mutY* background (purple) but enhanced in a Δ*recA* background (blue).

from bactericidal antibiotics by incorporating 8-oxo-dG into DNA, we tested the effect of mutating each polymerase on ampicillin cytotoxicity (fig. S6). Deletion of Δ*dinB* (DNA Pol IV) and Δ*umuDC* (DNA Pol V) reduced killing by ampicillin, which suggested that these two polymerases both contribute to ampicillin sensitivity, whereas mutations of *polA* (DNA Pol I) or *polB* (DNA Pol II) had no effect. The essential replication DNA polymerase Pol III is also involved in 8-oxo-dG incorporation because an antimutator allele of the catalytic subunit (*dnaE911*), which decreases the mutation frequency of a Δ*mutT* strain (*28*), also reduced ampicillin cytotoxicity (fig. S6). We then generated and tested a *dnaE911* Δ*dinB* Δ*umuDC* triple mutant and observed a striking reduction in sensitivity to all three classes of antibiotics (Fig. 2D and fig. S4B), consistent with Pol III, Pol IV, and Pol V's incorporation of more 8-oxo-dG than the cell can handle.

We hypothesized that the action of these polymerases under the conditions of elevated $OH^{\bullet}$ caused by the antibiotics could lead to increased 8-oxo-dG incorporation and, hence, a lethal MutM- and MutY-dependent DSB, as with DinB overproduction. We therefore treated a Δ*mutM* Δ*mutY* strain with the three different classes of antibiotics and, as anticipated, observed a significant decrease in killing, consistent with the action of these base excision repair enzymes resulting in DSBs (Fig. 3A and fig. S4C).

Deletion of Δ*recA*, which prevents DSB repair, has previously been shown to sensitize cells to antibiotics (*1*). To test whether the DSBs generated are repaired by the major RecA-dependent RecBCD pathway, we introduced a Δ*recB* mutant allele into cells and tested its effect on antibiotic cytotoxicity. The Δ*recB* mutant displayed approximately the same sensitivity as a Δ*recA* deletion, consistent with the hypothesis that the RecBCD pathway is used to repair the DSBs that are generated as a consequence of 8-oxo-dG incorporation (Fig. 3B).

To further support our hypothesis that DSBs, many of which are MutM and MutY dependent, are responsible for bactericidal cell death rather than being the result of DNA degradation in dead cells, we used the terminal deoxynucleotidyl transferase–mediated deoxyuridine triphosphate nick end labeling (TUNEL) assay. The TUNEL assay covalently attaches a fluorescent molecule to the 3′ terminus of a DNA molecule and, thus, can be used to directly measure DSBs. Thirty minutes after antibiotic treatment of wild-type cells, a time at which little cell death has occurred (Fig. 3C), we observed a qualitative increase in TUNEL-positive cells when analyzed by microscopy (Fig. 3D and fig. S7). We then quantified the number of TUNEL-positive cells in wild-type and Δ*mutM* Δ*mutY* populations after antibiotic treatment via flow cytometry (Fig. 3, E and G) and observed MutM- and MutY-dependent DSBs. Moreover, the median TUNEL signal for the Δ*mutM* Δ*mutY* strain is less than that of wild-type cells after treatment with antibiotics (Fig. 3, F and H). Collectively, these results are consistent with the hypothesis that bactericidal antibiotics lead to cell death largely by increasing the number of DSBs, a substantial fraction of which are MutM and MutY dependent.

Extension of common mechanism model. From the data described above, we are able to extend the model for a common mechanism of cell death induced by bactericidal antibiotics. Instead of generalized oxidative damage resulting in cell death, oxidation of the guanine nucleotide pool to 8-oxo-guanine results in several lethal outcomes. The approximately equal degree of protection from killing by β-lactams because of MutT overproduction, *dnaE911* Δ*dinB* Δ*umuDC*, and Δ*mutM* Δ*mutY* (Fig. 4) suggests that a substantial portion of the bactericidal effect of β-lactams is because of MutM- and MutY-mediated DSBs that are a consequence of Pol III, Pol IV, and Pol V's incorporation of 8-oxo-dG lesions into the daughter strand during replication (fig. S8). Penicillin, a β-lactam bactericidal antibiotic, was the first antibiotic described and was thought to kill cells primarily and specifically via inhibition of cell wall synthesis (*23*). It is noteworthy that our results suggest oxidation

