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### When the metabolism meets the cell cycle in bacteria

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*Published in:*  
Current opinion in microbiology

*DOI:*  
[10.1016/j.mib.2021.02.006](https://doi.org/10.1016/j.mib.2021.02.006)

*Publication date:*  
2021

*Document Version*  
Peer reviewed version

#### [Link to publication](#)

*Citation for pulished version (HARVARD):*

Beaufay, F, Coppine, J & Hallez, R 2021, 'When the metabolism meets the cell cycle in bacteria', *Current opinion in microbiology*, vol. 60, COMICR\_2012, pp. 104-113. <https://doi.org/10.1016/j.mib.2021.02.006>

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# Current Opinion in Microbiology

## When the metabolism meets the cell cycle in bacteria

--Manuscript Draft--

<b>Manuscript Number:</b>	COMICR-D-20-00098R2
<b>Full Title:</b>	When the metabolism meets the cell cycle in bacteria
<b>Article Type:</b>	60: Cell Regulation (2021)
<b>Short Title:</b>	Metabolic control of bacterial cell cycle
<b>Keywords:</b>	(p)ppGpp; DnaA; PykA; CCM; glycolysis; NstA; UgtP; OpgH; KidO; GdhZ
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<b>Abstract:</b>	Nutrients availability is the sinews of the war for single microbial cells, driving growth and cell cycle progression. Therefore, coordinating cellular processes with nutrients availability is crucial, not only to survive upon famine or fluctuating conditions but also to rapidly thrive and colonize plentiful environments. While metabolism is traditionally seen as a set of chemical reactions taking place in cells to extract energy and produce building blocks from available nutrients, numerous connections between metabolic pathways and cell cycle phases have been documented. The few regulatory systems described at the molecular levels show that regulation is mediated either by a second messenger molecule or by a metabolite and/or a metabolic enzyme. In the latter case, a secondary moonlighting regulatory function evolved independently of the primary catalytic function of the enzyme. In this review, we summarize our current understanding of the complex cross-talks between metabolism and cell cycle in bacteria.
<b>Author Comments:</b>	

1 **When the metabolism meets the cell cycle in bacteria**

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19 Running title: Metabolic control of bacterial cell cycle

20

21 Key words: (p)ppGpp, DnaA, PykA, CCM, glycolysis, NstA, UgtP, OpgH, KidO, GdhZ

22

23 **Highlights**

24 • Metabolism and cell cycle are interconnected at multiple levels in bacteria

25

26 • DNA replication and cytokinesis are processes subject to metabolic regulation

27

28 • DNA replication initiation rate and cell size can increase at high growth rate

29

30 • Metabolic enzymes bound to their substrates can interfere with activity of the  
31 replisome and the divisome

32

33

## 34 **Summary**

35 Nutrients availability is the sinews of the war for single microbial cells, driving growth  
36 and cell cycle progression. Therefore, coordinating cellular processes with nutrients  
37 availability is crucial, not only to survive upon famine or fluctuating conditions but also  
38 to rapidly thrive and colonize plentiful environments. While metabolism is traditionally  
39 seen as a set of chemical reactions taking place in cells to extract energy and produce  
40 building blocks from available nutrients, numerous connections between metabolic  
41 pathways and cell cycle phases have been documented. The few regulatory systems  
42 described at the molecular levels show that regulation is mediated either by a second  
43 messenger molecule or by a metabolite and/or a metabolic enzyme. In the latter case,  
44 a secondary moonlighting regulatory function evolved independently of the primary  
45 catalytic function of the enzyme. In this review, we summarize our current  
46 understanding of the complex cross-talks between metabolism and cell cycle in  
47 bacteria.

48

## 49 **Introduction**

50 Fluctuation in nutrients availability is likely the most common stress faced by single-  
51 cell microorganisms in their natural environments. Hence, both eukaryotic and  
52 prokaryotic cells use mechanisms to sense nutrient availability and accordingly  
53 regulate key steps in cell cycle progression [1,2]. DNA replication is an essential  
54 energy consuming process and it is therefore crucial for all living cells to proceed to  
55 DNA replication in optimal conditions. Likewise, cell division should take place only  
56 when chances of survival are high for daughter cells. In this review, we highlight the  
57 extensive cross-talks between metabolism and cell cycle described in bacterial model  
58 organisms. We also discuss the importance of cell cycle regulation by metabolism not  
59 only in the context of checkpoints when nutrients become scarce, but also in the case  
60 of steady state regulation that ensures cell size adaptation and homeostasis during  
61 balanced growth.

62

## 63 **Metabolic control of DNA replication and segregation**

64 *An ounce of prevention is worth a pound of cure*

65 In addition to being highly energy-demanding, DNA replication exposes ongoing  
66 replication forks to mutagenic damage by reactive species, including those generated  
67 by metabolic activities. It is therefore not surprising that cells use checkpoint  
68 mechanisms to monitor the metabolic status before starting chromosome replication  
69 and thereby minimize the risk of interrupting replication once started. A well-known  
70 metabolic regulator of DNA replication initiation is the hyperphosphorylated nucleotide  
71 guanosine penta- and tetra-phosphate, commonly referred to as (p)ppGpp, whose  
72 levels increase in response to nutrient starvation [3-6]. The role of (p)ppGpp has been

73 particularly well studied in the  $\gamma$ -proteobacterium *Escherichia coli* and the  $\alpha$ -  
74 proteobacterium *Caulobacter crescentus*. In *E. coli*, this molecule binds RNA  
75 polymerase (RNAP) [7,8], reducing the transcription of many genes such as *dnaA*  
76 coding for the DNA replication initiator protein [9] (**Figure 1**). Overexpression of *dnaA*  
77 *in trans* using a (p)ppGpp-insensitive promoter restores initiation of DNA replication in  
78 cells accumulating (p)ppGpp, suggesting that the neo-synthesis of active DnaA-ATP  
79 molecules is the limiting factor for initiating DNA replication under nutrient limitation  
80 [10,11]. However, the number of initiation events upon *dnaA* overexpression remains  
81 lower in cells that produce high (p)ppGpp levels than in unstressed cells harbouring  
82 basal low levels of (p)ppGpp. Interestingly, this discrepancy is suppressed in cells  
83 expressing a mutant RNAP that is blind to (p)ppGpp, suggesting that other transcripts  
84 whose levels are modulated by (p)ppGpp are involved in the DNA replication control  
85 [10]. Several other genes whose expression is reduced when (p)ppGpp accumulates  
86 might be involved, including *gidA* (tRNA modifying enzyme) located just next to *oriC*,  
87 *gyrA* and *gyrB* (DNA gyrase), *parC* and *parE* (Topoisomerase IV). The DNA gyrase  
88 (*gyrAB*) and the topoisomerase IV (*parCE*) act *in trans* to relax positive supercoils at  
89 *oriC*, thereby promoting DNA replication initiation. On the other hand, transcription  
90 initiation from *gidA* promoter, reading away from *oriC*, works *in cis* by introducing  
91 negative supercoils towards *oriC*, which also promotes initiation of DNA replication.  
92 (**Figure 1**) [12]. Interestingly, the expression of an inhibitor of DNA gyrase (*sbmC*) is,  
93 on the contrary, inhibited by (p)ppGpp [13], further supporting a negative control of  
94 *oriC* superhelicity by (p)ppGpp.

95 Even in the absence of nutrient deprivation, (p)ppGpp plays a critical role in  
96 coordinating growth with cell cycle progression [14,15]. Indeed, as a fast-growing

97 bacterium, *E. coli* adapts the rate of DNA replication initiation to growth rate by  
98 increasing the number of replicating chromosomes per cell cycle in nutrient-rich  
99 conditions. Hence, the *ori:ter* ratio – that represents the average number of DNA  
100 replication initiation events – is inversely proportional to the doubling time. But this  
101 correlation is abolished in cells unable to synthesize (p)ppGpp where multiple DNA  
102 replication forks occur even at slow growth rates [14]. Here again, this effect may arise  
103 from a transcriptional control given that RNAP mutations that mimic the effects of  
104 (p)ppGpp binding reinstate low *ori:ter* ratios at slow growth rate in the absence of  
105 (p)ppGpp [14].

