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The Response of *Escherichia coli* to the Alkylating Agents Chloroacetaldehyde and Styrene Oxide

Mark M. Muenter[a], **Ariel Aiken**[a], **Jadesola O. Akanji**[a], **Samir Baig**[a], **Sirine Bellou**[a], **Alyssa Carlson**[a], **Charles Conway**[a], **Courtney M. Cowell**[a], **Nicholas A. DeLateur**[a], **Alexis Hester**[a], **Christopher Joshi**[a], **Caitlin Kramer**[a], **Becky Leifer**[a], **Emma Nash**[a], **Macee Qi**[a], **Meghan Travers**[a], **Kelly C. Wong**[a], **Man Hu**[b], **Na Gou**[b,d], **Roger W. Giese**[c], **April Z. Gu**[b,d], and **Penny J. Beuning**[a,*]

[a]Department of Chemistry & Chemical Biology, Northeastern University, Boston, MA 02115 USA

[b]Department of Civil and Environmental Engineering, Northeastern University, Boston, MA 02115 USA

[c]Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115 USA

[d]School of Civil and Environmental Engineering, Cornell University, Ithaca, NY, 14850, USA

Abstract

DNA damage is ubiquitous and can arise from endogenous or exogenous sources. DNA-damaging alkylating agents are present in environmental toxicants as well as in cancer chemotherapy drugs and are a constant threat, which can lead to mutations or cell death. All organisms have multiple DNA repair and DNA damage tolerance pathways to resist the potentially negative effects of exposure to alkylating agents. In bacteria, many of the genes in these pathways are regulated as part of the SOS reponse or the adaptive response. In this work, we probed the cellular responses to the alkylating agents chloroacetaldehyde (CAA), which is a metabolite of 1,2-dichloroethane used to produce polyvinyl chloride, and styrene oxide (SO), a major metabolite of styrene used in the production of polystyrene and other polymers. Vinyl chloride and styrene are produced on an industrial scale of billions of kilograms annually and thus have a high potential for environmental exposure. To identify stress response genes in *E. coli* that are responsible for tolerance to the reactive metabolites CAA and SO, we used libraries of transcriptional reporters and gene deletion strains. In response to both alkylating agents, genes associated with several different stress pathways were upregulated, including protein, membrane, and oxidative stress, as well as DNA damage. *E. coli* strains lacking genes involved in base excision repair and nucleotide excision repair were sensitive to SO, whereas strains lacking *recA* and the SOS gene *ybfE* were sensitive to

*Address correspondence to: Penny J. Beuning, Department of Chemistry and Chemical Biology Northeastern University 360 Huntington Ave 102 Hurtig Hall Boston, MA 02115, Phone: 617-373-2865, Fax: 617-373-8795, beuning@neu.edu.

Conflict of interest statement

This work was carried out while M.M.M. was an employee of Rhodes Technologies, which played no role in the design, execution, or interpretation of the experiments reported here.

both alkylating agents tested. This work indicates the varied systems involved in cellular responses to alkylating agents, and highlights the specific DNA repair genes involved in the responses.

Keywords

transcriptional reporters; DNA repair; DNA damage; SOS response

1. Introduction

DNA damage poses a challenge to cells due to the possibilities that DNA lesions can lead to mutations or cell death [1]. DNA damage can form spontaneously or upon environmental exposures, for example to UV radiation and alkylating agents [1]. Alkylating agents are potentially genotoxic due to their ability to react at nucleophilic centers on DNA, forming adducts that can be cytotoxic or mutagenic, causing heritable mutations to occur in DNA, which can lead to cancer [1]. In this work, we probe the cellular effects of the alkylating agents chloroacetaldehyde (CAA) and styrene oxide (SO) in *E. coli* as a model system. We chose CAA and SO for our assays since they are direct-acting, share a common mechanism (alkylation), have been studied for their genotoxic properties, are readily available, and are important industrially [2–7].

CAA is a carcinogenic metabolite of vinyl chloride, forming several different DNA adducts including the cyclic base adducts 3,N^4-ethenocytosine (εC), 1,N^6-ethenoadenine (εA), N^2,3-ethenoguanine (εG), and 1,N^2-ethenoguanine (1,N^2-εG), with the A and C cyclic adducts the most predominant, and all of which can be mutagenic [8–10]. The εC adduct mostly produces C to A and C to T mutations, whereas εA results in mainly A to C mutations [10, 11]. In bacterial systems, εG has miscoding properties and can yield G to A transition mutations [10]. Mutagenic signatures of vinyl chloride exposure have been observed in the oncogenes H-*ras* and K-*ras* [12]. The εA and εG adducts can be removed by DNA glycosylases as part of the Base Excision Repair (BER) pathway [13, 14]. For example, the εA lesions are excised by the human and *E. coli* 3-methyladenine-DNA glycosylases and AlkA proteins, respectively [15–17]. *E. coli* AlkB and its human homologues ABH2 and ABH3 specifically repair base lesions, including the mutagenic exocyclic adducts εC, εA, and 1,N^2-εG by using an oxidative dealkylation mechanism known as Direct Repair (DR) [18–21].

SO, the principal metabolite of styrene, is a versatile electrophile that is able to react at various positions on DNA bases [5–7, 22, 23] either through the α- or β-carbon of the epoxide ring, resulting in a diversity of adducts. From studies with nucleosides *in vitro*, SO has been shown to react at the N7-, N^2-, and O^6-positions of deoxyguanine (dG), 1- and N^6-positions of deoxyadenine (dA), N^4-, N3-, and O^2-positions of deoxycytosine (dC), and the N3-position of thymine [22, 23]. *E. coli* strains harboring deletions of several DNA repair genes including the SOS-inducible genes *recA* and *uvrA*, the adaptive response genes *ada*, *alkB*, and *alkA*, and the 3-methyladenine repair gene *tag* were treated with SO and other reactive chemicals to evaluate growth [24]. SO caused extreme sensitivity of the *E. coli* strain lacking DNA damage repair genes relative to the wild-type strain [24]. Induction of

the SOS response as a result of *E. coli* treated with multiple epoxides including SO was also evaluated using the SOS-Chromotest, which revealed that most of the monosubstituted epoxides including SO resulted in SOS induction [25].