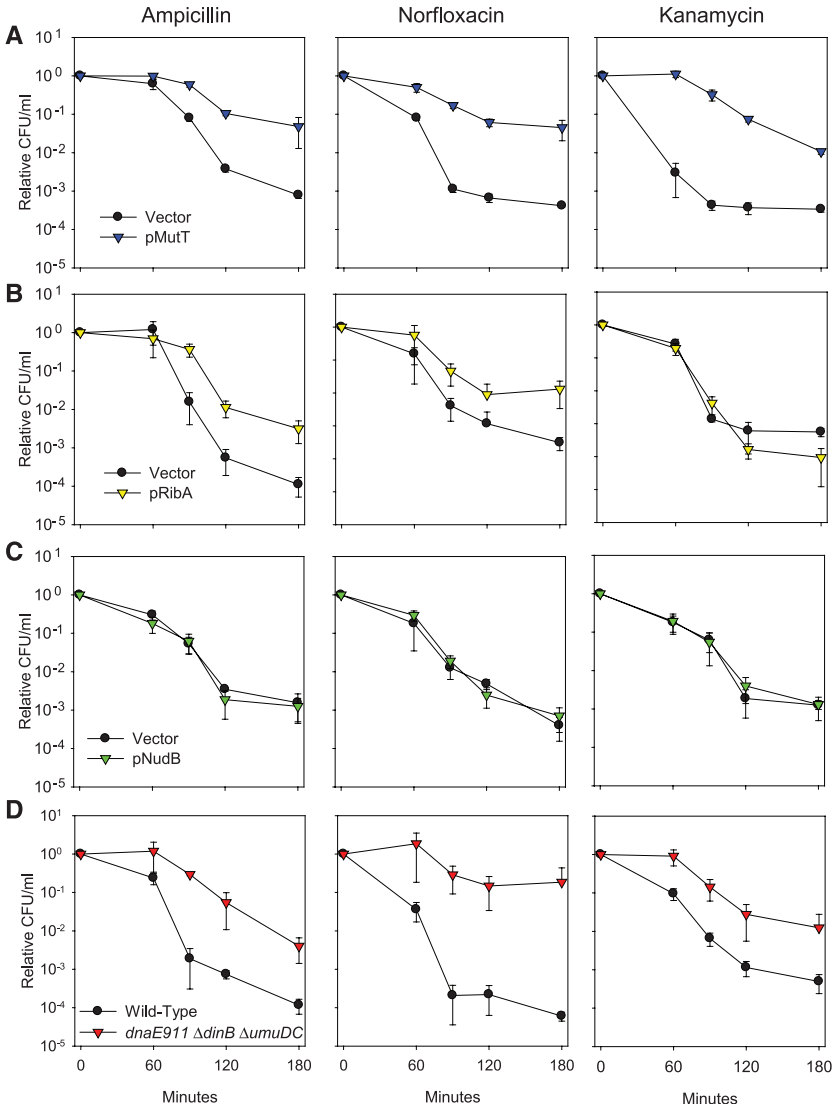

Fig. 2. The sensitivity of wild-type *E. coli* cells to killing by ampicillin, norfloxacin, and kanamycin is reduced when incorporation of 8-oxo-dG is minimized. (**A**) Overproduction of the 8-oxo-dGTP sanitizer MutT (blue) in wild-type MG1655 cells was sufficient to significantly reduce the sensitivity of cells to the bactericidal effects of all three classes of drugs compared with the vector control (black). (**B**) Overproduction of the alternative 8-oxo-dGTP sanitizer RibA (yellow) in MG1655 cells is also sufficient to reduce the sensitivity of cells to ampicillin and norfloxacin, but not kanamycin, probably because of its instability (fig. S5). (**C**) Overproduction of the 8-OH-dATP sanitizer NudB (green) does not reduce the antibiotic sensitivity. (**D**) A mutant strain that lacks the two Y-family DNA polymerases and expresses an antimutator replicative polymerase (*dnaE911* Δ*dinB* Δ*umuDC*) strain (red) is more resistant to killing by bactericidal antibiotics than wild-type cells (black).

of guanine nucleotides also contributes to cell death by penicillin (Fig. 4); this finding provides a new insight into the mechanism of action of the oldest known antibiotic. The approximately similar degrees of protection from killing by norfloxacin because of MutT overproduction and *dnaE911 ΔdinB ΔumuDC* indicate that an analogous mechanism is responsible for its bactericidal effects. However, the lesser degree of protection afforded by Δ*mutM* Δ*mutY* (Fig. 4) suggests that DNA gyrase inhibition additionally contributes to the DSBs caused by 8-oxo-dG incorporation (fig. S8).

