*Mini-review*

# (p)ppGpp and the bacterial cell cycle

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Genes of the Rel/Spo homolog (RSH) superfamily synthesize and/or hydrolyse the modified nucleotides pppGpp/ppGpp (collectively referred to as (p)ppGpp) and are prevalent across diverse bacteria and in plant chloroplasts. Bacteria accumulate (p)ppGpp in response to nutrient deprivation (generically called the stringent response) and elicit appropriate adaptive responses mainly through the regulation of transcription. Although at different concentrations (p)ppGpp affect the expression of distinct set of genes, the two well-characterized responses are reduction in expression of the protein synthesis machinery and increase in the expression of genes coding for amino acid biosynthesis. In *Escherichia coli*, the cellular (p)ppGpp level inversely correlates with the growth rate and increasing its concentration decreases the steady state growth rate in a defined growth medium. Since change in growth rate must be accompanied by changes in cell cycle parameters set through the activities of the DNA replication and cell division apparatus, (p)ppGpp could coordinate protein synthesis (cell mass increase) with these processes. Here we review the role of (p)ppGpp in bacterial cell cycle regulation.

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## 1. Introduction

The bacterial cell cycle can be divided chronologically from the time a cell is borne into at least three phases. These are (i) time taken to initiate DNA replication after cell division (birth of cell), called the 'B' phase and is akin to the G1 phase of eukaryotic cell; (ii) a period of DNA synthesis, which is called the 'C' phase and is akin to the S-phase of eukaryotic cells; (iii) time after the completion of DNA synthesis to division into two daughter cells, called the 'D' phase and which is akin to the G2-phase in eukaryotic cells. These three phases of cell cycle are evident in a slow growing bacterium where the initiation of a new round of DNA replication does not precede cell division (>60 min). In rich medium, bacteria can divide with a doubling time of 20 to 25 min which is much faster than the time required for DNA synthesis (C) and cell division (D) and this is made possible due to replication re-initiation before the completion of a previous round of replication. That is, replication initiation and DNA synthesis overlap with the segregation of replicated genome and cell division (Helmstetter 1996). In such scenario, the time interval between the two replication initiation events matches the doubling time of the cell. Here we review the role of the modified nucleotides, (p)ppGpp, whose concentration change with the growth rate (Cashel *et al.* 1996), in the regulation of cell cycle mainly from the studies carried out in the bacterial model organism *Escherichia coli*. To our knowledge, studies on the relationship between macromolecular composition or (p)ppGpp concentration with growth rate have not been performed in *Bacillus subtilis* unlike in *Escherichia coli* (Bremer and Dennis 1996). A cartoon of the cell cycle depicting the steps regulated by (p)ppGpp and discussed in this review is shown in figure 1.

## 2. Regulation in the B period

Under normal growth conditions, initiation of replication in *E. coli* occurs at the *oriC* locus and terminates at the *ter*

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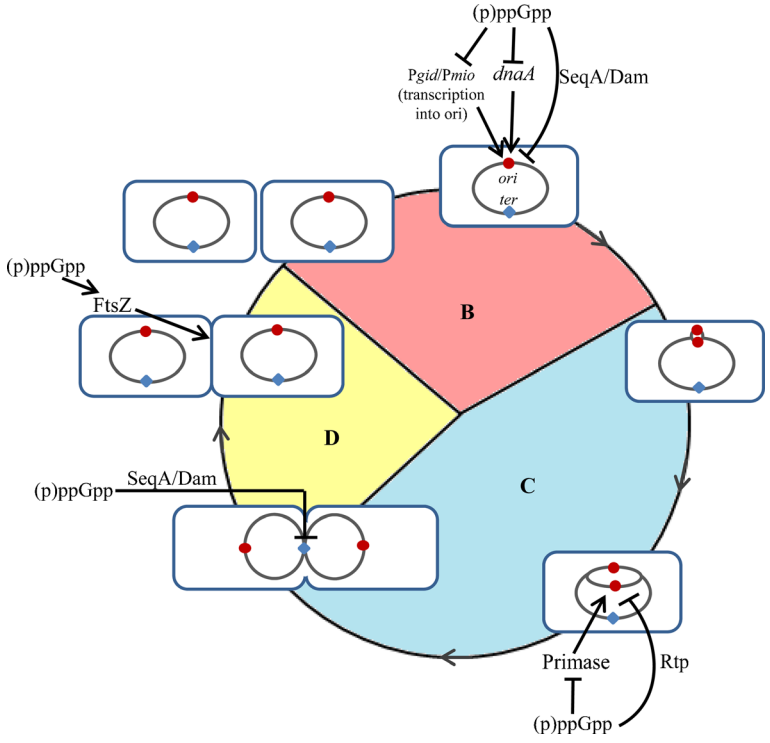


