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New discoveries linking transcription to DNA repair and damage tolerance pathways

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In *Escherichia coli*, the transcription elongation factor NusA is associated with all elongating RNA polymerases, where it functions in transcription termination and antitermination. Here, we review our recent results implicating NusA in the recruitment of DNA repair and damage tolerance mechanisms to sites of stalled transcription complexes.

Key words: NusA, DinB, excision repair, translesion DNA synthesis, stress-induced mutagenesis

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DNA damage can occur by a wide variety of both endogenous and exogenous sources, resulting in a variety of cellular problems including cell death. Mechanisms of DNA repair, which restore the DNA to its original undamaged state, and of DNA damage tolerance, which permit the replication of damaged DNA, are of great importance for allowing cells to cope with the lethal effects of DNA damage.[1] Here we review our recent results that suggest that NusA, in addition to its known roles in transcription elongation and termination, is important for coordinating the cellular responses to DNA damage in *Escherichia coli*.[2-4] Specifically, we propose that NusA couples transcription to both translesion DNA synthesis (TLS), enabling transcription-coupled TLS, and to nucleotide excision repair (NER), promoting a novel class of transcription-coupled repair.

NusA is an essential multidomain protein that functions in both termination and antitermination of transcription, and is associated with the RNA polymerase (RNAP) throughout the elongation and termination phases of transcription.[5-12] Originally reported in 1974, NusA forms an anti-termination complex with λN protein that is required for successful λ phage infection.[13] NusA is highly conserved throughout bacterial and archeal domains of life; however, to date no eukaryotic sequence or functional homolog has been identified.

The first indication that NusA may be involved in DNA repair and/or damage tolerance processes was the discovery that NusA physically interacts with the translesion DNA polymerase DinB (DNA pol IV).[2] Translesion DNA polymerases are a specialized class of DNA polymerases capable of replicating past DNA lesions that block the highly accurate, stringent replicative DNA polymerases, in a process termed translesion synthesis (TLS). TLS polymerases are conserved throughout all domains of life and although they display reduced fidelity on undamaged templates many TLS polymerases catalyze proficient DNA synthesis across from what are known as cognate lesions.[14,15] In *E. coli* there are two TLS DNA polymerases, DinB (pol IV) and $UmuD'_2C$ (pol V), both of which are transcriptionally induced by the SOS response to DNA damage.[1,16]

Either $dinB^+$ or $umuD^+C^+$ can serve as a multicopy suppressor of the temperature sensitivity of the *nusA11*(ts) strain.[2] This suppression requires the catalytic activities of these proteins and specifically the translesion synthesis abilities of DinB.[2] The fact that plasmids overexpressing *dinB* or *umuDC* partially suppress the temperature sensitivity of a *nusA11* mutant strain suggests that a key problem cells experience upon losing NusA function is a potentially lethal issue with their DNA.

These initial discoveries led us to propose a model of transcription-coupled TLS

(TC-TLS) where NusA associated with an RNA polymerase (RNAP) that has become stalled by a gap in the transcribed strand opposite to a lesion in the non-transcribed strand would recruit TLS polymerases to fill in the gap.[2] Repairing the gap would permit transcription of the gene by subsequent RNA polymerases, possibly even by the original RNA polymerase if it is retained during process. Such gaps can be generated by replication resuming at the site of the next Okasaki fragment on the lagging strand or replication re-start on the leading strand and have been estimated to average about 1,000 nucleotides in length.[17-20] Alternatively, UvrABC-dependent excision of lesions that are very close together but on opposing strands or incomplete repair of an intrastrand cross-link would generate 12–13 ntd gaps[1] that are opposite a lesion. While *E. coli* RNAP can inefficiently bypass a one nucleotide template strand gap[4,21] our studies show that RNAP cannot bypass a 14 ntd template strand gap,[4] suggesting that such gaps would indeed stall transcription. If a process of TC-TLS does exist, it might be of particular importance in mammals where some mRNAs can take many hours to transcribe[22] so that the consequences of encountering a template strand gap late in the transcriptional process would be severe.