This 8-oxo-dG–dependent DSB mechanism accounts for some of the bactericidal effects of kanamycin, as there is protection from killing by *dnaE911 ΔdinB ΔumuDC* and Δ*mutM* Δ*mutY*. However, our observation that the degree of protection caused by MutT overproduction is larger (Fig. 4) than that conferred by *dnaE911 ΔdinB ΔumuDC* or Δ*mutM* Δ*mutY*, together with the relatively modest increases in sensitivity conferred by the Δ*recA* and Δ*recB* mutants, suggests that an additional mechanism of cell killing is involved in the case of kanamycin. The reduced involvement of the 8-oxo-dG–dependent DSB mode of killing may, in part, be due to its translational inhibitory effects, which prevent the synthesis of SOS-regulated proteins (including the *dinB*- and *umuDC*-encoded DNA polymerases), other proteins required for stress responses, and antitoxins (leading to activation of their cognate toxins). Nevertheless, the strong suppression of kanamycin cytotoxicity by MutT overproduction suggests that, in addition to their known direct effect on the ribosome (*29*), a substantial amount of the cytotoxicity caused by aminoglycosides in vivo is due to oxidation of guanine nucleotides.

An intriguing hypothesis is that the protective effect of MutT overproduction is due to MutT's ability to sanitize the guanine ribonucleotide pool [8-oxo-guanosine triphosphate (8-oxo-rGTP) and 8-oxo-guanosine diphosphate (8-oxo-rGDP)] (*30*, *31*), as well as the guanine deoxynucleotide pool (fig. S8). RNA polymerase proficiently uses 8-oxo-rGTP as a substrate, incorporating 8-oxo-guanosine (8-oxo-rG) into transcripts at 1/10th the rate that it incorporates rG (*30*). These potentially altered transcripts could lead to mistranslated proteins, which is consistent with the previous observation that Δ*mutT* strains exhibit higher levels of protein carbonylation (*32*), a consequence of protein mistranslation. Moreover, the effect of 8-oxo-rGTP on protein mistranslation would be exacerbated by its misincorporation into ribosomal RNA and transfer RNA, which would be expected to further reduce the fidelity of protein synthesis. This potential for kanamycin-induced 8-oxo-rGTP–dependent mistranslation of cell envelope proteins could, in turn, cause more membrane alterations and could lead to increased drug uptake and further stimulation of the $OH^{\bullet}$ radical pathway through membrane stress two-component systems (Cpx) and