**Figure 1.** Cartoon of the bacterial cell cycle depicting (p)ppGpp-mediated regulation. Under slow growth conditions the bacterial cell cycle can be divided into at least three distinct stages: B refers to the period between cell division (birth of new cell) to initiation of chromosome replication; C refers to the period required for chromosome replication; and D refers to the period between completion of replication and the completion of cell division. The nature of (p)ppGpp-mediated regulation is depicted and these could be direct or indirect, see text for details. The arrow indicates positive regulation and the line ending with a 'T', negative regulation.

locus. DnaA protein is essential for the process of replication initiation at *oriC* (Messer and Weigel 1996). Overproduction of DnaA stimulates replication initiation so that the number of origins and the DNA content per unit mass increases (Atlung *et al.* 1987; Xu and Bremer 1988) and the cell mass at which initiation takes place decreases (Lobner-Olesen *et al.* 1989). In *E. coli*, but not in *Bacillus subtilis*, the cell-growth-dependent accumulation of DnaA-ATP to critical levels determines the timing of initiation (Hill *et al.* 2012). Although the level of DnaA-ATP protein is important, a recent study indicates it is not the only factor limiting initiation of replication in *E. coli* (Flatten *et al.* 2015). Transcription of *dnaA* operon is diminished following amino acid starvation and in the mutants with elevated basal (p)ppGpp levels. The growth-rate-regulated *dnaA* p2 promoter is the target of inhibition and proposed to be responsible for its growth rate regulation (Chiaramello and Zyskind 1989, 1990; Hernandez and Bremer 1990). It remains to be studied if the effect of (p)ppGpp is direct or indirect. Transcription by RNA polymerase (RNAP) is required at an early stage of initiation of replication *in vivo* (Lark 1972). Transcripts entering the *oriC* locus were, respectively, decreased or increased by conditions that increase or decrease (p)ppGpp (Rokeach and Zyskind 1986). Transcription from the *oriC* proximal P*gid* and P*mioC* promoters are required for the replication of *oriC* plasmids, and *in vitro*, transcription from these promoters is inhibited by (p)ppGpp (Ogawa and Okazaki 1991). In *E. coli*, the frequency of replication initiation is linked to the cell mass such that the initiation takes place at every copy of *oriC* at each successive doubling of a fixed unit mass (or volume) (Donachie 1968; Helmstetter and Leonard 1987; Bremer and Dennis 1996). Results suggestive of a role for (p)ppGpp in the regulation of initiation mass are, (i) following successive amino acid starvation, cells 30% smaller than unstarved newborn cells could still initiate replication while cell division was delayed (Grossman and Ron 1989); (ii) replication initiates at lower mass in cells lacking (p)ppGpp under fast growth conditions (Hernandez and Bremer 1993) and at higher cell mass in slow growth conditions (Wold *et al.* 1994). The stringent response blocks DNA replication outside the *ori* region in *B. subtilis* and at the origin in *E. coli* (Levine *et al.* 1991; Schreiber *et al.* 1995). *E. coli* cells subjected to the stringent response arrest with integer

number of chromosomes, indicating arrest of DNA replication initiation (Ferullo and Lovett 2008; Ferullo *et al.* 2009).

