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A Small-Molecule Inducible Synthetic Circuit for Control of the SOS Gene Network without DNA Damage

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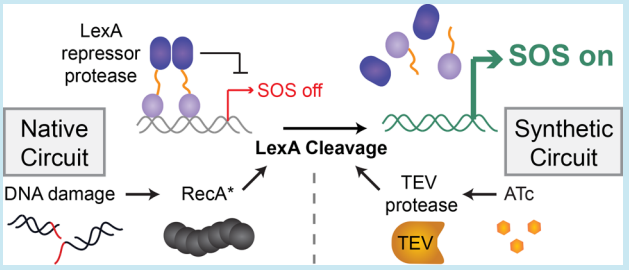
Supporting Information

ABSTRACT: The bacterial SOS stress-response pathway is a pro-mutagenic DNA repair system that mediates bacterial survival and adaptation to genotoxic stressors, including antibiotics and UV light. The SOS pathway is composed of a network of genes under the control of the transcriptional repressor, LexA. Activation of the pathway involves linked but distinct events: an initial DNA damage event leads to activation of RecA, which promotes autoproteolysis of LexA, abrogating its repressor function and leading to induction of the SOS gene network. These linked events can each independently contribute to DNA repair and mutagenesis, making it difficult to separate the contributions of the different events to observed phenotypes. We therefore devised a novel synthetic circuit to unlink these events and permit induction of the SOS gene network in the absence of DNA damage or RecA activation *via* orthogonal cleavage of LexA. Strains engineered with the synthetic SOS circuit demonstrate small-molecule inducible expression of SOS genes as well as the associated resistance to UV light. Exploiting our ability to activate SOS genes independently of upstream events, we further demonstrate that the majority of SOS-mediated mutagenesis on the chromosome does not readily occur with orthogonal pathway induction alone, but instead requires DNA damage. More generally, our approach provides an exemplar for using synthetic circuit design to separate an environmental stressor from its associated stress-response pathway.



KEYWORDS: *SOS pathway, LexA, stress response pathways, DNA repair, mutagenesis*

Prokaryotes are capable of dynamically responding to a diverse range of environmental stressors. This ability is often mediated by genetic circuits that can detect signals of stress and subsequently mount an appropriate response. The SOS pathway is a canonical example of such a stress-response pathway, mediating survival from genotoxic stress. The pathway is under the control of a single transcriptional regulator, LexA (Figure 1a).[1] In the absence of stress, LexA binds to the promoters of SOS pathway genes to repress the response. In *E. coli*, as many as 40 genes are regulated by LexA, with binding dictated by conserved SOS boxes that vary in strength and location relative to the promoters of SOS genes.[2] In the event of DNA damage, single stranded DNA (ssDNA) accumulates at stalled replication forks or double stranded DNA breaks and serves as the template for filamentation of the DNA damage sensor protein, RecA.[3] This activated RecA nucleoprotein filament (RecA*), in turn, triggers the self-cleavage (autoproteolysis) of LexA.[4] Cleaved LexA is no longer able to bind DNA, and the resulting derepression results in an orderly progression of SOS gene transcription based on the affinity of LexA for each respective SOS box.[5] The resulting cascade of genes expressed largely function to repair damaged DNA, but several, such as translesion DNA polymerases, are also associated with accelerated mutagenesis.[6] The SOS pathway has more recently been implicated in antibiotic tolerance and acquired resistance, sparking a renewed interest in understanding and manipulating its effects on evolutionary adaptation.[7−11]

Given its prominent role in both DNA repair and mutagenesis, the SOS pathway has been an area of intense interest for decades; however, a standing challenge in the field has been separating the roles of the upstream inciting events from those of the downstream induced SOS pathway genes. Separating the events preceding the SOS response is important because SOS pathway activation, as noted above, results from three linked but distinct events—DNA damage, RecA* activation, and LexA cleavage—and each of these processes plays discrete roles in the observed phenotypes. DNA damage, for example, can directly contribute to mutagenesis independent of SOS activation, and can also activate the general stress response pathway through RpoS and RpoE.[12,13] RecA* has additional functions beyond mediating LexA cleavage: RecA*

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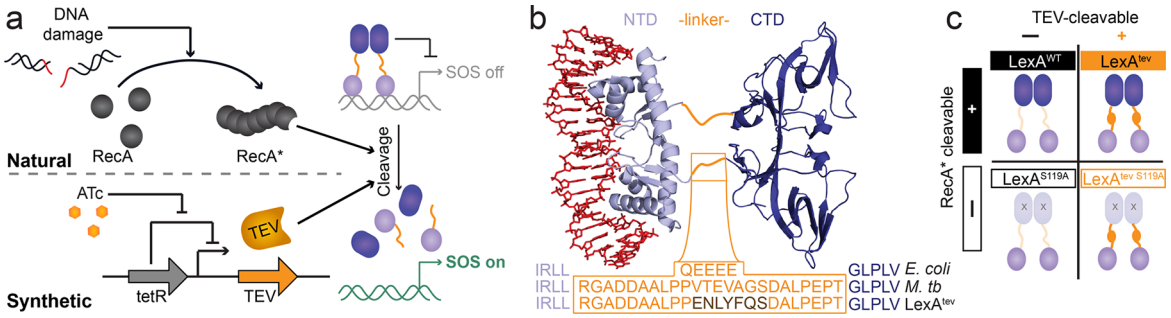

Figure 1. Schematic of engineered synthetic circuit. (a) In the native circuit (top), SOS genes are turned on after DNA damage activates filamentation of RecA, which induces LexA to undergo self-cleavage within its CTD. In the synthetic circuit design (bottom), the nontoxic small molecule anhydrotetracycline (ATc) induces expression of TEV protease, which instead cleaves LexA at a site introduced into its flexible linker. (b) Crystal structure of LexA protein bound to operator DNA adapted from PDB 3JS0.[48] The unstructured linker region is highlighted, with partial sequence alignment of LexA from *E. coli* and *M. tuberculosis*, along with the engineered linker sequence of LexA^{tev} containing the TEV recognition site. (c) Representation of four LexA variants examined in this study. The variants represent all combinations of RecA*- and/or TEV-mediated cleavage.

participates in homologous recombination, activates mobile genetic elements including prophage induction, and modulates the activity of error-prone polymerases, including serving as one component of the active DNA polymerase V complex.[14−19] As the last link in these integrated processes, LexA cleavage is ultimately responsible for the induction of SOS pathway genes.

Prior studies have employed classical genetic approaches to address the challenge of studying the biology of the SOS pathway independent of DNA damage and activated RecA, but each approach carries limitations. Deletion of *lexA*, for example, activates a constitutive hypermutable state;[20,21] however, unregulated overexpression of SOS effector genes obscures their role when induced at physiological levels. Similar findings and limitations are associated with a strain harboring *recA730*, an allele which constitutively generates RecA*.[21−23] A heat-labile *lexA41* allele, whose protein product degrades upon shifting to 42 °C, offers an alternative means to induce the SOS pathway in the absence of DNA damage; however, in addition to the added stressor of heat shock, this mutant LexA has numerous defects, including altered degradation kinetics and an inability to bind to certain SOS promoters, leading to incomplete split-phenotype SOS activation.[24] Introducing an undamaged F′ plasmid or phage genome into a UV-irradiated cell has also been used as an alternative means of separating SOS-associated mutagenesis from DNA damage. These studies suggest that targeted mutagenesis to damaged genomes exceeds untargeted mutagenesis; however, replication dynamics are likely different for exogenously introduced DNA than for the host genome.[25,26]

Given the importance of separating the upstream signals from the downstream SOS gene induction events, as well as the limitations of classical approaches, we considered whether modern synthetic biology approaches could be applied.[27,28] Envisioning the SOS pathway as a genetic circuit allowed us to conceive of an alternative circuit design, whereby the SOS response in *E. coli* could be induced without a requirement for DNA damage or RecA activation. We manipulated a variable region in LexA to introduce a new recognition site for tobacco etch virus protease (TEV), allowing us to control LexA cleavage by modulating expression of TEV with a nontoxic small molecule (Figure 1a). After validating our design biochemically, we demonstrated that bacterial strains harboring the synthetic circuit recapitulate the UV resistance phenotype of the native circuit. Furthermore, with the ability to now orthogonally investigate DNA damage, RecA activation, and LexA cleavage, we applied our synthetic circuit to demonstrate the importance of DNA damage for accelerating SOS-mediated mutagenesis. Our work offers a novel application of synthetic biology to separate a stressor from its associated stress-response pathway.