E. coli cells have a variety of mechanisms to repair DNA damage, many of which are regulated by the SOS response [26–28]. The SOS response leads to the LexA-, RecA-dependent upregulation of at least 57 genes, including those involved in DNA repair, DNA damage tolerance, and regulation of the cell cycle [1, 29]. In addition, the *E. coli* adaptive response is induced when cells are exposed to DNA-damaging alkylating agents and results in the direct reversal of DNA damage. The Ada protein, a DNA alkyltransferase, directly dealkylates damaged DNA and transfers the alkyl group to itself, leading to the expression of four genes: *ada*, *alkB*, *alkA*, and *aidB* [30, 31]. While human cells lack the LexA-mediated SOS response, most *E. coli* repair pathways have analogous systems in humans and other organisms [1, 32]. Moreover, many of the responses to genotoxic chemicals are conserved in *E. coli* and humans, so that interesting results with *E. coli* can, in turn, suggest areas of DNA repair systems in humans for study [33].

The focus of this work was to determine which *E. coli* genes contribute to survival upon exposure to CAA and SO. We first analyzed the expression of certain stress response genes upon exposure to each agent, using the established Transcriptional Effect Level Index (TELI) assay [34]. The main advantage of the TELI assay is to help to build better understanding of DNA damage responses and other cellular responses to stresses, by revealing correlations or lack of correlations for further study. Quantitative endpoints such as TELI, which incorporates temporal expression activities of multiple genes and gives more integrated DNA-damage and repair pathway activities, have been shown to be generally correlated with phenotypic genotoxicity endpoints [33–35]. The TELI gene expression library consists of each promoter of interest fused to the gene encoding green fluorescent protein (GFP) on a low-copy plasmid; the plasmid-based expression reporters as opposed to chromosomal integration may represent a potential challenge in interpreting the results [36]. Potentially the TELI assay will become useful to characterize in DNA damage responses in cells derived from individuals for different exposures, to learn which exposures are of greatest concern for an individual.

The TELI results of DNA damage responses to CAA and SO then informed our choice of bacterial strains in subsequent experiments. We investigated *E. coli* cellular survival in response to CAA and SO exposure by determining the sensitivity of a number of *E. coli* strains, each possessing single or multiple gene deletions. We find that multiple repair processes confer resistance to SO while only a few processes confer resistance to CAA.

2. Materials and Methods

2.1 Transcriptional Effect Level Index (TELI) Assays

Chloroacetaldehyde (CAA; f.c. 0.5 mg/mL in H_2O, TCI America) and styrene oxide (SO; f.c. 1.4 mg/mL in DMSO, TCI America) were prepared at 7x of the final concentratrions used for the TELI assay. A library of transcriptional fusions of the gene encoding green fluorescent protein (GFP) to the respective promoters for 114 stress-related genes in *E. coli*

MG1655 was employed in this assay, with detailed information for the library reported elsewhere [34, 36–38]. Within this library, each promoter fusion is expressed from a low-copy plasmid, pUA66 or pUA139, containing a kanamycin (Kan) resistance gene and a fast folding gfpmut2 gene, which allows continuous and real-time measurements of the promotor activities [34, 36]. The transcriptional effect level induced by the alkylating agents was measured according to the published protocol [34]. Quantitative end-point of the time-series response of a given gene, termed as transcriptional effect level index (TELI), is obtained by aggregating the induction of the altered gene expression level normalized over exposure time [34]. The TELI values indicate the relative change in expression of a given promotor upon treatment with alkylating agent relative to untreated control.

2.2 Strain Construction by Genetic Transduction

Knockout strains were obtained from the KEIO collection [39] in which gene deletions are constructed in BW25113; we constructed the AB1157 deletion strains via transduction using bacteriophage P1 [40]. The strains obtained from the *E. coli* Genetic Stock Center (CGSC) each had a Kan antibiotic resistance marker sequence in place of the gene to be studied (Table 1). The presence of the deletions in AB1157 was confirmed by PCR with primer pairs that amplify the region of interest for analysis by agarose gel electrophoresis and DNA sequencing (Eton and Macrogen, Cambridge, MA). Primer pairs used to verify transductions are summarized in Table 2.

2.3 Survival assays

E. coli strains were exposed separately to CAA at a concentration of 0.5 mg/mL prepared in sterile water or SO at 1.4 mg/mL prepared in dimethylformamide. The concentrations of each agent to be used for dosing were determined using WT AB1157 and PB109 (AB1157 Δ*alkB*) and represent the concentration at which moderate sensitivity was observed in PB109 relative to WT. Overnight cultures in LB (RPI or Affymetrix) were diluted 1:50 and incubated at 37 °C for 35–40 min. Once the OD_{600} reached 0.1 – 0.3, 1.5 mL of OD600 = 0.5 culture was harvested by centrifugation for 10 min at 3.8K RCF followed by removal of supernatant and resuspension of the pellets in 1 mL fresh LB. Next, 900 μL was transferred to the stock (S) microcentrifuge tube and 90 μL from the “S” tube transferred to the time 0 or control tube without treatment (T_0). A 90-μL aliquot of alkylating agent at the selected concentration was added to each (S) tube, mixed, and incubated at 37 °C with shaking. The T_0 samples were centrifuged for 3 min at 6K RCF, followed by removal of supernatant and resuspension of the pellet in 500 μL 0.85% saline, after which the samples were stored on ice. Time points were taken at 30 min (T_{30}), 60 min (T_{60}), and 90 min (T_{90}) and were processed as the T_0 time point. Serial dilutions (10-fold) of each time point were plated on LB-agar plates which were then incubated at 37 °C overnight. The data described herein represent the average of at least three trials; error bars represent standard deviation.

