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Review Article

The Emerging Role of (p)ppGpp in DNA Repair and Associated Bacterial Survival against Fluoroquinolones

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Abstract

(p)ppGpp binds to RNA polymerase, causing stalling at damaged DNA sites and subsequent backtracking, which facilitates the recognition and removal of damaged DNA by repair proteins. Additionally, (p)ppGpp regulates DNA repair proteins involved in the Save Our Soul response and mutagenic strand break repair pathways, which are crucial for repairing damages induced by Ultraviolet light and other DNA-damaging agents, including antibiotics. Through these repair pathways, (p)ppGpp plays a vital role in mending strand breaks induced by ciprofloxacin, a fluoroquinolone antibiotic. (p)ppGpp mediates bacterial survival by inhibiting the transcription of mismatch repair proteins while simultaneously upregulating error-prone polymerases mediated by stress-induced sigma factors, thereby facilitating mutagenesis. The function of (p)ppGpp in fine-tuning DNA repair proteins to support bacterial survival against antibiotics via stress-induced mutagenesis is an emerging topic in the field of antibiotic resistance research. Currently, limited information is available on how (p)ppGpp interconnects the various DNA repair pathways that directly influence bacterial resistance to antibiotics. (p)ppGpp is also known to promote bacterial persistence against ofloxacin, another fluoroquinolone, by regulating proteins that induce membrane depolarization. The overlapping functions of (p)ppGpp as a master regulator in DNA repair during stress and bacterial persistence are yet to be fully elucidated. This review focuses on recent publications highlighting (p)ppGpp as a potential link connecting DNA repair pathways to bacterial survival strategies against fluoroquinolone antibiotics.

Introduction

Bacteria constantly encounter starvation or stress in their host or environment, necessitating efficient DNA repair to maintain genome stability. Phosphorylated guanosines, known as (p)ppGpp, are synthesized during stress and starvation in bacteria and they act as signaling molecules that regulate the function of critical proteins thereby leading to a global reprogramming of almost all essential cellular processes. (p)ppGpp was first identified by Cashel and Gallant through thin-layer chromatography of nucleotide extracts from *Escherichia coli* (*E. coli*) bacterial cultures starved of amino acids.[1,2] They demonstrated that upon ribosomal stalling, caused by uncharged transfer RNA binding in the absence of amino acids, RelA, a ribosome-associated protein, synthesizes (p)ppGpp from guanosine triphosphate (GTP) or guanosine diphosphate. Since then, several research groups have worked to elucidate the function of (p)ppGpp through genetic and biochemical studies. (p)ppGpp plays a vital role in maintaining GTP/ppGpp homeostasis within cells.[3] It binds to several enzymes involved in nucleotide biosynthesis, leading to the inhibition of GTP biosynthesis during starvation or stringent response.[4–7] However, cells require substantial amounts of Guanosine triphosphate and Adenosine triphosphate (ATP) for replication and growth. During starvation and stringent response, ppGpp mediates an alternative pathway of GTP synthesis. This occurs through ppGpp binding to the transcription factor - xanthine dehydrogenase regulator, leading to upregulation of the purine salvage pathway mediated by the xanthine dehydrogenase enzyme, thus maintaining a basal level of the GTP pool. This basal supply of GTP substrate contributes to (p)ppGpp synthesis under stringent conditions.[8–10] Therefore the availability of (p)ppGpp during stringent conditions is dependent upon the regulation of GTP levels by the enzymes of purine metabolism that salvage purines.

(p)ppGpp binds to various cellular targets, inhibiting key processes such as transcription, translation, and replication. Notably, it binds to RNA polymerase, hindering transcription,[11–14] and to DnaG primase, inhibiting replication.[15] Furthermore, (p)ppGpp interacts with initiation factor-2 and elongation factor-G, impeding

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translation.[16,17] The regulatory mechanism of ppGpp also occurs at the promoter level, either by interacting with another transcription factor or by directly binding to RNA polymerase, which can then bind to the discriminator sequence present upstream of the transcription start site of genes. For example, (p)ppGpp-bound RNA polymerase binds to the promoter of *dnaA*, which codes for a replication initiation protein, thereby inhibiting its transcription.[18–20] Additionally, the sigma subunits of RNA polymerases are regulated by ppGpp-bound RNA polymerases at their promoters.[11,21] A similar regulation is found at the promoter regions of various stress response genes whose expression is influenced by ppGpp generated during stringent conditions.[22,23] Though (p)ppGpp acts as a master regulator of essential cellular processes, how they regulate various DNA repair proteins during stress or stringent conditions remains largely unexplored.

