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ppGpp Couples Transcription to DNA Repair in *E. coli*

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Abstract

The small molecule alarmone (p)ppGpp mediates bacterial adaptation to nutrient deprivation by altering the initiation properties of RNA polymerase (RNAP). ppGpp is generated in *E. coli* by two related enzymes, RelA and SpoT. We show that ppGpp is robustly, but transiently, induced in response to DNA damage and is required for efficient nucleotide excision DNA repair (NER). This explains why RelA/SpoT-deficient cells are sensitive to diverse genotoxic agents and UV radiation, whereas ppGpp induction renders them more resistant to such challenges. The mechanism of DNA protection by ppGpp involves promotion of UvrD-mediated RNAP backtracking. By rendering RNAP backtracking-prone, ppGpp couples transcription to DNA repair and prompts transitions between repair and recovery states.

Bacteria respond to starvation by rapidly accumulating guanosine-3',5'-(bis)pyrophosphate (ppGpp). This results in the inhibition of stable RNA (rRNA and tRNA) transcription and metabolic transition, known as the stringent response, allowing bacteria to conserve energy and adapt to adverse conditions (1–2). The global reprogramming of gene expression is a consequence of ppGpp binding to RNA polymerase (RNAP), causing destabilization of open promoter complexes (3–4). ppGpp acts synergistically with the transcription factor DksA, which also interacts with RNAP (5–6). Evidence suggests ppGpp also functions independently in preserving genomic integrity (7–10). Here we demonstrate that ppGpp is crucial for the process of nucleotide excision DNA repair (NER).

The majority of bulky DNA lesions are eliminated by NER (11). *E. coli* resistance to helix-distorting mutagens such as UV radiation, 4-nitroquinoline-1-oxide (4NQO), and nitrofurazone (NFZ), depends on the intracellular level of ppGpp (8–10) (Figs. 1A and S1). Cells lacking *relA* and *spoT* (ppGpp⁰) were sensitive to these stressors, whereas *relA256*

spoT203 cells, which produced excess ppGpp under normal exponential growth conditions, were more resistant than the parent control (Figs. 1A and S1). ppGpp was transiently induced in response to these challenges (Fig. 1B). Its level increased over 20 fold within the first 15 minutes of genotoxic stress, and then returned to basal levels within 45 minutes. Because DNA lesions caused by UV, 4NQO, and NFZ are eliminated predominantly by NER (12), we examined the potential role of ppGpp in NER.

RNAP facilitates the recognition of DNA damage by NER enzymes through transcription-coupled DNA repair (TCR) (13). ppGpp is a modulator of RNAP activity, and, therefore, could support NER by functioning in TCR. To test this, we compared the ability of wt and ppGpp-deficient cells to remove CPDs from the individual DNA strands of the *lac* operon (Figs. 1C and S2). wt IPTG-induced cells repaired CPDs in the transcribed strand of *lac* operon more rapidly than in the non-transcribed strand (14)(Figs. 1C and S2). In contrast, ppGpp⁰ cells displayed the same slow rate of repair on both strands, which was similar to the rate of repair of the non-transcribed strand in wt cell (Figs. 1C and S2). Thus, ppGpp is essential for TCR.

In the “forward translocation” TCR pathway, the translocase Mfd binds to the upstream edge of stalled RNAP (15) and pushes it forward against the lesion, thereby terminating transcription (16, 17). Mfd then recruits UvrA to the site of damage (16) to initiate NER. The “backtracking” pathway relies on the 3'-5' helicase UvrD, which binds RNAP and pulls it backward, away from the lesion site, thereby exposing the lesions to NER enzymes (18). To determine which of the two TCR pathways requires ppGpp we combined the *relA spoT* deletions with either *uvrD* or *mfd* (Fig. 2A). The sensitivity of *mfd relA spoT* to 4NQO, NFZ, or UV was approximately equal to that of the sum of individual *mfd* and ppGpp⁰ mutant strains. An additive effect of Mfd and ppGpp indicates that the two factors act in different repair pathways. In contrast, *uvrD relA spoT* mutant was as sensitive to genotoxic agents and UV as *uvrD* alone (Figs. 2A and S3). The epistatic relation between UvrD and ppGpp suggests that they act in the same, backtracking-mediated TCR pathway.