106 In *C. crescentus*, (p)ppGpp delays the G1-S transition and modulates the levels of  
107 DnaA and CtrA. CtrA is a response regulator activated by phosphorylation that  
108 regulates transcription of cell cycle genes and represses DNA replication initiation by  
109 binding the single *Caulobacter* origin of replication (*Cori*) [16]. Upon carbon or nitrogen  
110 starvation, DnaA levels decrease while elevated levels of active CtrA~P are maintained  
111 [17-22]. There is evidence that the decrease in DnaA levels involves both  
112 transcriptional and (post-)translational regulation. First, transcription of *dnaA*  
113 decreases when (p)ppGpp is bound to RNAP (Coppine & Hallez, unpublished).  
114 Second, translation of *dnaA* is inhibited upon nutrient starvation and this inhibition  
115 relies on a 5' untranslated region (5' UTR) but seems to be (p)ppGpp-independent [22].  
116 Finally, DnaA is degraded by at least two ATP-dependent proteases – Lon and ClpAP  
117 – and these proteolytic events require (p)ppGpp to some extent [20,21,23,24]. Since  
118 the nature of the nucleotide bound to DnaA influences its stability – with DnaA-ATP  
119 being somewhat more stable than DnaA-ADP [25]– it is tempting to speculate that one  
120 of the proteases might preferentially degrade one of the DnaA forms. In support of that,

121 Lon was recently shown to poorly degrade the hyperactive ATP-bound DnaA<sub>R357A</sub>  
122 mutant, suggesting that ClpAP might be required to clear active DnaA-ATP from  
123 starved cells, such as those entering into stationary phase [24]. In *E. coli*, a different  
124 phosphate-based metabolite, polyphosphate (PolyP), stimulates Lon-dependent  
125 proteolysis of DnaA-ADP. Since DnaA-ATP is constantly converted to DnaA-ADP,  
126 PolyP-Lon regulatory process leads to an inhibition of DNA replication initiation (**Figure**  
127 **1**) [26]. Although PolyP also modulates cell cycle progression in *C. crescentus*, it  
128 remains to be determined if this effect involves proteolytic events [27].

129 Many studies highlighted a tight link between the initiation step of chromosomal  
130 replication and global metabolic pathways such as the central carbon metabolism  
131 (CCM). For instance, the temperature-sensitivity of *E. coli* mutants defective in DNA  
132 replication initiation (*dnaA46* allele) is suppressed upon inactivation of genes involved  
133 in the acetate overflow pathway (**Figure 2**) [28]. These suppressor strains accumulate  
134 higher intracellular levels of acetate and addition of exogenous acetate to the growth  
135 medium is sufficient on itself to restore growth of *dnaA46* cells at high temperature [29].  
136 Interestingly, DnaA can be acetylated on a conserved Lysine residue in a growth-  
137 dependent pattern by the major acetyltransferase of *E. coli* (YfiQ), which uses Acetyl-  
138 CoA as a substrate, and this acetylation reduces DnaA activity [30]. Since inactivating  
139 *yfiQ* also suppresses *dnaA46* thermosensitivity [29], mutations in the acetate overflow  
140 pathway could decrease Acetyl-CoA levels, which in turn might reduce DnaA46  
141 acetylation, thereby triggering its activity. Interestingly, intracellular levels of Acetyl-  
142 CoA was shown in the yeast *Saccharomyces cerevisiae* to promote entry into the cell  
143 cycle by inducing acetylation of histones [31].

144 As a fast-growing bacterium, *B. subtilis* also adapts rate of DNA replication initiation to  
145 nutrient availability. Interestingly, inactivating the terminal part of glycolysis (e.g. *gapA*)  
146 (**Figure 2**) prevents cells from increasing their *ori:ter* ratio under fast-growing regimen  
147 [32]. Strikingly, several other metabolic pathways (e.g. fatty acid synthesis, respiration,  
148 ...) behave similarly, since their inactivation leads to a low *ori:ter* ratio even at high  
149 growth rates. Although the exact regulatory mechanism still needs to be uncovered, it  
150 requires an active DnaA protein and/or an intact *oriC*. Indeed, cells initiating DNA  
151 replication in an *oriC*- and/or DnaA-independent way are insensitive to growth rate [32].  
152 Finally, a citrate synthase (CitA) has been recently discovered in *C. crescentus* to  
153 trigger the G1-S transition by down-regulating CtrA~P activity [33]. In *C. crescentus*,  
154 the overall citrate synthase activity is catalysed by two paralogous enzymes (CitA and  
155 CitB) but only CitA regulates cell cycle progression. Although the enzymatic activity of  
156 CitA is dispensable for mediating cell cycle control, CitA presumably still monitors  
157 substrate availability. Indeed, the catalytically inactive mutants used in this study,  
158 CitA<sup>H303W</sup> and CitA<sup>H303A</sup> [33], still likely bind Acetyl-CoA and NADH with an affinity  
159 similar to wild type [34]. Thus, the CitA-dependent control of CtrA~P might require  
160 Acetyl-CoA and/or NADH binding rather than citrate synthase activity.

161

### 162 Better late than never

163 The elongation step of DNA replication is also subject to metabolic regulations. For  
164 example, in several bacterial model organisms such as *B. subtilis*, *E. coli* and  
165 *Staphylococcus aureus*, (p)ppGpp binds to the DNA primase DnaG (**Figure 1**), but this  
166 binding only leads to a replication arrest in *B. subtilis* [35-38]. Moreover, the loss-of-  
167 function mutations in the acetate overflow pathway (**Figure 2**) of *E. coli* described

168 above also suppress, although incompletely, the thermosensitivity of DNA replication  
169 elongation mutants (e.g. *dnaG*(Ts), *dnaN*(Ts)). In *B. subtilis*, loss-of-function mutations  
170 in genes involved in the terminal part of glycolysis – where redox reactions take place  
171 **(Figure 2)** – suppress the thermosensitivity of *dnaE*(Ts) alleles encoding the lagging  
172 strand DNA polymerase [39,40]. The same metabolic mutations suppress lethality of  
173 various thermosensitive DNA replication mutants such as *dnaG*(Ts) or *dnaC*(Ts)  
174 whereas mutations in genes involved in any other part of CCM – first preparatory part  
175 of glycolysis, Pentose Phosphate Pathway (PPP) and the Citric Acid Cycle (CAC)  
176 **(Figure 2)** – have no effect [39,40].

177 Despite the multiple genetic interactions identified between DNA replication and CCM,  
178 the molecular mechanisms behind these regulations are still poorly understood.  
179 Recently, pyruvate kinase (PykA, **Figure 2**) of *B. subtilis* has been shown to stimulate  
180 the DNA polymerase activity of DnaE *in vitro*, likely through a direct protein-protein  
181 interaction, however it also inhibits the helicase activity of DnaC [41]. PykA is  
182 responsible for the final step of glycolysis by catalysing the transfer of a phosphoryl  
183 group from PEP to ADP, generating pyruvate and ATP. Notwithstanding these  
184 counterintuitive effects seen *in vitro*, PykA may, as a moonlighting enzyme, directly  
185 determine the speed of the replication fork depending on substrate (PEP) availability  
186 by modulating replisome activities **(Figure 1)**.

187

188 Similarly to what happens in yeast [42], the relative abundance of metabolites  
189 fluctuates as a function of cell cycle in *C. crescentus* [43]. A corollary is that the redox  
190 state oscillates throughout the cell cycle as well. Indeed, new-born cells in G1 phase  
191 have a more reduced cytoplasm, which becomes oxidized during S phase, and then

192 returns to a more reduced state at the end of chromosome replication and the onset of  
193 cytokinesis [44]. The oxidized environment during DNA replication (S phase) promotes  
194 the activation of NstA, an inhibitor of topoisomerase IV (ParCE), through the formation  
195 of intermolecular disulfide bonds between NstA monomers [44] (**Figure 1**). Thus, the  
196 oscillation of the redox state throughout the *Caulobacter* cell cycle restricts the  
197 decatenation activity of the topoisomerase IV to late predivisional cells, and inhibits  
198 this activity during active replication.

199

## 200 **Metabolic control of cytokinesis**

### 201 *The more you eat, the bigger you are*

202 Fast-growing bacteria such as *E. coli*, *B. subtilis*, *Salmonella typhimurium* or  
203 *Pseudomonas aeruginosa* adapt their cell size according to nutrient availability [2,45-  
204 47]. For example, *E. coli* cells grown in rich medium are twice as long as cells cultivated  
205 in nutrient-poor conditions (**Figure 3A**). Both *B. subtilis* and *E. coli* coordinate growth  
206 rate with cell division by monitoring UDP-glucose levels thanks to non-orthologous  
207 glucosyltransferases, respectively UgtP and OpgH [48,49]. The binding of their  
208 substrate – UDP-glucose which accumulates in cells under nutrient-rich conditions –  
209 stimulates direct interaction with FtsZ, a highly conserved tubulin-like protein that  
210 assembles at the division site as a scaffolding structure called the Z-ring [48,50]. The  
211 cytoplasmic protein, UgtP, prevents Z-ring assembly in a concentration-dependent  
212 manner and the membrane-associated protein, OpgH, acts as a non-competitive  
213 inhibitor, sequestering FtsZ. Both proteins effectively raise the apparent critical  
214 concentration for FtsZ assembly and GTP hydrolysis in the presence of elevated UDP-  
215 glucose [48] (**Figure 3B**). In addition to activation by substrate binding, UgtP levels are

216 regulated by Clp-dependent proteolysis with *clpC* and *clpE* expression being induced  
217 under nutrient-poor conditions [51]. It is noteworthy that although *E. coli* and *B. subtilis*  
218 cells lacking *opgH* or *ugtP*, respectively, are smaller than wild-type cells, they still  
219 present a narrow Gaussian cell size distribution, suggesting that the metabolic control  
220 of cell size is superimposed on the mechanism responsible for cell size homeostasis.