Bacterial DNA repair pathways,[24–26] operate meticulously to rectify lesions in their genome caused by diverse DNA-damaging agents. This review delves into the involvement of (p)ppGpp in some of the major DNA repair pathways including nucleotide excision repair, mismatch repair, and mutagenic strand break repair. However, further studies investigating the in vivo role of (p)ppGpp in these pathways in genome maintenance under cellular context are still needed. Additionally, this review article explores bacterial stress survival mechanisms involving various DNA repair pathways, such as the Save Our Soul response, stress-induced mutagenesis, ciprofloxacin (CPX)-induced mutagenesis, which aid bacterial survival in the presence of antibiotics.[27,28] The overlapping function of (p)ppGpp in membrane depolarization that leads to bacterial cell survival in the presence of antibiotics is not completely understood. However, studying this function will help us understand how (p)ppGpp can contribute to various mechanisms of antibiotic resistance. The viewpoints presented and the questions raised in this review will help guide future research in understanding the role of (p)ppGpp in these DNA repair mechanisms and their relation to antibiotic associated bacterial survival.

Cooperative function of RNA polymerase and ppGpp plays an important role in nucleotide excision repair (NER)

NER eliminates bulky DNA lesions, such as cyclobutane pyrimidine dimers (CPDs) and 6,4-photoproducts, induced by Ultraviolet (UV) radiation. There are two major NER pathways: global genomic NER and transcription-coupled NER (TC-NER). Global genomic NER removes UV-induced DNA lesions throughout the genome, affecting both non-transcribed and transcribed strands. In contrast, TC-NER specifically targets the transcribed strand. TC-NER begins with the stalling of RNA polymerase at DNA lesions.[29,30] *In vivo* studies using excision repair sequencing showed a higher transcribed strand/non-transcribed strand repair ratio, indicating that transcribed strands were repaired much faster than non-transcribed strands in wild-type *E. coli* cells upon UV exposure.[31,32] However, analysis of DNA damage and repair of CPDs at single nucleotide resolution using the CPD-seq technique revealed that CPD repair by the TC-NER pathway occurs globally across all regions of the genome,[33] including sense strands, antisense strands, and intergenic regions where transcription by RNA polymerase is required.[34–36] The process of induction of genome-wide transcription by UV irradiation is termed as "pervasive transcription". Also, based on *in vivo* and *in vitro* techniques, Mutation Frequency Decline (Mfd), a forward translocase protein was considered to be critical for TC-NER.[31,32] *In vivo* live cell imaging studies identified that the Mfd protein associates with RNA polymerase to aid the transcription elongation process during normal growth even in the absence of DNA damage.[37] Cryo-electron microscopy studies revealed that Mfd protein binding to DNA induces structural changes in Mfd, leading to Mfd-UvrA binding via the ATPase motif IVa and exhibiting translocase activity via motif Ic.[38] Single-molecule imaging in *E. coli* cells elucidated that ATP hydrolysis by UvrA is required for the Mfd-$UvrA_2$ complex interaction with DNA. UvrB loading onto the template strand at sites of stalled RNA polymerases is synchronized with the dissociation of Mfd from DNA.[39] Additionally, a comparison of UV-irradiated *E. coli* cells and NER-deficient cells indicated that the concentration of UvrA increases during the Save Our Soul (SOS) response in wildtype cells, aiding Mfd turnover and recruitment at sites of UV lesions where RNA polymerase stalls.[37,39–41] However, solid evidence for this mechanism remains to be elucidated.[34,35] Mfd was considered sufficient for transcription-coupled NER in *E. coli*,[31,32] but *Δmfd* mutants were not found to be as sensitive to UV radiation,[42–44] suggesting that Mfd might not be the most critical player in regulating TC-NER in *E. coli*.[35] Therefore, evidences so far indicated that Mfd protein fundamentally functions during the process of transcription apart from playing a role in the nucleotide excision repair pathway.

