Roles for the transcription elongation factor NusA in both DNA repair and damage tolerance pathways in *Escherichia coli*

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The process of nucleotide excision repair (NER) acts to remove a wide variety of DNA lesions and in *Escherichia coli* is mediated through the concerted action of the *uvrA*, *uvrB*, and *uvrC* gene products (1). The process of transcription-coupled repair (TCR) targets NER to actively transcribed genes, resulting in preferential repair of the transcribed strand relative to the nontranscribed strand (2–4). In *E. coli*, the *mfd* gene product couples the process of NER to transcription, and has been shown to be responsible for the strand specific repair of UV-induced lesions (5–7). We have recently reported that the highly conserved TLS polymerase DinB (DNA pol IV), a member of the class of specialized DNA polymerases that can replicate damaged DNA, interacts physically and genetically with the transcription elongation factor NusA (8, 9). Δ*mfd* strains are sensitive to DNA-damaging agents, nitrofurazone (NFZ) and 4-nitroquinolone-1-oxide but not to UV radiation. Gene expression profiling suggests that this sensitivity is unlikely to be due to an indirect effect on gene expression affecting a known DNA repair or damage tolerance pathway. We demonstrate that an N2-furfuryl-dG (N2-f-dG) lesion, a structural analog of the principal lesion generated by NFZ, blocks transcription by *E. coli* RNA polymerase (RNAP) when present in the transcribed strand, but not when present in the nontranscribed strand. Our genetic analysis suggests that NusA participates in a nucleotide excision repair (NER)-dependent process to promote NFZ resistance. We provide evidence that transcription plays a role in the repair of NFZ-induced lesions through the isolation of RNAP mutants that display altered ability to survive NFZ exposure. We propose that NusA participates in an alternative class of TCR involved in the identification and removal of a class of lesion, such as the N2-f-dG lesion, which are accurately and efficiently bypassed by DinB in addition to recruiting DinB for TLS at gaps encountered by RNAP.

### Results

**nusA** **Mutant Strains Are Sensitive to DNA-Damaging Agents.** To further investigate the role for NusA in DNA repair/damage tolerance pathways, we explored the possibility that *nusA* mutants might render cells sensitive to exposure to DNA-damaging agents. Strikingly, we observed that at the permissive temperature (30 °C), *nusA11*(ts) strains are specifically sensitive to the DNA-damaging agents NFZ and 4-NQO, but not to UV, methyl methanesulfonate (MMS) (Fig. 1), ethyl methanesulfonate (EMS), or hydrogen peroxide. This sensitivity to NFZ and 4-NQO can be complemented by providing *nusA*+ in trans at the permissive temperature (Fig. SI A and B). The greater sensitivity of a *nusA11* mutant strain compared with that of a Δ*mfd* strain implies that NusA participates in a dinB-independent, as well as a dinB-dependent, role in promoting survival after exposure to NFZ or 4-NQO.

The fact that the *nusA11* mutation does not sensitize cells to UV, MMS, EMS, or hydrogen peroxide indicates that the expression of genes involved in the various DNA repair and damage tolerance pathways that enable cells to cope with lesions induced by these agents—nucleotide excision repair, base excision repair, recombinational repair, and *umuDC*-mediated TLS (reviewed in ref. 1)—is not perturbed. Additionally, at the permissive temperature, *nusA11* mutant strains display wild-type levels of UV-induced mutagenesis (9), suggesting that SOS induction and DNA pol IV (UmuD–C) are operating normally. Collectively, these data suggest that the sensitivity to NFZ and 4-NQO observed in

### Author contributions:

S.E.C., R.A.M., R.L., and G.C.W. designed research; S.E.C., C.A.L., and R.A.M. performed research; M.A.K. and J.J.C. contributed new reagents/analytic tools; S.E.C., R.A.M., R.L., and G.C.W. wrote the paper. The authors declare no conflict of interest. This article is a PNAS Direct Submission. See Commentary on page 15314.

### References


a **nusA** mutant strain is not likely due to an indirect effect of gene expression on a DNA repair or damage tolerance process. We also performed microarray analyses to assess the genome-wide changes in gene expression that occur in a **nusA** background at the permissive temperature. We did not observe changes in the expression of any genes known to be involved in DNA repair or damage tolerance, but rather differential expression of genes whose products are involved in a variety of aspects of cellular metabolism (Table S1 and Table S2). These findings motivated us to investigate the alternative hypothesis that NusA might play a hitherto unsuspected role in DNA repair.