221

### 222 Pyruvate or fatty acids on the menu to keep one's figure

223 Pyruvate is another important metabolite that modulates cell division in *B. subtilis* [52]  
224 (**Figure 2**). Indeed, *pykA* mutations that prevent synthesis of pyruvate from PEP,  
225 suppress the thermosensitivity of the *ftsZ(ts1)* allele and lead to cell division defects in  
226 an otherwise wild-type background, with cells harbouring several Z-rings as well as  
227 minicells [52]. More strikingly, addition of exogenous pyruvate to the growth medium  
228 not only restores the thermosensitivity of *ftsZ(ts1)* in a *pykA* mutant background but  
229 also suppresses cell division defects of *pykA* mutant cells. Although evidence suggests  
230 a role of the E1 $\alpha$  subunit of the pyruvate dehydrogenase (PDH-E1 $\alpha$ ), the mechanism  
231 and the proxy by which pyruvate levels control FtsZ dynamics remain unknown.

232 Fatty acid biosynthesis (**Figure 2**) is another metabolic pathway described to regulate  
233 cell size in different microorganisms. Indeed, the inactivation of early steps of fatty acid  
234 biosynthesis ( $\Delta fabH$ ) in *E. coli* decreases the rate of inner membrane lipid biogenesis,  
235 which leads to a ~70% reduction of cellular volume, [53]. Strikingly, this effect seems  
236 to be specific to fatty acid biosynthesis since inhibiting the synthesis any other  
237 membrane constituents either reduces cell size in a lipid-dependent way or does not  
238 impact cell size [54]. For a long time, nutrient availability has been proposed to be  
239 coupled to the rate of fatty acid biosynthesis in *E. coli* [55,56], but again the underlying

240 mechanism and the exact role played by FabH in this process remain to be determined.  
241 The proximity between PykA, PDH and FabH on the metabolic map (**Figure 2**) raises  
242 the interesting hypothesis of a possible link between the observed cell division defects  
243 of all these mutants and the initial step of fatty acids metabolism. In support of that,  
244 fatty acids were recently shown to be a key molecular determinant of cell size control  
245 in fast-growing prokaryotic and eukaryotic microorganisms [54].

246

#### 247 *pHine tuning cytokinesis, the acid test*

248 External stimuli such as pH variation can also control cell size as reported in *E. coli*, *S.*  
249 *aureus*, *Streptococcus pneumoniae* and *C. crescentus* [57-59]. For instance,  
250 compared to growth in neutral pH, *E. coli* cells grown under acidic conditions have 25%  
251 less volume, and cells grown in alkaline conditions have 20% more volume (**Figure**  
252 **3A**). Growth in acidic media stimulates cytokinesis by favouring the recruitment of the  
253 late cell division protein FtsN to the division machinery, which triggers constriction and  
254 septal wall synthesis. Therefore, cells grown in acidic conditions are shorter than their  
255 counterparts grown in alkaline conditions. Similarly, in *Salmonella*, external pH  
256 modulates the activity of two peptidoglycan (PG) synthase paralogs, PBP3 and  
257 PBP3sal. These PG transpeptidases actively participate in septum synthesis and  
258 promote cell division in the acidic environment of the phagosome during infection [60].  
259 In *C. crescentus*, glutathione levels oscillate throughout the cell cycle and indirectly  
260 influence cytokinesis [43]. Mutants unable to synthesize glutathione display defects in  
261 cytokinesis that were primarily attributed to dysregulation of the potassium efflux  $K^+/H^+$   
262 antiporter, KefB, whose activity is inhibited by glutathione [43]. However, in the  
263 absence of glutathione, whether cytokinesis is impacted by a reduction of intracellular

264 K<sup>+</sup> or a more acidic pH remains to be determined knowing that both cations affect FtsZ  
265 dynamics *in vitro* and cell size *in vivo* [58,61-63].

266

### 267 Dividing when sated

268 *C. crescentus* uses GdhZ (a NAD-dependent glutamate dehydrogenase) and KidO (a  
269 NAD(H)-binding protein) to coordinate cytokinesis with metabolism by monitoring  
270 glutamate and NADH cellular supplies [64,65]. When bound to substrate, glutamate or  
271 NAD<sup>+</sup> for GdhZ and NADH for KidO, these proteins act in synergy to negatively  
272 regulate the Z-ring structure. KidO prevents lateral interactions between FtsZ  
273 protofilaments while GdhZ shrinks protofilaments by stimulating the GTPase activity of  
274 FtsZ. As substrate binding is required for GdhZ and KidO to regulate FtsZ dynamics  
275 [64,65], localization of both regulators in the vicinity of the Z-ring during constriction  
276 might further enhance their concerted action by funnelling the NADH generated by  
277 GdhZ to KidO (**Figure 3B**). In addition, GdhZ and KidO activities are restricted to the  
278 early and late stages of the cell cycle thanks to the degradation of both regulators by  
279 the ClpXP protease. This temporal regulation prevents premature assembly of the cell  
280 division machinery in new-born cells and stimulates the disassembly of the Z-ring at  
281 the end of the cell cycle [64,65]. Like pyruvate, glutamate is also a central cellular  
282 metabolite, located at the edge of the nitrogen cycle and the CAC (**Figure 2**). By  
283 coordinating cytokinesis with metabolic activity (i.e. nutrient availability), cells ensure  
284 completion of cytokinesis and release of progeny when growth conditions are optimal.  
285 Interestingly, the cell division control mediated by GdhZ seems conserved among  $\alpha$ -  
286 proteobacteria, at least in the facultative intracellular pathogen *Brucella abortus* [66].

287

288 **Concluding remarks**

289 The number of genetic interactions between DNA replication or cytokinesis and  
290 metabolic mutants strongly suggests that these essential processes are  
291 interconnected, with some metabolic reactions linked to multiple steps of the cell cycle.  
292 The inactivation of the highly conserved pyruvate kinase encoding gene *pykA* can fully  
293 suppress the lethality of DNA replication elongation mutants (e.g. *dnaE(Ts)*) in *E. coli*  
294 [52] as well as the thermosensitivity displayed by *B. subtilis ftsZ(ts1)* cells [52]. Whether  
295 the pyruvate kinase regulates both cell cycle stages in the same species remains to  
296 be tested but the central position of pyruvate for several metabolic pathways  
297 (neoglucogenesis, amino acids synthesis, CAC, fatty acids synthesis) makes this  
298 metabolite a perfect candidate to monitor nutrient availability (**Figure 2**).

299 But what are the underlying mechanisms? How does metabolism influence cell cycle  
300 progression? One can speculate that a metabolite whose concentration rapidly  
301 changes upon stress, alone or bound to an enzyme as a substrate or a ligand, directly  
302 interacts with a component of the replisome to regulate its activity. In support of that,  
303 metabolic enzymes were found in high-throughput protein-protein interactions screens  
304 as physical partners of replisome components both in *E. coli* and *B. subtilis* [67,68].  
305 Additionally, the viability of thermosensitive DNA replication mutants was greatly  
306 improved when the growth medium was supplemented with CCM metabolites [69].  
307 Alternatively, the metabolite whose concentration changes upon stress might be used  
308 as a substrate for enzymes that mediate post-translational modifications (acetylation,  
309 phosphorylation, ...) of a replisome component, such as the acetylation of DnaA [30].  
310 The composition of the cytoplasmic membrane is another non-exclusive proxy used to  
311 transduce the metabolic status to the replisome, at least for the initiation step since

312 acidic phospholipids have been shown to stimulate DnaA activation by regenerating  
313 DnaA-ATP [70,71].