Transcript cleavage factors GreA and GreB interfere with UvrD-mediated TCR, as they compete with UvrD-mediated backtracking (18). Inactivation of these anti-backtracking factors greatly suppressed the sensitivity of ppGpp⁰ cells to killing by genotoxic agents and UV (Figs. 2B and S4), arguing that ppGpp acts as a pro-backtracking factor in UvrD-mediated TCR. In bacteria, transcription and translation are coupled. The leading ribosome directly controls the rate of transcription elongation by preventing RNAP backtracking (19) thereby also interfering with UvrD-mediated TCR (18). Analogous to the situation with Gre-deficiency, we show that slowing ribosome translocation with a sublethal dose of chloramphenicol (Cm) rendered ppGpp⁰ cells more resistant to genotoxic chemicals and UV (Figs. 2C and S5).

DksA has been shown to promote ppGpp activities *in vivo* and *in vitro* (5, 6). Accordingly, DksA-deficient cells were sensitive to 4NQO, NFZ, and mitomycin C, whereas inactivation of GreA fully suppressed this sensitivity (8) (Figs. 2D and S6). Moreover, DksA overexpression suppressed the sensitivity to genotoxic stress in the absence of ppGpp (Fig.

S7). Collectively, these *in vivo* results argue that ppGpp, in conjunction with DksA, promotes UvrD-mediated TCR by facilitating RNAP backtracking.

To test whether ppGpp assists UvrD in promoting RNAP backtracking, we examined the effect of ppGpp and UvrD on transcription elongation in a reconstituted single round runoff assay (Figs. 3A and S8). UvrD forced RNAP to stall at many sites, so that only a small fraction of transcription complexes reached the end of the template to form a full-length runoff product (18). The majority of observed paused or arrested complexes were the result of UvrD-induced backtracking, hence their sensitivity to GreB (Fig. 3B; Table S1). Addition of 100 μ M ppGpp, a concentration corresponding to the cellular level of ppGpp during the stringent response, greatly stimulated UvrD-mediated backtracking: less than 20% of RNAP molecules traversed the middle of the template in the presence of ppGpp together with UvrD (Fig. 3A, lane 6), whereas more than 50% of RNAPs did so in the presence of UvrD alone (Fig. 3A, lane 3). DksA+ppGpp further promoted the pro-backtracking activity of UvrD (Fig. 3A, lanes 7-9). DksA alone potentiated UvrD-mediated backtracking only slightly (Fig. 3A, lanes 10-12).

The structural model of the ppGpp-RNAP complex suggests that ppGpp facilitates partial opening of the RNAP clamp (4), thereby potentially rendering RNAP prone to backtracking. To test this prediction, we monitored the front edge of a stable elongation complex stalled at position +32 (EC32) by probing it with exonuclease III (Figs. 3C and S8). The footprinting of EC32 showed that RNAP was almost evenly distributed between post and pre-translocated states (lane 3). ppGpp shifts the equilibrium toward the pre-translocated state (lanes 4). Longer incubation with exonuclease III resulted in progressive backtracking, which was more prominent in the presence of ppGpp than in its absence (lanes 5 and 6).

To confirm that ppGpp also facilitates RNAP backtracking *in vivo*, we utilized a plasmid (p1EC) in which the Lac repressor bound to its operator site blocks an isolated EC approximately 70 nt downstream of the transcription start site (Fig. 3D) (20). To monitor the effect of ppGpp and UvrD on the positioning of the halted EC, we performed *in situ* DNA footprinting using the single-strand-specific probe, chloroacetaldehyde (CAA). Cells transformed with pEC1 alone, or together with the UvrD overexpression plasmid (pUvrD), were treated with serine hydroxamate (SHX) to induce the stringent response (Fig. S8). ppGpp induction caused the roadblocked EC to backtrack over a longer distance (Fig. 3D, compare lanes 3 and 4): CAA reactive sites were more prominent upstream of the footprint and the reactivity of the downstream margin of the bubble was diminished. Backtracking was substantially more extensive in the SHX-treated cells that overexpressed UvrD (lane 5). SHX failed to promote backtracking in UvrD-deficient cells (Fig. S9). Thus, as shown *in vitro*, ppGpp also facilitates UvrD-mediated RNAP backtracking *in vivo*. Consistently, SHX-pretreated cells are more resistant to genotoxic stress (Fig. S10).

