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Current Opinion in Microbiology

When the metabolism meets the cell cycle in bacteria

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Abstract:	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.
Author Comments:	

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

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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 γ -proteobacterium *Escherichia coli* and the α -
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_{R357A}
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^{H303W} and CitA^{H303A} [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 α subunit of the pyruvate dehydrogenase (PDH-E1 α), 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⁺ 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⁺ 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 α -
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^{Ntr}), 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.

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326

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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^{ATP}, 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^{ATP} is converted to DNA^{ADP}
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; α -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

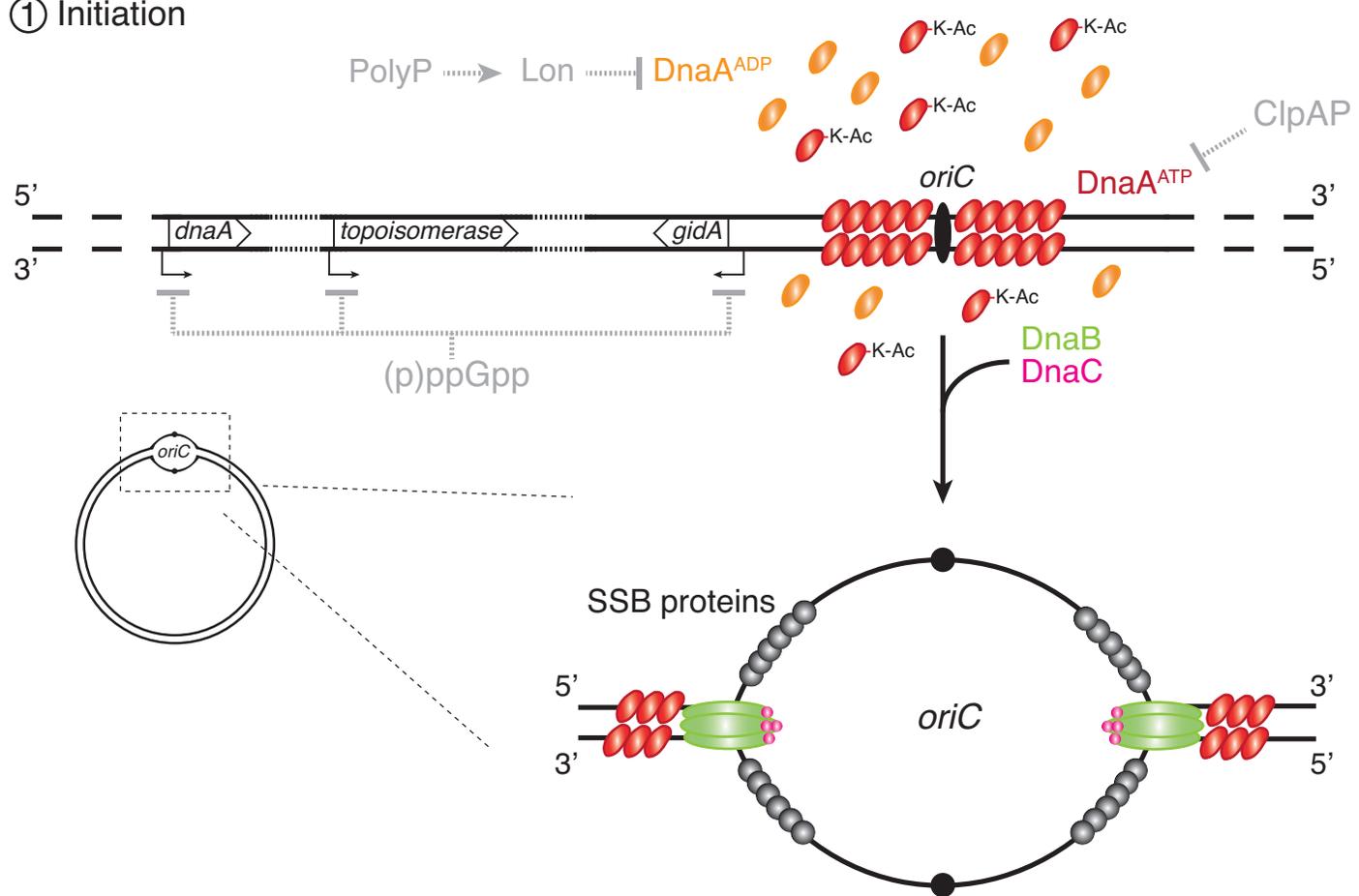
615 grey and metabolites used as a proxy for this regulation (e.g. UDP-Glucose) are
616 represented in light blue. The replicating DNA molecules are represented in black. (B)
617 Proteins coordinating metabolism with cell division interfere with Z-ring dynamics by
618 using different molecular mechanisms. 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⁺, 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