RESULTS AND DISCUSSION

Design of a LexA Variant Capable of Orthogonal Regulation. To obtain orthogonal control over the SOS pathway, we envisioned engineering an *E. coli* LexA variant that could be rendered dysfunctional inside the cell, not by self-cleavage but by an alternative protease. Our design began with an examination of the LexA sequence conservation across more than 400 different species (Figure S1). LexA is a homodimer consisting of an N-terminal DNA binding domain (NTD) and a C-terminal serine protease domain (CTD) connected by a flexible linker (Figure 1b). Although the functional NTD and CTD are generally well-conserved across prokaryotes, we noted that the linker region is highly divergent, spanning from its shortest length of 5 amino acids in *E. coli* up to 50 amino acids in some species. This natural diversity in length and sequence led us to hypothesize that the short linker in *E. coli* could tolerate modification and provide a locus for introducing an exogenous protease cleavage site without significantly perturbing the function of the NTD and CTD.

Reasoning that a longer linker could increase accessibility for an exogenous protease, we replaced the linker from *E. coli* with the 24 amino acid linker from *M. tuberculosis*. Within this scaffold, we then centrally embedded the optimized recognition site for TEV (Figure 1b).[29] We expressed and purified this engineered LexA variant, hereafter called LexA^{tev}. To abolish LexA self-cleavage, we also made a corresponding S119A mutant, inactivating the catalytic active-site serine (LexA$^{tev\ S119A}$).[30] This variant could potentially be activated by the synthetic, but not the native, circuit. With native LexA (LexA^{WT}) and the protease-inactive S119A variant (LexA^{S119A}), these four variants thus covered the full range of RecA*-induced and/or TEV-cleavable systems (Figure 1c).

Our alteration to the LexA linker would ideally be non-perturbing to NTD and CTD function, while permitting TEV cleavage. To compare our novel LexA^{tev} and LexA$^{tev\ S119A}$ proteins with their native-linker counterparts, we began by quantitatively comparing their DNA binding and protease functions. Using an electrophoretic mobility shift assay

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(EMSA) for DNA binding activity, we found that association of LexA^{tev} with SOS operator DNA was comparable to that of LexA^{WT} (K_d 65 ± 21 and 47 ± 15 nM, respectively), and similar results were noted for the S119A variants (Table 1, Figure S2A). The DNA-binding of LexA^{tev} was specific to SOS operator DNA, as the affinity was similar in the presence of a large excess of nonspecific DNA and no binding was observed to a scrambled operator duplex (Figure S2B). To evaluate self-cleavage activity, the proteins were incubated with activated RecA* and cleavage products were analyzed (Figure 2a). As anticipated, both LexA^{S119A} and LexA$^{tev\ S119A}$ were unreacted, while LexA^{WT} and LexA^{tev} were both proficient in RecA*-stimulated self-cleavage. Both of the RecA*-cleavable proteins behaved similar to one another in the presence of either SOS operator DNA or scrambled DNA (Figure S3). LexA can also undergo an efficient RecA*-independent, Ser119-mediated, self-cleavage reaction when exposed to high pH *in vitro*, and this reaction can be readily quantified by fitting the reaction progress curve to a first-order kinetic model (Table 1, Figure S4).[31,32] Under these conditions, we found that LexA^{WT} and LexA^{tev} cleaved with similar efficiencies (0.014 ± 0.003 and 0.019 ± 0.003 min^{-1}, respectively), while LexA^{S119A} and LexA$^{tev\ S119A}$ showed no demonstrable cleavage at any time point observed, as expected. Equally important for our synthetic circuit design, we found that, when incubated with purified TEV, both LexA^{tev} and LexA$^{tev\ S119A}$ were readily cleaved *in vitro*, while LexA^{WT} and LexA^{S119A} remained intact (Figure 2b). TEV-mediated cleavage was not affected by the presence of DNA containing either an SOS operator box or a scrambled SOS operator sequence (Figure S3). These results together confirmed our hypothesis that altering the linker, which has previously been subjected only to limited investigation,[33] can add new functionality while preserving native characteristics. Our results suggest that this linker could provide a locus for exploring other alterations in the future.

Table 1. Biochemical Characterization of Purified LexA Variants[a]

LexA variant	K_d (nM)	k (min^{-1})
LexA^{WT}	47 ± 15	0.019 ± 0.003
LexA^{S119A}	43 ± 17	N.D.
LexA^{tev}	65 ± 21	0.014 ± 0.003
LexA$^{tev\ S119A}$	37 ± 9	N.D.

[a]The reported values are the binding constants of LexA with operator DNA and the rates of alkaline-induced self-cleavage. Values are the mean ± SD from at least 3 independent experiments. N.D., not detected.

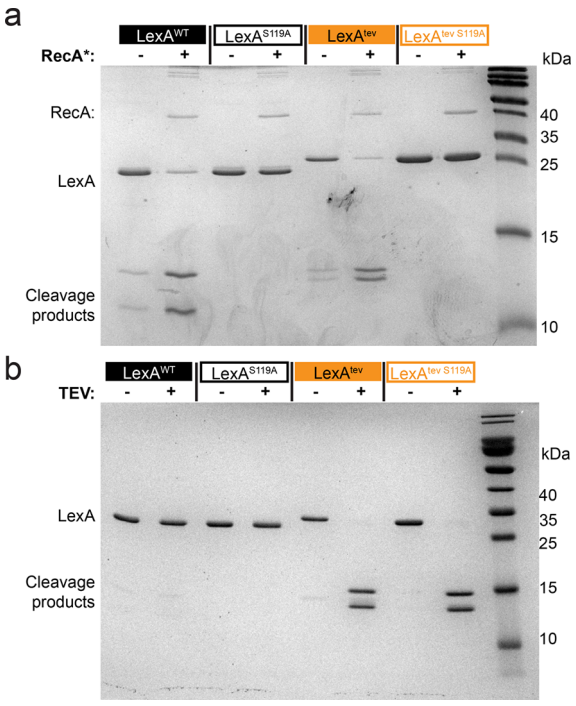

Figure 2. Biochemical validation of LexA cleavage by native or synthetic circuits. (a) Purified LexA proteins were incubated with or without activated RecA* and visualized by SDS-PAGE. (b) Analogous incubations with TEV were carried out and resolved by SDS-PAGE.

Construction of Synthetic Circuit Inside Cells. To advance our synthetic circuit strategy into cells, we engineered our LexA variants into the native *lexA* locus by scarless recombineering (Table S1).[34] The parent strain for the majority of our studies was *E. coli* MG1655 Δ*sulA* (hereafter *lexA*^{WT}), as recombineering at the *lexA* locus requires a transient Δ*lexA* state and the Δ*lexA sulA*$^+$ strain is nonviable. In this background, the *lexA* locus was replaced with a cassette containing chloramphenicol resistance and an I-SceI cut site for efficient scarless recombineering. Subsequent replacement of this locus with *lexA*^{S119A} serves as an SOS-off strain, unable to self-cleave and therefore constitutively repressed.[21] Two analogous strains were generated: one encoding our synthetic LexA variant that is also proficient in self-cleavage (*lexA*^{tev}) and a second that harbors both the variant linker and the S119A mutation (*lexA*$^{tev\ S119A}$), which should render LexA only capable of orthogonal activation by TEV. Notably, once the *lexA* locus has been replaced in the recombineering process, *sulA* can potentially be reintroduced. While some prior studies have shown that deletion of *sulA* does not significantly alter SOS survival phenotypes,[21] to address the possible influence of *sulA* deletion in our synthetic circuit strain, we also restored *sulA* in the *lexA*$^{tev\ S119A}$ strain (called *lexA*$^{tev\ S119A}$ *sulA*$^+$).