## 3. Regulation in the C period

Replication termination protein (Rtp)-mediated arrest approximately 100–200 kbp to the left and right of *oriC* was noted in *B. subtilis* following the stringent response (Levine *et al.* 1995; Autret *et al.* 1999). However, Rtp-independent arrest is also observed past this site and throughout the chromosome following amino acid starvation (Wang *et al.* 2007); the replication forks were non-disruptively arrested and likely from the direct inhibition of primase activity by (p)ppGpp. Like the *B. subtilis* primase, activity of *E. coli* primase is inhibited by (p)ppGpp *in vitro* (Maciag *et al.* 2010); unlike in *B. subtilis*, inhibition by ppGpp is stronger than by pppGpp. Only modest inhibition of replication elongation following stringent response is observed in *E. coli*, the effect is exacerbated in the *gppA* mutant that increases the pppGpp to ppGpp ratio, however, the magnitude of the effect is less than that observed in *B. subtilis* (Denapoli *et al.* 2013). The low concentration of GTP during stringent response in *B. subtilis* is due to direct and potent inhibition of its biosynthesis by (p)ppGpp and could be a factor contributing to the more severe inhibition (Lopez *et al.* 1981; Kriel *et al.* 2012). An increase in the (p)ppGpp to ppGpp ratio after stringent response is seen in a hypomorphic *obgE* mutant and is associated with replication defect (Persky *et al.* 2009). The modest effect on replication reported in *E. coli* is consistent with the integer number of chromosomes observed after stringent response (Ferullo and Lovett 2008); a similar study has not been conducted in *B. subtilis*.

## 4. Regulation in the D period

The study by Ferullo and Lovett (2008) indicates stringent response inhibits chromosome segregation in addition to replication initiation. Depletion of ObgE, a ribosome associated GTPase that binds (p)ppGpp, similarly inhibits segregation (Foti *et al.* 2007). Following the stringent response, frequency of marker near *oriC* is same as that near *ter*, suggesting completion of ongoing replication and the absence of initiation. However, when chromosome segregation was studied by fluorescence microscopy using *parS* sequence and GFP-ParB, the number of foci at *ter* region was half of that noted for the *ori* region, indicating prolonged cohesion of the DNA at the *ter* region following the completion of replication. During recovery from the stringent response, in cells with multiple nucleoids, segregation occurred first at the mid-cell and later at the quarter positions. The authors suggest the prolonged cohesion at the termini could be a means to enforce sequential segregation by restraining segregation of recent sister chromosomes until all others have segregated (Ferullo and Lovett 2008). Stringent arrest of chromosome replication is not observed in the *dam* or *seqA* background, and is partially restored in genetic backgrounds that specifically prevent SeqA action at the *oriC* or mimic SeqA action at the oriC (in absence of SeqA). The authors propose that the DNA methylation and SeqA binding to the non-origin loci is necessary to enforce a full stringent arrest, affecting both the initiation of replication and the chromosome segregation (Ferullo and Lovett 2008).

Characterization of the ppGpp$^0$ strain showed it to filament upon nutritional down shift, suggesting a role for (p)ppGpp in the process of cell division (Xiao *et al.* 1991). Insights of its role in cell division came from the studies carried out in the D'Ari lab to understand the function of PBP2 (penicillin binding protein 2), which is a murein synthase encoded by *pbpA* and required for the lateral elongation of murein (peptidoglycan). When PBP2 is inactivated using the β-lactam antibiotic mecillinam, cells become spherical, growth ceases and the cells die. It was found that the cell death resulted from the inability of the cells to divide, and the elevated levels of the division proteins FtsQ, FtsA and FtsZ rescued the growth (Vinella *et al.* 1993). RelA mediated elevation of (p)ppGpp by the partial inactivation of aminoacyl-tRNA synthetase that mimics amino acid starvation also restored growth (Vinella *et al.* 1992). Using the truncated RelA peptide fragment that constitutively produces (p)ppGpp, it was shown that the elevation of (p)ppGpp was sufficient to rescue growth. Mecillinam resistance could be obtained in the slow growth conditions where (p)ppGpp levels are high (Joseleau-Petit *et al.* 1994) and in the *relA* mutant strains due to increased SpoT-mediated (p)ppGpp synthesis following iron deprivation (Vinella *et al.* 2005). Together the results indicate that a threshold level of (p)ppGpp is required to confer mecillinam resistance. Since increase in expression of the *ftsQAZ* operon or increase in the (p)ppGpp levels permit coccal growth, it was speculated that the latter positively regulates the expression of the cell division genes. Studies have failed to provide convincing evidence for the regulation of these genes by (p)ppGpp either at the level of transcription or FtsZ protein content (Navarro *et al.* 1998).