A stringent RNAP allele, *rpoB**35, carries a point mutation (H1244Q) in the beta subunit that mimics the presence of ppGpp (7). *rpoB**35 suppressed the sensitivity of ppGpp⁰ cells to genotoxic agents and UV (8) (Figs. 2E and S11). In the absence of UvrD, however, cells harboring *rpoB**35 were unable to effectively suppress their sensitivity to DNA damaging agents, and were almost as sensitive as *uvrD*(-) cells (Fig. S12), suggesting that *rpoB**35

acted via UvrD-mediated TCR. To test this directly, we examined the effect of UvrD and ppGpp on *rpoB**35 RNAP *in vitro*. RpoB*35 was purified and used in a single-round assay (Fig. 3A). In the absence of UvrD, the RpoB*35 enzyme elongated slightly faster than wild-type enzyme (21), and was less responsive to certain strong pauses (compare Fig. 3A, lanes 1 and 13) (21). However, RpoB*35 became much more prone to backtracking in the presence of UvrD (Fig. 3A, lanes 14 and 15). The extent of UvrD-mediated backtracking by RpoB*35 was comparable to that of wild-type enzyme in the presence of ppGpp and DksA (compare Fig. 3A, lanes 8, 9 and 14, 15). Moreover, addition of ppGpp (with or without DksA) to the RpoB*35 reaction had no further effect on UvrD-mediated backtracking (lanes 17 and 18). We thus conclude that RpoB*35 mimics the effect of ppGpp on wild-type RNAP. These results provide direct support for the UvrD/ppGpp-mediated TCR and explain how ppGpp couples transcription to DNA repair.

Here we implicate ppGpp as an important component of UvrD-mediated TCR (Fig. S13). Our *in vivo* and *in vitro* data indicate that ppGpp and UvrD act together to promote backtracking, and that this pro-backtracking activity accounts for most of their phenotypes with respect to DNA damage. Modeling suggests that RNAP undergoes a conformational change upon binding ppGpp, widening its ‘claw-pincers’ (4) and rendering it prone to backtracking (22). In the absence of stress, the *E. coli* cell contains $\sim 3 \times 10^3$ molecules of UvrD (23), at an approximate 1:1 ratio with RNAP. Considering the high affinity of UvrD for RNAP ($K_d = 35$ nM), most of the UvrD should be sequestered by RNAP under normal growth conditions. However, for UvrD to act as a helicase capable of backtracking it must form a dimer (18, 24). Such dimers are not stable ($K_d \sim 470$ nM) (25). As the induction of UvrD during the SOS response is only 2-3 fold (26), UvrD dimerization is expected to occur only intermittently during stress. Thus, by lowering the energy barrier necessary to cause backtracking, ppGpp widens the critical window of opportunity for UvrD to act in TCR. DksA contributes significantly to UvrD/ppGpp-mediated TCR (Figs. S6 and S7), because it stabilizes RNAP in the backtracking-prone state imposed by ppGpp (Fig. 3A) (4) and competes with anti-backtracking Gre factors for the same binding site on RNAP (6).