We completed the design of our synthetic circuit by introducing TEV into the cells. To control TEV expression, we placed the gene on a medium-copy plasmid under the control of the anhydrotetracycline (ATc)-inducible *tetA* promoter, along with a copy of its associated *tetR* repressor gene. To help minimize any effects from leaky TEV expression under uninduced conditions, we also added a ssrA degron tag to the C-terminus of TEV.[35] A complete sequence and map of the pMG81 plasmid containing TEV protease can be found in Figure S5.

With strains harboring both the chromosomal *lexA* variants and inducible TEV plasmid, we first wanted to examine whether native circuit functions were retained in the expected manners; specifically, if LexA^{tev} could bind and repress SOS promoters in unstressed cells, and if the strains with preserved CTD protease activity could undergo SOS induction in response to DNA damage. To monitor derepression of the SOS gene network, we employed a GFP reporter plasmid under the control of the *recA* promoter (P_{recA}-GFP); for consistency, all cell-based assays were performed in the presence of both this plasmid as well as the TEV-expression plasmid.[36] Mid log cultures were either left untreated or irradiated with UV light (50 J/m^2), and GFP fluorescence was monitored after 1 h by flow cytometry (Figure 3a). In the

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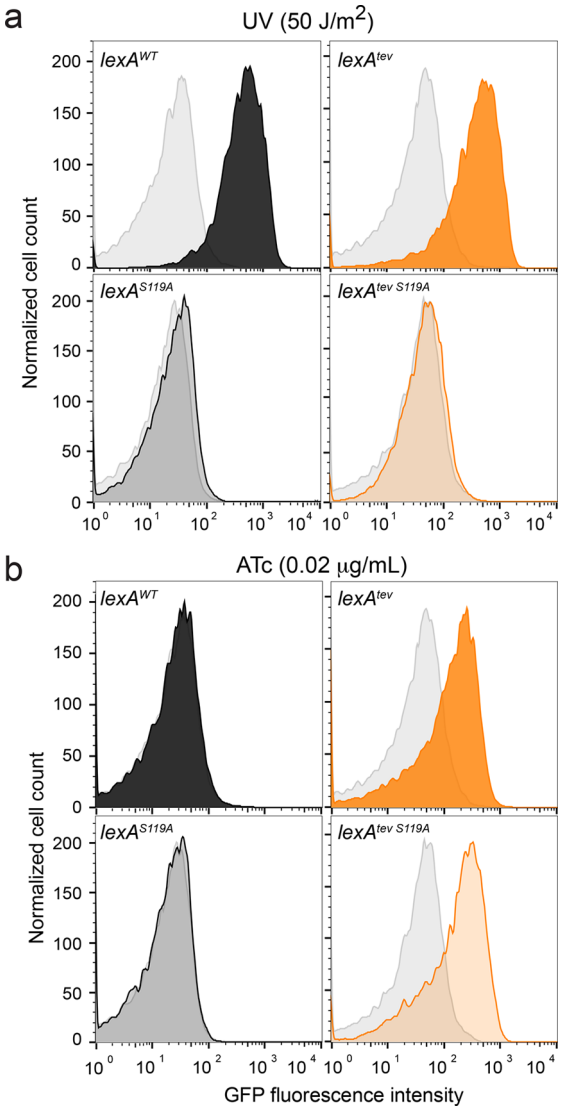

Figure 3. Induction of native and synthetic SOS circuits *in vivo*. Strains engineered at the native *lexA* locus and containing an ATc-inducible TEV expression plasmid were evaluated for GFP expression from an SOS reporter plasmid 1 h after exposure to (a) UV damage or (b) ATc. Representative histograms show single-cell GFP fluorescence profiles for unexposed (light gray) or exposed cells (dark gray and orange).

absence of UV exposure, $lexA^{tev}$ and $lexA^{tev\ S119A}$ strains had low baseline GFP expression, similar to that of $lexA^{WT}$ and $lexA^{S119A}$. In response to UV light, however, $lexA^{tev}$ and $lexA^{WT}$ strains demonstrated a ∼1-log shift in mean fluorescence intensity compared to the undamaged controls, while $lexA^{S119A}$ and $lexA^{tev\ S119A}$ strains showed no DNA damage-associated GFP expression. To examine a wider range of SOS-controlled genes, we repeated these experiments with reporters containing three additional promoters representing a range of induction kinetics: P_{recN}-GFP, P_{lexA}-GFP, and P_{sulA}-GFP. Similarly to P_{recA}-GFP, these three reporter plasmids demonstrate a rightward shift in mean fluorescence intensity compared to controls in the $lexA^{WT}$ and $lexA^{tev}$ strains, while there was no GFP expression associated with DNA damage in the $lexA^{S119A}$ and $lexA^{tev\ S119A}$ strains (Figure S6). These results have two implications. First, given the low baseline fluorescence intensity in unstressed cells, both $LexA^{tev}$ and $LexA^{tev\ S119A}$ bind to SOS operator DNA in cells and suppress expression comparably to $LexA^{WT}$. Second, similar levels of GFP expression with $LexA^{tev}$ and $LexA^{WT}$ upon irradiation confirms that the native SOS pathway is preserved in those strains.

After confirming the preserved function of the native circuit, we next evaluated the function of the synthetic circuit. Using the $lexA^{tev\ S119A}$ strain, we initially examined expression of TEV and cleavage of $LexA^{tev\ S119A}$ by Western blotting. Steady state levels of this LexA variant were comparable to those in the $lexA^{WT}$ strain (Figure S7A). We added a range of concentrations of ATc to a mid log culture of $lexA^{tev\ S119A}$ and analyzed TEV and LexA levels after 10 and 60 min (Figure S7B). TEV was undetectable at baseline, and sustained expression starting within 10 min could be observed at or above 0.02 μg/mL. Concurrently, under these conditions full-length $LexA^{tev}$ becomes undetectable within 10 min, which is in the time range observed with native circuit activation by UV light.[37] Following the rapid degradation of LexA, we consistently observe some reaccumulation of LexA at 60 min. As both LexA and TEV are autoregulated at their respective promoters, this reaccumulation may represent the changing equilibrium with increased synthesis of new LexA, concurrent with decreased production of TEV protease. Notably, no LexA cleavage products were detected by Western blot, suggesting rapid degradation after cleavage, analogous to that observed when native LexA self-cleaves.[38] Given that we observed a mild growth deficit at ATc doses at or above 0.2 μg/mL, we chose to use a standard concentration of 0.02 μg/mL ATc for initial studies on our synthetic circuit. However, these data suggest the potential for a titratable response with further refinement. Together, these data support that TEV can be activated rapidly following ATc addition and that TEV can cleave engineered $LexA^{tev}$ and promote its depletion.