RNA polymerase backtracking, a mechanism where RNA polymerase slides in reverse orientation, is essential for regulation of gene transcription and maintenance of genome stability.[45] In bacteria, transcription fidelity and prevention of collisions between transcription and replication processes depend on RNA polymerase backtracking of transcription complex containing mis-incorporated bases by binding to the transcription factor DksA in the presence of the signaling molecule guanosine tetraphosphate (ppGpp).[46] Although the backtracking of RNA polymerase aids proofreading, excessive backtracking, such as in the case of arrested elongation complexes, can occasionally cause codirectional collisions. These collisions may lead to double-strand breaks, posing a threat to bacterial survival.[47] Notably, ppGpp accumulates upon exposure to DNA-damaging agents (like UV radiation),[48] binds to RNA polymerase, induces backtracking activity, and coordinates the transition of RNA polymerase between transcription elongation and NER.[49,50] Cryo-electron microscopy studies revealed two ppGpp binding sites within RNA polymerase structures.[49,50] ppGpp binding to Site 1 is required for RNA polymerase backtracking during NER, while binding to Site 2, together with the transcription factor DksA, inhibits transcription initiation.[49,50] During nutrient starvation and stringent response, ppGpp binding to Site 2 inhibits transcription initiation at promoter regions.[51,52] Upon encountering bulky lesions caused by UV light or other damaging agents like nitrofurazone (NFZ) or 4-nitroquinoline-1-oxide (4NQO), ppGpp binding to Site 1 facilitates RNA polymerase backtracking in association with UvrD (a helicase) in an additive fashion promoting NER.[48] The combined action of ppGpp and UvrD in backtracking RNA polymerase facilitates the recruitment of $UvrA_2BC$ excision nuclease to the lesion site, leading to damage excision.[34–36,44,48] *In vitro* biochemical experiments implicated UvrD's helicase action in unwinding the excised oligo and displacing $UvrA_2BC$ from DNA,[53] but this concept warrants further *in vivo* experiments.[35] DNA polymerase I can exclusively perform this helicase function in the absence of UvrD.[54] Mutants of *relA* and *spoT*, which are deficient in ppGpp synthesis, are extremely sensitive to UV, 4NQO, and NFZ damage due to the failure of RNA polymerase backtracking, leading to compromised repair. Deletion of transcription elongation factors GreA and GreB, which act as anti-backtracking factors, can rescue the mutant phenotypes associated with (p)ppGpp and UvrD proteins by mitigating the compromised repair