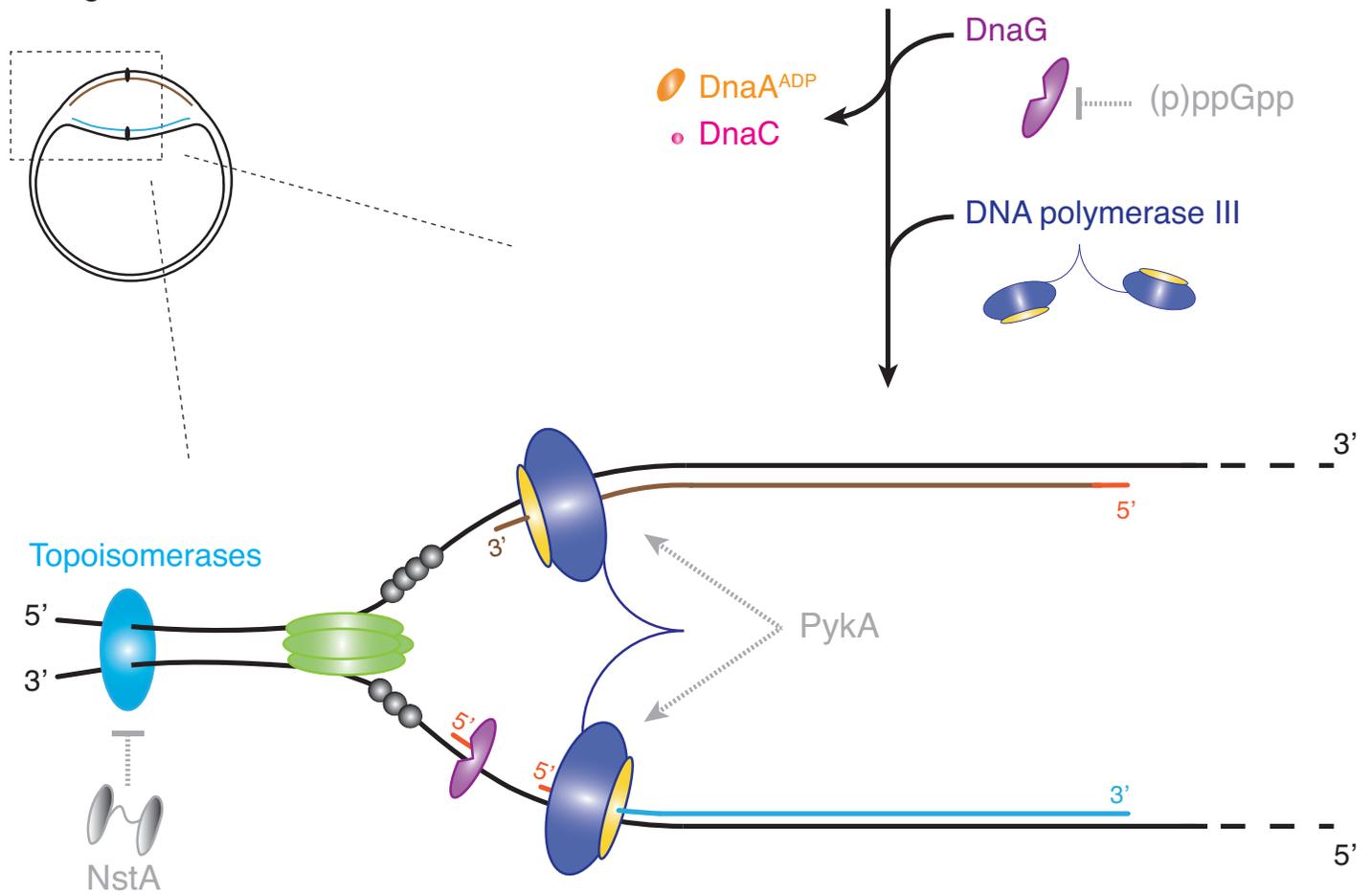
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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