We proceeded to ask whether the P_{recA}-GFP SOS reporter could be activated through our novel ATc-inducible circuit. Analogous to the experimental setup with UV-mediated induction, mid log cultures either remained untreated or were induced with 0.02 μg/mL ATc, and GFP fluorescence was monitored (Figure 3b). At 1 h after ATc addition, $lexA^{tev}$ and $lexA^{tev\ S119A}$ strains showed a ∼0.7-log increase in mean fluorescence intensity, while $lexA^{WT}$ and $lexA^{S119A}$ strains demonstrated no change in fluorescence. The expanded series of reporters likewise showed an ATc-dependent increase in GFP fluorescence from P_{recN}-GFP, P_{lexA}-GFP and P_{sulA}-GFP in our $lexA^{tev}$ and $lexA^{tev\ S119A}$ strains, indicative of broad pathway activation across a range of LexA-controlled promoters (Figure S6). To correlate our GFP fluorescence at 0.02 μg/mL ATc with a quantifiable UV dose, we compared the extent of GFP expression through the synthetic circuit to that of the native circuit across a range of UV doses (Figure S6). The degree of SOS activation most closely correlated to that seen with ∼5 J/m^2 UV light. We attributed this degree of SOS activation to several factors, including the rate of TEV synthesis after ATc addition or the cleavage rate of TEV protease *versus* that of LexA self-cleavage. Taken together, the above results show that our synthetic circuit design achieved the desired aims. The $lexA^{tev}$ strain offers us two ways to activate the SOS response: orthogonally, by adding ATc, or natively, by inducing DNA damage. Uniquely, the $lexA^{tev\ S119A}$ strain can only activate the SOS response by orthogonal means, thus bypassing the requirement for DNA damage and RecA*.

Small-Molecule Inducible UV Resistance. Having demonstrated ATc-inducible activation of several SOS reporter

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genes, we next asked whether our synthetic circuit could recapitulate the global phenotypes normally associated with SOS gene network activation. We chose first to test our strains in the classic model of survival and DNA repair after UV damage. The strains were streaked across LB agar plates in the presence or absence of ATc and were subsequently exposed to a gradient of UV light from 0 to 120 J/m^2. In the absence of ATc, the strains with intact native activation pathways, *lexA*^{WT} and *lexA*^{tev}, survived UV doses up to ~100 J/m^2 (Figure 4a). By

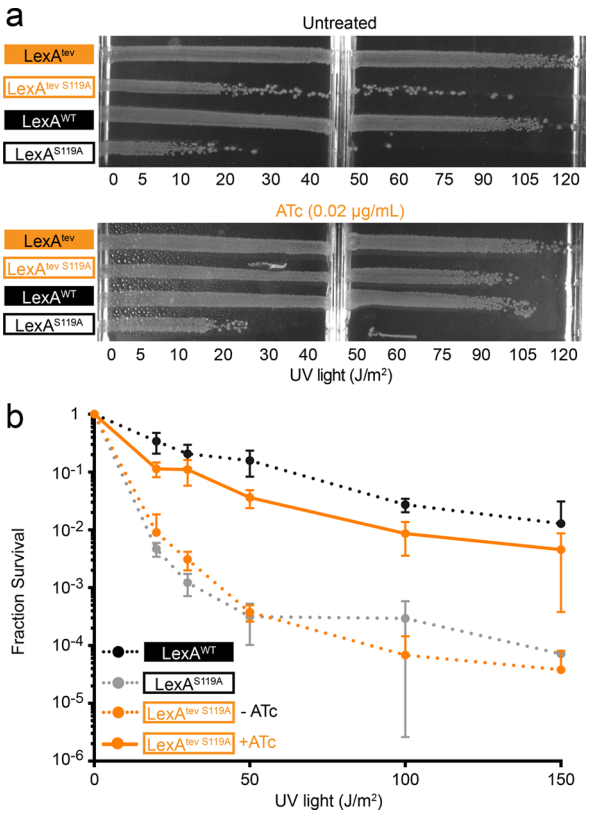

Figure 4. Survival in response to UV damage. (a) Strains were streaked across LB agar without ATc (top) or with 0.02 μg/mL ATc (bottom). The plates were exposed to a gradient of UV light from 0 to 120 J/m^2 and plates were imaged after 24 h. (b) Survival curves for mid log cultures of engineered strains following UV light exposure. Viable colony forming units were determined under each condition and the fraction survival was determined relative to the unexposed cells. Error bars represent SEM from at least 4 independent determinations.

contrast, *lexA*^{S119A} and *lexA*$^{tev\ S119A}$ were largely unable to survive doses above 10−20 J/m^2. With *lexA*$^{tev\ S119A}$, we observed rare colonies that could tolerate higher doses of UV irradiation, which could represent sporadic leaky expression of TEV. In the presence of ATc, these patterns for survival remain unchanged for *lexA*^{WT}, *lexA*^{S119A}, and *lexA*^{tev}. However, as predicted by our circuit design, in the presence of ATc, *lexA*$^{tev\ S119A}$ shows a >5-fold increase in the tolerated UV dose, demonstrating our desired small-molecule inducible UV resistance pattern.

To further quantify UV resistance, we next examined survival curves across a range of UV doses (Figure 4b). A mid log phase culture was split into media containing ATc or no ATc and then immediately irradiated. Serial dilutions were plated onto LB agar, and viable cells were counted after overnight incubation. Relative to *lexA*^{WT} where the native SOS pathway is intact, *lexA*^{S119A} showed enhanced killing by ~2−3 log across UV doses. In the absence of ATc, the *lexA*$^{tev\ S119A}$ strain followed a similar survival curve to *lexA*^{S119A}. In the presence of ATc, however, *lexA*$^{tev\ S119A}$ showed a significant ~1.5−2 log increase in the surviving fraction across all UV doses. We also repeated the survival curve analysis with *lexA*$^{tev\ S119A}$ *sulA*$^{+}$ and found similar levels of small-molecule inducible UV-resistance in comparison to *lexA*$^{tev\ S119A}$ strain (Figure S8A). As with the P$_{recA}$-GFP reporter studies, the synthetic circuit does not fully achieve the functional levels seen in the *lexA*^{WT} strain. It is possible that because our synthetic circuit best mimics a UV dose of 5 J/m^2, mild suppression of the response compared to the *lexA*^{WT} strain accounts for the incomplete rescue. Nonetheless, these data support the functionality of SOS-mediated DNA repair and survival following activation of the synthetic circuit. While prior studies have successfully designed gene circuits controlling individual or small groups of genes,[27] our study offers a novel example of small-molecule control over an entire stress response gene network in bacteria.

Small-Molecule Inducible Mutagenesis through the Synthetic Circuit. Turning our attention to another hallmark of the SOS response, we assessed mutagenesis by utilizing a rifampin resistance assay. As resistance to the antibiotic rifampin is acquired through any one of a number of specific point mutations in the *rpoB* chromosomal gene locus, this assay has been classically used as a reliable proxy for the overall mutation frequency across the genome.[39] We focused our analysis on *lexA*^{WT}, which can only activate the SOS pathway through the native circuit, and *lexA*$^{tev\ S119A}$, which can only activate through the synthetic circuit. Mid log phase cultures were split into media with or without ATc. The cultures were then immediately damaged with increasing doses of UV light, and serial dilutions were plated after 1 h. The total population size without UV damage was determined after overnight growth on plates with nonselective media, and the number of rifampin-resistant colonies was counted after 48 h of growth on media containing selective levels of rifampin.

