Linking transcription with DNA repair, damage tolerance, and genome duplication

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The transcription of genes by RNA polymerases (RNAPs) is far from a smooth ride. Not only is template sequence-dependent pausing of RNAPs a frequent occurrence (1), lesions within the transcribed strand present major barriers to continued movement of RNAPs, with potentially disastrous consequences for gene expression (2). To make matters worse, such stalled transcription complexes mask the DNA damage from recognition and removal by repair systems (3) (Fig. 1*A*). Transcription-coupled repair (TCR) provides a solution to this problem by recruiting repair enzymes to RNAPs blocked by lesions, the outcome being preferential repair of DNA damage within the transcribed strand of expressed genes (4, 5). The transcription repair coupling factor in *Escherichia coli*, Mfd, is the best-characterized example of such coupling and has provided a paradigm for TCR in other organisms (6) (Fig. 1*B*). A report in PNAS indicates that a second, very different, type of TCR also exists. Cohen et al. (7) demonstrate that a transcription elongation factor, NusA, promotes an Mfd-independent pathway of TCR in *E. coli*, providing an explanation for the mild damage sensitivity of cells lacking Mfd and suggesting that a second mechanism of TCR could be operative in other organisms. Their work also points to NusA as being a central player in co-ordination of transcription, DNA repair, damage tolerance, and genome stability.

This newly identified function of NusA is unexpected. NusA has long been known to be an RNAP elongation factor that modulates transcription pausing and termination (8). Recent work by the Walker laboratory (9, 10) also identified a possible role of NusA in recruiting a translesion synthesis DNA polymerase, DinB, to transcription complexes. Translesion synthesis (TLS) provides an important mechanism of damage tolerance in which specialized DNA polymerases with reduced fidelity replicate past DNA lesions that otherwise block the high-fidelity replicative polymerases used to duplicate most of the genome. Walker and colleagues suggested a model in which gaps arise within the transcribed strand of genes opposite a DNA lesion as a result of passage of a replication fork and/or abortive DNA repair (9, 10). Such gaps block progression of transcribing RNAPs, confirmed by
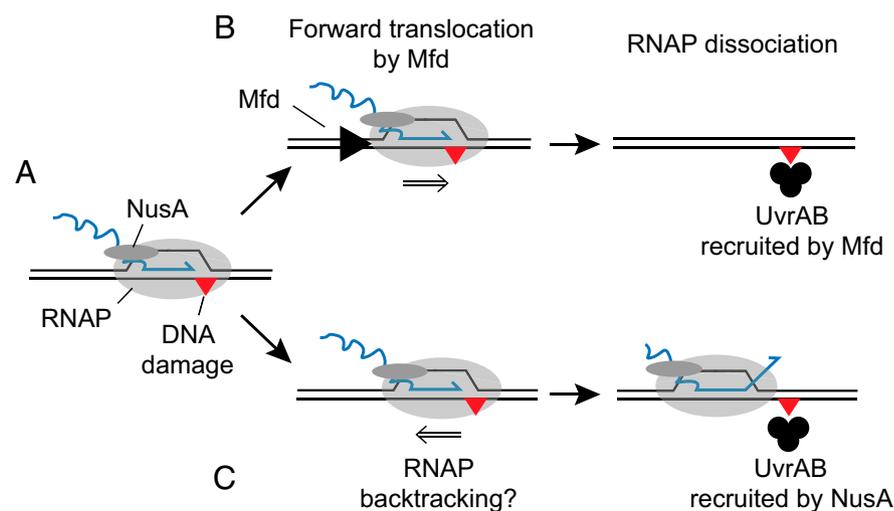

Fig. 1. Two different types of transcription-coupled repair might target RNA polymerases blocked by DNA damage. (*A*) DNA lesions within the template strand block transcription by RNA polymerase. (*B*) *E. coli* Mfd, a DNA translocase, induces forward movement and dissociation of blocked RNAPs. This results in unmasking of the DNA damage and lesion removal by Mfd-recruited nucleotide excision repair enzymes. (*C*) Specific types of DNA lesion may be sensed directly by RNAP to induce backtracking of the RNAP and exposure of the DNA lesion. Recruitment of excision repair enzymes by NusA might then facilitate repair of the lesion.

Cohen et al. (7), but recruitment of DinB might allow DNA synthesis past the lesion and repair of the gap, allowing completion of transcription. NusA might therefore promote tolerance of DNA damage by the transcription machinery in a process termed "transcription-coupled translesion synthesis" (9).

The new study in PNAS (7) indicates that NusA might promote not only tolerance but also repair of DNA damage. Lack of NusA activity confers hypersensitivity to a variety of DNA-damaging agents, especially nitrofurazone (7). This sensitivity is not caused by the absence of either the transcription elongation factor activity of NusA or NusA-promoted translesion synthesis by DinB. Instead, physical and functional interaction of NusA with UvrA, a key component of the nucleotide excision repair machinery, indicates that NusA promotes lesion removal by nucleotide excision repair (7). The known presence of NusA in transcription elongation complexes (11) implied a direct link between NusA-promoted excision repair and transcription, a link confirmed by the isolation of mutations in RNAP that confer either resistance or sensitivity to nitrofurazone in a NusA- and UvrA-dependent manner (7).

Importantly, absence of either DinB or Mfd did not alter the pattern of nitrofurazone resistance/sensitivity of these RNAP mutants, lending strong support to the view that NusA promotes DNA damage repair in addition to DinB-catalyzed damage tolerance and that this repair occurs independently of Mfd.