The striking recessive sensitivity of **nusA** mutant strains to NFZ at the permissive temperature indicates that it is a partial loss-of-function mutation. The analysis of a strain completely lacking **nusA** is not feasible in standard *E. coli* genetic backgrounds, because **nusA** is essential for viability. However, it is possible in a specialized genetic background lacking horizontally transferred DNA (23, 24). In such a strain (MDS42), we observe that both **nusA** and Δ**nusA** mutations result in sensitivity to NFZ and 4-NQO (Fig. 2A and D). However, the complete loss of **nusA** additionally results in sensitivity to UV and MMS (Fig. 2), supporting the notion that the **nusA** allele is a partial loss-of-function mutant. Providing **nusA** in trans complements the NFZ, UV, and MMS sensitivity as well as the growth defect of a Δ**nusA** strain (Fig. S1C). Microarray analysis of the **nusA** deletion strain (23) did not reveal any statistically significant changes in the expression of genes whose products have been implicated in DNA repair. Although we cannot unambiguously rule out the possibility that the increased sensitivity to killing by these DNA-damaging agents is due to an effect on gene expression, these data are consistent with the hypothesis that **nusA** participates directly in a process that promotes cellular survival after challenge with DNA damage.

**Fig. 1.** **nusA** mutants are specifically sensitive to NFZ and 4-NQO. (A) Percent survival of strains treated with 0–15 μM NFZ. All graphs in this figure and the following figure were performed at the permissive temperature (30 °C), and error bars represent the SD determined from at least three independent cultures. (B) Percent survival of strains treated with 0–17.5 μM 4-NQO. At 30 °C the sensitivity of the Δ**dinB** strain to NFZ and 4-NQO is less than the degree of sensitivity observed at 37 °C (10). (C) Percent survival of strains irradiated with 0–45 J/m² UV. (D) Percent survival of strains treated with 0–0.08% MMS.

**Fig. 2.** Comparison of **nusA**11 and Δ**nusA** mutations in MDS42. (A–C) Percent survival of strains treated with the DNA-damaging agents NFZ (μM), UV, and MMS, respectively, at 30 °C. For all graphs in this figure, error bars represent the SD determined from at least three independent cultures. (D–F) Percent survival of strains treated with the DNA-damaging agents NFZ (μM), UV, and MMS, respectively, at 37 °C. **N^2^**-furfuryl-dG Lesion Blocks Transcription by *E. coli* RNAP. If the specific sensitivity of **nusA**11 mutant strains after exposure to NFZ and 4-NQO were due to a failure to repair a specific class of lesion introduced by these agents, what could these lesions be? A possible answer is suggested by our previous observations that DinB carries out preferential and accurate TLS with **N^2^**-furfuryl-dG (N²-f-DG), a mimic of the major adduct formed by NFZ (10, 25). Given that DinB is present in considerable excess over the replicative DNA polymerase in both SOS-uninduced cells (250 DinB/10² pol III) and SOS-induced cells (2,500 DinB/10² pol III) (26, 27), it seems likely that N²-dG lesions with properties similar to N²-f-DG would be readily bypassed by DinB, resulting in their continued presence in the genome where they could potentially hinder transcription.

To test the hypothesis that this type of lesion would obstruct transcription, we monitored *E. coli* RNAPs ability to use a template containing the N²-f-DG lesion in vitro. The presence of an N²-f-DG lesion on the transcribed strand completely blocked transcription (Fig. 3), whereas the presence of the same lesion on the nontranscribed strand had little effect on transcription (Fig. S2A). Generation of a 3′CPM-terminated transcript allowed us to map the position of the transcript generated when N²-f-DG is present on the transcribed strand, showing that transcription is stalled four nucleotides (ntd) upstream of the lesion (Fig. S2B).
Stalling of RNAP at such a lesion in the transcribed strand could be a detection mechanism that then allows repair proteins to subsequently be recruited.

We also monitored the ability of RNAP to bypass template strand gaps, which we propose stall transcription in our model of TC-TLS. We observed that *E. coli* RNAP is able to bypass a 1-ntd gap, with similar efficiencies to those previously published (~45% bypass) (28). However, unlike T7 RNAP (28, 29), transcription by *E. coli* RNAP is unable to bypass a larger, 14-ntd gap (~2% bypass) (Fig. 3). Even with prolonged incubation time, RNAP is not capable of bypassing either the N2-f-dG adduct or 14-ntd gapped templates (Fig. S3). Moreover, addition of purified NusA or NusA11 to the reactions did not directly alter RNAPs ability to transcribe through these modified templates (Fig. S4). This observation indicates that NusA does not act by modulating RNAPs ability to carry out transcription over a lesion or a gap in the transcribed strand but instead suggests that NusA might play a role in the recruitment of factors, such as DNA repair systems or DinB for TLS, to sites of RNAP stalled by an N2-f-dG lesion or by a gap.