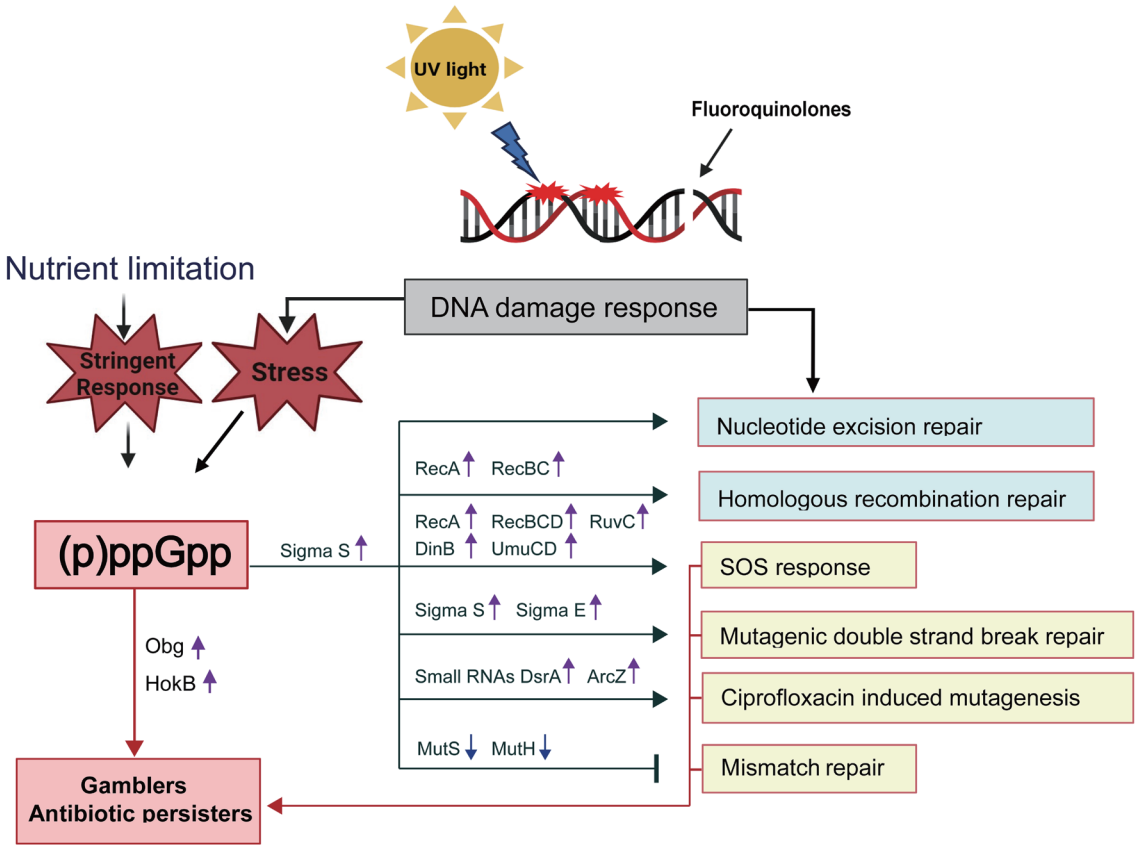

Fig. 1. (p)ppGpp-dependent repair pathways (black arrows) illustrating the role of (p)ppGpp and the set of proteins regulated by (p)ppGpp in each repair pathway. Red arrows indicate the DNA repair pathways that are known to aid bacterial survival as persisters and gamblers in the presence of fluoroquinolones. Blue color arrow indicates downregulation, while purple color indicates upregulation in the presence of (p)ppGpp. (Created with Biorender.com) ArcZ, small RNA that regulates ArcA/B regulon; DinB, DNA polymerase IV named for its DNA Damage, inducible role; DsrA, small RNA named from Downstream Regulatory RNA A; HokB, Toxin protein belonging to the toxin, antitoxin system (name derived from 'Host Killing'); MutS/MutH, mismatch repair proteins that play a role in preventing mutations; Obg, GTPase protein named after its association with SpoO sporulation factor of Bacillus; RecA, Recombinase A; RecBCD, Recombinase BCD; RuvC, recombination protein that resolves UV induced damaged complexes that undergo recombination; SOS, Save Our Soul; UmuCD, translesion synthesis polymerases named after their role in UV mutagenesis.

mechanism.[44,48] NusA, another transcription elongation factor, assists in UvrD-facilitated RNA polymerase backtracking upon encountering a DNA lesion.[44,48,55] Recently, RNA polymerase was identified as playing a major role in the ribonucleotide excision repair pathway,[56] however, the function of (p)ppGpp in this repair pathway is yet to be studied. Overall, nucleotide excision repair is orchestrated by RNA polymerase with the inevitable co-action of ppGpp at a global level upon exposure to DNA-damaging agents, including UV light (Fig. 1). However, the faithful regeneration of the damaged genome after repair might depend upon the prevailing environment (nutrient-rich or nutrient-deficient) and the degree of damage caused by UV light or genotoxic agents. Overall, the function of (p)ppGpp in nucleotide excision repair in bacterial genome maintenance is yet to be fully understood. An impeccable comprehensive analysis of DNA damage and repair upon exposure to various DNA damages that induce bulky adducts in the genome during stringent response might help understand the functional role of (p)ppGpp by nucleotide excision repair pathway in bacteria.

