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# Control of bacterial transcription, translation and replication by (p)ppGpp

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The small nucleotides pppGpp and ppGpp (or (p)ppGpp) are rapidly synthesized in response to nutritional stress. In *Escherichia coli*, the enzymes RelA and SpoT are triggered by different starvation signals to produce (p)ppGpp. In many Gram-positive bacteria this is carried out by RelA and two small homologs. (p)ppGpp, along with the transcription factor DksA, has profound effects on transcription initiation in *E. coli*. (p)ppGpp/DksA exert differential effects on promoters by playing upon their intrinsic kinetic parameters, and by facilitating the utilization of alternative sigma factors. (p)ppGpp also regulates replication and translation. These studies highlight (p)ppGpp as a key factor in bacterial physiology that responds rapidly to diverse stresses, by shutting down growth and priming cellular defensive and adaptive processes.

## Addresses

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## Introduction

Guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp) – collectively known as (p)ppGpp, were first identified by Michael Cashel 40 years ago [1] and references therein). These nucleotides were found to accumulate rapidly in *E. coli* cells starved for amino acids, and inhibit synthesis of ribosomal and transfer RNAs [1]. Subsequently (p)ppGpp was found to be induced in other bacteria and plants by multiple stress conditions, in response to which they shut down growth and trigger adaptive responses [1,2]. The ability of cells to produce these small nucleotides profoundly affects cellular processes including transcription, replication and translation, and is important for virulence induction [3–8], differentiation [9] and persistence [10]. The (p)ppGpp-induced starvation response is called the strin-

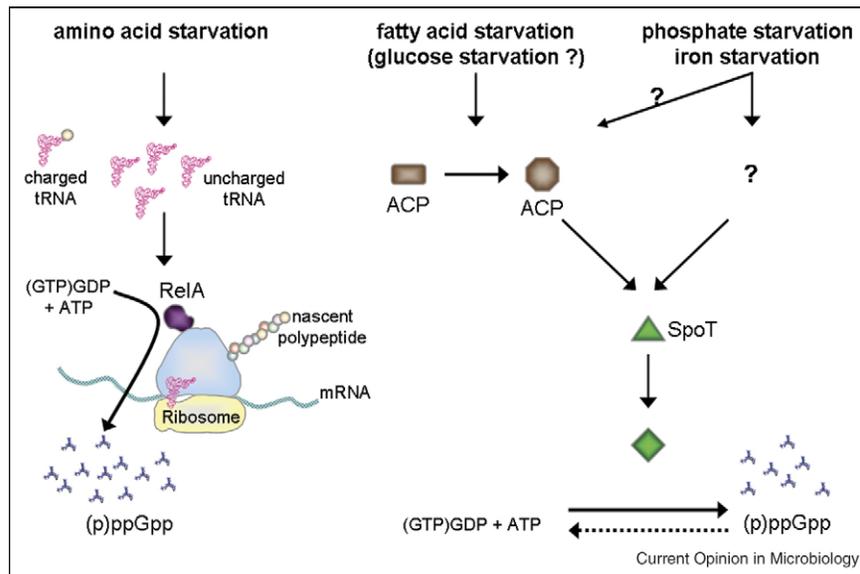
gent response, although (p)ppGpp is likely involved in homeostatic growth control as well. Studies first carried out in *E. coli* established the paradigm of (p)ppGpp-mediated regulation. Ensuing studies in other organisms have revealed variations in both the metabolism of (p)ppGpp and its physiological effects. Here, we outline the progress made over the past few years. A common theme of these studies is the importance of (p)ppGpp because of its ability to modify global cellular metabolism nearly instantaneously in response to changes in the external environment, thus optimizing growth and promoting survival.

## (p)ppGpp metabolism

The first (p)ppGpp synthase to be discovered was the RelA protein that associates with ribosomes in *E. coli* [1]. During amino acid starvation, the binding of uncharged tRNAs to the ribosomal ‘A’ site stalls protein synthesis, enabling an idling reaction in which RelA synthesizes pppGpp/ppGpp from GTP/GDP, respectively, using ATP [1]. Despite the low abundance of RelA (~1/200 ribosomes), up to mM levels of (p)ppGpp are produced rapidly. This is possible because (p)ppGpp synthesis causes dissociation of RelA from the ribosome, allowing RelA to potentially shuttle to another stalled ribosome and repeat the reaction [11].