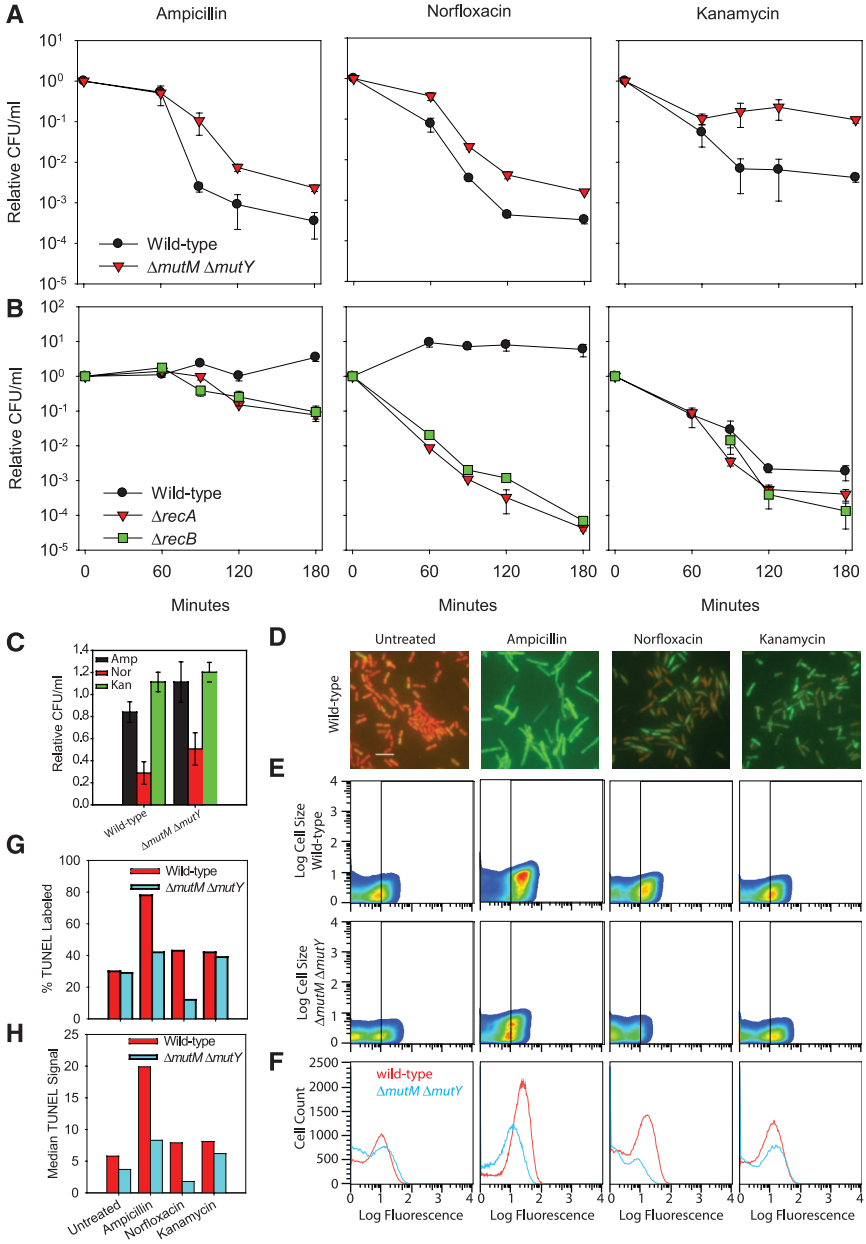

Fig. 3. Bactericidal antibiotics cause lethal DSBs. (**A**) A Δ*mutM* Δ*mutY* strain is less sensitive than wild-type cells to bactericidal antibiotic killing. (**B**) Deletion of Δ*recA* and Δ*recB* sensitizes cells to killing by bactericidal antibiotics. Relative CFU/ml of wild-type (black), Δ*recA* (red), and Δ*recB* (green) cells treated with 2 μg/ml ampicillin, 25 ng/ml norfloxacin, and 3 μg/ml kanamycin. (**C**) Neither ampicillin nor kanamycin treatments for 30 min significantly reduced the number of viable wild-type or Δ*mutM* Δ*mutY* cells. Norfloxacin treatment for 30 min results in a reduction in viable cell number for both wild-type (29% survival) and Δ*mutM* Δ*mutY* cells (51% survival). (**D**) Representative fields of wild-type cells after 30 min of treatment. Cells containing a DSB (TUNEL-positive, green) were overlaid on the propidium iodine staining of all cells (red). Scale bar, 5 μM. (**E**) Pseudocolor plot of cell size versus TUNEL fluorescence for untreated and treated wild-type and Δ*mutM* Δ*mutY* cells at 30 min. The vertical line at 10 fluorescence units is the cutoff of TUNEL-positive cells, which results in 30% of untreated wild-type cells being TUNEL-positive consistent with our microscopy results (~28% TUNEL-positive cells: 81 green out of 292 cells). The number of TUNEL-positive wild-type cells increases after antibiotic treatment (top), and Δ*mutM* Δ*mutY* cells (bottom) have fewer TUNEL-positive cells than wild-type. Both cell size (forward scatter) and fluorescence signal (TUNEL signal) are in arbitrary units (A.U.). (**F**) The distributions of the fluorescence intensity of wild-type (red) and Δ*mutM* Δ*mutY* (blue) TUNEL-stained cells suggest that Δ*mutM* Δ*mutY* cells have fewer dsDNA breaks. (**G**) The percentage of positive TUNEL-labeled cells (≥10 A.U.) determined in (E) is plotted for both wild-type (red) and Δ*mutM* Δ*mutY* (blue). (**H**) Histogram of the median TUNEL signal for the wild-type and Δ*mutM* Δ*mutY* populations shown in (F).

changes in metabolic function (Arc) (*29*). Thus, kanamycin treatment could potentially result in a catastrophic cycle of mistranslation driven by 8-oxo-rGTP.

It is also possible that 8-oxo-rGTP and 8-oxo-rGDP contribute to cell killing by interfering with the functioning of guanosine triphosphatases (GTPases), 13 of which are conserved in 75% of bacteria and most of which have critical functions in translation (*33*). For example, the reduced ability of the essential *E. coli* GTPase, Era, to hydrolyze 8-oxo-rGTP, compared with GTP (fig. S9), could alter the ratio between its GTP- and GDP-bound forms. In addition, oxidation of guanosine pentaphosphate or tetraphosphate [(p)ppGpp] could potentially interfere with the proper operation of the bacterial stringent response (*34*). Such bactericidal effects stemming from oxidation of the guanine ribonucleotide pool likely contribute to bactericidal effects of ampicillin and norfloxacin as well, but they appear to be less important than those mediated by the oxidation of the guanine deoxyribonucleotide pool.