Downregulation of mismatch repair proteins by (p)ppGpp

MutS, MutL, and MutH are crucial proteins in the mismatch repair system, responsible for recognizing misincorporated bases in the DNA resulting from spontaneous deamination reactions, DNA synthesis during replication, and repair synthesis following recombination events.[57–61] Defects in the mismatch repair system lead to the accumulation of mutations in the genome.[62–64] MutH plays a role in methyl-directed mismatch repair by recognizing hemimethylated DNA and cleaving the newly synthesized unmethylated strand containing the mismatched base.[65] In the absence of DNA adenine methylase, which methylates DNA at GATC sequences, MutH fails to recognize the newly synthesized DNA, resulting in cleavage of both parent and daughter strands.[66] MutL aids the UvrD helicase in unwinding the strand with the mismatch, after which the resulting single strand is bound by single-strand binding protein.[67,68] Exonucleases, including ExoI, ExoVII, ExoX, and RecJ, act on the cleaved strand, followed by repair synthesis by DNA polymerase III and ligation by DNA ligase to complete the repair process.[57,58]

During the stationary phase, the concentrations of MutS and MutH decrease approximately tenfold in *E. coli* cells.[69] Although the downregulation of *mutL* has not been identified, there is a limitation in the availability of functional MutL protein during the stationary phase.[70] Furthermore, in the presence of RpoS, a general stress response regulator, the transcript levels of *mutS* and *mutH*

decrease about four-fold and two-fold, respectively (Fig. 1).[71] While the regulatory mechanisms of MutS, MutH, and other mismatch repair proteins during the exponential and stationary phases are yet to be studied,[72] it should be noted that RpoS synthesis and activation are positively regulated by (p)ppGpp during the stationary phase or in response to limited nutrients in the media. The anti-adapter proteins IraD, IraP, and IraM, which stabilize the sigma (S) factor by preventing its degradation by ClpXP proteases, are upregulated by ppGpp during DNA damage, phosphate starvation, and magnesium starvation, respectively.[73–75] The ppGpp-mediated stabilization of sigma (S) could be a potential reason for the downregulation of mismatch repair during starvation. Notably, bacteria utilize the mismatch repair system to genetically adapt by modulating their mutation rates to survive challenging environmental conditions.[64,76] The role of (p)ppGpp in the regulation of mismatch repair pathway might occur (i) through stabilization of RpoS and (ii) through downregulation of MutS and MutH. However, further studies are required to verify the role of (p)ppGpp in regulation of the mismatch repair pathway proteins that lead to mutagenesis in the bacterial genome.