In addition to amino acid starvation, (p)ppGpp can be induced by other stress conditions, including deprivation of phosphorus, iron, carbon source or fatty acids in a manner that depends on a second protein, SpoT ([1,12,13<sup>••</sup>,14] and references therein) (Figure 1). SpoT both synthesizes (p)ppGpp and hydrolyzes it to GTP/GDP and pyrophosphate. It was recently demonstrated that the acyl carrier protein ACP, an essential cofactor in fatty acid metabolism, physically interacts with SpoT in *E. coli* [13<sup>••</sup>]. This interaction is required for the accumulation of (p)ppGpp in response to fatty acid starvation by shifting the balance between the synthetic and hydrolytic activities of SpoT. The authors raise the interesting possibility that this pathway might also relay information about carbon levels to SpoT, since carbon deprivation would affect glycolysis, leading to fatty acid starvation. The unusually mobile structure of ACP enables it to interact with multiple partners, some outside the realm of fatty acid biosynthesis, and thus ACP might convey diverse inputs to SpoT. These observations, coupled with data that other proteins (such as G-proteins) [15,16] interact with SpoT, support an intriguing model wherein SpoT is the key player that

Figure 1



Metabolism of (p)ppGpp in *E. coli*. (p)ppGpp is synthesized by two enzymes, RelA and SpoT, each of which responds to different environmental cues. Amino acid starvation causes accumulation of uncharged tRNAs, which bind to the ribosomal A site and trigger RelA-mediated synthesis of (p)ppGpp from (GTP)GDP and ATP. SpoT synthesizes and hydrolyzes (p)ppGpp through distinct active sites. Fatty acid starvation or potentially, glucose starvation, triggers a conformational change in the acyl carrier protein (ACP), which binds to SpoT and shifts the balance of its activity towards (p)ppGpp synthesis. Phosphate or iron starvation also results in (p)ppGpp accumulation through modulation of SpoT activity.

monitors the physiological state of *E. coli* cells and tunes levels of (p)ppGpp accordingly.

The Gram-positive bacteria often lack SpoT homologs, but have a single RelA homolog which possesses the ability to both synthesize and hydrolyze (p)ppGpp [17,18]. Structural studies of the catalytic fragment of the bi-functional RelA enzyme from *Streptococcus equisimilis* show that it modulates (p)ppGpp levels through distinct yet negatively coordinated active sites [19]. RelA was considered the only gene involved in the stringent response in these bacteria until recently, when two small RelA homologs capable of (p)ppGpp-synthesis were identified in the Gram-positive bacteria *Streptococcus mutans* [20<sup>\*</sup>] and *Bacillus subtilis* [21<sup>\*</sup>]. Their apparent homologs are found in many Gram-positive bacteria [20<sup>\*</sup>,21<sup>\*</sup>], raising the possibility that tripartite regulation by RelA and two other homologs might be the prevailing mode of (p)ppGpp-metabolism in these bacteria, with each homolog potentially incorporating different cues from the environment.

### (p)ppGpp and regulation of transcription

(p)ppGpp induces profound transcriptional alterations, including the repression of stable RNA (rRNA and tRNA) synthesis and the induction of stress response factors and genes required for amino acid biosynthesis and transport. Microarray profiling shows that the (p)ppGpp-mediated transcriptional re-programming encompasses several hundred genes in each organism [9,22–24]. This phenomenon