nusA11 mutant strains, at the permissive temperature, are highly sensitive to nitrofurazone (NFZ) and 4-nitroquinolone-1-oxide (4-NQO) but not to ultraviolet (UV) radiation or methylmethane sulfonate (MMS). Δ*nusA* mutant strains, which can be constructed in a strain background lacking horizontally transferred DNA,[23,24] are additionally sensitive to UV and MMS.[4] Gene expression profiling suggests that the sensitivity of *nusA* mutant strains to DNA damage is not an indirect effect on the expression of genes known to be involved in DNA repair or damage tolerance[4,23] and support the alternative hypothesis that NusA might play a hitherto unsuspected role in DNA repair.

Why the specific sensitivity of *nusA11* mutant strains to NFZ and 4-NQO? NFZ and 4-NQO primarily generate lesions on the N^2-position of guanine and DinB carries out preferential and accurate TLS over N^2-furfuryl-dG (N^2-f-dG),[25,26] a mimic of the major adduct formed by NFZ as well as certain other N^2-dG adducts.[27-29] We demonstrate that the N^2-f-dG lesion blocks transcription by *E. coli* RNA polymerase (RNAP) when present in the transcribed strand, but not when present in the non-transcribed strand. Given that DinB is present in considerable excess over the replicative DNA polymerase in both SOS-uninduced cells (250 DinB/10–20 pol III) and SOS-induced cells (2,500 DinB/10–20 pol III),[30,31] we have proposed that N^2-dG lesions with properties similar to N^2-f-dG represent of a class of "stealth" lesions that would not hinder the process of DNA replication because they can be so efficiently bypassed by DinB (DNA pol IV). However, the continued presence of such lesions in the genome would then obstruct transcription.

In addition to a *dinB*-dependent pathway to promote survival after NFZ, physical and genetic interactions between NusA and UvrA suggest that NusA participates in a *uvrABC*-dependent process.[4] The *uvrA*, *uvrB* and *uvrC* gene products mediate the nucleotide excision repair (NER) pathway that acts to repair a wide variety of DNA lesions.[32] NER can occur both globally and coupled to transcription. In the latter case, it results in the preferential repair of the transcribed strand relative to the non-transcribed strand, through a process known as transcription-coupled repair (TCR).[33,34] Genetic analysis suggests that NusA and Mfd, a transcription-coupling repair factor in *E. coli*, function in separate pathways to promote resistance to NFZ and 4-NQO and in partially redundant pathways to promote survival after exposure to UV.[4] The *rpoB* gene encodes the β catalytic subunit of RNAP. Our demonstration that specific *rpoB* mutants that confer sensitivity or resistance to NFZ are dependent on *nusA*$^+$ and *uvrA*$^+$, but not *dinB*$^+$ or *mfd*$^+$, suggests that the *nusA*-dependent, *uvr*-dependent process of NFZ resistance identified is a previously unrecognized form of transcription-coupled repair that functions independently of Mfd.[4]

Even in the absence of exogenous DNA damage *nusA11* mutant strains display partial SOS induction that is greater in stationary phase than during exponential growth and a striking increase in RecA-GFP foci particularly in stationary phase.[4] It is possible that a metabolite generated at higher levels in stationary phase cells than in exponentially growing cells generates DNA damage that is requires *nusA*$^+$ for repair. Alternatively, active replication during exponential growth may mask any defects in *nusA*-dependent transcriptional repair of these endogenous lesions through the recruitment of DNA repair and damage tolerance 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.[3] At the permissive temperature *nusA11* mutant strains display an ~470-fold reduction in stress-induced mutagenesis in the standard Lac$^+$ reversion system and a similar striking abolition of mutagenesis in a modified assay monitoring resistance to tetracycline.[3] In contrast, *nusA11* mutant strains display wild-type levels of UV-induced mutagenesis.[3] A possible role for NusA under stressed conditions could arise from a deficiency in DNA repair that results in unrepaired endogenous lesions being present in the transcribed strand. Alternatively, the very limited amount of replication allowed might result in DNA replication intermediates that have more single stranded gaps than in exponentially growing cells. The process of transcription-coupled trans-lesion synthesis we have hypothesized[2] might therefore be one mechanism that could contribute to the generation of stress-induced mutations by recruiting TLS DNA polymerases to fill these gaps.

