JB Accepted Manuscript Posted Online 20 March 2017
J. Bacteriol. doi:10.1128/JB.00039-17

Metabolism shapes the cell

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Running Header: Metabolic regulation of growth and division

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ABSTRACT

More than five decades of work support the idea that cell envelope synthesis, including the inward growth of cell division, is tightly coordinated with DNA replication and protein synthesis through central metabolism. Remarkably, no unifying model exists to account for how these fundamentally disparate processes are functionally coupled. Recent studies demonstrate that proteins involved in carbohydrate and nitrogen metabolism can moonlight as direct regulators of cell division, coordinate cell division and DNA replication, and even suppress defects in DNA replication. In this minireview, we focus on studies illustrating the intimate link between metabolism and regulation of peptidoglycan (PG) synthesis during growth and division, and we identify the following three recurring themes: 1) Nutrient availability, not growth rate, is the primary determinate of cell size; 2) The degree of gluconeogenic flux is likely to have a profound impact on metabolites available for cell envelope synthesis, so growth medium selection is a critical consideration when designing and interpreting experiments related to morphogenesis; 3) Perturbations in pathways relying on commonly shared and limiting metabolites, like undecaprenyl phosphate (Und-P), can lead to pleotropic phenotypes in unrelated pathways.

INTRODUCTION

To accurately partition chromosomes and other cell contents during reproduction, cells must possess mechanisms to organize repeated cycles of cell growth, chromosome replication, and division. Eukaryotes orchestrate this coordination using the cell cycle and separate growth, DNA synthesis, and cytokinesis into distinct, temporally sequestered phases. Bacteria, by contrast, simultaneously increase in cell size and replicate DNA before (or concurrent with) cell division. Elucidating the molecular mechanisms prokaryotes employ to achieve spatiotemporal organization of these intertwined yet functionally disparate processes is of considerable interest to scientists seeking to understand bacterial reproduction, and many outstanding questions remain to be answered. For example, how is DNA replication kept in sync with changing growth and division rates? How are cell dimensions maintained or actively rearranged in response to environmental or developmental cues? What signals do cells sense to switch between increasing in cell size and dividing during the cell cycle? Relatedly, how are these signals transduced to activate/deactivate the distinct machineries required for each process?

Perhaps one of the biggest mysteries remaining in bacterial cell biology relates to understanding the regulatory crosstalk that must occur to integrate central metabolism with macromolecular biosynthesis. Nutrients are converted into the stored energy and precursors used to synthesize macromolecules like DNA and peptidoglycan (PG), so it is no surprise that nutrient availability has a profound impact on growth capacity. However, a growing body of evidence also suggests that metabolites and metabolic enzymes may play a more direct role in regulating critical aspects of cell growth and division than previously appreciated. These findings raise the intriguing possibility that metabolism itself may be the major determinant in shaping the underlying organization of the bacterial cell.

Actively growing bacteria respond rapidly to changing conditions by adjusting their overall shape and size. When nutrients are unrestricted, bacteria often capitalize on the available resources by increasing in cell size and reproducing more often. For rod-shaped bacteria including *Escherichia coli* and *Bacillus subtilis*, cell size is determined by both the length and width of the cell envelope. During steady-state growth, rapidly growing cells are generally longer and sometimes wider than their slower growing counterparts (1-4), at least when nutrients are unrestricted (3). The positive correlation between cell size and growth rate is likely due to nutrient availability rather than the growth rate itself, because the relationship can be broken under conditions where growth rate is controlled by restricting nutrients. For example, in minimal media with different tryptophan concentrations, *E. coli* cells growing at steady-state are largest (by volume) at concentrations of tryptophan that result in ~1/2 the maximal growth rate achieved with non-limiting tryptophan (5).

During balanced growth, cell size is remarkably homogenous across a population, suggesting that the signals cuing growth and division cycles are regulated and not random. Single cell experiments performed on *E. coli* and *Caulobacter crescentus* show that cells achieve cell size homeostasis not by triggering cell division when a specific cell volume is achieved, rather by elongating a constant amount (and thus adding a constant volume) before dividing (6-9). Precise division at mid-cell allows for a homogenous population size to be maintained over time (7). A more recent study demonstrates that when bacteria grow, surface area and cell volume scale together across a variety of bacteria (10). The authors of this study also provide data implicating levels of a limiting PG precursor as the likely signal for cuing cell division, thus providing a possible mechanistic basis to describe how bacteria may integrate central metabolism with growth and division (10).