The *lexA*^{WT} strain demonstrated a low baseline and a UV dose-dependent increase in the number of rifampin-resistant mutants relative to the starting population (Figure 5a). In the *lexA*$^{tev\ S119A}$ strain, in the absence of ATc the frequency of resistant mutants remained low, in part due to the potent killing of the strain by UV light, with growth not detected above 20−30 J/m^2. However, when the *lexA*$^{tev\ S119A}$ strain was treated with ATc concurrently with UV damage, mutagenesis was largely restored to levels seen with *lexA*^{WT}, and showed a similar dose-dependence. As with DNA repair, minor differences can be observed between the *lexA*$^{tev\ S119A}$ and *lexA*^{WT} strains, particularly at the highest dose. Most of this can be accounted for by the small decrease in survival of the *lexA*$^{tev\ S119A}$ strain compared to the *lexA*^{WT} strain; indeed, when the number of resistant mutants was normalized to overall cell viability after irradiation, the *lexA*$^{tev\ S119A}$/ATc^{+} condition gives a mutation frequency per viable cell that is even higher than that of the *lexA*^{WT} strain (Table S2). As with our UV survival experiments, we evaluated the impact of *sulA* on the function of our synthetic circuit and once again observed >1-log increase in ATc-inducible mutagenesis in the *lexA*$^{tev\ S119A}$ *sulA*$^{+}$ strain (Figure S8B), similar to the levels observed in the absence of *sulA*. Overall, we conclude that synthetic circuit activation allows for small-molecule inducible mutagenesis in the setting of UV damage that is comparable to native pathway activation.

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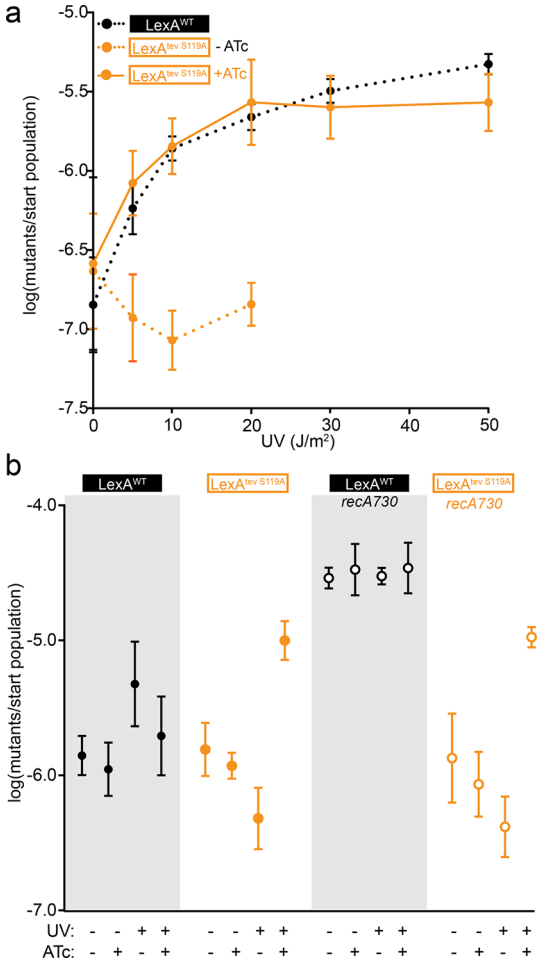

Figure 5. Separating the signals in SOS mutagenesis. (a) After exposure to various doses of UV light in the presence or absence of ATc, the *lexA*^{WT} or *lexA*$^{tev\ S119A}$ strains were plated on selective rifampin-containing media and the number of resistant colonies was quantified relative to an undamaged population of cells plated on nonselective media. Error bars represent the standard error across at least 6 independent replicates. (b) Rifampin resistant colonies were quantified relative to an undamaged population following exposure to either UV light (10 J/m^2) and/or ATc (0.02 μg/mL). Open circles indicate the presence of the *recA730* constitutively active allele.

Genetic and Environmental Requirements for SOS-Mediated Mutagenesis. Finally, we used our synthetic circuit to separate the roles of DNA damage, RecA activation, and LexA cleavage in SOS-mediated mutagenesis. As noted earlier, some studies have indicated that mutagenesis can occur in the absence of exogenous DNA damage when the SOS pathway is artificially hyperactivated—for example, with *lexA* deletion,[21] overexpression of the error prone polymerases,[40] or constitutive RecA activation through either *recA730* or *recA441* mutations.[21,41] However, other studies have indicated a role for DNA damage in SOS mutagenesis: when undamaged DNA, such as F′-plasmids or phage, is introduced into bacteria undergoing a UV-induced, native SOS response, mutagenesis appears to occur at only low rates on the undamaged, foreign DNA.[25,26] Our synthetic circuit offers an opportunity to reconcile these differences by studying the inducible SOS response, rather than a constitutive response, and examining SOS mutagenesis on the chromosomal *rpoB* locus, rather than foreign DNA.

To examine the requirements for SOS mutagenesis, we therefore evaluated the mutation frequency with UV alone (at a fixed 10 J/m^2), ATc alone, or both together (Figure 5b). With the *lexA*^{WT} strain, mutagenesis was observed with UV light in the presence or absence of ATc, although the mutational frequency is slightly decreased in the presence of ATc. As overall viability is not significantly altered (Table S2), this dampening could be due to a metabolic cost associated with TEV production. Importantly, our data with *lexA*$^{tev\ S119A}$ suggests that SOS pathway activation with ATc in the absence of UV is not sufficient for heightened mutagenesis. In this strain, we observed that the number of rifampin-resistant mutants increased when treated with UV and ATc together, but not with either one alone (Figure 5b). This aligns well with prior studies, which used a heat-labile *lexA41* allele to simulate LexA cleavage and degradation without DNA damage; in this case, mutagenesis was similarly impaired, although concerns about altered interactions with SOS promoters limited the authors' conclusions.[24,42]

Our results imply that either DNA damage and/or RecA* are necessary for mutagenesis, beyond simply acting as the signals involved in inducing LexA cleavage. DNA damage could provide a nidus for SOS mutagenesis, while the alternative functions of RecA*, including its role as one component of the active DNA polymerase V complex,[19] could be important for SOS mutagenesis. To examine if activated RecA* is sufficient to restore SOS mutagenesis without DNA damage, we generated *lexA*^{WT} and *lexA*$^{tev\ S119A}$ strains in the background of a *recA730* allele, which encodes for an E38L mutation that renders RecA constitutively active.[43] The *lexA*$^{tev\ S119A}$ *recA730* showed a similar responsiveness to ATc addition as the *lexA*$^{tev\ S119A}$ strain (Figure S9), suggesting that the strain offered similar orthogonal control over LexA cleavage. Evaluating these strains, as previously established,[21−23] *lexA*^{WT} *recA730* shows a high baseline mutation frequency irrespective of DNA damage (Figure 5b). This finding likely reflects complete pathway derepression and aberrantly high concentrations of activated error-prone polymerases. With the *lexA*^{tev} *recA730* strain, we observed the expected low mutation frequency at baseline, as the noncleavable LexA^{S119A} exerts a dominant repression on SOS genes even in the presence of activated RecA*. Interestingly, in the presence of ATc and absence of UV, there is no increase in mutational frequency in the *lexA*$^{tev\ S119A}$ *recA730* strain. As with *lexA*$^{tev\ S119A}$ *recA*^{WT}, only in the presence of ATc and UV light are rifampin-resistant mutants readily recovered.

Taken together, these data indicate that both DNA damage and LexA cleavage are necessary for SOS mutagenesis, even in the presence of activated RecA*. These results support a model where DNA damage is necessary to provide a nidus of mutation, upon which SOS-mediated mechanisms can build; only with both DNA damage and LexA cleavage can we observe appreciable mutagenesis at the chromosomal *rpoB* locus. One limitation of our approach is our inability to demonstrate if RecA* is necessary for mutagenesis, as DNA damage in the presence of *recA*^{WT} also activates RecA. Future improvements of our synthetic circuit could involve separation of DNA damage from RecA activation, chromosomal integration of TEV, or added autoregulatory capacities in the circuit. Nevertheless, our study represents a step beyond previous genetic approaches, which now allows more deliberate manipulation of the SOS stress response pathway in a near-native system.