**Identification of a NusA-Dependent, uvr-dependent Process for NFZ Resistance.** Two prior observations led us to consider the possibility that NusA might play a role in the recruitment of nucleotide excision repair (NER) machinery to an RNAP that has been stalled by an NFZ-induced lesion. First, a high-throughput protein interaction screen identified NusA as an interaction partner of UvrA (30), which we have confirmed by far Western blotting (Fig. 4A). Additionally, Δuvis strains are sensitive to NFZ, and uvr-dependent NER is the predominant mechanism for processing NFZ-induced DNA damage in *E. coli* (31).

Epistasis analysis with respect to NFZ sensitivity of *nusA11* and ΔnusA alleles revealed that ΔnusA is largely epistatic to NFZ. Because NusA is a component of elongating RNA polymers, it seems possible that the uvr-dependent process that the *nusA11* mutation might be affecting could be a type of transcription-coupled nucleotide excision repair of lesions introduced by NFZ. However, we observe an additive relationship for both NFZ and 4-NQO sensitivity with Δmfd and *nusA11* alleles, implying that NusA and Mfd function in separate pathways (Fig. 4C), and suggesting the possibility that NusA is required for an alternative type of Mfd-independent transcription-coupled nucleotide excision repair. In contrast epistasis analysis with respect to UV sensitivity of ΔnusA and Δmfd alleles revealed a synergistic relationship in which the double mutant was much more sensitive than either of the single mutants (Fig. 4D). This suggests that, in addition to any roles with Mfd in promoting TCR of UV-induced lesions, NusA additionally plays a role in more generally directing NER (*Discussion*).

**RNA Polymerase Mutants Display an Altered Ability to Deal with NFZ.** To search for additional in vivo evidence that transcription might play a role in directing uvr-dependent NER of lesions introduced by NFZ, we screened the previously described plasmid-borne mutant libraries of *rpoB* (32), which encodes for the β catalytic subunit of RNAP, for the ability to cause either NFZ sensitivity (NFZ2) or NFZ resistance (NFZ3). We isolated three single mutants: the *NFZ2* mutant *rpoB* (D185Y) and the *NFZ3* mutants *rpoB* (V287A) and *rpoB* (D320N). The *NFZ2* mutant *rpoB* (D185Y) displayed a 10-fold sensitivity to NFZ compared with an *rpoB*− plasmid control, whereas the *NFZ3* mutants *rpoB* (V287A) and *rpoB* (D320N) displayed a 10-fold resistance (Fig. 4E).

We observed that, when expressed in a ΔdinB strain (Fig. S5B) or Δmfd (Fig. 4F) background, these *rpoB* mutants displayed the same pattern of NFZ2 or NFZ3, although the relative degree of NFZ2 or NFZ3 differs from that observed in a wild-type background, indicating that these gene products do not play a role in this phenomenon. Strikingly, when expressed in a *nusA11* background, this pattern was altered because these *rpoB* mutants had largely lost their ability to confer NFZ2 or NFZ3 (Fig. 4G and Fig. S5A). Similarly, in a ΔuvrA background, expression of these *rpoB* mutants also resulted in loss of the original pattern of relative sensitivity or resistance (Fig. 4H). These results indicate that the original pattern of NFZ sensitivity or resistance depends on *nusA* and *uvrA*. The fact that mutating a core component of RNA polymerase affects the *nusA*-dependent, uvr-dependent process of NFZ resistance we have identified provides additional evidence that this process could be a previously unrecognized form of transcription-coupled repair that functions independently of Mfd.