314 Whatever the mechanism transducing the signal from metabolism to influence the cell  
315 cycle is, it relies on metabolites whose intracellular concentrations fluctuate upon  
316 environmental changes and thereby report environmental status (e.g. UDP-Glucose  
317 for the central carbon metabolism or glutamine for nitrogen metabolism). For instance,  
318 *Caulobacter* and *Sinorhizobium meliloti* cells monitor intracellular glutamine levels as  
319 a proxy for nitrogen availability with the help of the nitrogen-related  
320 phosphotransferase system (PTS<sup>Ntr</sup>), which leads to (p)ppGpp accumulation upon  
321 glutamine deprivation [72-74]. Rather than only gears providing energy and building  
322 blocks, increasing evidence supports metabolism as overseeing major cellular  
323 processes such as DNA replication and cytokinesis. Now we need to understand how  
324 these regulatory phenomena work at the molecular level.

325 **References**

326

- 327 1. Ewald JC: **How yeast coordinates metabolism, growth and division.** *Curr Opin Microbiol*  
328 2018, **45**:1-7.
- 329 2. Wang JD, Levin PA: **Metabolism, cell growth and the bacterial cell cycle.** *Nat Rev*  
330 *Microbiol* 2009, **7**:822-827.
- 331 3. Ferullo DJ, Lovett ST: **The stringent response and cell cycle arrest in Escherichia coli.**  
332 *PLoS Genet* 2008, **4**:e1000300.
- 333 4. Hallez R, Delaby M, Sanselicio S, Viollier PH: **Hit the right spots: cell cycle control by**  
334 **phosphorylated guanosines in alphaproteobacteria.** *Nat Rev Microbiol* 2017,  
335 **15**:137-148.
- 336 5. Irving SE, Choudhury NR, Corrigan RM: **The stringent response and physiological roles**  
337 **of (pp)pGpp in bacteria.** *Nat Rev Microbiol* 2020.
- 338 6. Ronneau S, Hallez R: **Make and break the alarmone: regulation of (p)ppGpp**  
339 **synthetase/hydrolase enzymes in bacteria.** *FEMS Microbiol Rev* 2019, **43**:389-400.
- 340 7. Ross W, Sanchez-Vazquez P, Chen AY, Lee JH, Burgos HL, Gourse RL: **ppGpp Binding**  
341 **to a Site at the RNAP-DksA Interface Accounts for Its Dramatic Effects on**  
342 **Transcription Initiation during the Stringent Response.** *Mol Cell* 2016, **62**:811-823.
- 343 8. Ross W, Vrentas CE, Sanchez-Vazquez P, Gaal T, Gourse RL: **The magic spot: a ppGpp**  
344 **binding site on E. coli RNA polymerase responsible for regulation of**  
345 **transcription initiation.** *Mol Cell* 2013, **50**:420-429.
- 346 9. Chiaramello AE, Zyskind JW: **Coupling of DNA replication to growth rate in Escherichia**  
347 **coli: a possible role for guanosine tetraphosphate.** *J Bacteriol* 1990, **172**:2013-  
348 2019.
- 349 10. Riber L, Lobner-Olesen A: **Inhibition of Escherichia coli chromosome replication by**  
350 **rifampicin treatment or during the stringent response is overcome by de novo**  
351 **DnaA protein synthesis.** *Mol Microbiol* 2020.
- 352 • This work shows that *de novo* synthesis of DnaA, from a (p)ppGpp-insensitive promoter, in  
353 *E. coli* cells experiencing high (p)ppGpp levels allows DNA replication initiation. This suggests  
354 that (p)ppGpp arrests chromosome replication initiation essentially by limiting production of  
355 active DnaA, at least in *E. coli*.
- 356 11. Sinha AK, Lobner-Olesen A, Riber L: **Bacterial Chromosome Replication and DNA**  
357 **Repair During the Stringent Response.** *Front Microbiol* 2020, **11**:582113.
- 358 12. Kraemer JA, Sanderlin AG, Laub MT: **The Stringent Response Inhibits DNA Replication**  
359 **Initiation in E. coli by Modulating Supercoiling of oriC.** *mBio* 2019, **10**.
- 360 13. Traxler MF, Summers SM, Nguyen HT, Zacharia VM, Hightower GA, Smith JT, Conway T:  
361 **The global, ppGpp-mediated stringent response to amino acid starvation in**  
362 **Escherichia coli.** *Mol Microbiol* 2008, **68**:1128-1148.

- 363 14. Fernandez-Coll L, Maciag-Dorszynska M, Tailor K, Vadia S, Levin PA, Szalewska-Palasz  
364 A, Cashel M: **The Absence of (p)ppGpp Renders Initiation of Escherichia coli**  
365 **Chromosomal DNA Synthesis Independent of Growth Rates.** *mBio* 2020, **11**.
- 366 • This paper demonstrates that basal levels of (p)ppGpp coordinate DNA replication initiation  
367 with growth rate in *E. coli*.
- 368 15. Imholz NCE, Noga MJ, van den Broek NJF, Bokinsky G: **Calibrating the Bacterial Growth**  
369 **Rate Speedometer: A Re-evaluation of the Relationship Between Basal ppGpp,**  
370 **Growth, and RNA Synthesis in Escherichia coli.** *Front Microbiol* 2020, **11**:574872.
- 371 16. Quon KC, Yang B, Domian IJ, Shapiro L, Marczyński GT: **Negative control of bacterial**  
372 **DNA replication by a cell cycle regulatory protein that binds at the chromosome**  
373 **origin.** *Proc Natl Acad Sci U S A* 1998, **95**:120-125.
- 374 17. Boutte CC, Crosson S: **The complex logic of stringent response regulation in**  
375 **Caulobacter crescentus: starvation signalling in an oligotrophic environment.**  
376 *Mol Microbiol* 2011, **80**:695-714.
- 377 18. Britos L, Abeliuk E, Taverner T, Lipton M, McAdams H, Shapiro L: **Regulatory response**  
378 **to carbon starvation in Caulobacter crescentus.** *PLoS One* 2011, **6**:e18179.
- 379 19. Gonzalez D, Collier J: **Effects of (p)ppGpp on the progression of the cell cycle of**  
380 **Caulobacter crescentus.** *J Bacteriol* 2014, **196**:2514-2525.
- 381 20. Gorbatyuk B, Marczyński GT: **Regulated degradation of chromosome replication**  
382 **proteins DnaA and CtrA in Caulobacter crescentus.** *Mol Microbiol* 2005, **55**:1233-  
383 1245.
- 384 21. Lesley JA, Shapiro L: **SpoT regulates DnaA stability and initiation of DNA replication**  
385 **in carbon-starved Caulobacter crescentus.** *J Bacteriol* 2008, **190**:6867-6880.
- 386 22. Leslie DJ, Heinen C, Schramm FD, Thuring M, Aakre CD, Murray SM, Laub MT, Jonas K:  
387 **Nutritional Control of DNA Replication Initiation through the Proteolysis and**  
388 **Regulated Translation of DnaA.** *PLoS Genet* 2015, **11**:e1005342.
- 389 23. Jonas K, Liu J, Chien P, Laub MT: **Proteotoxic stress induces a cell-cycle arrest by**  
390 **stimulating Lon to degrade the replication initiator DnaA.** *Cell* 2013, **154**:623-636.
- 391 24. Liu J, Francis LI, Jonas K, Laub MT, Chien P: **CipAP is an auxiliary protease for DnaA**  
392 **degradation in Caulobacter crescentus.** *Mol Microbiol* 2016, **102**:1075-1085.
- 393 25. Wargachuk R, Marczyński GT: **The Caulobacter crescentus Homolog of DnaA (HdaA)**  
394 **Also Regulates the Proteolysis of the Replication Initiator Protein DnaA.** *J*  
395 *Bacteriol* 2015, **197**:3521-3532.
- 396 26. Gross MH, Konieczny I: **Polyphosphate induces the proteolysis of ADP-bound**  
397 **fraction of initiator to inhibit DNA replication initiation upon stress in Escherichia**  
398 **coli.** *Nucleic Acids Res* 2020, **48**:5457-5466.
- 399 •• In this paper, the authors show that polyphosphate exclusively binds to DnaA-ADP, which  
400 selectively stimulates Lon-dependent proteolysis of the ADP bound form of DnaA in *E. coli*.