Codirectional collisions between the replication fork and backtracked RNAP often lead to DNA double strand breaks (21). However, because ppGpp accumulates only during the critical phase of genotoxic stress (Fig. 1B), when it also inhibits replication (9, 27), such detrimental collisions are effectively avoided. The timely decline of ppGpp triggers the recovery process (Fig. S13), which relies on the concerted action of anti-backtracking factors, GreAB, active ribosomes, and Mfd that compete with pro-backtracking UvrD (Figs. 2, S4, S5, S14, S15) (19, 28). Thus, ppGpp not only helps to activate UvrD-TCR, but also assures that the process is deactivated promptly so that transcription may recover and conflicts with replication are avoided.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Magnusson LU, Farewell A, Nystrom T. Trends Microbiol. 2005; 13:236–242. [PubMed: 15866041]
2. Potrykus K, Murphy H, Philippe N, Cashel M. Environ Microbiol. 2011; 13:563–575. [PubMed: 20946586]
3. Barker MM, Gaal T, Josaitis CA, Gourse RL. J Mol Biol. 2001; 305:673–688. [PubMed: 11162084]
4. Zuo Y, Wang Y, Steitz TA. Mol Cell. 2013; 50:430–436. [PubMed: 23623685]
5. Paul BJ, et al. Cell. 2004; 118:311–322. [PubMed: 15294157]
6. Parshin A, et al. Proc Natl Acad Sci U S A. 2015; 112:E6862–6871. [PubMed: 26604313]
7. McGlynn P, Lloyd RG. Cell. 2000; 101:35–45. [PubMed: 10778854]
8. Trautinger BW, Jaktaji RP, Rusakova E, Lloyd RG. Mol Cell. 2005; 19:247–258. [PubMed: 16039593]
9. DeNapoli J, Tehranchi AK, Wang JD. Mol Microbiol. 2013; 88:93–104. [PubMed: 23461544]
10. Madison KE, et al. Mol Microbiol. 2014; 92:28–46. [PubMed: 24612328]
11. Reardon JT, Sancar A. Prog Nucleic Acid Res Mol Biol. 2005; 79:183–235. [PubMed: 16096029]
12. Batty DP, Wood RD. Gene. 2000; 241:193–204. [PubMed: 10675030]
13. Kamarthapu V, Nudler E. Curr Opin Microbiol. 2015; 24:15–20. [PubMed: 25596348]
14. Mellon I, Hanawalt PC. Nature. 1989; 342:95–98. [PubMed: 2554145]
15. Westblade LF, et al. Nucleic Acids Res. 2010; 38:8357–8369. [PubMed: 20702425]
16. Selby CP, Sancar A. Science. 1993; 260:53–58. [PubMed: 8465200]
17. Park JS, Marr MT, Roberts JW. Cell. 2002; 109:757–767. [PubMed: 12086674]
18. Epshtein V, et al. Nature. 2014; 505:372–377. [PubMed: 24402227]
19. Proshkin S, Rahmouni AR, Mironov A, Nudler E. Science. 2010; 328:504–508. [PubMed: 20413502]
20. Epshtein V, Toulmé F, Rahmouni AR, Borukhov S, Nudler E. EMBO J. 2003; 22:4719–4727. [PubMed: 12970184]
21. Dutta D, Shatalin K, Epshtein V, Gottesman ME, Nudler E. Cell. 2011; 146:533–543. [PubMed: 21854980]
22. Sekine S, Murayama Y, Svetlov V, Nudler E, Yokoyama S. Mol Cell. 2015; 57:408–421. [PubMed: 25601758]
23. Arthur HM, Eastlake PB. Gene. 1983; 25:309–316. [PubMed: 6319240]
24. Maluf NK, Fischer CJ, Lohman TM. J Mol Biol. 2003; 325:913–935. [PubMed: 12527299]
25. Maluf NK, Lohman TM. J Mol Biol. 2003; 325:889–912. [PubMed: 12527298]
26. Siegel EC. Mol Gen Genet. 1983; 191:397–400. [PubMed: 6355763]
27. Schreiber G, Ron EZ, Glaser G. Curr Microbiol. 1995; 30:27–32. [PubMed: 7765879]
28. Schalow BJ, Courcelle CT, Courcelle J. J Bacteriol. 2012; 194:2637–2645. [PubMed: 22427630]
29. Sarubbi E, Rudd KE, Cashel M. Mol Gen Genet. 1988; 213:214–222. [PubMed: 2460731]

Fig. 1. ppGpp is required for genotoxic stress survival and TCR
(A) ppGpp⁰ cells (*relA spoT*) or cells producing excessive amount of ppGpp (*rel256spoT203*) (29), were treated with 4NQO, NFZ or UV. Data from three independent experiments are presented as the mean $\pm$ s.e.m.; **P<0.01, *P<0.05. Representative efficiencies of colony formation are shown in Fig. S1. (B) MG1655 cells were treated with 4NQO or NFZ for the indicated time. ppGpp was detected by thin layer chromatography. The same area (rectangle) in each lane was used for ppGpp quantitation. Values are the means ($\pm$ SE) of three independent experiments. (C) Strand-specific repair of the *lacZ* operon (6.6 kb of *Apa* I-*Sst* II fragment) was measured at 0, 10, 20, 30, and 40 minutes after UV irradiation (50 J/m²). Data from three independent experiments (Fig. S2) are presented as the mean $\pm$ s.e.m. T - transcribed strand; NT - non-transcribed strand.