Broad implications. Our model has two broad implications for the effectiveness of bactericidal antibiotics. First, in addition to a bacterial cell's intrinsic permeability to a drug and its ability to excrete it through drug pumps, our results suggest that its complement of DNA polymerases, DNA repair enzymes, and nucleotide sanitizers, such as MutT, could also play a role in a bacterium's intrinsic susceptibility to antibiotics. It is known that *E. coli* cells maintain a constant level of MutT after antibiotic stress (*1*), which suggests that a fitness cost may be associated with up-regulation of nucleotide sanitizers, perhaps decreasing mutagenesis that could lead to multidrug resistance (*35*). Second, the enhanced utilization of 8-oxo-guanine in nucleic acid transactions resulting in bacterial cell death could provide an avenue for identifying targets for the use of antimicrobial adjuvants. In the last few decades, only a handful of new classes of antibiotics have been introduced, which has led many to lament that the antibiotic pipeline is broken (*36*, *37*). Although new antimicrobial therapies are needed, adjuvants have the potential to extend the usefulness of current therapies. Our results suggest, for example, that bactericidal antibiotics could be potentiated by targeting proteins involved in repairing dsDNA breaks, e.g., inhibiting RecA or RecBCD, or by influencing the incorporation of 8-oxo-guanine into the DNA and RNA or the consequences of this incorporation.

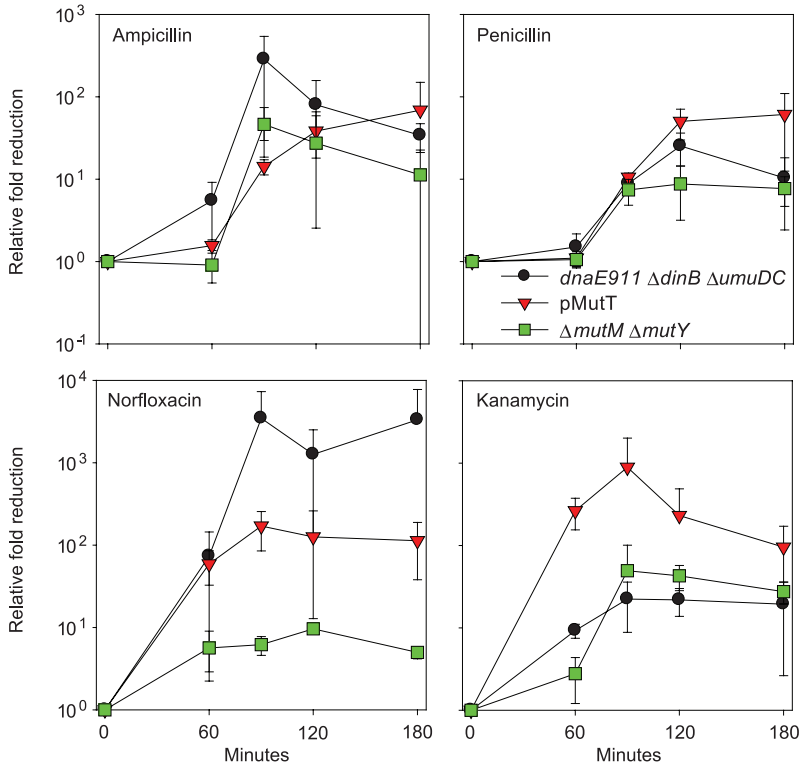

Fig. 4. Relative protective effect against killing because of MutT overproduction, mutation of three DNA polymerases (*dnaE911 ΔdinB ΔumuDC*), and *ΔmutM ΔmutY* for β-lactams, norfloxacin, and kanamycin suggests oxidation of guanine mediates bactericidal antibiotic–induced cell death. For β-lactams (ampicillin and penicillin), a similar fold rescue is observed for all three conditions. For norfloxacin, a similar degree of protection is afforded by MutT overproduction and mutation of the DNA polymerases, but a lesser degree of protection is observed for *ΔmutM ΔmutY*. For kanamycin, the greater fold rescue associated with MutT overproduction compared with *dnaE911 ΔdinB ΔumuDC* and *ΔmutM ΔmutY* suggests oxidation of the guanine to 8-oxo-rGTP is additionally contributing to cell death.

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Supplementary Materials

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