Role of (p)ppGpp in recombinational repair and mutagenic strand break repair

Recombinational repair is crucial for cells to repair strand breaks that may occur during physiological replication-transcription conflicts or exposure to antibiotics that induce strand breaks.[49,77–79] Both single-strand breaks and double-strand breaks are repaired through recombinational repair mechanisms. The Recombinase BCD (RecBCD) proteins play a key role by recognizing double-strand breaks in the DNA and initiating recombination events at the site of the Chi sequence, which is a hotspot for homologous recombination in *E. coli* (crossover hot spot instigator).[80–82] Additionally, RecBCD proteins assist in loading the Recombinase A (RecA) protein onto single-stranded DNA, thereby initiating strand invasion and subsequent recombination events.[81,83,84] The backtracking action of RNA polymerase upon ppGpp binding has been identified as being involved in double-strand break repair in *E. coli* upon exposure to the antibiotic phleomycin. Phleomycin sensitizes ppGpp null mutants and RNA polymerase Site 1 mutants similarly, suggesting that ppGpp binding to Site 1 of RNA polymerase could be involved in mending double-strand breaks.[85] Double-strand break-induced error-prone repair processes in the presence of ppGpp might help bacteria adapt to environmental stresses.[86] In *E. coli*, ppGpp and pppGpp inhibit replication by binding directly to DnaG, a primase essential for replication elongation.[80,87–89] Additionally, *in vitro* and *in vivo* experiments show that ppGpp inhibits the promoter of *dnaA*, downregulating its transcription and consequently inhibiting replication initiation.[18,19] This allows cells to take some time to repair the damaged genome and restore the normal functions upon encountering favorable conditions. Studies have shown that (p)ppGpp induction using serine hydroxamate leads to the accumulation of single stranded DNA (ssDNA) of plasmid pHV16101-1 in *B. subtilis*. This increased ssDNA accumulation occurs because (p)ppGpp binds to primase DnaG and inhibits its activity during the replication process.[15] Furthermore, (p)ppGpp synthesized during UV-induced DNA damage stress prevents replication-transcription conflicts by mediating replication inhibition at lesion sites.[80] Recombinase FOR (RecFOR) proteins are another set of recombination proteins involved in repairing gapped single-strand breaks and plasmid recombination events by loading RecA at these sites. RuvABC is a Holliday junction-specific resolvase that resolves harmful recombination intermediates formed during UV irradiation in *E. coli* and its absence causes cell death. However, in the absence of Ruv proteins, increased levels of (p)ppGpp rescue cells from death upon UV exposure.[80] RecG is another helicase/resolvase involved in the resolution of Holliday junction intermediates and other recombination intermediates not resolved by Ruv proteins. RecG also plays a role in replication fork progression by mediating (p)ppGpp-dependent modulation of RNA polymerase.[90] The binding of (p)ppGpp to RNA polymerase destabilizes stalled RNA polymerases at UV lesion sites and promotes RecFOR-mediated loading of RecA, thereby activating fork regression. Such activation promotes lesion bypass by translesion polymerases, thus avoiding strand breaks but resulting in mutagenesis and increased survival.[91] When cells are exposed to phleomycin, double-strand breaks are induced in the genome; ppGpp, together with UvrD, aids backtracking of RNA polymerase, which assists in double-strand break repair. The RecA protein facilitates the double-strand break repair mechanism.[85] Also, (p)ppGpp might promote mutagenic double-strand break repair during stress, which requires both homologous recombination repair proteins and SOS response proteins such as LexA, RecA, RecB, RecC, RuvA, RuvB, and RuvC.[92] Additionally, in the mutagenic double-strand break repair pathway, ppGpp mediates the regulation of sigma S protein during starvation or stationary phase through the upregulation of error-prone polymerases pol IV and pol V, which aid in mutagenic DNA break repair.[93] Sigma E, another stress response protein activated by ppGpp, promotes spontaneous breakage of DNA. It has been reported that Sigma E is essential for both double-strand break repair and stress-induced mutagenesis.[94] Therefore, (p)ppGpp might play a role in homologous recombination pathway by regulating expression of recombination repair proteins that might help restore genome integrity. Upon exposure to certain antibiotics like phleomycin or ciprofloxacin, (p)ppGpp is also shown to promote mutagenic double-strand break repair leading to a compromised genome.

ppGpp is required for an efficient SOS response

UV radiation in *E. coli* induces single-strand breaks, triggering the SOS response. This response activates the cleavage of LexA protein by RecA, leading to the derepression of genes typically inhibited by LexA.[95,96] The gene products of these derepressed genes are involved in repairing DNA damage and restoring genome stability. Although the SOS response aims to restore the genome, severe or prolonged DNA damage can result in mutagenesis of the genomic landscape.[97–99] The SOS response in *E. coli* involves a series of sequential events,[100] engaging approximately fifty genes, including *lexA, recA, polII, polIV (DinB), polV (umuCD), and sulA*. Studies have shown that the stringent response induces genes such as *recA, ruvA,* and *umuD*, whose gene products also function in the SOS response (Fig. 1).[11,101] Furthermore, deletion mutants of *relA* or *spoT* display a delayed SOS response in *E. coli*.[100] The control of ppGpp synthesis by the RelA protein during stress suggests a potential overlapping role of (p)ppGpp in the SOS and stringent responses. Recent findings from Rosenberg's group identified that ppGpp binding to Site 1 of RNA polymerase is crucial for the SOS response that promotes CPX-induced mutagenesis.[79] Additionally, research by Nudler's group identified that ppGpp binding to Site 1 of RNA polymerase facilitates nucleotide excision repair (NER), the failure of which renders bacterial cells sensitive to UV and other genotoxic agents, namely NFZ and 4NQO.[50] Therefore, (p)ppGpp binding to RNA polymerase at Site 1 is essential for (i) the nucleotide excision repair pathway, which serves as the first line of defense against DNA damage induced by UV light, and