Fig. 2. ppGpp contributes to the UvrD-mediated (backtracking) TCR pathway

(A) MG1655 cells lacking either Mfd or UvrD or RelA/SpoT, or combinations of Mfd/RelA/SpoT and UvrD/RelA/SpoT were exposed to the indicated amounts of 4NQO and NFZ. Data from three independent experiments are presented as the mean $\pm$ s.e.m.; **P<0.01, *P<0.05, ns - non-significant. Representative efficiencies of colony formation are shown in Fig. S3. (B) Inactivating anti-backtracking factors GreA and GreB suppresses ppGpp⁰ sensitivity to 4NQO, NFZ, and UV. Data from three independent experiments are presented as the mean $\pm$ s.e.m. (P<0.01). Representative efficiencies of colony formation are shown in Fig. S4. (C) Slowing ribosomal translocation with a sub-lethal concentration of chloramphenicol suppresses ppGpp⁰ sensitivity to 4NQO or UV. Data from three independent experiments are presented as the mean $\pm$ s.e.m. (P<0.01). Representative efficiencies of colony formation are shown in Fig. S5. (D) Inactivating GreA suppresses the sensitivity of DksA-deficient cells to genotoxic stress caused by mitomycin C, 4NQO, or NFZ. Data from three independent experiments are presented as the mean $\pm$ s.e.m. (P<0.01). Representative efficiencies of colony formation are shown in Fig. S6. (E) The *rpoB*³⁵ allele suppresses ppGpp⁰ sensitivity to 4NQO, NFZ and UV. Data from three independent experiments are presented as the mean $\pm$ s.e.m. (P<0.01). Representative efficiencies of colony formation are shown in Fig. S11.

Fig. 3. ppGpp promotes UvrD-mediated RNAP backtracking *in vitro* and *in vivo*
 (A) EC20 was formed by WT RNAP or RpoB*35 (lanes 13-18) at the T7A1 DNA template and then chased in the presence of specified amounts of UvrD, ppGpp and/or DksA where added to the chase reaction. The pro-backtracking activity of UvrD was assessed as a ratio (%) between the total amounts of RNA products located above and below the arbitrary midsection line indicated by red asterisks. Mean values $\pm$ s.e.m. ($P < 0.05$) from three independent experiments are shown in Table S1. (B) EC20 (lane 1) immobilized on Co^{++} -beads was chased with (lanes 3-6) or without UvrD+ppGpp (lane 2), followed by washing (lane 4). GreB without (lanes 5 and 6) or with NTPs (lane 6; second chase) was added. Red lines connect exemplary corresponding RNA from arrested ECs before and after GreB treatment (lanes 4, 5) to illustrate transcript cleavage. The red asterisk denotes the same position as in (A). (C) The top and bottom panels resolve the same probes to show protection of the ^{32}P -end-labeled non-template DNA strand of EC32 from ExoIII digestion

in the presence or absence of ppGpp. Blue boxes correspond to the areas of ExoIII footprinting at the front edge of EC32. The weight of the red lines illustrates the intensity of the footprint signal. Bands corresponding to the pre- and post-translocated states, as well as backtracked species, are indicated. The extent of backtracking (%) was measured as the ratio between all backtracked signals and the total RNA signal in the corresponding lane. **(D)** The p1EC constructs (left) (19) and primer extension analyses (right). CAA modifications on the non-template strand of p1EC (lanes 2, 3), p1EC in the presence of the ppGpp inducer, serine hydroxamate (SHX) (lane 4), or p1EC1+SHX+pUvrD (lane 5). The lac operator (Lac) and transcription bubble are indicated. Red lines show the position of blocked EC. Experimental workflows for A, B, C, and D are shown in Fig. S8.