3. Results

In order to determine the cellular pathways responsible for tolerance to CAA and SO, we determined the expression of stress response genes and assessed the survival of a number of *E. coli* strains containing deletions of genes that were likely to confer survival. The strains

chosen for this work contained deletions of genes that represented a range of DNA repair functions as well as some of the genes implicated in cellular responses to CAA and SO from TELI experiments.

3.1 Transcriptional Effect Level Index (TELI) Assays reveal stress responses to alkylating agents

The TELI assay is a toxicogenomic assay that indicates which genes show changed expression in response to specific treatments [34]. TELI values determined for each stress-related gene category can indicate the involvement of different stress response and damage repair pathways [34]. In the case of the TELI assay, the promoters of specific stress-related genes are fused to the *gfp* gene on a low-copy plasimid. The accumulation of GFP fluorescence in the strains serves as a reporter for the rate of transcription initiation from the promoter region.

The TELI assay utilizes a library of transcriptional fusions of GFP to the promoters of 114 *E. coli* stress-related genes, encompassing several categories including protein stress (including *clpB*, *dnaK*, *entC*, *grpE*, and *lon*), membrane stress (including *amiC*, *emrA*, and *marR*), oxidative stress (including *oxyR*, *soxR*, and *sodA*), general stress (including *uspA* and *rpoE*), and DNA damage (including *lexA*, *recA*, *umuD*, and *uvrA*) [34], revealing those promoters that respond when cells are exposed to, in this work, the alkylating agents CAA and SO. Overall, CAA and SO induce multiple stress responses and yield higher TELI values in the DNA damage category compared to the other stress categories, which is consistent with the known abilities of both agents to damage DNA (Figure 1) [3, 5–7]. The expression of multiple genes (Figures 2 and 3) in the DNA damage stress category of the TELI library was induced upon exposure to either CAA or SO, with SO exposure inducing expression of more genes and at higher expression levels than CAA.

Within the TELI library of 114 stress-related genes employed, there are 36 DNA damage and repair genes represented. Of these 36 genes, we chose a sub-set of 29 genes, as well as three additional genes, that are known to contribute to DNA damage repair pathways and acquired strains lacking these genes for cell survival assays (Table 1). Within the subset of genes chosen for survival assays, *ada*, *dinG*, *mutM*, *mutS*, *mutT*, *nfo, recE*, *recN*, *sbmC, sulA*, *symE*, *umuD, uvrC*, and *ybfE* all yielded moderate to high TELI response signals upon exposure to CAA. Upon exposure to SO, as high or higher TELI responses were observed for these genes as well as *dam*, *dinB*, *mfd*, *mug*, *mutY*, *ruvA*, *uvrA*, *uvrD*, *uvrY*, and *yebG* (Figures 2 and 3). We find that CAA and SO exposure induces genes that play multiple roles in responding to DNA damage, with SO inducing genes that represent a wider range of DNA stress categories and inducing higher expression in general. Furthermore, although expression of the *lexA* gene was induced weakly by CAA and more strongly by SO, as determined by TELI (Figure 2), it was not selected for sensitivity assays since, as the master regulator of the SOS reponse, deletion of *lexA* is likely to render difficulties in data interpretation in these experiments [45, 46].

3.2 Survival Assays

To identify the DNA damage response pathways that contribute to survival upon exposure to CAA and SO, we used a library of *E. coli* strains harboring gene deletions. We selected a subset of the genes upregulated in the TELI experiments as well as several others likely to contribute to survival (Table 3). The *E. coli* mutants were assayed versus WT separately for survival upon treatment with CAA (Figure 4) and SO (Figure 5).

Upon exposure to CAA, strains lacking *recA* and *ruvA* showed the lowest survival; both genes contribute to homologous recombination, although RecA is also important for SOS induction [29] and translesion synthesis by *E. coli* pol V [47, 48]. Strains lacking *ybfE*, an SOS-induced gene of unknown function, and *recN*, which is involved in recombination repair (Table 3), also showed a decrease in survival, although the observed change for *recN* was not statistically significant. Strikingly, none of the other strains tested exhibited sensitivity to CAA.

Upon treatment with SO, a number of the strains tested exhibited sensitivity. The strain lacking *recA* yielded the greatest sensitivity to SO, as it did upon CAA treatment. The strain lacking *ybfE* was also highly sensitive to SO. Deletion of both Y-family DNA polymerase genes, *dinB* and *umuDC*, resulted in modest sensitivity to SO. Strains lacking the *uvrA* or *uvrD* genes involved in nucleotide excision repair were sensitive to SO, as were strains lacking the DNA glycosylase *mutM* and nucleases *nfo*, *recE*, and *recJ*. Deletion of the SOS-regulated genes *sbmC*, *symE* and *yebG* also conferred sensitivity to SO.

We observed a general correlation between TELI and survival assays in that SO induced expression of more genes and to a higher level than CAA and more mutant strains were sensitive to SO than to CAA. Several of the genes we studied were observed by TELI to be highly expressed upon exposure to SO and their deletion conferred sensitivity to SO, including strains lacking *recE*, *symE*, and *sbmC*. Notable exceptions for both CAA and SO were *recA*, *ruvA*, *dinG* and *umuD*. Both *recA* and *ruvA* promoters showed low to undetectable expression in the TELI assay (Figure 1); the strain lacking *recA* was sensitive to both CAA and SO but Δ*ruvA* was only sensitive to CAA (Figure 5). Whereas both *dinG* and *umuD* promoters showed activity in the TELI assay, deletion of either of these genes did not confer sensitivity to CAA or SO. The lack of cellular sensivity could be due to the ability of DNA repair pathways to compensate for each other; since these pathways are criticial for survival, a given lesion can often be repaired by more than one route [1]. In addition, deletion of *umuD*, a translesion synthesis manager protein [76], alone does not cause sensitivity, but a strain lacking genes for both Y-family translesion DNA polymerases *umuDC* and *dinB* is moderately sensitive to SO.