- 401 27. Boutte CC, Henry JT, Crosson S: **ppGpp and polyphosphate modulate cell cycle**  
402 **progression in *Caulobacter crescentus***. *J Bacteriol* 2012, **194**:28-35.
- 403 28. Maciag M, Nowicki D, Janniére L, Szalewska-Palasz A, Wegrzyn G: **Genetic response to**  
404 **metabolic fluctuations: correlation between central carbon metabolism and DNA**  
405 **replication in *Escherichia coli***. *Microb Cell Fact* 2011, **10**:19.
- 406 29. Tymecka-Mulik J, Boss L, Maciag-Dorszynska M, Matias Rodrigues JF, Gaffke L, Wosinski  
407 A, Cech GM, Szalewska-Palasz A, Wegrzyn G, Glinkowska M: **Suppression of the**  
408 ***Escherichia coli* dnaA46 mutation by changes in the activities of the pyruvate-**  
409 **acetate node links DNA replication regulation to central carbon metabolism.**  
410 *PLoS One* 2017, **12**:e0176050.
- 411 30. Zhang Q, Zhou A, Li S, Ni J, Tao J, Lu J, Wan B, Li S, Zhang J, Zhao S, et al.: **Reversible**  
412 **lysine acetylation is involved in DNA replication initiation by regulating activities**  
413 **of initiator DnaA in *Escherichia coli***. *Sci Rep* 2016, **6**:30837.
- 414 31. Shi L, Tu BP: **Acetyl-CoA induces transcription of the key G1 cyclin CLN3 to promote**  
415 **entry into the cell division cycle in *Saccharomyces cerevisiae***. *Proc Natl Acad Sci*  
416 *U S A* 2013, **110**:7318-7323.
- 417 32. Murray H, Koh A: **Multiple regulatory systems coordinate DNA replication with cell**  
418 **growth in *Bacillus subtilis***. *PLoS Genet* 2014, **10**:e1004731.
- 419 33. Berge M, Pezzatti J, Gonzalez-Ruiz V, Degeorges L, Mottet-Osman G, Rudaz S, Viollier  
420 PH: **Bacterial cell cycle control by citrate synthase independent of enzymatic**  
421 **activity**. *Elife* 2020, **9**.
- 422 • This paper describes a moonlighting function for one of the three citrate synthase paralogs,  
423 which controls cell cycle progression in *C. crescentus* by regulating the activity of the cell cycle  
424 regulator CtrA.
- 425 34. Pereira DS, Donald LJ, Hosfield DJ, Duckworth HW: **Active site mutants of *Escherichia***  
426 ***coli* citrate synthase. Effects of mutations on catalytic and allosteric properties.**  
427 *J Biol Chem* 1994, **269**:412-417.
- 428 35. Denapoli J, Tehranchi AK, Wang JD: **Dose-dependent reduction of replication**  
429 **elongation rate by (p)ppGpp in *Escherichia coli* and *Bacillus subtilis***. *Mol*  
430 *Microbiol* 2013, **88**:93-104.
- 431 36. Maciag M, Kochanowska M, Lyzen R, Wegrzyn G, Szalewska-Palasz A: **ppGpp inhibits**  
432 **the activity of *Escherichia coli* DnaG primase**. *Plasmid* 2010, **63**:61-67.
- 433 37. Rymer RU, Solorio FA, Tehranchi AK, Chu C, Corn JE, Keck JL, Wang JD, Berger JM:  
434 **Binding mechanism of metalNTP substrates and stringent-response alarmones**  
435 **to bacterial DnaG-type primases**. *Structure* 2012, **20**:1478-1489.
- 436 38. Wang JD, Sanders GM, Grossman AD: **Nutritional control of elongation of DNA**  
437 **replication by (p)ppGpp**. *Cell* 2007, **128**:865-875.
- 438 39. Janniére L, Canceill D, Suski C, Kanga S, Dalmais B, Lestini R, Monnier AF, Chapuis J,  
439 Bolotin A, Titok M, et al.: **Genetic evidence for a link between glycolysis and DNA**  
440 **replication**. *PLoS One* 2007, **2**:e447.

- 441 40. Nouri H, Monnier AF, Fossum-Raunehaug S, Maciag-Dorszynska M, Cabin-Flaman A,  
442 Kepes F, Wegrzyn G, Szalewska-Palasz A, Norris V, Skarstad K, et al.: **Multiple links**  
443 **connect central carbon metabolism to DNA replication initiation and elongation**  
444 **in *Bacillus subtilis***. *DNA Res* 2018, **25**:641-653.
- 445 41. Horemans S, Pitoulis M, Holland A, Soultanas P, Janniere L: **Glycolytic pyruvate kinase**  
446 **moonlighting activities in DNA replication initiation and elongation**. *bioRxiv* 2020.
- 447 42. Cai L, Tu BP: **Driving the cell cycle through metabolism**. *Annu Rev Cell Dev Biol* 2012,  
448 **28**:59-87.
- 449 43. Hartl J, Kiefer P, Kaczmarczyk A, Mittelviehhaus M, Meyer F, Vonderach T, Hattendorf B,  
450 Jenal U, Vorholt JA: **Untargeted metabolomics links glutathione to bacterial cell**  
451 **cycle progression**. *Nat Metab* 2020, **2**:153-166.
- 452 •• This paper describes, for the first time in bacteria, that abundance of ~400 metabolites,  
453 including glutathione, fluctuates along the *C. crescentus* cell cycle. The authors also show that  
454 glutathione indirectly controls cytokinesis by regulating the activity of a potassium efflux  
455 system.
- 456 44. Narayanan S, Janakiraman B, Kumar L, Radhakrishnan SK: **A cell cycle-controlled**  
457 **redox switch regulates the topoisomerase IV activity**. *Genes Dev* 2015, **29**:1175-  
458 1187.
- 459 •• In this work, the authors show that the redox state of the cytoplasm oscillates along the cell  
460 cycle and that the oscillating redox level is used by *Caulobacter* cells to constrain the  
461 activity of the topoisomerase IV at the end of the S phase.
- 462 45. Cooper S, Helmstetter CE: **Chromosome replication and the division cycle of**  
463 ***Escherichia coli* B/r**. *J Mol Biol* 1968, **31**:519-540.
- 464 46. Donachie WD, Begg KJ: **Cell length, nucleoid separation, and cell division of rod-**  
465 **shaped and spherical cells of *Escherichia coli***. *J Bacteriol* 1989, **171**:4633-4639.
- 466 47. Schaechter M, Maaloe O, Kjeldgaard NO: **Dependency on medium and temperature of**  
467 **cell size and chemical composition during balanced growth of *Salmonella***  
468 ***typhimurium***. *J Gen Microbiol* 1958, **19**:592-606.
- 469 48. Hill NS, Buske PJ, Shi Y, Levin PA: **A moonlighting enzyme links *Escherichia coli* cell**  
470 **size with central metabolism**. *PLoS Genet* 2013, **9**:e1003663.
- 471 • This paper along with [49•] show that two unrelated glucosyltransferases, OpgH in *E. coli* and  
472 UgtP in *B. subtilis*, coordinate cell size with central metabolism by regulating FtsZ dynamics.  
473 In both cases, the glucosyltransferase-dependent regulation of Z-ring assembly requires the  
474 binding of the substrate (UDP-glucose). Together, these papers illustrate the convergent  
475 evolution of analogous systems to mediate metabolic control of cell size in rapidly growing  
476 bacteria.
- 477 49. Weart RB, Lee AH, Chien AC, Haeusser DP, Hill NS, Levin PA: **A metabolic sensor**  
478 **governing cell size in bacteria**. *Cell* 2007, **130**:335-347.
- 479 • See comments for [48•]