Intriguingly, mapping these *rpoB* mutations on the crystal structure of *T. thermophilus* RNAP elongation complex (33) revealed that all three were located in the leading part of RNA polymerase that would first encounter a lesion in double-stranded DNA during the process of transcription (Fig. S5C). The crystal structure predicts that when RNAP stalls at the −4 position relative to the N2-f-dG lesion in the transcribed strand, the N2-f-dG

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Fig. 4. Interactions with NER and a role for transcription. (A) Far-Western blot demonstrates that NusA interacts with UvrA. Cell lysates harboring the empty vector (lane 1) or overexpressing UvrA (lane 2) were separated by SDS/ PAGE, transferred to a PVDF membrane, and incubated with purified NusA. α-NusA antibodies detected the binding of NusA to a 100-kDa migrating protein specifically in the UvrA (103 kDa) overexpressing lane. (B) Percent survival of strains treated with 0–15 μM NFZ at 30 °C. Squares, wild type/ AB1157; circles, nusA11 (SEC164); inverted triangles, ∆uvrA (SEC 316); diamonds, nusA11ΔuvrA (SEC181). In this and all graphs in this figure, error bars represent the SD determined from at least three independent cultures. (C) nusA17 and ∆mfd strains display an additive phenotype with respect to NFZ (filled bars) or 4-NQO (striped bars) sensitivity at 10 μM at 30 °C. (D) Percent survival of strains, MDS42, ∆nusA, ∆mfd (SEC1629), and ∆nusA ∆mfd double mutants (SEC1276), to UV irradiation demonstrates that the ∆nusA ∆mfd double mutant is much more sensitive than either of the single mutants. (E) Sensitivity of rpoB mutants expressed in AB1157 to 10 μM NFZ at 37 °C. (F) Sensitivity of rpoB mutants expressed in a ∆mfd background to 10 μM NFZ at 37 °C. (G) Sensitivity of rpoB mutants expressed in a nusA11 mutant background to 12.5 μM NFZ at 30 °C. (H) Sensitivity of rpoB mutants expressed in a ∆uvrA background to 10 μM NFZ at 37 °C. Despite differences in the survival of ∆uvrA strains expressing the rpoB variants, the ability of each rpoB mutant to confer NFZ or NFZ as observed in a uvrA strain background is lost.

increase of SOS induction (34) in exponential phase nusA11

cells (0.8%) compared with 0.2% in wild-type cells (Fig. S6 A–C) and a 25-fold increase in stationary-phase nusA11

cells (~2.5%) compared with wild type (~0.1%) (Fig. 5 A–C). As expected, lexA (Del) cells, lacking the LexA repressor, display SOS induction in 100% of cells in both exponential and stationary phase. Additionally, we observed that nusA11

cells were somewhat elongated compared with nusA− cells, with a smaller population displaying extreme filamentation, >30 times the size of nusA− cells, specifically in stationary phase (Fig. S6 D and E). The distribution of RecA-GFP foci of exponentially growing nusA11

cells is similar to that of nusA− cells (0–5 foci per cell) (35) (Fig. S6 F–J). In contrast, in stationary-phase cells, grown at the permissive temperature, RecA-GFP foci are observed in ~2% of wild-type cells and ~19% of nusA11

cells. If wild-type strains are irradiated with UV, all cells then have RecA-GFP foci (Fig. 5 D–F).

Discussion

We propose that, in addition to its postulated role in TC-TLS (8), NusA plays a key role in a previously unrecognized pathway of transcription-coupled NER that is distinct from the well-characterized Mfd-dependent pathway. This NusA-dependent transcription-coupled repair pathway (NusA-TCR) is important for the repair of a class of DNA lesion typified by the N2-DG adduct, a structural analog of the major NFZ-induced lesion. Such lesions could be considered “stealth lesions” in that they can be readily bypassed during DNA replication because of the high levels of DinB relative to the replicative DNA polymerase, but then absolutely block transcription when present in the transcribed strand. NusA-TCR would help prioritize the cell’s NER resources to maximally benefit transcription while also facilitating the recognition and repair of lesions that are otherwise more difficult to detect (Fig. 6). There are 20 molecules of UvrA/SOS-induced and 250 molecules of UvrA/SOS-induced cell (1), in many cases there would be more lesions than UvrA molecules.

We speculate that the RNAP βlobe, which contains the NFZ8 D185R substitution, may facilitate RNAP backtracking upon encountering a lesion or gap in the template DNA so as to expose downstream DNA. NER may then be recruited to the DNA via contacts to NusA and possibly to the lineage-specific insertion β4 (36) in which the NFZ2 V287A and D320N substitutions are located. Precedence exists for RNAP backtracking to expose a downstream DNA priming site for DNA polymerases during M13 phage replication (37).