4. Discussion

In this work, we used assays of gene expression and cellular sensitivity to identify repair pathways that play pivotal roles in tolerance to DNA damage induced by the alkylating agents CAA and SO. There are several types of DNA damage caused by the alkylating agents studied here. For example, DNA exposure to CAA has been shown to result in formation of the mutagenic exocyclic adducts εC, εA, εG, and 1,N^2-εG [8–11, 19, 77]. *E.*

coli AlkB repairs εC, εA, and 1,N^2-εG adducts, similar to the repair profile of the human homolog ABH2 [9, 18–21, 78, 79]. The εC and 1,N^2-εG lesions are also repaired by base excision repair proteins double-stranded uracil-DNA glycosylase (UDG) and mug, respectively; 1,N^2-εG does not appear to be a substrate for Nfo or AlkB [9, 15]. Some DNA glycosylase genes, such as *ung* and *mutM*, exhibit altered expression upon cellular exposure to CAA (Figure 2). We observed only modest sensitivity to CAA of a strain lacking *alkB*, which could be due to the presence of compensating repair pathways. Indeed, the most sensitive strains to CAA here were those lacking recombination repair genes *recA* and *ruvA*.

SO forms adducts via either its epoxide α- or β-position at multiple sites of dA, dC, and dG, and at the N3 position of dT [5–7, 22, 23]. The dG adduct can undergo subsequent depurination, resulting in an abasic site [80], consistent with the finding that endonuclease III is involved in repair of SO-induced damage in human cells [81], and our observation that several BER-associated genes showed TELI responses upon exposure to SO (Figure 2). In *E. coli*, elevated SO concentrations promoted acetic acid formation, membrane permeability, and cell lysis, and a reduction in colony growth and formation, which together are likely the reasons for the more robust stress response to SO in the TELI experiment [82]. It was observed in *in vivo* bacterial replication assays that DNA containing most SO lesions could be replicated, but DNA containing some of these same lesions could not be replicated with purified replicative DNA polymerases [83, 84]. This previous work was carried out prior to the discovery of the biochemical activity of Y-family translesion DNA polymerases; indeed, we observed that deletion of both Y-family polymerases *umuDC* and *dinB* sensitizes cells to SO (Figure 5), suggestion that they play a role in bypass of SO-induced DNA lesions.

Deletions of the genes *recA*, involved in recombination and repair, and *ybfE*, a gene of unknown function, conferred sensitivity to both CAA and SO. Although *recA* promoter activity was essentially unchanged upon CAA exposure, there was low but detectable expression upon exposure to SO. A similar observation was made for the *ruvA* promoter, which showed slightly higher expression when exposed to SO than to CAA. One possible reason for the lack of *recA* promoter activity in TELI is that *recA* is one of the most abundant proteins in the cell and thus it has been proposed that sufficient RecA is present for some of its functions without induction [85]. Similarly, of two related carcinogens, N-hydroxy-N-2-aminofluorene and acetoxy-N-2-acetylaminofluorene, only the latter induced RecA [86]. Using TELI, similar effects were observed with two genotoxic nanomaterials, nano-silver and nano-TiO_2_a, in which nano-silver did not induce RecA whereas nano-TiO-2_a led to robust induction [33–35]. Although the promoters of *recA* and *ruvA* are not appreciably activated by exposure to either agent, the roles of *recA* and *ruvA* in recombination and DNA repair [60, 65, 66, 87] appear to be important for survival upon exposure to these agents. The strains lacking *recA* and *ybfE* showed the largest degree of sensitivity upon exposure to both agents. The critical and multifaceted roles of these genes, particularly *recA*, in stress resposes are also highlighted by their contributions to survival upon UV- and X-irradation, as well as exposure to a number of antibiotics and other damaging agents [88–93]. Although the function of *ybfE* is unknown, the decrease in cell survival observed for the mutant strain when exposed to either agent suggests that it plays a key role in DNA damage tolerance; indeed, the *ybfE* gene is known to be regulated by LexA [74, 94] and was upregulated by both CAA and SO.

Although there was little to no expression observed by TELI of the *recA* and *ruvA* genes upon CAA exposure, other specific repair pathways were induced by both CAA and SO and include the SOS-inducible gene *sulA*, the DNA repair gene *ykfG*, the mismatch repair gene *mutS*, the recombination and repair genes *recE* and *recN*, the DNA gyrase inhibitor *sbmC*, and the SOS-induced putative antitoxin *symE*. The *recE* gene encodes exonuclease VIII and digests DNA in the $5' \rightarrow 3'$ direction, yielding dsDNA with $3'$- ssDNA overhangs and *recE* mutations cause *recB*, *recC*, and *sbcA* mutants to become recombination-deficient [95]. By TELI, the *recE* promoter showed increased expression upon exposure to both CAA and SO. We further find by TELI that the *sbcB* promoter shows increased expression upon exposure to both agents; *sbcB* encodes exonuclease I, which is involved in the RecBCD pathway [96]. We find that the *recN* promoter showed increased TELI response, but the strain harboring a *recN* deletion was not sensitive to either CAA or SO. Taken together, this work implicates recombination repair in cellular responses to CAA and SO.

The promoters of the glycosylases *mutM*, *mug*, and the nuclease *nfo* each showed increased TELI responses upon exposure to CAA and SO and the strains lacking *mutM* and *nfo* showed sensitivity to SO. Increased expression of the *mug*, *mutM*, and *nfo* promotors as determined by TELI and the lowered survival of the *mutM* and *nfo* mutants suggests the involvement of BER in repair of SO-induced lesions, consistent with the finding that SO-induced lesions lead to formation of abasic sites [80]. Finally, deletion of *uvrA* and *uvrD*, both involved in NER, resulted in sensitivity to SO, presumably due to the presence of bulky styrene adducts and consistent with a previous report that lack of *uvrA* confers modest sensitivity to SO [97]. Like *recA*, genes involved in NER also confer survival upon exposure to a wide range of agents including radiation and several antibiotics [88–92].