(ii) an efficient SOS response during ciprofloxacin-induced mutagenesis. If the nucleotide excision repair pathway fails to restore genomic integrity, an SOS response is triggered in UV-exposed cells. However, whether the induction of the SOS response during UV-induced DNA repair also requires (p)ppGpp remains an open question. Nonetheless, the role of (p)ppGpp in inducing the SOS response appears to be crucial for ciprofloxacin-induced mutagenesis, which contributes to antibiotic resistance in bacteria.

Stress-induced mutagenesis

Stress-induced mutagenesis involves cells sensing various growth-limiting factors in the environment and,[102] in turn, activating generalized stress response proteins, such as alternative sigma factors, SOS response proteins, and other DNA repair proteins, including a specific set of error-prone polymerases that induce mutations in the genome. Although this pathway compromises genome integrity, it offers the advantage of increased cell survival. In stress-induced mutagenesis, (p)ppGpp downregulates mismatch repair proteins and high-fidelity polymerases, which is necessary for bacterial adaptation and survival against antibiotics.[49,103] ppGpp and DksA promote the translation and stabilization of Sigma S protein, which subsequently upregulates the transcription of small RNAs DsrA and ArcZ and also the IraP protein.[104] The small RNA DsrA enhances *rpoS* messenger RNA (mRNA) transcription by binding to its inhibitory stem-loop structure in the 5′-UTR and prevents *rpoS* mRNA degradation by RNase E,[105] while the IraP protein stabilizes RpoS protein expression. This upregulation of RpoS, a global regulator of the general stress response, induces a switch from high-fidelity polymerases to error-prone polymerases responsible for stress-induced mutagenesis and survival.[106] Therefore, (p)ppGpp is a master regulator of stress-induced mutagenesis pathway without which bacterial cells might succumb to death upon exposure to stress conditions including DNA damaging agents and antibiotics.

CPX-induced mutagenesis and bacterial survival against fluoroquinolone antibiotics as persisters and gamblers

CPX is a fluoroquinolone antibiotic that binds to topoisomerase II and induces strand breaks.[79] At minimal antibiotic concentration of CPX, around 20% of the cell subpopulation shows an increased number of mutations upon survival against this antibiotic. The Rosenberg group identified that in this subpopulation of bacteria, termed gamblers, cells risk genome mutability compared to the rest of the population.[77] During this process, CPX-induced strand breaks initiate the SOS response, which leads to increased reactive oxygen species within the cells due to impaired aerobic respiration or electron transport chain, triggering the stringent response.[77] This is followed by the concerted action of (p)ppGpp and DksA, favoring RpoS activation and the expression of error-prone polymerases as observed in stress-induced mutagenesis. Fluorescent cell sorting experiments identified that the gambler subpopulation of bacteria exhibits an active general stress response. The sigma S active gambler cell subpopulation can generate 400-fold more mutants compared to the sigma S inactive population upon exposure to CPX.[77,79] This survival is dependent on the adaptive mutations facilitated by DNA repair pathways that rely on (p)ppGpp. The absence of the stringent response-induced (p)ppGpp, leads to bacterial cell death upon treatment with fluoroquinolone antibiotics. Biochemical and genetic studies have shown that the binding of ppGpp at two distinct sites in the beta subunit of RNA polymerase is essential for this mutagenesis and survival mechanism. While binding of ppGpp to Site 1 is essential for the SOS DNA-damage response that aids in the backtracking of RNA polymerase during elongation, the binding of ppGpp along with DksA to Site 2 on RNA polymerase initiates transcription by sigma S (σ^S), a global regulator of the general stress response or stringent response. Stringent cells give rise to a higher number of Amp^R and Rif^R mutants compared to stringent off cells. (p)ppGpp binding-mediated backtracking of RNA polymerase also leads to the pausing of RecBCD nuclease activity on the double-strand break, ensuring the loading of RecA on ssDNA, forming a RecA-activated nucleoprotein filament that induces the SOS response and subsequent double-strand break repair. In the presence of (p)ppGpp, Sigma S protein, together with recombination proteins such as RecA, RecBCD, RuvC, polIV, polV, and polII, aids in this mutagenesis process via mutagenic double-strand break repair. It should be noted that CPX-induced mutagenesis occurs at highly transcribed regions of the genome,[79] associated with higher occupancy of RNA polymerase. Therefore, RNA polymerase and ppGpp are crucial to CPX-induced mutagenesis that occurs during transcription in bacteria, aiding the survival of the gambler subpopulation (Fig. 1).[107]