Evidence for positive regulation of cell division by (p)ppGpp also came from studying the genetic suppressors that show RelA-dependent rescue of the heat-sensitive cell division defect of the *ftsZ84* allele in low salt medium (Powell and Court 1998). Constitutive (p)ppGpp synthesis was sufficient for restoring growth and was associated with 3- to 4-fold increase in FtsZ84 levels, although activation of transcription from the isolated pQ or pZ promoters was not observed. In the *ftsZ84* mutant lacking (p)ppGpp, i.e. in the Δ*relA* Δ*spoT* (ppGpp$^0$) genetic background, *rpoD**

mutations that convert RNAP into a form that behaves as though it has been modified by (p)ppGpp conferred RpoS-independent suppression. Since RNAP is a well studied target of (p)ppGpp (Reddy *et al.* 1995; Cashel *et al.* 1996; Chatterji *et al.* 1998; Mechold *et al.* 2013; Ross *et al.* 2013; Zuo *et al.* 2013), models were proposed by which (p)ppGpp could directly or indirectly regulate the transcription of the *ftsQAZ* operon in the native chromosomal context. Compared to the wild type or the *relA* mutant, the size of $ppGpp^0$ cells are significantly longer and the FtsZ content normalized to cell mass is reduced, indicating positive regulation of FtsZ by basal (p)ppGpp under normal growth conditions. Consistent with the lowered FtsZ content, cell septation in the $ppGpp^0$ cells becomes extremely vulnerable to SulA-mediated inhibition as seen following the deletion of *lon* (Nazir and Harinarayanan 2015). It seems, in the physiological context, functions regulated by basal (p)ppGpp are masked by the existence of redundant regulation and synthetic lethal screens could be used to identify such functions, as exemplified by an earlier study (Harinarayanan *et al.* 2008).

Negative correlation is observed between cell size and (p)ppGpp levels, slow growing cells and those entering stationary phase in rich media are smaller in size and have higher (p)ppGpp concentration (Schaechter *et al.* 1958; Cashel *et al.* 1996). One report documented an increase in FtsZ levels associated with decrease in growth rate (Aldea *et al.* 1990) consistent with a positive regulatory role for (p)ppGpp in FtsZ expression. However, another study found FtsZ levels to remain unchanged with respect to growth rate in *B. subtilis* and *E. coli* (Weart and Levin 2003) and evidence for a nutrient-dependent inhibitor of FtsZ ring formation under fast growth conditions has been presented in *E. coli* and *B. subtilis* (Weart *et al.* 2007; Chien *et al.* 2012; Hill *et al.* 2013).

## 5. Concluding remark

It is well established that a change in the steady state growth rate follows from the changes in the parameters associated with the different periods of the cell cycle. (p)ppGpp concentration varies inversely with the growth rate (Lazzarini *et al.* 1971; Friesen *et al.* 1975; Sokawa *et al.* 1975; Potrykus *et al.* 2011) and changing the basal (p)ppGpp concentration inversely affects growth rate in defined growth medium (Sarubbi *et al.* 1988). The latter finding suggests an active role for (p)ppGpp in cell cycle regulation. This is further evident from the studies reviewed here that show changing cellular (p)ppGpp levels affect different aspects of the cell cycle. Since the protein synthesis machinery is regulated by (p)ppGpp (Cashel *et al.* 1996), it is well suited to co-ordinate cell cycle to the rate of protein synthesis and thereby to the cell mass. As (p)ppGpp shows concentration dependent effects, studying cell cycle following incremental changes in (p)ppGpp levels could reveal new regulatory aspects of this molecule.