In summary, we confirmed that a broader range of DNA damage genes were expressed upon exposure to SO, with a number of the same genes expressed upon exposure to CAA by the TELI assay. The survival assays also yielded a broader range of DNA damage response genes that confer resistance toward SO, which could be due to the greater diversity of DNA damage induced by SO. The goal of this work was to identify genes important for responses to the two alkylating agents CAA and SO and to point out aspects of DNA damage responses that need further study. For example, the lack of correlation between TELI gene expression and cellular sensitivity for *dinG* is unexpected, and deletion of *ybfE*, a gene of unknown function, confers sensitivity to both CAA and SO. In the long term, the technology might be applied to exposure of cell cultures from individuals to learn which chemicals are most toxic on an individual basis.

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Highlights

- Numerous stress responses are induced by chloroacetaldehyde and styrene oxide
- DNA damage is most prominent category of stress responses
- Strains lacking *recA, ruvA*, and *ybfE* are sensitive to chloroacetaldehyde
- Strains lacking *recA*, *mutM*, *nfo*, *uvrA*, *uvrD*, *ybfE* are sensitive to styrene oxide

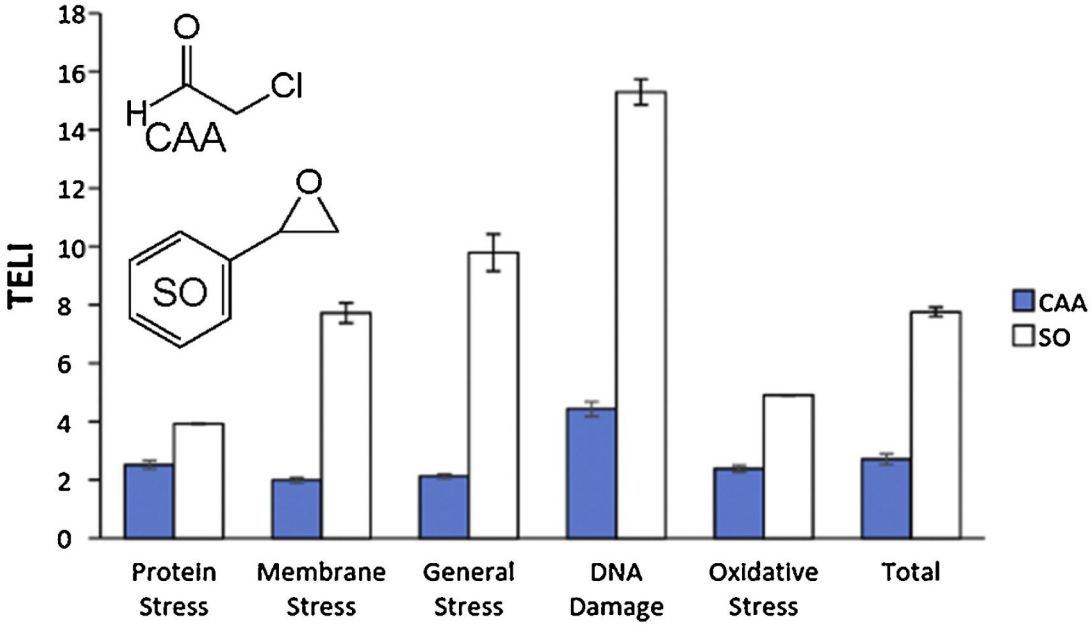

Figure 1.

Overall TELI response versus various stress categories upon exposure to CAA (0.5 mg/mL in H2O) and SO (1.4 mg/mL in DMSO). Values represent the average of TELI values for all genes in the category indicated.

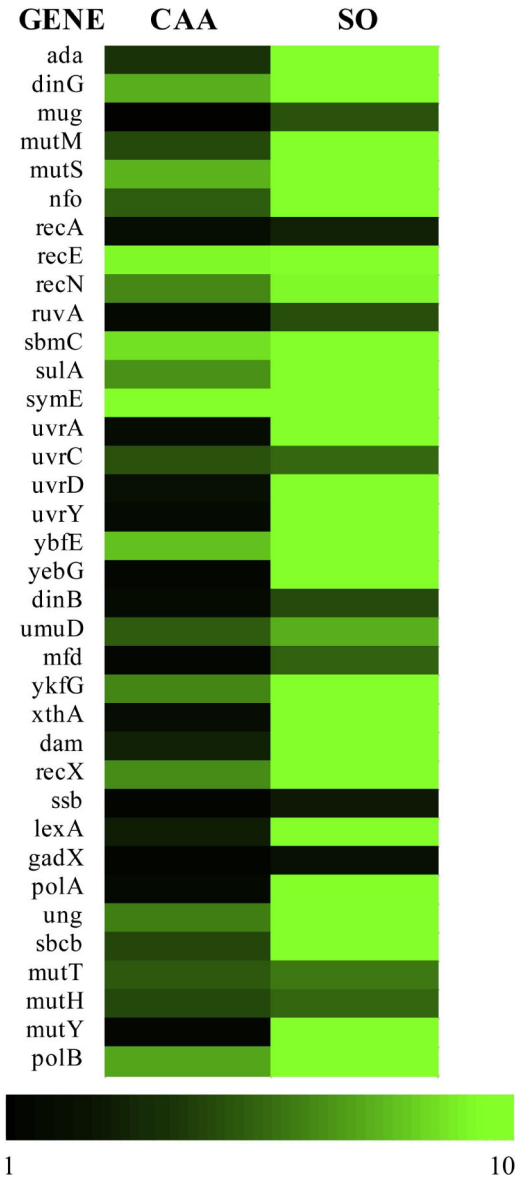

Figure 2.

TELI fluorescence heat map of DNA damage and DNA stress response genes from *E. coli* MG1655 exposed to CAA (0.5 mg/mL in H2O) and SO (1.4 mg/mL in DMSO). The TELI values range from 1 (black = promoter of gene not upregulated and GFP not produced) to 10 (bright green = promoter of gene upregulated and GFP produced), as indicated.