A significant role for NusA in recruitment of NER machinery to damaged DNA raises an interesting structural question given the known interactions of NusA on the face of RNAP opposite to the downstream DNA entering an elongating complex. E. coli NusA contacts the RNAP α-subunit CTD via the C-terminal NusA acidic repeat domains (AR1 and AR2) (38) and contacts the RNA exit channel via its N-terminal domain (39, 40); these contacts position the S1 domain and G181 (nusA11 is G181D) near the β′-lobe, in which a suppressor of nusA11 (rpoC10; E402K) has been mapped (22, 41). In contrast, the NFZ2 substitution in the βlobe, the NFZ2 substitutions in β4, and the downstream DNA are ~150 Å from the RNA exit channel and ~125 Å from the position of oCTD attachment to RNAP via a flexible linker. Could NusA target NER over these distances? The combined length of the flexible α-subunit linker, the oCTD, and the NusA AR domains is at least 120 Å. Furthermore, the linearly arranged domains of NusA span >150 Å from N to C terminus. Because the NER machinery also must span some distance, it appears to be plausible that they could be re-recruited to the downstream side of RNAP by NusA tethered either to the oCTD via AR2 or to exiting RNA and the RNAP exit channel via the NusA NTD.

Although elegant biochemical studies of Mfd-dependent TCR have offered detailed insights into the mechanism by which it repairs UV-induced DNA damage (5–7), it is striking that, in contrast to mutation of the mammalian transcription-coupling repair factor (42, 43), ∆mfd mutants display only a modest in-

adduct would be located in the minor groove of the dsDNA ahead of the transcription bubble.

Induction of DNA Damage in nusA11. To test whether the NusA-dependent repair process we had postulated is important for processing endogenous lesions, we examined whether untreated nusA11 strains at the permissive temperature show indications that they have suffered DNA damage. We observed a 4-fold
increase in sensitivity to UV (44). This has led to the inference that TCR is much less important in bacteria than eukaryocytes. Our results support an alternative interpretation: TCR is as important in bacteria as it is in eukaryotes, but its importance has been underestimated in the past because of the existence of an alternative Mfd-independent pathway of TCR that had not yet been recognized. Interestingly, the N2†-fG lesion stalls transcription at the −4 position in contrast to UV lesions that enter the active site of the RNAP (45–48). These observations may suggest a possible explanation for why the nusA11 mutation differentially affects TCR of the two classes of lesions.

The sensitivity of ΔnusA mutant strains to other agents such as UV and MMS suggests that NusA could also play a role in the transcription-coupled repair of lesions introduced by these agents as well. Our epistasis analysis does not exclude the possibility that NusA works in conjunction with Mfd to promote TCR of UV-induced lesions but does indicate that NusA plays a role in directing NER in a manner that is independent of Mfd. Interestingly, the sequenced genomes of several cancer cell lines have suggested the existence of an additional class of NER that is dependent transcriptional recruit factors to stalled replication forks. If this were the case, NusA-dependent transcriptional recruitment of repair factors in stationary phase might be much more important in helping cells cope with these endogenous lesions because of the absence of replication. This defect in processing endogenously generated DNA damage may be a contributing factor to the reduction of stress-induced mutagenesis, a measure of mutagenesis in non-growing or very slowly growing cells, observed in a nusA11 mutant background (9). We proposed that this defect may be a reflection of a deficiency in DNA repair or an inability to recruit DinB for TC-TLS under stressed conditions.

Our model of NusA-TCR complements our previously described model of TC-TLS (8), which proposes that NusA associated with elongating RNA polymerases can recruit TLS polymerases to fill in gaps opposite to lesions in the transcribed strand to allow for the continuation of transcription. An alternative class of TCR, NusA-dependent TCR, where NusA participates in a previously unrecognized branch of the TCR pathway. NusA is capable of recruiting NER to sites of stalled RNAPs to repair DNA lesions on the transcribed strand.
tant strain, in a manner that requires the catalytic activities of DinB and UmuDC (8), suggests that a key problem cells experience upon losing NusA function is a potentially lethal issue with their DNA. Thus, in addition to transcription termination or antitermination, an important biological role for NusA may be to coordinate DNA repair and damage tolerance systems to resolve problems that arise when transcription is attempted on damaged DNA.

Materials and Methods

Strains and plasmids used in this study are listed in Table S3. DNA damage sensitivity assays, microarray analysis, protein purification, in vitro transcription, far-Western blotting, RNAP mutant screening, and live-cell microscopy are based on published methods. A detailed description of these procedures can be found in SI Materials and Methods.

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