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## References

Aldea M, Garrido T, Pla J and Vicente M 1990 Division genes in *Escherichia coli* are expressed coordinately to cell septum requirements by gearbox promoters. *EMBO J.* **9** 3787–3794

Atlung T, Lobner-Olesen A and Hansen FG 1987 Overproduction of DnaA protein stimulates initiation of chromosome and minichromosome replication in *Escherichia coli. Mol. Gen. Genet.* **206** 51–59

Autret S, Levine A, Vannier F, Fujita Y and Seror SJ 1999 The replication checkpoint control in *Bacillus subtilis*: identification of a novel RTP-binding sequence essential for the replication fork arrest after induction of the stringent response. *Mol. Microbiol.* **31** 1665–1679

Bremer H and Dennis PP 1996 Modulation of chemical composition and other parameters of the cell by growth rate; in *Escherichia coli and Salmonella: cellular and molecular biology* 2nd ed. (eds) FC Neidhardt, JL Ingraham, ECC Lin, KB Low, B Magasanik, WS Reznikoff, M Riley, M Schaechter and HE Umbarger (Washington, DC: ASM Press) pp 1553–1569

Cashel M, Gentry D, Hernandez VJ and Vinella D 1996 The stringent response; in *Escherichia coli and Salmonella: cellular and molecular biology* 2nd ed. (eds) FC Neidhardt, JL Ingraham, ECC Lin, KB Low, B Magasanik, WS Reznikoff, M Riley, M Schaechter and HE Umbarger (Washington, DC: ASM Press) pp 1458–1496

Chatterji D, Fujita N and Ishihama A 1998 The mediator for stringent control, ppGpp, binds to the beta-subunit of *Escherichia coli* RNA polymerase. *Genes Cells* **3** 279–287

Chiaramello AE and Zyskind JW 1989 Expression of *Escherichia coli dnaA* and *mioC* genes as a function of growth rate. *J. Bacteriol.* **171** 4272–4280

Chiaramello AE and Zyskind JW 1990 Coupling of DNA replication to growth rate in *Escherichia coli*: a possible role for guanosine tetraphosphate. *J. Bacteriol.* **172** 2013–2019

Chien AC, Hill NS and Levin PA 2012 Cell size control in bacteria. *Curr. Biol.* **22** R340–R349

Denapoli J, Tehranchi AK and Wang JD 2013 Dose-dependent reduction of replication elongation rate by (p)ppGpp in *Escherichia coli* and *Bacillus subtilis. Mol. Microbiol.* **88** 93–104

Donachie WD 1968 Relationship between cell size and time of initiation of DNA replication. *Nature* **219** 1077–1079

Ferullo DJ and Lovett ST 2008 The stringent response and cell cycle arrest in *Escherichia coli*. *PLoS Genet.* **4** e1000300

Ferullo DJ, Cooper DL, Moore HR and Lovett ST 2009 Cell cycle synchronization of *Escherichia coli* using the stringent response, with fluorescence labeling assays for DNA content and replication. *Methods* **48** 8–13

Flatten I, Fossum-Raunehaug S, Taipale R, Martinsen S and Skarstad K 2015 The DnaA protein is not the limiting factor for initiation of replication in *Escherichia coli*. *PLoS Genet.* **11** e1005276

Foti JJ, Persky NS, Ferullo DJ and Lovett ST 2007 Chromosome segregation control by *Escherichia coli* ObgE GTPase. *Mol. Microbiol.* **65** 569–581

Friesen JD, Fiil NP and von Meyenburg K 1975 Synthesis and turnover of basal level guanosine tetraphosphate in *Escherichia coli*. *J. Biol. Chem.* **250** 304–309

Grossman N and Ron EZ 1989 Apparent minimal size required for cell division in *Escherichia coli*. *J. Bacteriol.* **171** 80–82

Harinarayanan R, Murphy H and Cashel M 2008 Synthetic growth phenotypes of *Escherichia coli* lacking ppGpp and transketolase A (*tktA*) are due to ppGpp-mediated transcriptional regulation of *tktB*. *Mol. Microbiol.* **69** 882–894

Helmstetter CE 1996 Initiation of chromosome replication; in *Escherichia coli and Salmonella: cellular and molecular biology* 2nd ed. (eds) FC Neidhardt, JL Ingraham, ECC Lin, KB Low, B Magasanik, WS Reznikoff, M Riley, M Schaechter and HE Umbarger (Washington, DC: ASM Press) pp 1627–1639

Helmstetter CE and Leonard AC 1987 Coordinate initiation of chromosome and minichromosome replication in *Escherichia coli*. *J. Bacteriol.* **169** 3489–3494