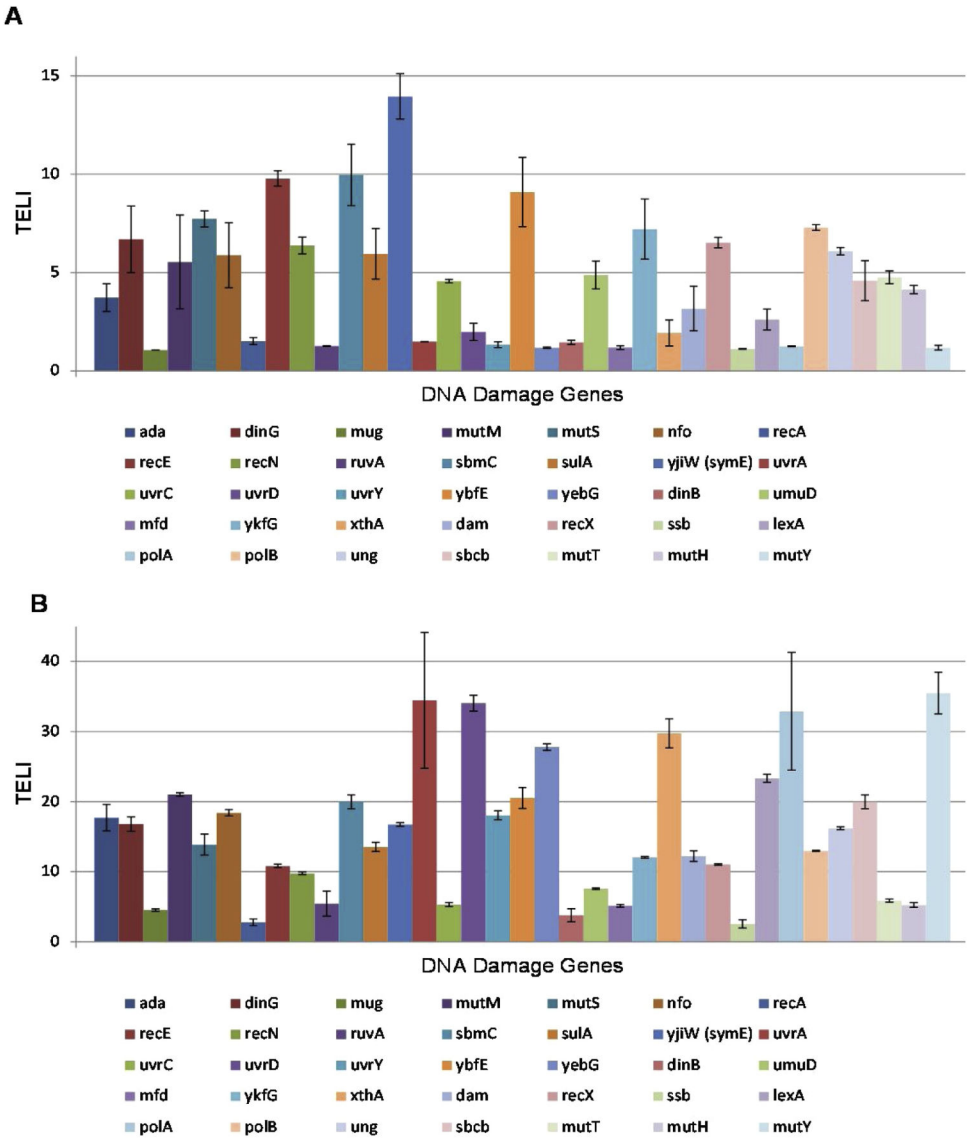

Figure 3.

TELI versus DNA damage gene promoters from *E. coli* MG1655 exposed to (A) CAA (0.5 mg/mL in H2O) and (B) SO (1.4 mg/mL in DMSO).

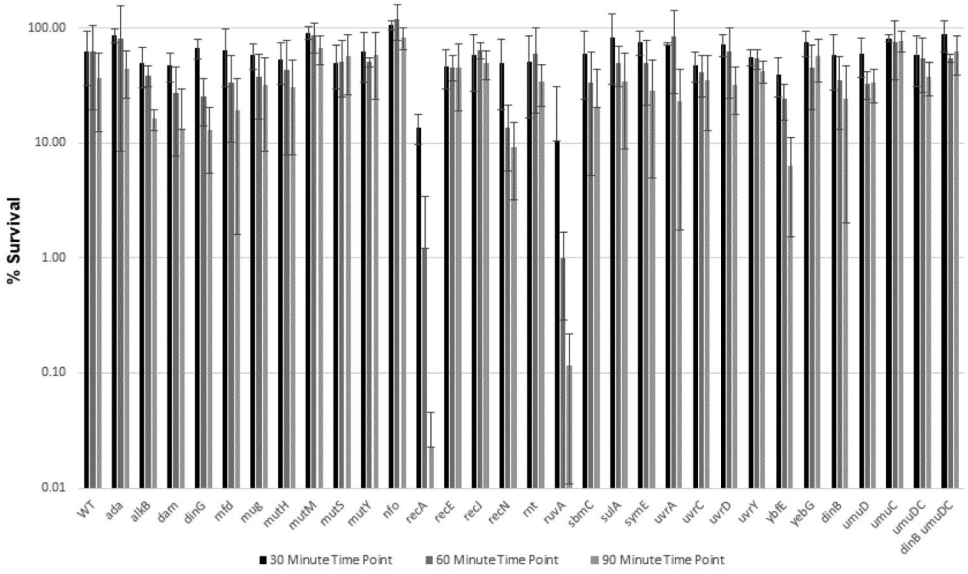

Figure 4.

Summary bar graph of percent survival of *E. coli* WT AB1157 and AB1157 mutant strains versus time upon 30 min, 60 min, and 90 min exposure to 0.5 mg/mL CAA.