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CONCLUSION

In this study, we have generated a synthetic circuit for inducible control over the SOS pathway independent of DNA damage and RecA*. Our engineered strain shows small-molecule inducible DNA repair and mutagenesis, and opens the possibility of addressing a wide variety of significant questions related to SOS function. For example, one significant advantage of our approach is that the circuit now permits temporal separation of DNA damage from the induction of the SOS response, which can be applied to examine the dynamics of cell death and DNA repair after DNA damage. Furthermore, other phenotypes that have been associated with SOS function, such as the generation of persisters,[44] biofilm formation,[45] or acquired antibiotic resistance[8] can potentially be examined independent of DNA damage. Future refinement of the synthetic circuit may allow for titratability of the response across a dose range of ATc or examine pathway inactivation after washout of ATc. More generally, the concept of reprogramming stress response pathways with synthetic biology approaches can allow for separation of environmental changes from their associated responses to probe other aspects of bacterial adaptation, survival, and evolution.

MATERIALS AND METHODS

LexA Alignment. A comprehensive database of LexA homologues was generated using the ConSurf Server Database, which utilizes CS-BLAST of the SWISS-PROT protein databank to calculate sequence homology and conservation of protein structures.[46] LexA homologues were aligned and percent identity calculated using Jalview sequence alignment tool.[47]

LexA^{tev} Cloning and Expression. For experiments with purified LexA, a previously described expression vector was used as a basis for mutagenesis, encoding LexA with an N-terminal His-tag with or without the S119A mutation.[32] Linker variations were introduced by overlap extension PCR. Proteins were expressed in *E. coli* BL21 pLysS, followed by one-step purification using the His-tag as previously described.[32]

***In Vitro* Protein Analysis.** An EMSA was used to quantitatively determine the binding efficiency of LexA proteins to the SOS consensus sequence.[48] 44 bp oligonucleotides containing either the SOS consensus sequence or a scrambled control were 5′-radiolabeled with ^{32}P using T4 polynucleotide kinase, then annealed to their complementary unlabeled strand. Serial dilutions of LexA were incubated with 100 pM of the radiolabeled duplex in 70 mM Tris (pH 7.6), 10 mM $MgCl_2$ (Buffer A) at 25 °C either alone or with a 1000-fold excess (1 ng/μL) of unlabeled sonicated salmon sperm DNA, and samples were separated on a native 8% acrylamide gel at 25 °C in 0.5× Tris/Borate/EDTA buffer. The gels were then imaged on a Typhoon imager. Quantification was performed on ImageJ software by quantifying the ratio of bound to unbound DNA normalized to background phosphorescence. The data were fit to one site specific binding *via* Prism software.

To qualitatively examine LexA autocleavage, 5 μM purified LexA variants were incubated in Buffer A with 20 μg/mL $RecA_f$ (New England Biolabs, NEB) preactivated by coincubation with 10 μM ATPγS and 10 μM ssDNA, either alone or in the presence of 10 μM SOS operator or scrambled operator DNA. After 2 h at 37 °C, reactions were quenched with 2× Laemmli buffer. For alkali-induced cleavage, 5 μM LexA was incubated in a 1:1 ratio with 2× autocleavage buffer (100 mM CAPS, 100 mM Tris, 300 mM NaCl, pH 10.6) for 2 h before quenching with Laemmli buffer. For TEV-mediated cleavage, 5 μM LexA proteins were incubated for 2 h with 0.8 μM TEV in 50 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, and 1 mM DTT, with or without 10 μM of SOS operator or scrambled operator DNA. Samples were separated on either 12% SDS-PAGE gel (for TEV-cleavage) or 20% SDS-PAGE gels (for RecA*-cleavage) in 1× Tris-Glycine-SDS (TGS) Buffer (Biorad), visualized using Coomassie stain, and imaged on a Gel Doc XR+ (Biorad). Band intensity was quantified using ImageJ software and first order cleavage kinetics were determined by Prism software.

Strain Generation for Cell-Based Assays. A *lexA::Ics:cat* (Cam^R), ΔsulA::FRT MG1655 *E. coli* strain previously used for the generation of the *lexA^{S119A}* strain served as the parent for recombineering.[21] The PCR-amplified gene fragment containing *lexA^{tev}* or *lexA$^{tev\ S119A}$* and ∼1000 bp of flanking DNA were amplified and used in the recombineering protocol described previously.[21] The *sulA* gene was reintroduced by $P1_{vir}$ transduction of a Δ*torT::[FRT-kan-FRT]*(Kan^R) strain obtained through the Keio collection,[49] as *torT* is a nearby gene with high linkage to *sulA*. The *recA730* allele was introduced by $P1_{vir}$ transduction from the donor SS4247 strain as previously described.[21] All strains were confirmed by PCR amplification and sequencing of the relevant loci. A comprehensive list of strains and genotypes used can be found in Table S1.

All cell-based protocols were performed in strains cotransformed with both a GFP reporter plasmid maintained by kanamycin selection and an inducible TEV plasmid maintained by ampicillin selection. The GFP reporter plasmids used (P_{recA}-GFP, P_{recN}-GFP, P_{sulA}-GFP, P_{lexA}-GFP) were obtained from the Open Biosystems *E. coli* promoter collection (Thermo Scientific).[36] For the TEV expression plasmid, the parent strain for cloning was a medium-copy pMG81 vector (Goulian Lab collection) encoding the *tetR* and *tetA* gene locus under the control of a bidirectional TetR-regulated promoter. An autolysis refractory N-terminal maltose binding protein (MBP) fusion of TEV was amplified out from RK1043 (Addgene),[50] appending an *ssrA* tag in the process.[35] This gene was then cloned using Gibson assembly into pMG81, replacing the *tetA* gene locus.

Western Blotting. Overnight cultures of strains were diluted 1:100 in 15 mL of 1× MM (1× M9 minimal media salts, 10 mM $MgSO_4$, 1 mM $CaCl_2$, 0.2% glucose, and 0.1% Casamino acids, with 30 μg/mL kanamycin and 100 μg/mL ampicillin for plasmid maintenance) and shaken at 37 °C. At mid log phase, cultures were either untreated or ATc was added at a concentration range from 0.005−0.2 μg/mL and cells were incubated at 37 ° C. At 0, 10, and 60 min, 1 mL of culture was removed, pelleted and resuspended in 100 μL of media. 50 μL was mixed with 50 μL of 2× Laemmli buffer, denatured at 95 °C, and run on a 12% SDS-PAGE denaturing gel. The gel was transferred to a polyvinylidene fluoride (PVDF) membrane using the iBlot Gel Transferring System (Invitrogen). Membranes were probed with mouse anti-LexA (Santa Cruz, sc-365999) 1:1000 or goat anti-MBP (NEB) 1:50 000, followed, respectively, by horse radish peroxidase(HRP)-conjugated goat anti-mouse or rabbit anti-goat (1:2000, Santa Cruz). Membranes were imaged on a Amersham Imager 600 system after being exposed to Immobilon Western HRP substrate (Millipore).

Cell-Based Fluorescent Assays. Overnight cultures of strains were diluted 1:100 in 1× MM and shaken at 37 °C. At mid log phase, 100 μL of strains in a 96-well plate were exposed

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to UV light at the doses noted and/or ATc (0.02 μg/mL). Recovery and activation of SOS genes was allowed for 1 h before cell were pelleted by centrifugation at 7600*g* and resuspended in 1× phosphate-buffered saline (PBS) with 4% paraformaldehyde. Single-cell GFP fluorescence intensity was analyzed using a FACSCalibur (BD Biosciences) and analyzed using FlowJo software.

DNA Repair and Mutagenesis Assays. Overnight cultures of strains were diluted 1:100 in 1× MM and shaken at 37 ° C. At mid log phase, cultures were streaked across LB agar supplemented with 100 μg/mL ampicillin and 30 μg/mL kanamycin, and either no ATc or ATc at 0.02 μg/mL. Streaked strains were allowed 5−10 min settling into the agar and then exposed to a gradient of UV light with the plates partially covered to alter exposure intensity. The plates were analyzed following 24 h at 37 °C.