Our proposed model of NusA-dependent TCR suggests that NusA, independently of Mfd, is also able to recruit NER machinery to sites of RNAP stalled by a lesion.[4,35] This NusA-dependent TCR pathway is important for the repair of a class of DNA lesion typified by the N^2-f-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. Intriguingly, many types of endogenous DNA damage, including lesions generated as a byproduct of by lipid peroxidation, are N^2-dG adducts[36] and may have similar properties as those generated by NFZ suggesting that "stealth lesions" may be more ubiquitous than damage generated by exogenous sources, such as UV.

Although elegant biochemical studies of Mfd-dependent TCR have offered detailed insights into the mechanism by which it repairs UV-induced DNA damage in the transcribed strand,[37-39] it is striking that Δ*mfd* mutants display only a very modest increase in sensitivity to UV.[40] This has led to the inference that TCR is much less important in bacteria than eukaryotes, a conclusion rationalized by the argument that eukaryotic cells spend more time in a quiescent state compared to bacteria. However, in the environment bacteria are not growing under lab conditions of plentiful nutrients, so this rationalization is inconsistent with the lifestyles of most bacteria in nature. Our results suggest an alternative interpretation: TCR is as important in bacteria as it is in eukaryotes, but its importance has not been appreciated in the past because the existence of an alternative Mfd-independent pathway of TCR had not yet been recognized. TC-TLS and NusA-TCR would provide a way of prioritizing the use of the cells translesion DNA synthesis and NER resources to benefit maximally transcription and suggests an additional reason for the conservation of *nusA*$^+$ throughout bacteria and archea.

The sensitivity of Δ*nusA* mutant strains to agents such as UV and MMS suggest that NusA could also play a role in the transcription-coupled repair of lesions introduced by these agents as well. It remains a possibility that NusA works in conjunction with Mfd to promote TCR of UV-induced lesions; however, epistasis analysis suggests that NusA also plays a role in directing NER in a manner that is independent of Mfd. It is possible that NusA-dependent NER is a variant of TCR that can remove lesions from both strands analogous to the system inferred to exist in mammalian cells.[41-43] Interestingly, the N^2-f-dG lesion stalls transcription at the -4 position[4] in contrast to UV lesions which enter the active site of the RNAP.[44-47] These observations may suggest a possible explanation for why the *nusA11* mutation differentially affects TCR of the two classes of lesions. Moreover, it is not clear whether we have yet identified all the factors that would be required for NusA-dependent TCR. If RNAP were to backtrack upon encountering the N^2-f-dG lesion, it is possible that this backtracking may be sufficient to expose enough of the DNA to allow binding of UvrA. However, it is also possible that RNAP might need to be moved or dislodged from the DNA in order for NER to bind to the damaged substrate. NusA does not contain any obvious domains that would promote such activities and thus another factor could be needed. Curiously, temperature sensitive mutations of the essential transcription termination factor *rho* render cells sensitive to UV.[48] It would be interesting to assess the roles for Rho and other Nus factors in particular NusG, which also associates with elongating RNAPs[5] and is dispensible for viability in a reduced genome background,[23] in promoting survival after treatment with NFZ and 4-NQO and in stress-induced mutagenesis.

Taken together we propose two new linkages between transcription and DNA repair/damage tolerance processes: a novel pathway of transcription-coupled TLS and a new class of transcription-coupled repair. We further propose that in bacteria NusA, a component of all elongating RNA polymerases, plays a key role in linking transcription to these DNA processing pathways. However, we may have only uncovered the tip of the iceberg and expect that future experiments will test these models and provide exciting new details into the mechanisms by which NusA participates in both DNA repair and damage tolerance pathways.

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