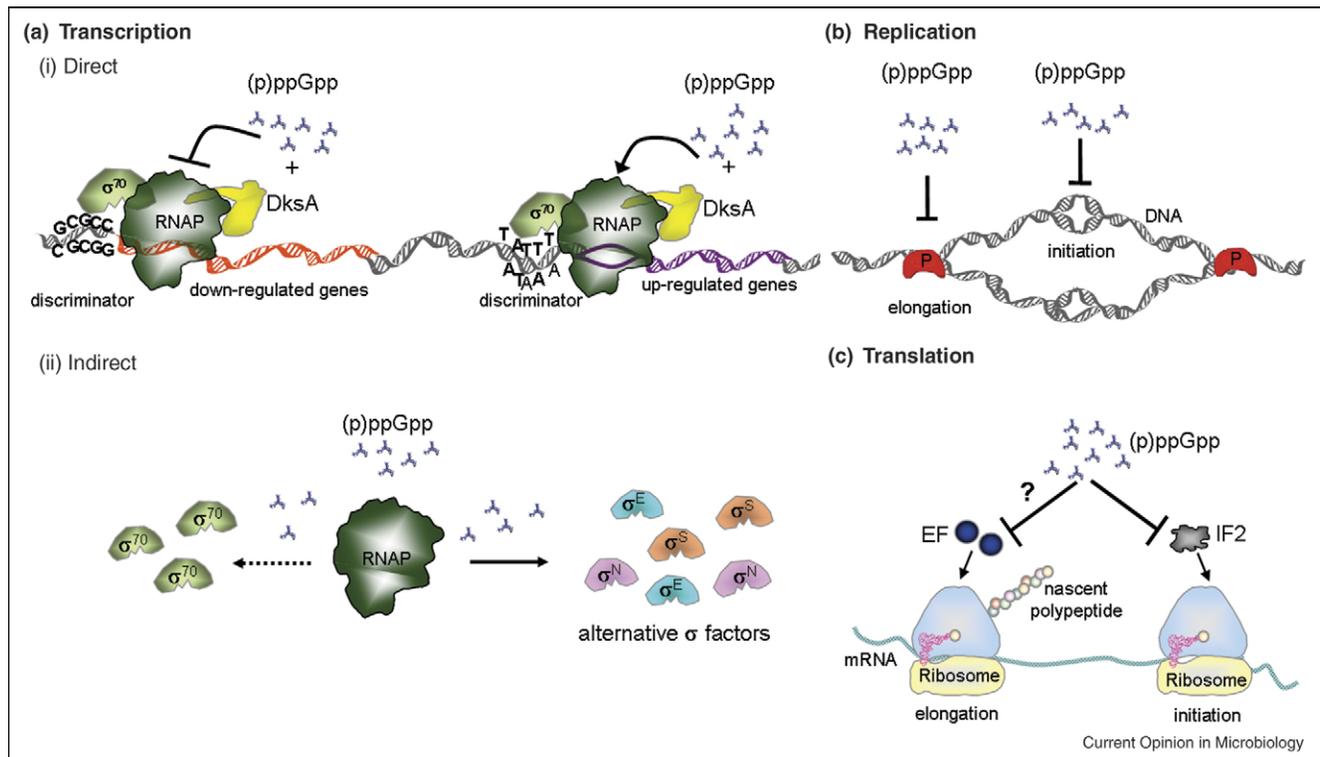
can even be visualized microscopically as loss of RNAP foci at rRNA operons and appearance of a more diffuse signal throughout the *E. coli* nucleoid [25]. Despite the global nature of this change in gene expression, much of it can be explained by the interactions between RNAP, (p)ppGpp, promoters, sigma factors, and a recently identified cofactor called DksA (Figure 2).

### DksA

(p)ppGpp has strong effects on transcription initiation *in vivo*, yet the repression of rRNA transcription by (p)ppGpp *in vitro* does not have the same magnitude, and there appears to be no *in vitro* effect on amino acid synthesis promoters. This dilemma was resolved when the Gourse group discovered that addition of a co-factor DksA (named after its original discovery as a suppressor of *dnaK*) to purified *in vitro* reactions could re-capitulate the *in vivo* effects [26,27]. DksA is required for the *in vivo* effect of (p)ppGpp on transcription from both rRNA and amino acid synthesis promoters, and therefore is crucial for the stringent response in *E. coli*.

The discovery of the role of DksA provides a new context for studying how (p)ppGpp affects transcription initiation. ppGpp can bind RNAP and directly regulate its function. However, a point of contention is whether the binding site identified in the ppGpp – *Thermus thermophilus* RNAP co-crystal [28] is the one responsible for its effects on transcription [57]. DksA is structurally similar to the transcription factor GreA/B [29] which