For UV survival curves, 200 μL of mid log phase cultures were transferred to 24-well plates to increase surface area and exposed to UV light and/or ATc (0.02 μg/mL). After UV exposure, cultures were immediately serially diluted in 1× PBS ± ATc, and then plated on LB agar plates with 100 μg/mL ampicillin, 30 μg/mL kanamycin, and ±0.02 μg/mL ATc. Plates were counted through a combination of manual counting and OpenCFU software.

For analysis of mutagenesis, overnight cultures of strains were diluted 1:1000 in 1× MM and shaken at 37 °C. At mid log phase, 1 mL cultures were transferred to 24-well plates and then exposed to UV light at doses ranging from 5 to 50 J/m^2 and/or 0.02 μg/mL ATc. Cell recovery was allowed for 1 h shaking at 37 ° C following damage. Cultures were serially diluted in 1× PBS and plated on LB agar with ampicillin and kanamycin for viable cell counts and selective LB agar with ampicillin, kanamycin, and rifampin (100 μg/mL) to quantify the number of rifampin resistant mutants. Resistant colonies were tabulated after 48 h of growth. The mutagenesis assays with UV alone, ATc alone, or both, were performed with 4 mL cultures in 6-well plates to increase the limit of detection. The frequency of resistant mutants per starting population was determined by normalizing the number of resistant colonies to an average of three undamaged control cell counts on nonselective media ± ATc. The frequency of resistant mutants per viable cell count was determined by normalizing the number of resistant colonies to an average of three UV-damaged or undamaged controls plated on nonselective media.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.7b00108.

Figure S1. Alignment of LexA sequences; Figure S2. DNA binding of LexA variants; Figure S3. LexA cleavage in the presence of DNA; Figure S4. Quantification of self-cleavage rates of LexA^{WT} and LexA^{tev}; Figure S5. Plasmid map and sequence of the TEV containing pMG81 vector; Figure S6. GFP reporters for induction of the native and synthetic SOS circuits *in vivo*; Figure S7. Western blotting of LexA and TEV; Figure S8. UV survival and mutagenesis in a *sulA*$^+$ strain. Figure S9; GFP reporters for synthetic circuit activation in strains with the *recA730* allele; Table S1. *E. coli* MG1655 strain variants used in this study; Table S2. Rifampin resistance following native and synthetic circuit activation (PDF)

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J.K., M.J.C., M.Y.L., C.Y.M. M.G., and R.M.K. designed the experiments; J.K. performed all the experiments with assistance from M.J.C., M.Y.L., and C.Y.M.; J.K. and R.M.K. wrote the manuscript with input from all authors.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ATc, anhydrotetracycline; CTD, LexA C-terminal domain; EMSA, electrophoretic mobility shift assay; MBP, maltose binding protein; NTD, LexA N-terminal domain; ssDNA, single-stranded DNA; RecA*, activated RecA nucleoprotein filament; TEV, tobacco etch virus protease

■ REFERENCES

(1) Butala, M., Žgur-Bertok, D., and Busby, S. J. W. (2009) The bacterial LexA transcriptional repressor. *Cell. Mol. Life Sci. 66*, 82−93.

(2) Schnarr, M., Oertel-Buchheit, P., Kazmaier, M., and Granger-Schnarr, M. (1991) DNA binding properties of the LexA repressor. *Biochimie 73*, 423−431.

(3) Cox, M. M. (2007) Regulation of bacterial RecA protein function. *Crit. Rev. Biochem. Mol. Biol. 42*, 41−63.

(4) Little, J. W. (1991) Mechanism of specific LexA cleavage: autodigestion and the role of RecA coprotease. *Biochimie 73*, 411−422.

(5) Mohana-Borges, R., Pacheco, A. B. F., Sousa, F. J. R., Foguel, D., Almeida, D. F., and Silva, J. L. (2000) LexA repressor forms stable dimers in solution. The role of specific DNA in tightening protein-protein interactions. *J. Biol. Chem. 275*, 4708−4712.

(6) Courcelle, J., Khodursky, a, Peter, B., Brown, P. O., and Hanawalt, P. C. (2001) Comparative gene expression profiles following UV exposure in wild-type and SOS-deficient Escherichia coli. *Genetics 158*, 41−64.

(7) Erill, I., Campoy, S., and Barbé, J. (2007) Aeons of distress: An evolutionary perspective on the bacterial SOS response. *FEMS Microbiol. Rev. 31*, 637−656.

(8) Cirz, R. T., Chin, J. K., Andes, D. R., De Crécy-Lagard, V., Craig, W. a., and Romesberg, F. E. (2005) Inhibition of mutation and combating the evolution of antibiotic resistance. *PLoS Biol. 3*, 1024−1033.

(9) Lu, T. K., and Collins, J. J. (2009) Engineered bacteriophage targeting gene networks as adjuvants for antibiotic therapy. *Proc. Natl. Acad. Sci. U. S. A. 106*, 4629−4634.

(10) Culyba, M. J., Mo, C. Y., and Kohli, R. M. (2015) Targets for Combating the Evolution of Acquired Antibiotic Resistance. *Biochemistry 54*, 3573−3582.

