Gre-family factors modulate DNA damage sensing by Deinococcus radiodurans RNA polymerase

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Supplementary Information

Figure S1. Analyzed DNA lesions. The modified nucleotide positions are shown with pink ovals.

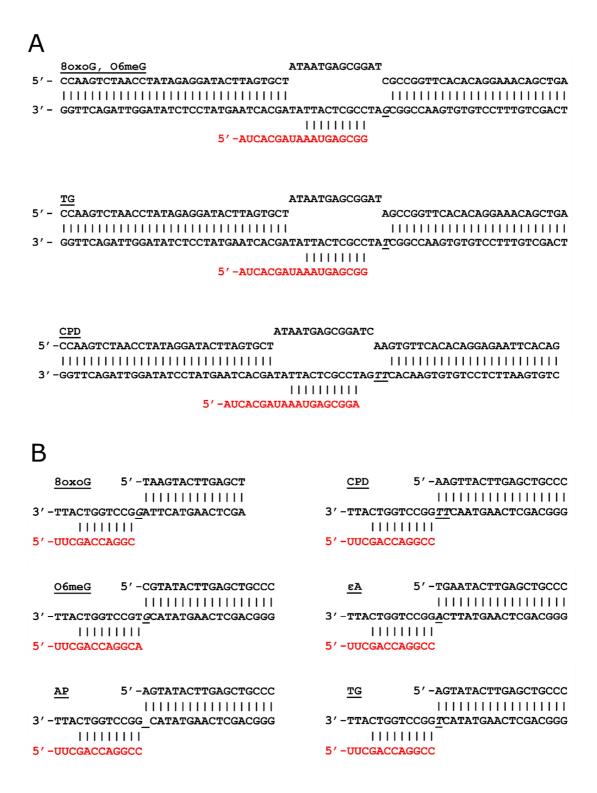


Figure S2. Structure of analyzed TECs. (A) Complete TECs containing 8oxoG, O6meG, TG and CPD. (B) Minimal TECs containing 8oxoG, O6meG, the AP-site, TG, CPD and εA. RNA is shown in red. Positions of modified nucleotides in the template DNA strand are shown in underlined italics.

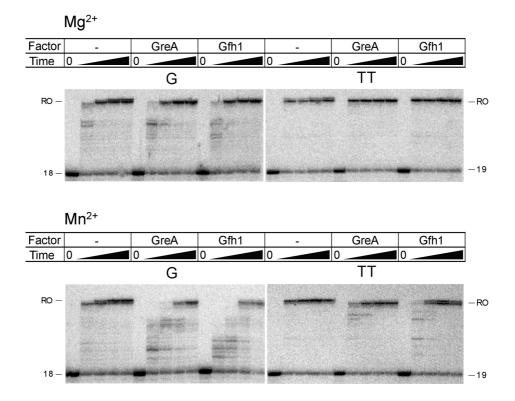


Figure S3. RNA synthesis by *Dra* RNAP in control TECs corresponding to the 80xoG and CPD templates in the absence or in the presence of GreA and Gfh1. The reactions were performed for 10", 30", 1', and 20' in the presence of 10 μ M of NTPs at 30 °C for the G template, and 100 μ M of NTPs at 37 °C for the TT template, in transcription buffers containing either Mg²⁺ or Mn²⁺. Positions of the starting RNA (18/19 nt) and full-length run-off (RO) transcripts are shown on the sides of the gels. GreA and Gfh1 were added to 5 μ M where indicated.

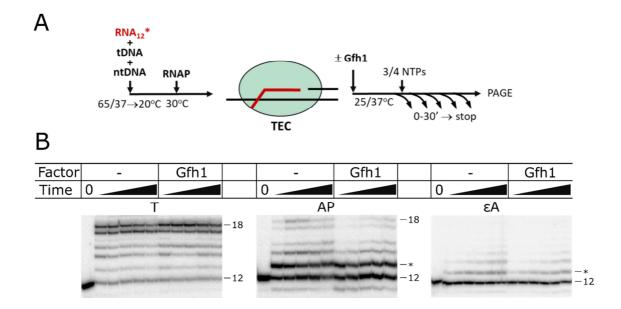


Figure S4. Analysis of transcription in minimal TECs containing the AP-site, εA and in corresponding control TEC. (A) Scheme of the experiment. (B) Analysis of translesion RNA synthesis. The reactions were performed for 10", 30", 1', 3', and 30' in the presence of 1 mM ATP, UTP and GTP at 25 °C for the AP-site or in the presence of 1 mM ATP, UTP, GTP and CTP at 37 °C for εA . Gfh1 was added to 5 μM where indicated. Positions of the starting RNA (12 nt), read-through transcripts (18 nt) and the sites of lesions (asterisks) are indicated. In the case of the control minimal TEC, most complexes efficiently extend RNA to the expected position (18 nt). The presence of the AP-site or εA strongly inhibits transcription.

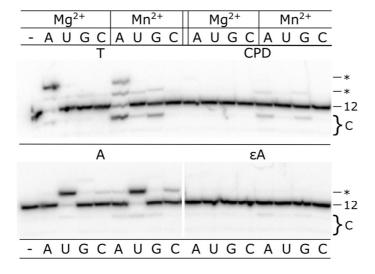


Figure S5. Fidelity of nucleotide incorporation by Dra RNAP on the CPD, εA and corresponding control templates at low nucleotide concentrations. The reactions were performed under the same conditions as in Figure 2 (at 37 °C) but at 10 μ M NTPs. Positions of the starting 12 nt RNA, the sites of lesions, and the cleavage products (C) are indicated.