- 480 50. Chien AC, Zareh SK, Wang YM, Levin PA: **Changes in the oligomerization potential of**  
481 **the division inhibitor UgtP co-ordinate Bacillus subtilis cell size with nutrient**  
482 **availability**. *Mol Microbiol* 2012, **86**:594-610.
- 483 51. Hill NS, Zuke JD, Buske PJ, Chien AC, Levin PA: **A nutrient-dependent division**  
484 **antagonist is regulated post-translationally by the Clp proteases in Bacillus**  
485 **subtilis**. *BMC Microbiol* 2018, **18**:29.
- 486 52. Monahan LG, Hajduk IV, Blaber SP, Charles IG, Harry EJ: **Coordinating bacterial cell**  
487 **division with nutrient availability: a role for glycolysis**. *mBio* 2014, **5**:e00935-  
488 00914.
- 489 53. Yao Z, Davis RM, Kishony R, Kahne D, Ruiz N: **Regulation of cell size in response to**  
490 **nutrient availability by fatty acid biosynthesis in Escherichia coli**. *Proc Natl Acad*  
491 *Sci U S A* 2012, **109**:E2561-2568.
- 492 54. Vadia S, Tse JL, Lucena R, Yang Z, Kellogg DR, Wang JD, Levin PA: **Fatty Acid**  
493 **Availability Sets Cell Envelope Capacity and Dictates Microbial Cell Size**. *Curr*  
494 *Biol* 2017, **27**:1757-1767 e1755.
- 495 •• Data presented in this paper show that fatty acid synthesis is used as a universal  
496 mechanism, at least for fast-growing prokaryotic and eukaryotic microorganisms (*E. coli*,  
497 *B. subtilis* and *S. cerevisiae*), to determine cell size in a growth rate-dependent way.
- 498 55. Li SJ, Cronan JE, Jr.: **Growth rate regulation of Escherichia coli acetyl coenzyme A**  
499 **carboxylase, which catalyzes the first committed step of lipid biosynthesis**. *J*  
500 *Bacteriol* 1993, **175**:332-340.
- 501 56. Takamura Y, Nomura G: **Changes in the intracellular concentration of acetyl-CoA and**  
502 **malonyl-CoA in relation to the carbon and energy metabolism of Escherichia coli**  
503 **K12**. *J Gen Microbiol* 1988, **134**:2249-2253.
- 504 57. Heinrich K, Leslie DJ, Morlock M, Bertilsson S, Jonas K: **Molecular Basis and Ecological**  
505 **Relevance of Caulobacter Cell Filamentation in Freshwater Habitats**. *mBio* 2019,  
506 **10**.
- 507 58. Mueller EA, Westfall CS, Levin PA: **pH-dependent activation of cytokinesis modulates**  
508 **Escherichia coli cell size**. *PLoS Genet* 2020, **16**:e1008685.
- 509 • This study characterizes a molecular mechanism that allows cell size adaptation to pH. The  
510 authors show that recruitment of the late cell division protein FtsN to the divisome is favored at  
511 acidic pH, thereby explaining why *E. coli* cells grown under alkaline conditions are longer than  
512 the ones grown at acidic pH.
- 513 59. Perez AJ, Cesbron Y, Shaw SL, Bazan Villicana J, Tsui HT, Boersma MJ, Ye ZA, Tovpeko  
514 Y, Dekker C, Holden S, et al.: **Movement dynamics of divisome proteins and**  
515 **PBP2x:FtsW in cells of Streptococcus pneumoniae**. *Proc Natl Acad Sci U S A* 2019,  
516 **116**:3211-3220.
- 517 60. Castanheira S, Cestero JJ, Rico-Perez G, Garcia P, Cava F, Ayala JA, Pucciarelli MG,  
518 Garcia-Del Portillo F: **A Specialized Peptidoglycan Synthase Promotes Salmonella**  
519 **Cell Division inside Host Cells**. *mBio* 2017, **8**.

- 520 •• This paper shows that *Salmonella* cells use two PBP3 paralogs to mediate cell division  
521 depending on the conditions, a traditional one used during planktonic growth outside from the  
522 host and an acidic-sensitive one used during growth in acidified phagosome inside the host.
- 523 61. Ahijado-Guzman R, Alfonso C, Reija B, Salvarelli E, Mingorance J, Zorrilla S, Monterroso  
524 B, Rivas G: **Control by potassium of the size distribution of Escherichia coli FtsZ**  
525 **polymers is independent of GTPase activity.** *J Biol Chem* 2013, **288**:27358-27365.
- 526 62. Mendieta J, Rico AI, Lopez-Vinas E, Vicente M, Mingorance J, Gomez-Puertas P:  
527 **Structural and functional model for ionic (K(+)/Na(+)) and pH dependence of**  
528 **GTPase activity and polymerization of FtsZ, the prokaryotic ortholog of tubulin.**  
529 *J Mol Biol* 2009, **390**:17-25.
- 530 63. Tadros M, Gonzalez JM, Rivas G, Vicente M, Mingorance J: **Activation of the**  
531 **Escherichia coli cell division protein FtsZ by a low-affinity interaction with**  
532 **monovalent cations.** *FEBS Lett* 2006, **580**:4941-4946.
- 533 64. Beaufay F, Coppine J, Mayard A, Laloux G, De Bolle X, Hallez R: **A NAD-dependent**  
534 **glutamate dehydrogenase coordinates metabolism with cell division in**  
535 **Caulobacter crescentus.** *EMBO J* 2015, **34**:1786-1800.
- 536 • In this paper, the authors show that the catabolic glutamate dehydrogenase (GdhZ) of *C.*  
537 *crescentus* bound to its substrate (NAD<sup>+</sup> or glutamate) interacts with FtsZ to trigger its  
538 GTPase activity, thereby stimulating Z-ring disassembly. They also show that the  
539 oxidoreductase-like protein KidO bound to NADH inhibits formation of lateral  
540 interactions between FtsZ protofilaments.
- 541 65. Radhakrishnan SK, Pritchard S, Viollier PH: **Coupling prokaryotic cell fate and division**  
542 **control with a bifunctional and oscillating oxidoreductase homolog.** *Dev Cell*  
543 2010, **18**:90-101.
- 544 66. Beaufay F, De Bolle X, Hallez R: **Metabolic control of cell division in alpha-**  
545 **proteobacteria by a NAD-dependent glutamate dehydrogenase.** *Commun Integr*  
546 *Biol* 2016, **9**:e1125052.
- 547 67. Butland G, Peregrin-Alvarez JM, Li J, Yang W, Yang X, Canadien V, Starostine A, Richards  
548 D, Beattie B, Krogan N, et al.: **Interaction network containing conserved and**  
549 **essential protein complexes in Escherichia coli.** *Nature* 2005, **433**:531-537.
- 550 68. Noirot-Gros MF, Dervyn E, Wu LJ, Mervelet P, Errington J, Ehrlich SD, Noirot P: **An**  
551 **expanded view of bacterial DNA replication.** *Proc Natl Acad Sci U S A* 2002,  
552 **99**:8342-8347.
- 553 69. Krause K, Maciag-Dorszynska M, Wosinski A, Gaffke L, Morcinek-Orlowska J, Rintz E,  
554 Bielanska P, Szalewska-Palasz A, Muskhelishvili G, Wegrzyn G: **The Role of**  
555 **Metabolites in the Link between DNA Replication and Central Carbon Metabolism**  
556 **in Escherichia coli.** *Genes (Basel)* 2020, **11**.
- 557 70. Saxena R, Finland N, Patil D, Sharma AK, Crooke E: **Crosstalk between DnaA protein,**  
558 **the initiator of Escherichia coli chromosomal replication, and acidic**  
559 **phospholipids present in bacterial membranes.** *Int J Mol Sci* 2013, **14**:8517-8537.
- 560 71. Sekimizu K, Kornberg A: **Cardiolipin activation of dnaA protein, the initiation protein**  
561 **of replication in Escherichia coli.** *J Biol Chem* 1988, **263**:7131-7135.

- 562 72. Goodwin RA, Gage DJ: **Biochemical characterization of a nitrogen-type**  
563 **phosphotransferase system reveals that enzyme El(Ntr) integrates carbon and**  
564 **nitrogen signaling in Sinorhizobium meliloti.** *J Bacteriol* 2014, **196**:1901-1907.
- 565 73. Ronneau S, Caballero-Montes J, Coppine J, Mayard A, Garcia-Pino A, Hallez R:  
566 **Regulation of (p)ppGpp hydrolysis by a conserved archetypal regulatory**  
567 **domain.** *Nucleic Acids Res* 2019, **47**:843-854.
- 568 74. Ronneau S, Petit K, De Bolle X, Hallez R: **Phosphotransferase-dependent**  
569 **accumulation of (p)ppGpp in response to glutamine deprivation in Caulobacter**  
570 **crescentus.** *Nat Commun* 2016, **7**:11423.  
571

572 **Acknowledgements**

573 Work in the R.H. lab is supported by the Fonds de la Recherche Scientifique – FNRS  
574 (F.R.S. – FNRS) with an Incentive Grant for Scientific Research (MIS F.4516.19F) and  
575 a Welbio Starting Grant (WELBIO-CR-2019S-05). F.B. was holding a FRIA fellowship  
576 from the Fonds de la Recherche Scientifique - FNRS (F.R.S. – FNRS). R.H. is a F.R.S.  
577 – FNRS Research Associate.