Hernandez VJ and Bremer H 1990 Guanosine tetraphosphate (ppGpp) dependence of the growth rate control of *rrnB* P1 promoter activity in *Escherichia coli*. *J. Biol. Chem.* **265** 11605–11614

Hernandez VJ and Bremer H 1993 Characterization of RNA and DNA synthesis in *Escherichia coli* strains devoid of ppGpp. *J. Biol. Chem.* **268** 10851–10862

Hill NS, Kadoya R, Chattoraj DK and Levin PA 2012 Cell size and the initiation of DNA replication in bacteria. *PLoS Genet.* **8** e1002549

Hill NS, Buske PJ, Shi Y and Levin PA 2013 A moonlighting enzyme links *Escherichia coli* cell size with central metabolism. *PLoS Genet.* **9** e1003663

Joseleau-Petit D, Thevenet D and D'Ari R 1994 ppGpp concentration, growth without PBP2 activity, and growth-rate control in *Escherichia coli*. *Mol. Microbiol.* **13** 911–917

Kriel A, Bittner AN, Kim SH, Liu K, Tehranchi AK, Zou WY, Rendon S, Chen R, *et al*. 2012 Direct regulation of GTP homeostasis by (p)ppGpp: a critical component of viability and stress resistance. *Mol. Cell* **48** 231–241

Lark KG 1972 Evidence for the direct involvement of RNA in the initiation of DNA replication in *Escherichia coli* 15T. *J. Mol. Biol.* **64** 47–60

Lazzarini RA, Cashel M and Gallant J 1971 On the regulation of guanosine tetraphosphate levels in stringent and relaxed strains of *Escherichia coli*. *J. Biol. Chem.* **246** 4381–4385

Levine A, Vannier F, Dehbi M, Henckes G and Seror SJ 1991 The stringent response blocks DNA replication outside the *ori* region in Bacillus subtilis and at the origin in *Escherichia coli*. *J. Mol. Biol.* **219** 605–613

Levine A, Autret S and Seror SJ 1995 A checkpoint involving RTP, the replication terminator protein, arrests replication downstream of the origin during the stringent response in *Bacillus subtilis*. *Mol. Microbiol.* **15** 287–295

Lobner-Olesen A, Skarstad K, Hansen FG, von Meyenburg K and Boye E 1989 The DnaA protein determines the initiation mass of *Escherichia coli* K-12. *Cell* **57** 881–889

Lopez JM, Dromerick A and Freese E 1981 Response of guanosine 5'-triphosphate concentration to nutritional changes and its significance for *Bacillus subtilis* sporulation. *J. Bacteriol.* **146** 605–613

Maciag M, Kochanowska M, Lyzen R, Wegrzyn G and Szalewska-Palasz A 2010 ppGpp inhibits the activity of *Escherichia coli* DnaG primase. *Plasmid* **63** 61–67

Mechold U, Potrykus K, Murphy H, Murakami KS and Cashel M 2013 Differential regulation by ppGpp versus pppGpp in *Escherichia coli*. *Nucleic Acids Res.* **41** 6175–6189

Messer W and Weigel C 1996 Initiation of chromosome replication; in *Escherichia coli and Salmonella: cellular and molecular biology* 2nd ed. (eds) FC Neidhardt, JL Ingraham, ECC Lin, KB Low, B Magasanik, WS Reznikoff, M Riley, M Schaechter and HE Umbarger (Washington, DC: ASM Press) pp 1579–1601

Navarro F, Robin A, D'Ari R and Joseleau-Petit D 1998 Analysis of the effect of ppGpp on the *ftsQAZ* operon in *Escherichia coli*. *Mol. Microbiol.* **29** 815–823

Nazir A and Harinarayanan R 2015 Inactivation of cell division protein FtsZ by SulA makes Lon indispensable for the viability of a ppGpp$^0$ strain of *Escherichia coli*. *J. Bacteriol.* **198** 688–700

Ogawa T and Okazaki T 1991 Concurrent transcription from the *gid* and *mioC* promoters activates replication of an *Escherichia coli* minichromosome. *Mol. Gen. Genet.* **230** 193–200

Persky NS, Ferullo DJ, Cooper DL, Moore HR and Lovett ST 2009 The ObgE/CgtA GTPase influences the stringent response to amino acid starvation in *Escherichia coli*. *Mol. Microbiol.* **73** 253–266