① Initiation



② Elongation



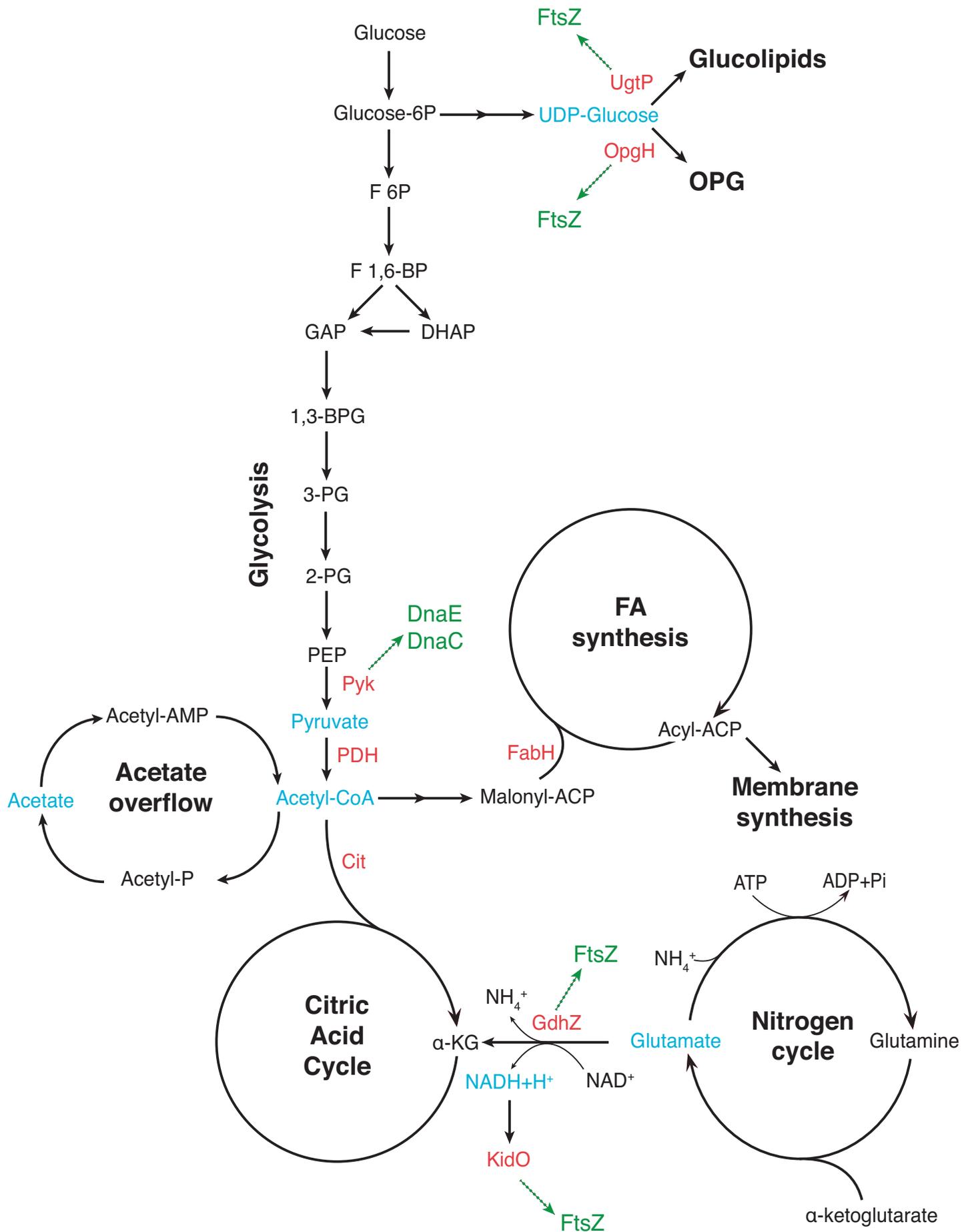
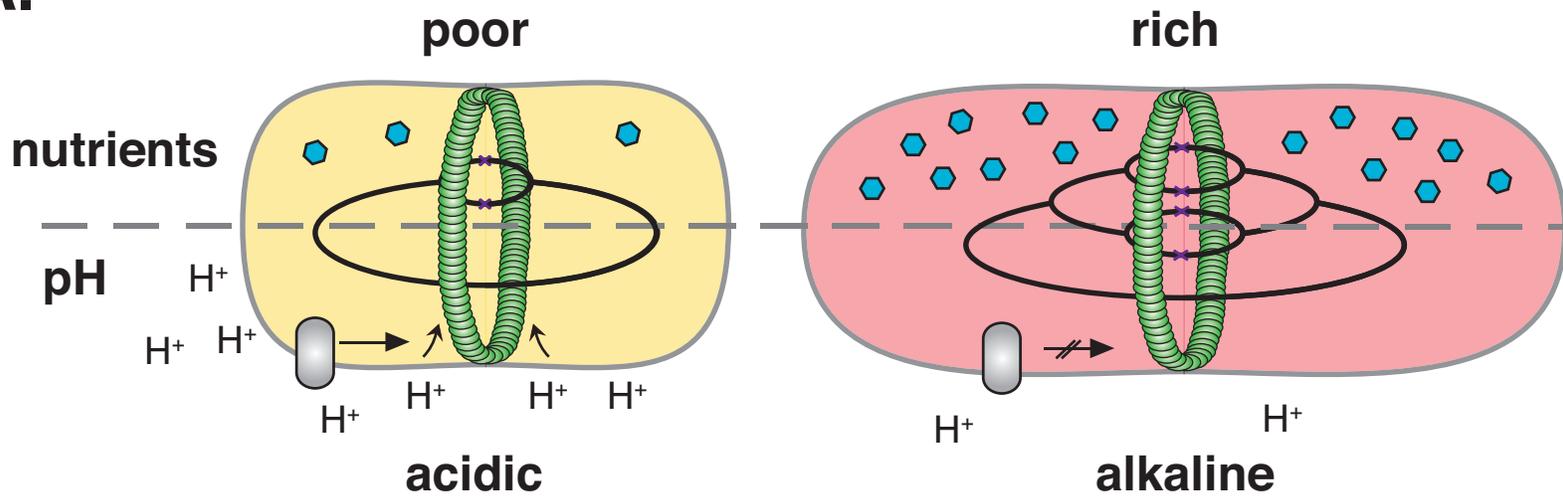


Figure 3

A.



B.