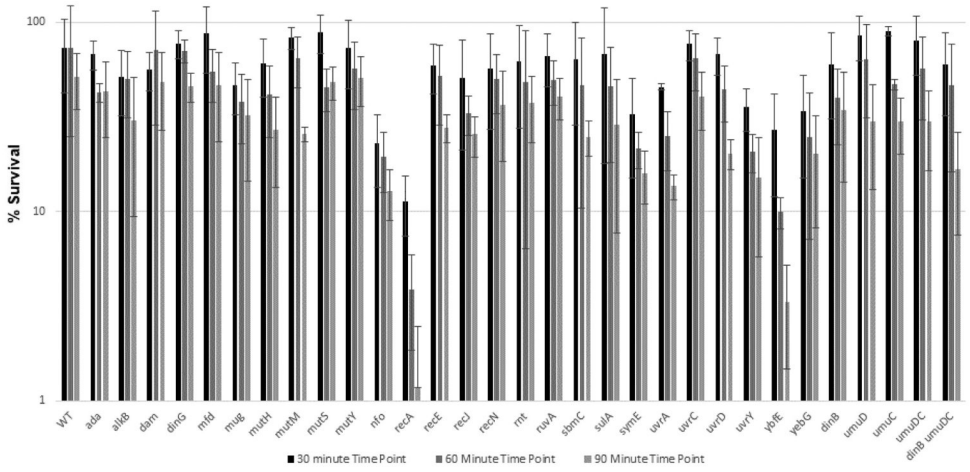

Figure 5.

Summary bar graph of percent survival of *E. coli* WT AB1157 and AB1157 mutant strains versus time upon 30 min, 60 min, and 90 min exposure to 1.4 mg/mL SO.

Table 1.

Strains used in this work.

Bacterial Strain	Relevant Genotype	Source
AB1157	*thr-1 araC-14 leuB-6(Am) Δ(gpt-proA)62 lacY1 tsx-33 qsr-0 glnV44(AS) galK2(Oc) LAM-Rac-0 hisG4(Oc) rfbC1 mgl-51 rpoS396(Am) rpsL31 kdgK51 xylA5 mtl-1 argE3(Oc) thi-1*	Laboratory stock
MG1655	F^{-}-λ^{-}-*ilvG*$^{-}$-*rfb*-50 *rph*-1	Laboratory stock
PB108	AB1157 Δ*ada*::kan	PI (JW2201–1) [39] to AB1157, this work
PB109	AB1157 Δ*alkB*::kan	PI (JW2200–1) [39] to AB1157, this work
AB1157 Δ*dinB*	AB1157 Δ*dinB*	[41, 42]
PB110	AB1157 Δ*dinG*::kan	PI (JW0784–1) [39] to AB1157, this work
PB130	AB1157 Δ*mfd*::kan	PI (JW1100–l) [39] to AB1157, this work
PB111	AB1157 Δ*mug*::kan	PI (JW3040–1) [39] to AB1157, this work
PB112	AB1157 Δ*mutM*::kan	PI (JW3610–2) [39] to AB1157, this work
PB113	AB1157 Δ*mutS*::kan	PI (JW2703–2) [39] to AB1157, this work
PB129	AB1157 Δ*mutY*::kan	PI (JW2928–1) [39] to AB1157, this work
PB114	AB1157 Δ*nfo*::kan	PI (JW2146–1) [39] to AB1157, this work
PB115	AB1157 Δ*recA*::kan	PI (JW2669–1) [39] to AB1157, this work
PB116	AB1157 Δ*recE*::kan	PI (JW1344–1) [39] to AB1157, this work
PB101	AB1157 Δ*recJ*::kan	PI (JW2860–1) [39] to AB1157; Graham Walker
PB117	AB1157 Δ*recN*::kan	PI (JW5416–1) [39] to AB1157, this work
PB118	AB1157 Δ*rnt*::kan	PI (JW1644–5) [39] to AB1157, this work
PB119	AB1157 Δ*ruvA*::kan	PI (JW1850–2) [39] to AB1157, this work
PB120	AB1157 Δ*sbmC*::kan	PI (JW1991–2) [39] to AB1157, this work
PB121	AB1157 Δ*sulA*::kan	PI (JW0941–1) [39] to AB1157, this work
PB122	AB1157 Δ*symE*::kan	PI (JW4310–1) [39] to AB1157, this work
PB100	AB1157 Δ*umuC*	Graham Walker
AB1157 Δ*umuD*	AB1157 Δ*umuD*	Graham Walker
GW8017	AB1157 Δ*umuDC*	[43]
SEC136	AB1157Δ*dinB* Δ*umuDC*	Susan Cohen; Graham Walker
PB123	AB1157 Δ*uvrA*	Graham Walker
PB124	AB1157 Δ*uvrC*::kan	PI (JW1898–l) [39] to AB1157, this work
PB125	AB1157 Δ*uvrD*::kan	PI (JW3786–5) [39] to AB1157, this work
PB126	AB1157 Δ*uvrY*::kan	PI (JW1899–1) [39] to AB1157, this work
PB127	AB1157 Δ*ybfE*::kan	PI (JW5816–1) [39] to AB1157, this work
PB128	AB1157 Δ*yebG*::kan	PI (JW1837–1) [39] to AB1157, this work

Table 2.

Primers used to verify transductions performed for this work.