578

579 **Conflict of interest**

580 The authors declare that they have no conflict of interest.

581

582 **Figure legends**

583

584 **Figure 1** Overview of the initiation (1) and elongation (2) steps of DNA replication in  
585 bacteria

586 (1) The initiator protein DnaA bound to ATP (DNA<sup>ATP</sup>, red) binds the single origin of  
587 replication (*oriC*) to separate DNA strands and helps, together with single-strand  
588 binding proteins (dark grey), in recruiting the helicase (DnaB, green) in complex with  
589 the helicase loader (DnaC, pink). (2) The DNA primase (DnaG, purple) is recruited to  
590 initiate transcription of short RNA primers (red lines), DNA<sup>ATP</sup> is converted to DNA<sup>ADP</sup>  
591 and DnaC is released from the initiation complex. The multisubunit DNA polymerase  
592 III (dark blue) together the sliding clamp (DnaN, yellow) starts to synthesize DNA  
593 continuously from the leading strand (brown line) and discontinuously from the lagging  
594 strand (blue line). The topoisomerase IV and the DNA gyrase concomitantly introduce  
595 negative supercoiling upstream of the DNA polymerase III.

596 The regulation mediated by (p)ppGpp, PolyP, Lon, ClpAP, PykA and NstA, described  
597 in the text, are represented in light grey with dashed lines.

598

599 **Figure 2** Schematic overview of the metabolic routes involved in the metabolic control  
600 of cell cycle in bacteria

601 Regulatory enzymes are indicated in red, while metabolites used as a proxy for cell  
602 cycle control are represented in light blue. The cell cycle components targeted by the  
603 metabolic enzymes are indicated in green. OPG, Osmoregulated periplasmic glucans;  
604 F 6-P, Fructose 6-phosphate; F 1,6-BP, Fructose 1,6-biphosphate; DHAP,  
605 Dihydroxyacetone-P; GAP, Glyceraldehyde-3-P; 1,3 BPG, 1,3-Bisphosphoglycerate;  
606 3-PG, 3-P-Glycerate; 2-PG, 2-P-Glycerate; PEP, Phosphoenolpyruvate;  $\alpha$ -KG, alpha-  
607 ketoglutarate. Pyk, Pyruvate kinase; PDH, Pyruvate dehydrogenase; Cit, Citrate  
608 synthase; FA, Fatty acids.

609

610 **Figure 3** Molecular mechanisms used by bacteria to coordinate metabolism with cell  
611 division

612 (A) The size of fast-growing bacteria grown in rich conditions (e.g. high intracellular  
613 concentration of UDP-Glucose) or at alkaline pH can be twice longer than the ones  
614 grown in poor conditions or at acidic pH. The Z-ring is represented in green, FtsN in

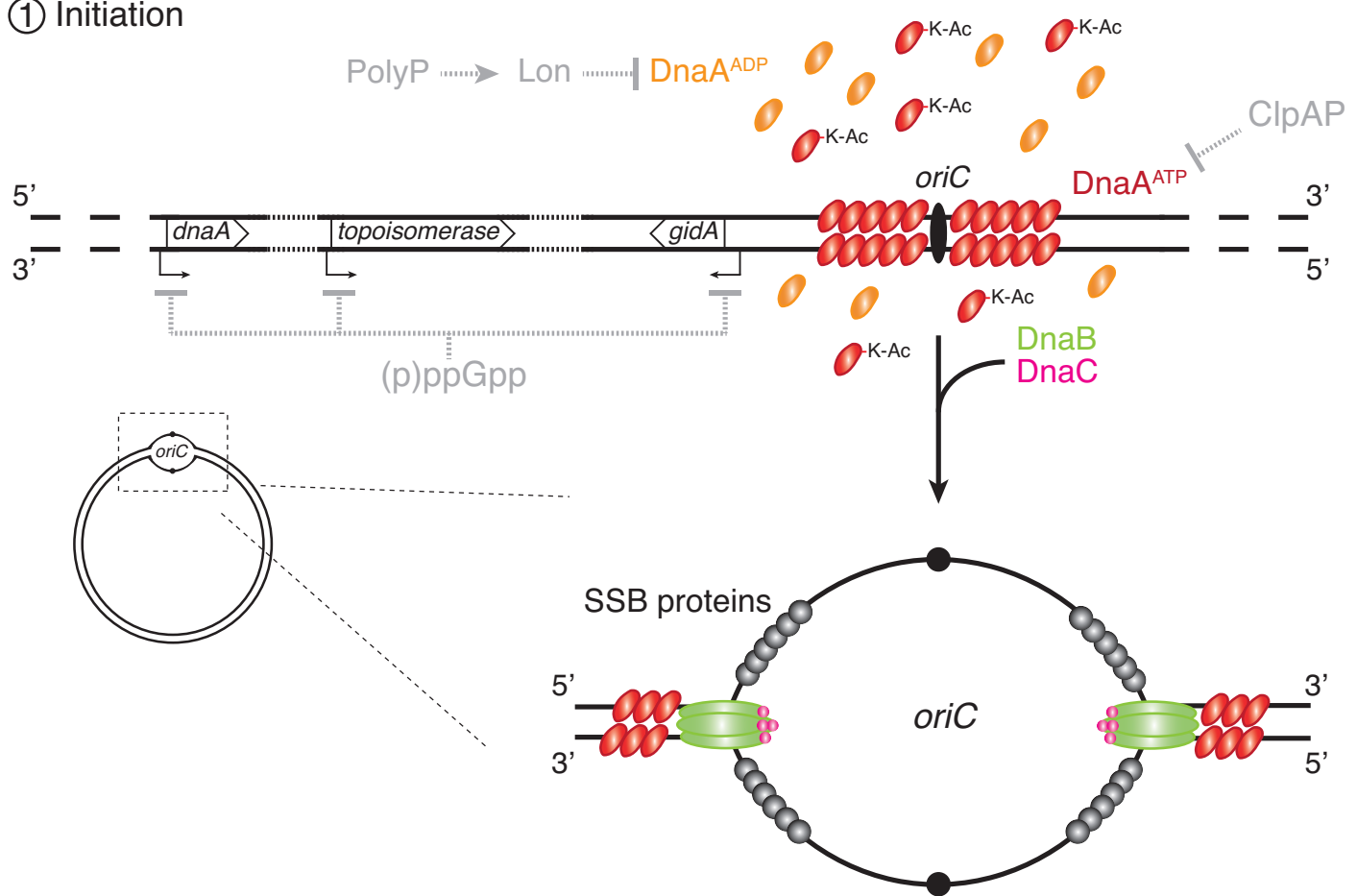
615 grey and metabolites used as a proxy for this regulation (e.g. UDP-Glucose) are  
616 represented in light blue. The replicating DNA molecules are represented in black. (B)  
617 Proteins coordinating metabolism with cell division interfere with Z-ring dynamics by  
618 using different molecular mechanisms. UgtP in *B. subtilis* and OpgH in *E. coli* interfere  
619 with the Z-ring dynamics by respectively severing or sequestering FtsZ molecules  
620 (green) only when the UDP-Glucose (light blue hexagon) is highly concentrated. GdhZ  
621 bound to its substrate (glutamate, light blue square) or its cofactor (NAD<sup>+</sup>, light orange  
622 star) shrinks FtsZ protofilaments by stimulating its GTPase activity while KidO bound  
623 to NADH (light blue star) interferes with the lateral interactions between FtsZ  
624 protofilaments.