Antibiotic persistence in the presence of the minimal inhibitory concentration of ofloxacin,[27] another fluoroquinolone antibiotic, has been shown to induce membrane depolarization in a subpopulation of cells that exhibit increased expression of the toxin HokB. The Obg protein, a universally conserved GTPase found in bacteria, has been identified to trigger persistence by inducing HokB protein expression, a process that requires (p)ppGpp (Fig. 1).[108] Moreover, the ObgE protein has also been identified to function as a checkpoint regulator of replication. Genetic studies indicated that ObgE acts in conjunction with RecA and RecB repair proteins to prevent strand breaks and fork regression during replication arrest upon exposure to replication inhibitors or during the stringent response.[109,110] However, the *in vivo* role of (p)ppGpp binding to ObgE in resolving the replication fork conflicts that might arise during the stringent response is yet to be studied. While several factors affect persister formation in bacteria, the (p)ppGpp signaling molecule plays a significant role in persister formation in most bacterial species, although there are some exceptions.[111] The mechanism of Obg-mediated antibiotic persistence in the presence of (p)ppGpp is not yet completely understood. Additionally, it remains unexplored whether the gambler subpopulation and antibiotic persister subpopulation arise during exposure to CPX within the host, where the concentration of the antibiotic might vary in different tissues of the body.[112–117] It should be noted that (p)ppGpp levels increase in human and mouse gut microbes during the fasting phase,[118] which might enhance adaptive mutations and aid in the survival of antibiotic-resistant microbes, including persisters and gamblers.[28] It is intriguing to investigate if the gambler subpopulation carrying adaptive mutations can subsequently multiply into an antibiotic-resistant population upon prolonged exposure to the minimal antibiotic concentration of CPX. Since (p)ppGpp is involved in both gambler cell and antibiotic persister cell formation in the presence of fluoroquinolones,[119] ppGpp could potentially serve as a link that connects DNA repair pathways to bacterial survival against antibiotics (Fig. 1).

Limitations

It should be noted that (p)ppGpp independent mechanisms of antibiotic resistance development in bacteria are not discussed in this review.

Conclusions

The stress signaling molecule (p)ppGpp primarily binds to RNA polymerase, inducing backtracking upon encountering DNA lesions. This interaction promotes nucleotide excision repair (NER) and the Save our Soul response by recruiting repair proteins. (p)ppGpp also regulates proteins belonging to the homologous recombination repair pathway that help maintain genome integrity upon exposure to DNA damaging agents. Consequently, prolonged stress may favor mutagenic repair pathways that involve downregulation of mismatch repair proteins, thereby compromising genomic integrity. Stress-induced mutagenesis in the presence of antibiotics like ciprofloxacin (CPX), leads to adaptive mutations in the genome potentially passing antibiotic resistance to successive generations. The involvement of several DNA repair pathways discussed above underscores the complex interplay between (p)ppGpp-mediated DNA repair pathways and antibiotic resistance. Understanding these regulatory mechanisms is essential for developing effective strategies to combat antibiotic resistance.

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Conflict of interest

None.

Author contributions

SS is the sole author of the manuscript.

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