Figure 2



(p)ppGpp regulates transcription, replication and translation. **(a) Transcription:** **(i)** (p)ppGpp/DksA directly inhibit the transcription of rRNA genes by destabilizing the promoter-RNAP open complexes formed during initiation. These promoters form inherently unstable open complexes with RNAP. On the contrary, genes that are upregulated by (p)ppGpp/DksA have promoters that form stable open complexes with RNAP. (p)ppGpp/DksA directly induce expression of these genes, likely by increasing the rate of formation of RNAP-promoter open complexes (shown as a purple bubble). **(ii)** (p)ppGpp indirectly activates the expression of many stress-induced genes by releasing RNAP from  $\sigma^{70}$ -dependent promoters, and facilitating the use of alternative  $\sigma$  factors (such as  $\sigma^S$ ,  $\sigma^E$  and  $\sigma^N$ ). **(b) Replication:** (p)ppGpp rapidly inhibits replication initiation (in *E. coli*) and elongation (in *B. subtilis*) in response to nutrient stress. Initiation is regulated indirectly through inhibition of transcription. Elongation is regulated by (p)ppGpp independent of transcription, and the likely target is the primase (P). **(c) Translation:** (p)ppGpp inhibits translation by binding and interfering with the functions of the initiation factor IF2, and also potentially inhibiting the elongation factors EF-Tu and EF-G (EF).

binds RNAP by extending a coiled-coil domain into the regulatory 'secondary' channel of the polymerase [30]. DksA likely binds RNAP similarly [29]. However, neither the details of how DksA binds to RNAP, nor how it stimulates the effects of (p)ppGpp on RNAP, are clear at this point. Furthermore, there are situations when DksA affects RNAP activity independently of (p)ppGpp [31].

#### rRNA vs. amino acid biosynthesis promoters

The insight into DksA function explains the discrepancies between the *in vivo* and *in vitro* effects of (p)ppGpp. Yet what determines which promoters are upregulated and which are downregulated by (p)ppGpp? Studies comparing rRNA and amino acid biosynthesis promoters offer a coherent model that regulation occurs in a promoter-specific manner, and that the distinguishing characteristics appear to be the inherent kinetic parameters of the promoters [27,32,33,34]. The rRNA promoters have a GC-rich

'discriminator' sequence between the -10 element and the transcription start site, that has a sequence-specific suboptimal interaction with the conserved 1.2 region of the  $\sigma$ -subunit of RNAP. These promoters form extremely unstable open complexes with RNAP during transcription initiation [34,35]. ppGpp/DksA lower the stability of all open complexes and thus the intrinsically unstable rRNA open complexes are further destabilized and transcription initiation is inhibited [33]. Unlike rRNA promoters, the amino acid biosynthesis promoters have an AT-rich discriminator whose sequence allows optimal binding with the 1.2 region of  $\sigma$ . Once open complexes are formed at amino acid biosynthesis promoters, they are intrinsically stable enough to cope with the (p)ppGpp-mediated destabilization. Meanwhile, (p)ppGpp/DksA increase the rate of formation of open complexes at these promoters [27], thus stimulating transcription initiation. At rRNA promoters this rate is sufficiently high and does not require (p)ppGpp/DksA-dependent stimulation.

### Indirect effects on transcription

In addition to direct induction, (p)ppGpp can indirectly activate amino acid biosynthesis promoters by releasing RNAP from rRNA promoters [32,35]. It is possible that *in vivo*, both direct and indirect pathways are operative, potentially to different levels at different promoters. The proportionate contribution of each pathway at individual promoters remains to be determined.

An important mechanism by which (p)ppGpp induces global changes in transcription initiation is by altering the utilization of sigma factors. (p)ppGpp frees RNAP from  $\sigma^{70}$ -dependent genes to shift the transcriptional balance towards genes dependent on alternative  $\sigma$  factors [23,24,36–39,40<sup>\*</sup>]. In the case of the stationary-phase-specific sigma factor  $\sigma^S$ , in addition to the indirect effect mentioned above [38], (p)ppGpp also induces  $\sigma^S$  transcription [1,24], and increases  $\sigma^S$  stability [41<sup>\*\*</sup>].  $\sigma^S$  levels can be stabilized by the protein IraP that counteracts RssB, an adaptor protein that delivers  $\sigma^S$  to the ClpXP protease for degradation. (p)ppGpp promotes the stability of  $\sigma^S$  in response to phosphate starvation by increasing the transcription of *iraP*, which has an AT-rich discriminator sequence [41<sup>\*\*</sup>,42]. (p)ppGpp/DksA also appear to affect the activity of the extracytoplasmic stress factor  $\sigma^E$  by both directly and indirectly activating  $\sigma^E$ -dependent transcription [40<sup>\*</sup>].