Potrykus K, Murphy H, Philippe N and Cashel M 2011 ppGpp is the major source of growth rate control in *E. coli*. *Environ. Microbiol.* **13** 563–575

Powell BS and Court DL 1998 Control of *ftsZ* expression, cell division, and glutamine metabolism in Luria-Bertani medium by the alarmone ppGpp in *Escherichia coli*. *J. Bacteriol.* **180** 1053–1062

Reddy PS, Raghavan A and Chatterji D 1995 Evidence for a ppGpp-binding site on *Escherichia coli* RNA polymerase: proximity relationship with the rifampicin-binding domain. *Mol. Microbiol.* **15** 255–265

Rokeach LA and Zyskind JW 1986 RNA terminating within the *E. coli* origin of replication: stringent regulation and control by DnaA protein. *Cell* **46** 763–771

Ross W, Vrentas CE, Sanchez-Vazquez P, Gaal T and Gourse RL 2013 The magic spot: a ppGpp binding site on *E. coli* RNA polymerase responsible for regulation of transcription initiation. *Mol. Cell* **50** 420–429

Sarubbi E, Rudd KE and Cashel M 1988 Basal ppGpp level adjustment shown by new *spoT* mutants affect steady state growth rates and *rrnA* ribosomal promoter regulation in *Escherichia coli*. *Mol. Gen. Genet.* **213** 214–222

Schaechter M, Maaloe O and Kjeldgaard NO 1958 Dependency on medium and temperature of cell size and chemical composition during balanced grown of *Salmonella typhimurium*. *J. Gen. Microbiol.* **19** 592–606

Schreiber G, Ron EZ and Glaser G 1995 ppGpp-mediated regulation of DNA replication and cell division in *Escherichia coli*. *Curr. Microbiol.* **30** 27–32

Sokawa Y, Sokawa J and Kaziro Y 1975 Regulation of stable RNA synthesis and ppGpp levels in growing cells of *Escherichia coli*. *Cell* **5** 69–74

Vinella D, D'Ari R, Jaffe A and Bouloc P 1992 Penicillin binding protein 2 is dispensable in *Escherichia coli* when ppGpp synthesis is induced. *EMBO J.* **11** 1493–1501

Vinella D, Joseleau-Petit D, Thevenet D, Bouloc P and D'Ari R 1993 Penicillin-binding protein 2 inactivation in *Escherichia coli* results in cell division inhibition, which is relieved by FtsZ overexpression. *J. Bacteriol.* **175** 6704–6710

Vinella D, Albrecht C, Cashel M and D'Ari R 2005 Iron limitation induces SpoT-dependent accumulation of ppGpp in *Escherichia coli*. *Mol. Microbiol.* **56** 958–970

Wang JD, Sanders GM and Grossman AD 2007 Nutritional control of elongation of DNA replication by (p)ppGpp. *Cell* **128** 865–875

Weart RB and Levin PA 2003 Growth rate-dependent regulation of medial FtsZ ring formation. *J. Bacteriol.* **185** 2826–2834

Weart RB, Lee AH, Chien AC, Haeusser DP, Hill NS and Levin PA 2007 A metabolic sensor governing cell size in bacteria. *Cell* **130** 335–347

Wold S, Skarstad K, Steen HB, Stokke T and Boye E 1994 The initiation mass for DNA replication in *Escherichia coli* K-12 is dependent on growth rate. *EMBO J.* **13** 2097–2102

Xiao H, Kalman M, Ikehara K, Zemel S, Glaser G and Cashel M 1991 Residual guanosine 3',5'-bispyrophosphate synthetic activity of *relA* null mutants can be eliminated by *spoT* null mutations. *J. Biol. Chem.* **266** 5980–5990

Xu YC and Bremer H 1988 Chromosome replication in *Escherichia coli* induced by oversupply of DnaA. *Mol. Gen. Genet.* **211** 138–142

Zuo Y, Wang Y and Steitz TA 2013 The mechanism of *E. coli* RNA polymerase regulation by ppGpp is suggested by the structure of their complex. *Mol. Cell* **50** 430–436

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