**Declaration of interests**

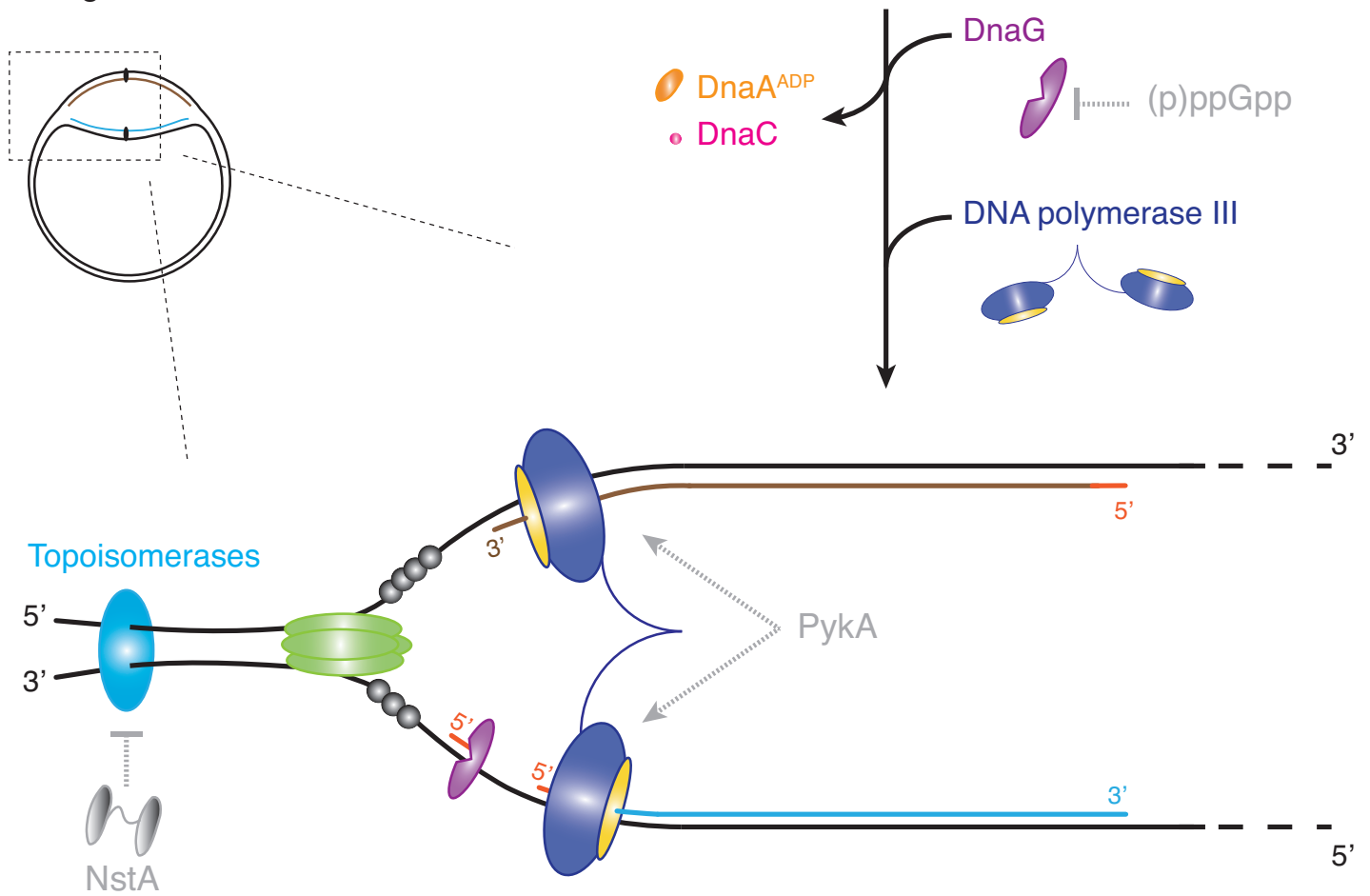
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

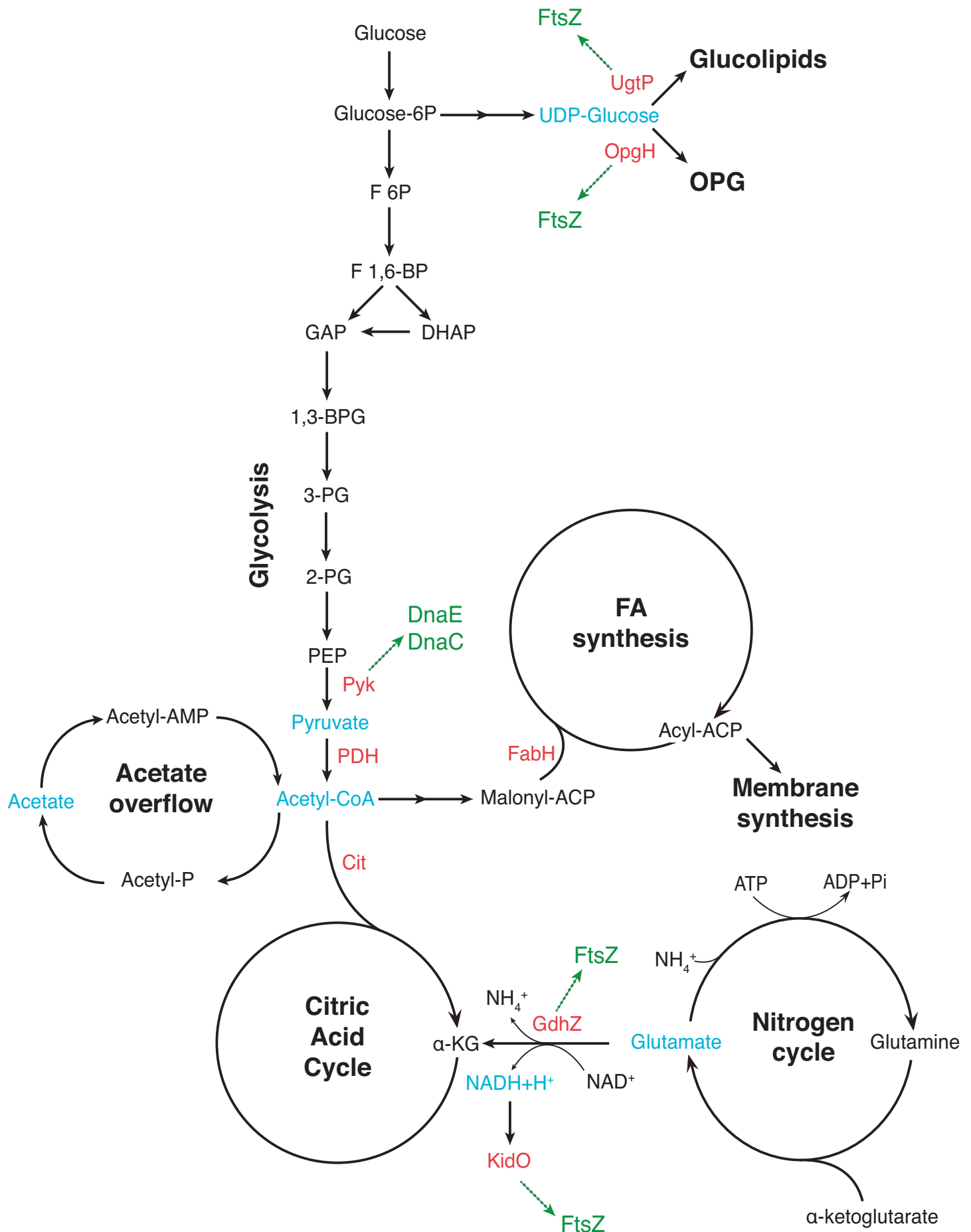
The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

## ① Initiation



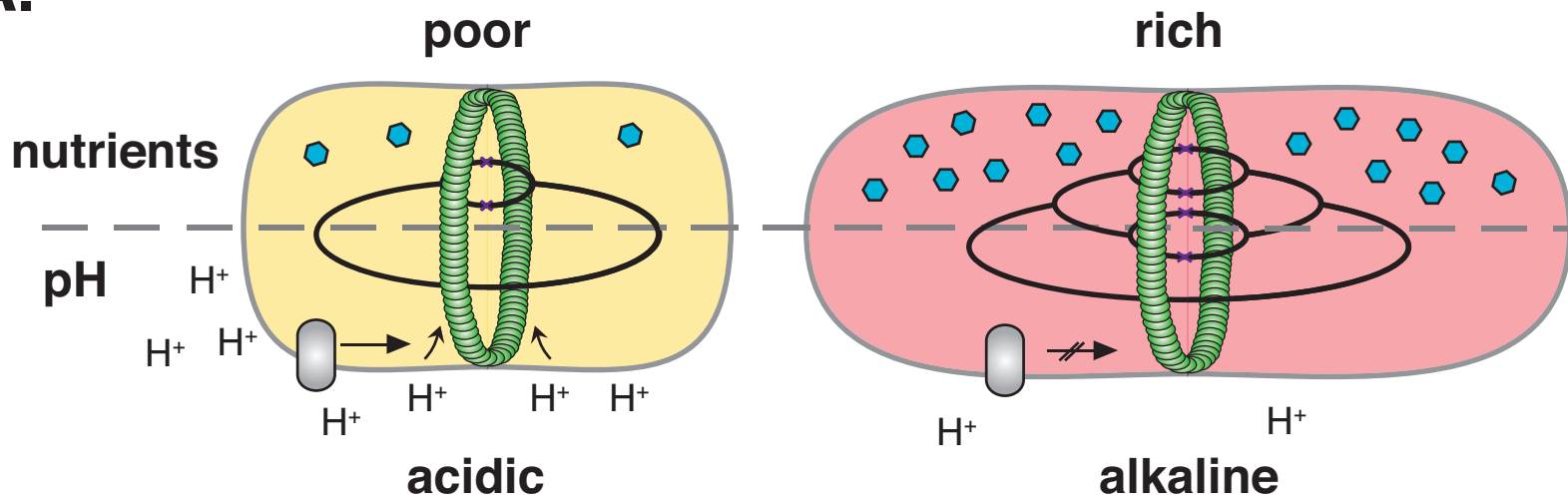
## ② Elongation





## Figure 3

A.



B.