In conclusion, (p)ppGpp in concert with DksA affects transcription initiation, by differentiating between the intrinsic kinetic properties of promoters, and by freeing RNAP for utilization by alternative sigma factors, resulting in global alterations of gene expression in *E. coli*.

### Other bacteria

In other bacteria, the mode of transcriptional regulation by (p)ppGpp can be different from *E. coli*. For example, in *B. subtilis*, the effect of (p)ppGpp on rRNA transcription appears to be indirect and independent of DksA homologs [43]. The *B. subtilis* rRNA promoters are insensitive to (p)ppGpp *in vitro*, but like *E. coli* rRNA promoters, are sensitive to the concentration of the initiating nucleotide (GTP in *B. subtilis* and GTP, CTP or ATP in *E. coli*). Upon amino acid starvation in *B. subtilis*, (p)ppGpp inhibits production of GTP by targeting the enzyme inosine monophosphate dehydrogenase that catalyzes an early step in GTP biosynthesis. Consequently, the rRNA promoters are downregulated because of decreased GTP.

### (p)ppGpp and regulation of translation

(p)ppGpp inhibits translation by repressing transcription of the protein synthesis machinery including tRNA, rRNA and ribosomal proteins. In addition, (p)ppGpp might inhibit the activity of the translation

elongation factors EF-Tu and EF-G *in vitro* [1]. Recent evidence [44<sup>\*</sup>] shows that (p)ppGpp also interacts with the initiation factor IF2 and inhibits translation initiation by preventing IF2-dependent formation of both the 30s translation initiation complex and the initiation di-peptide. Both GTP and ppGpp bind the same site on IF2, however the negatively charged 3'-diphosphate moiety of ppGpp protrudes out of IF2, potentially interfering with its function. The authors propose a model in which IF2 oscillates between a GTP-bound active form during growth and a ppGpp-bound inactive form under nutrient starvation, thus acting as a metabolic sensor to control translation accordingly.

### (p)ppGpp and genome integrity/evolvability

A fundamental requirement for genome integrity is accurate genome duplication. This in turn requires the process of DNA replication to be regulated upon the onset of nutritional or other stresses. Not surprisingly, the stringent response affects DNA replication. In *E. coli*, replication initiation is inhibited by (p)ppGpp [45,46]. In *B. subtilis* replication elongation is inhibited by (p)ppGpp [46] irrespective of the position of the replication forks along the chromosome [47<sup>\*\*</sup>]. The (p)ppGpp-induced arrest is likely to be mediated through primase and does not lead to recruitment of the recombination protein RecA. Thus replication can be rapidly suspended until favorable conditions are restored. It remains to be examined whether this replication control helps to protect genome integrity during transient stress and/or sporulation, or enables evolvability through stress-induced mutagenesis, which is known to be facilitated by (p)ppGpp [48,49].

In *E. coli*, (p)ppGpp is proposed to help maintain genomic integrity by resolving conflicts between replication and transcription [50]. Stalling of RNAP at DNA lesions can lead to backed-up arrays of RNAP that pose formidable blocks to replication. There is evidence for the model that (p)ppGpp acts by destabilizing stalled RNAP elongation complexes, thus decreasing the likelihood that they impede replication fork progression [50]. A somewhat conflicting view comes from the observation that (p)ppGpp also induces transcription elongation pausing [51,52], which can hinder replication and trigger DNA damage responses. Indeed, the SOS response is induced during the stringent response in *E. coli* [24]. There is also evidence for a connection between the G-proteins (e.g. Obg, IF2) that are regulated by (p)ppGpp [44<sup>\*</sup>,53], and DNA metabolism and/or chromosome dynamics [54,55]. Although a consensus has not been reached to sufficiently explain these observations, an intriguing possibility is that by bringing replication, transcription, translation and DNA repair pathways together, (p)ppGpp modulates the genomic stability and evolvability of microbes in response to stress [56].

## Conclusion

Information flow in cells depends on replication, transcription and translation. By directly or indirectly modulating these processes, their interactions and their responses to environmental changes, (p)ppGpp is crucial for the survival and propagation of many bacterial species. The studies discussed here have provided important insights and also revealed the confounding complexity of bacterial physiology. Complete understanding of how (p)ppGpp, the 'magic spot' affects cellular physiology remains an exciting challenge for basic and applied research.

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