These data indicate that two mechanisms exist to couple nucleotide excision repair to transcription in *E. coli*. Why might two mechanisms be better than one? Although transcription can proceed right up to a UV light-induced pyrimidine dimer within the transcribed strand, resulting in nucleotide insertion across from the 3′ T residue (3), Cohen et al. demonstrate that a mimic of the major adduct formed by nitrofurazone stalls RNAP four nucleotides upstream of the lesion (7). Different types of DNA damage therefore generate blocked transcription complexes with very different structures that may be preferentially repaired by different TCR

pathways. In support of this view, the translocase activity of Mfd is important for its role in TCR, allowing Mfd to induce dissociation of blocked RNAP and so unmasking DNA lesions for repair enzymes (12) (Fig. 1*B*). NusA, in contrast, is not a motor protein and therefore presumably cannot directly clear blocked RNAP away from sites of DNA damage. Instead, Cohen et al. speculate that NusA-dependent TCR may proceed via sensing of DNA damage by RNAP itself and subsequent backward translocation of RNAP to expose the damage for NusA-promoted repair (Fig. 1*C*). These differences in Mfd and NusA activity may impact on the types of blocked transcription complex structures that these two TCR systems preferentially deal with.

Much remains to be discovered regarding the molecular details of how NusA promotes TCR and the interplay between the NusA and Mfd pathways. However, regardless of mechanism, this new study provides important insight into how the impact of DNA damage on transcription is minimized. Recent analyses of cancer cell lines have hinted at a type of nucleotide excision repair that is directed at both transcribed and nontranscribed strands of expressed genes rather than the transcribed strand only (13, 14). Cohen et al.

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suggest that NusA-dependent nucleotide excision repair could be just such a variant of TCR (7), although NusA-targeted repair of both the nontranscribed and the transcribed strand remains to be demonstrated. Rescue of blocked RNAPs by NusA-directed TCR may also impact on efficient duplication of the genome, given the ability of transcription complexes to inhibit movement of replication forks (15, 16). Notably, although Mfd can displace a stalled RNAP involved in a head-on collision with a replication fork in vitro (17), absence of Mfd has a limited impact on resolution of conflicts between gene expression and genome duplication in vivo (18). Analysis of loss of both NusA and Mfd is needed to clarify the importance of TCR for efficient genome duplication. It is clear, however, that NusA is emerging as a key factor in multiple aspects of DNA and RNA metabolism.

1. Davenport RJ, Wuite GJ, Landick R, Bustamante C (2000) Single-molecule study of transcriptional pausing and arrest by E. coli RNA polymerase. *Science* 287: 2497–2500.
2. Tornaletti S, Hanawalt PC (1999) Effect of DNA lesions on transcription elongation. *Biochimie* 81:139–146.
3. Selby CP, Sancar A (1990) Transcription preferentially inhibits nucleotide excision repair of the template DNA strand in vitro. *J Biol Chem* 265:21330–21336.
4. Bohr VA, Smith CA, Okumoto DS, Hanawalt PC (1985) DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. *Cell* 40: 359–369.
5. Mellon I, Hanawalt PC (1989) Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand. *Nature* 342:95–98.
6. Savery NJ (2007) The molecular mechanism of transcription-coupled DNA repair. *Trends Microbiol* 15:326–333.
7. Cohen SE, et al. (2010) Roles for the transcription elongation factor NusA in both DNA repair and damage tolerance pathways in *Escherichia coli*. *Proc Natl Acad Sci USA* 107:15517–15522.
8. Roberts JW, Shankar S, Filter JJ (2008) RNA polymerase elongation factors. *Annu Rev Microbiol* 62:211–233.
9. Cohen SE, Godoy VG, Walker GC (2009) Transcriptional modulator NusA interacts with translesion DNA polymerases in *Escherichia coli*. *J Bacteriol* 191: 665–672.
10. Cohen SE, Walker GC (2010) The transcription elongation factor NusA is required for stress-induced mutagenesis in *Escherichia coli*. *Curr Biol* 20:80–85.
11. Mooney RA, et al. (2009) Regulator trafficking on bacterial transcription units *in vivo*. *Mol Cell* 33:97–108.
12. Park JS, Marr MT, Roberts JW (2002) *E. coli* transcription repair coupling factor (Mfd protein) rescues arrested complexes by promoting forward translocation. *Cell* 109:757–767.
13. Pleasance ED, et al. (2010) A comprehensive catalogue of somatic mutations from a human cancer genome. *Nature* 463:191–196.
14. Pleasance ED, et al. (2010) A small-cell lung cancer genome with complex signatures of tobacco exposure. *Nature* 463:184–190.
15. French S (1992) Consequences of replication fork movement through transcription units *in vivo*. *Science* 258:1362–1365.
16. Azvolinsky A, Giresi PG, Lieb JD, Zakian VA (2009) Highly transcribed RNA polymerase II genes are impediments to replication fork progression in *Saccharomyces cerevisiae*. *Mol Cell* 34:722–734.
17. Pomerantz RT, O'Donnell M (2010) Direct restart of a replication fork stalled by a head-on RNA polymerase. *Science* 327:590–592.
18. Trautinger BW, Jaktaji RP, Rusakova E, Lloyd RG (2005) RNA polymerase modulators and DNA repair activities resolve conflicts between DNA replication and transcription. *Mol Cell* 19:247–258.