(11) Kohanski, M. A., Dwyer, D. J., and Collins, J. J. (2010) How antibiotics kill bacteria: from targets to networks. *Nat. Rev. Microbiol. 8*, 423−35.
(12) Merrikh, H., Ferrazzoli, A. E., Bougdour, A., Olivier-Mason, A., and Lovett, S. T. (2009) A DNA damage response in Escherichia coli involving the alternative sigma factor, RpoS. *Proc. Natl. Acad. Sci. U. S. A. 106*, 611−6.
(13) Al Mamun, A. A. M., Lombardo, M., Shee, C., Lisewski, A. M., Gonzalez, C., Lin, D., Nehring, R. B., Saint-ruf, C., Gibson, J. L., Frisch, R. L., Lichtarge, O., Hastings, P. J., and Rosenberg, S. M. (2012) Identity and Function of a Large Gene Network Underlying Mutagenic Repair of DNA breaks. *Science 338*, 1344−1348.
(14) Bichara, M., Meier, M., Wagner, J., Cordonnier, A., and Lambert, I. B. (2011) Postreplication repair mechanisms in the presence of DNA adducts in Escherichia coli. *Mutat. Res., Rev. Mutat. Res. 727*, 104−122.
(15) Roberts, J. W., Roberts, C. W., and Craig, N. L. (1978) Escherichia coli recA gene product inactivates phage λ repressor. *Proc. Natl. Acad. Sci. U. S. A. 75*, 4714−4718.
(16) Tang, M., Shen, X., Frank, E. G., O'Donnell, M., Woodgate, R., and Goodman, M. F. (1999) UmuD′(2)C is an error-prone DNA polymerase, Escherichia coli pol V. *Proc. Natl. Acad. Sci. U. S. A. 96*, 8919−8924.
(17) Godoy, V. G., Jarosz, D. F., Simon, S. M., Abyzov, A., Ilyin, V., and Walker, G. C. (2007) UmuD and RecA Directly Modulate the Mutagenic Potential of the Y Family DNA Polymerase DinB. *Mol. Cell 28*, 1058−1070.
(18) Patel, M., Jiang, Q., Woodgate, R., Cox, M. M., and Goodman, M. F. (2010) A New Model for SOS-induced Mutagenesis: How RecA Protein Activates DNA Polymerase V. *Crit. Rev. Biochem. Mol. Biol. 45*, 171−184.
(19) Jiang, Q., Karata, K., Woodgate, R., Cox, M. M., and Goodman, M. F. (2009) The active form of DNA polymerase V is UmuD′2 C−RecA−ATP. *Nature 460*, 359−363.
(20) Pacelli, L. Z., Edmiston, S. H., and Mount, D. W. (1979) Isolation and Characterization of Amber Mutations in the lexA Gene of Escherichia coli K-12. *J. Bacteriol. 137*, 568−573.
(21) Mo, C. Y., Manning, S. A., Roggiani, M., Culyba, M. J., Samuels, A. N., Sniegowski, P. D., Goulian, M., and Kohli, R. M. (2016) Systematically Altering Bacterial SOS Activity under Stress Reveals Therapeutic Strategies for Potentiating Antibiotics. *mSphere 1*, e00163−16.
(22) Witkin, E. M., McCall, J. O., Volkert, M. R., and Wermundsen, I. E. (1982) Constitutive expression of SOS functions and modulation of mutagenesis resulting from resolution of genetic instability at or near the recA locus of Escherichia coli. *Mol. Gen. Genet. 185*, 43−50.
(23) Fijalkowska, I. J., Dunn, R. L., and Schaaper, R. M. (1997) Genetic requirements and mutational specificity of the Escherichia coli SOS mutator activity. *J. Bacteriol. 179*, 7435−7445.
(24) Peterson, K. R., and Mount, D. W. (1987) Differential repression of SOS genes by unstable LexA41 (Tsl-1) protein causes a "split-phenotype" in Escherichia coli K-12. *J. Mol. Biol. 193*, 27−40.
(25) Christensen, R. B., Christensen, J. R., Koenig, I., and Lawrence, C. W. (1985) Untargeted mutagenesis induced by UV in the lacI gene of Escherichia coli. *Mol. Gen. Genet. 201*, 30−34.
(26) Ichikawa-Ryo, H., and Kondo, S. (1975) Indirect mutagenesis in phage lambda by ultraviolet preirradiation of host bacteria. *J. Mol. Biol. 97*, 77−92.
(27) Khalil, A. S., and Collins, J. J. (2010) Synthetic biology: applications come of age. *Nat. Rev. Genet. 11*, 367−379.
(28) Callura, J. M., Dwyer, D. J., Isaacs, F. J., Cantor, C. R., and Collins, J. J. (2010) Physiology Using Synthetic Riboregulators. *Proc. Natl. Acad. Sci. U. S. A. 107*, 15898−15903.
(29) Phan, J., Zdanov, A., Evdokimov, A. G., Tropea, J. E., Peters, H. K., Kapust, R. B., Li, M., Wlodawer, A., and Waugh, D. S. (2002) Structural basis for the substrate specificity of tobacco etch virus protease. *J. Biol. Chem. 277*, 50564−50572.
(30) Slilaty, S. N., and Little, J. W. (1987) Lysine-156 and serine-119 are required for LexA repressor cleavage: a possible mechanism. *Proc. Natl. Acad. Sci. U. S. A. 84*, 3987−3991.
(31) Little, J. W. (1984) Autodigestion of lexA and phage lambda repressors. *Proc. Natl. Acad. Sci. U. S. A. 81*, 1375−1379.
(32) Mo, C. Y., Birdwell, L. D., and Kohli, R. M. (2014) Specificity determinants for autoproteolysis of LexA, a key regulator of bacterial SOS mutagenesis. *Biochemistry 53*, 3158−3168.
(33) Oertel-Buchheit, P., Schmidt-Dorr, T., Grnader-Schnarr, M., and Schnarr, M. (1993) Spacing Requirements Between LexA Operator Half-sites can be Relaxed by Fusing the LexA DNA Binding Domain with some Alternative Dimerization Domains. *J. Mol. Biol. 229*, 1−7.
(34) Blank, K., Hensel, M., and Gerlach, R. G. (2011) Rapid and highly efficient method for scarless mutagenesis within the Salmonella enterica chromosome. *PLoS One 6*, e15763.
(35) McGinness, K. E., Baker, T. A., and Sauer, R. T. (2006) Engineering Controllable Protein Degradation. *Mol. Cell 22*, 701−707.
(36) Zaslaver, A., Bren, A., Ronen, M., Itzkovitz, S., Kikoin, I., Shavit, S., Liebermeister, W., Surette, M. G., and Alon, U. (2006) A comprehensive library of fluorescent transcriptional reporters for Escherichia coli. *Nat. Methods 3*, 623−628.
(37) Sassanfar, M., and Roberts, J. W. (1990) Nature of the SOS-inducing signal in Escherichia coli: The involvement of DNA replication. *J. Mol. Biol. 212*, 79−96.
(38) Neher, S. B., Flynn, J. M., Sauer, R. T., and Baker, T. a. (2003) Latent ClpX-recognition signals ensure LexA destruction after DNA damage. *Genes Dev. 17*, 1084−1089.
(39) Garibyan, L., Huang, T., Kim, M., Wolff, E., Nguyen, A., Nguyen, T., Diep, A., Hu, K., Iverson, A., Yang, H., and Miller, J. H. (2003) Use of the rpoB gene to determine the specificity of base substitution mutations on the Escherichia coli chromosome. *DNA Repair 2*, 593−608.
(40) Kim, S. R., Maenhaut-Michel, G., Yamada, M., Yamamoto, Y., Matsui, K., Sofuni, T., Nohmi, T., and Ohmori, H. (1997) Multiple pathways for SOS-induced mutagenesis in Escherichia coli: an overexpression of dinB/dinP results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. *Proc. Natl. Acad. Sci. U. S. A. 94*, 13792−7.
(41) Miller, J. H., and Low, K. B. (1984) Specificity of mutagenesis resulting from the induction of the SOS system in the absence of mutagenic treatment. *Cell 37*, 675−682.
(42) Ennis, D., Peterson, K., and Mount, D. (1988) Increased expression of the Escherichia coli umuDC operon restores SOS mutagenesis in lexA41 cells. *Mol. Gen. Genet. 213*, 541−544.
(43) Lin, L. L., and Little, J. W. (1988) Isolation and characterization of noncleavable (Ind-) mutants of the LexA repressor of Escherichia coli K-12. *J. Bacteriol. 170*, 2163−2173.
(44) Dörr, T., Lewis, K., and Vulić, M. (2009) SOS response induces persistence to fluoroquinolones in Escherichia coli. *PLoS Genet. 5*, e1000760.
(45) Bernier, S. P., Lebeaux, D., DeFrancesco, A. S., Valomon, A., Soubigou, G., Coppée, J. Y., Ghigo, J. M., and Beloin, C. (2013) Starvation, Together with the SOS Response, Mediates High Biofilm-Specific Tolerance to the Fluoroquinolone Ofloxacin. *PLoS Genet. 9*, e1003144.
(46) Goldenberg, O., Erez, E., Nimrod, G., and Ben-Tal, N. (2009) The ConSurf-DB: Pre-calculated evolutionary conservation profiles of protein structures. *Nucleic Acids Res. 37*, 323−327.
(47) Waterhouse, A. M., Procter, J. B., Martin, D. M. A., Clamp, M., and Barton, G. J. (2009) Jalview Version 2-A multiple sequence alignment editor and analysis workbench. *Bioinformatics 25*, 1189−1191.
(48) Zhang, A. P. P., Pigli, Y. Z., and Rice, P. A. (2010) Structure of the LexA-DNA complex and implications for SOS box measurement. *Nature 466*, 883−886.
(49) Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., and Mori, H. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout

DOI: 10.1021/acssynbio.7b00108
ACS Synth. Biol. 2017, 6, 2067−2076

mutants: the Keio collection. *Mol. Syst. Biol.*, DOI: 10.1038/msb4100050.

(50) Kapust, R. B., Tözsér, J., Fox, J. D., Anderson, D. E., Cherry, S., Copeland, T. D., and Waugh, D. S. (2001) Tobacco etch virus protease: mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency. *Protein Eng., Des. Sel. 14*, 993–1000.

DOI: 10.1021/acssynbio.7b00108
ACS Synth. Biol. 2017, 6, 2067–2076