Primer Name	Primer Sequence
KanFor[1]	CAGTCATAGCCGAATAGCCT
KanRev[1]	CGGCCACAGTCGATGAATCC
ada-check5	GGAGAAAGCTAAAGAGGTTGTTCG
ada-check3	GGCTGGCAACGTCATTAATATCGC
alkB-check5	CGTGGTGATGGCACACTTTCCG
alkB-check3	CAGACAAGTACAAGAAGTTCCATGC
dam-seq5	CGATCTGAAGTAATCAAGGTTATCTCC
dam-seq3	CCAGCTGCCAGGGCTTTTGCGG
dinG-check5	CGCATTATGTTGGTGGTTATTGCG
ding-check3	GGACTTCACCATTGAGCATATGAGC
mfd-seq5	GGCGATTCAATTTGCTAAACCATGTC
mfd-seq3	GGTGACAGTGTCGGATAGTGCAGG
mug-check5	CCAAGCGATTATGAGTCGCCTGC
mug-check3	CGTAGCTTCCTGGACGATTAATCG
mutM-check5	GCTTTGATGTAACAAAAAACCTCGC
mutM-check3	GCGATGTCACCCATTTCCTGCC
mutS-check5	GCGTACTTGCTTCATAAGCATCACG
mutS-check3	CCCATATATGGCGATAGTGATGGG
mutY-seq5	CCGGATGCAAGCATGATAAGGCC
mutY-seq3	GACCTTCTGCTTCACGTTGCAGG
nfo-check5	CCTGTTAACCCGCTATCATTACCG
nfo-check3	GCTGAATCAGCGGGTTACGCCG
recA-check5	CGTATGCATTGCAGACCTTGTGG
recA-check3	GCGTACCGCACGATCCAACAGG
recE-check5	GCAAGATCATTCACTGAACAAAACG
recE-check3	GCACGGTTTCCCTGAGTTTTTTGC
recN-check5	CCTGATTCGTCGCTGTGATTACC
recN-check3	CGCATAGTGATTTGATTCCTTTTCG
rnt-check5	GGTTATCGCGTTTGTGGAAGATCC
rnt-check3	CCTGAATCATTGCATGTCATCAGGC
ruvA-check5	CCATTTTTCAGTTCATCGAGACACC
ruvA-check3	CCAACATACTCTTCCAGTAATTTGG
sbmC-check5	CCTTTCTTTTGCAGCAGACTGGC
sbmC-check3	GGCAGGAGCGAAAAAATTGAAAGG
sulA-check5	GGTATTCAATTGTGCCCAACG TTGC
sulA-check3	GGATCTGCTCAATATTAACTCTACC

Primer Name	Primer Sequence
symE-check5	GCATCGCTAATCACAATCACTATTCC
symE-check3	GCTAAGCCTCTATTATCGCTTTCG
uvrC-check5	GCTCAATCTCAGTCCGAAAACGG
uvrC-check3	GGATGACACGGAACAGTGTAAGC
uvrD-check5	CGGTTGGCATCTCTGACCTCGC
uvrD-check3	GGCAACGCTATCCTTTTGTCACC
uvrY-check5	CGTGACCATAACTGTGGACAATCG
uvrY-check3	GCGACATAGATAACCGTACCACCA
ybfE-check5	CGTCGCTATCTCAATGATTAACGC
ybfE-check3	CGGTATTACCGGTGTCGCTGCC
yebG-check5	GCCTAATAACATCACGCGAGTTGC
yebG-check3	CCGACTTGCTGGTTTCATTATTGG

[1] As reported in [44]

Table 3.

Summary of *E. coli* DNA stress response genes tested here and their functions [49]

Gene(s)/References	DNA Stress Category	Gene Function
ada [31]	Adaptive Response (AR)	DNA alkylation repair; O^6-methylguanine-DNA methyltransferase
alkB [50]	DR and AR	DNA alkylation repair; alpha-ketoglutarate- and Fe(II)-dependent dioxygenase
dam [51]	Alkylation	DNA-(adenine-N^6)-methyltransferase
dinB [52, 53]	Pol IV (SOS)	DNA polymerase IV (Y-family DNA polymerase); translesion DNA synthesis; damage bypass
dinG [54]	Recombination repair	ATP-dependent DNA helicase; putative repair and recombination enzyme; unwinding of DNA
mfd [55]		Transcription repair coupling factor; translocase
mug [56]	MMR	Stationary phase mismatch/uracil DNA glycosylase
mutH [57]	MMR	Methyl-directed mismatch repair
mutM [58]	BER	formamidopyrimidine DNA glycosylase; GC to TA
mutS [57]	MMR	Methyl-directed mismatch repair protein
mutY	BER	Adenine glycosylase, GA repair
nfo [59]	Oxidative stress	AP endodeoxyribonuclease IV; member of *soxRS* regulon; endonuclease IV
recA [60]	SOS, recombination repair	Binds ssDNA to form nucleoprotein filament during SOS mutagenesis; homologous recombination
recE [61]	Recombination repair	exonuclease VIII, ds DNA exonuclease, 5’ → 3’ specific; recombination and repair
recJ [60, 62]	Recombination repair	ssDNA 5’ → 3’ exonuclease; recombination and repair
recN [61, 63]	Recombination repair	Recombination and repair
rnt [64]	RNA Degradation	RNase T; RNA processing; degrades RNA
ruvA [65, 66]	Recombination repair	Drives branch migration of Holliday structures during recombination and repair
sbmC [67]	Relieve strain	DNA gyrase inhibitor, SOS regulated
sulA [68]	SOS	SOS cell division inhibitor; Inhibits cell division and FtsZ ring formation; LexA regulon
symE [69, 70]	unknown	Hypothetical protein; RNA antitoxin
umuDC [52, 53]	SOS	SOS mutagenesis and repair; DNA polymerase V (Y-family DNA polymerase complex); DNA translesion synthesis; damage bypass
uvrA [71]	NER	excision nuclease subunit A; Component of UvrABC Nucleotide Excision Repair Complex
uvrC [71]	NER	Excision nuclease subunit C; repair of UV damage to DNA
uvrD [71]	NER	DNA-dependent ATPase I and helicase II; Component of UvrABC Nucleotide Excision Repair Complex; unwinds forked DNA structures; dismantles the RecA nucleoprotein filament
uvrY [72]	DNA Binding	DNA binding response regulator; controls the expression of *csrB*/*C* sRNAs; hydrogen peroxide resistance
xthA [73]	DNA Repair	Exodeoxyribonuclease III and AP endodeoxyribonuclease VI; RNase H activity; DNA repair
ybfE [74]	SOS	LexA-regulated protein; unknown function
yebG [75]	SOS	DNA damage-inducible gene; SOS regulon