In bacteria, growth and division cycles are largely coordinated by the actin-like protein MreB (and its paralogs) and/or the tubulin-like protein FtsZ. In many bacteria, MreB is primarily associated with cell elongation and FtsZ with cell division. However, these functional generalizations are probably oversimplified for bacteria as a whole, as the roles of MreB and FtsZ can overlap (11-14) and even be completely reversed (15-17). MreB and FtsZ are hypothesized to act as scaffolds, directing sites of new PG synthesis and old PG turnover (18). MreB and FtsZ are part of larger, multiprotein complexes (the elongasome and divisome)(19, 20) that ultimately define cellular dimensions by imposing spatial constraints on elongation and cell division (21, 22). The dependency of cell size on nutrient availability indicates that metabolism itself plays a critical role in controlling MreB and FtsZ activity, although possible mechanisms for this regulation are only beginning to emerge. One of the emerging themes discussed in more detail in this minireview is that the type of medium in which bacteria are cultured (particularly whether the medium primarily supports glycolytic or gluconeogenic growth regimes) can have a profound impact on cell shape and cell shape mutants, and thus is an important consideration when designing and interpreting studies.

Metabolism and Cell Elongation

Mecillinam resistance

The close relationship between metabolism and cell elongation is illustrated by studies with mutants resistant to mecillinam (amdinocillin), a β-lactam antibiotic that selectively targets the cell elongation transpeptidase PBP2 (23-25). In *E. coli* (as well as many other Gram-negatives) treatment with mecillinam results in rounded cells that lyse (25, 26). Mecillinam resistance can be conferred by mutations in genes in the *mrd* and *mre* loci that encode

components of the elongasome such as PBP2 and MreB (24), as well as in several dozen other targets, many of which have been shown to result in increased levels of the growth rate regulating molecule (p)ppGpp (synthesized by RelA and SpoT in *E. coli*)(27). Although mecillinam resistance has been studied for more than three decades, the mechanism by which enhanced (p)ppGpp levels leads to mecillinam resistance has not been elucidated. In *E. coli*, overexpression of the cell division proteins FtsQ, FtsA, and FtsZ (but not FtsZ alone) or artificially induced synthesis of (p)ppGpp bypasses the lethality associated with mecillinam treatment, so it has been proposed that increased (p)ppGpp levels result in an enhanced cell division capacity that allows the wider cells to divide (28, 29). However, subsequent studies demonstrated that increased FtsZ levels are not responsible for the mecillinam resistance, at least for the mutants that have been examined (30, 31), indicating that another mechanism confers resistance.

One way that (p)ppGpp's effects could confer resistance is through modulation of carbon metabolism. In *E. coli*, (p)ppGpp has been shown in combination with the RpoS regulator DskA to activate expression of *csrB* and *csrC,* genes encoding antagonists of the global carbon storage regulator CsrA (32). Downregulation of CsrA represses expression of glycolytic genes (*pgi, pfkA, pfkB, tpi, eno, pykF,* and *pykA*), promotes expression of genes important for flux through gluconeogenesis (*pck, fbp, pps,* and *pgm*) (33, 34), and leads to elevated levels of several intermediates in cell envelope synthesis, including phosphoenolpyruvate (PEP)(35, 36). Since gluconeogenesis is the primary pathway for generating precursors for cell envelope biogenesis, the higher levels of (p)ppGpp may result in a gain of function in PG synthesis by enhancing the available PG precursor pool. A similar relationship to enhanced gluconeogenesis and PG precursors could also explain why *crp* and *cya* mutants are resistant to mecillinam (37-39), since

145 in *E. coli* CRP-cAMP represses expression of genes involved in shifting flux through
146 gluconeogenesis, including *pck* (40, 41).

147 One mecillinam resistant *E. coli* mutant that depends on SpoT but not RelA for
148 resistance possesses a mutation in *aroK*. *aroK* encodes a shikimate kinase that catalyzes the
149 ATP dependent conversion of shikimate to shikimate-3-phosphate in the aromatic amino acid
150 synthesis pathway (31). The mutant also shows partial rescue of the filamentation and lethality
151 effects associated with *ftsZ84* (Ts), a temperature sensitive allele of *ftsZ*, indicating that *aroK*
152 inactivation acts as a general suppressor of defects in cell envelope synthesis. Based on the fact
153 that FtsZ levels were not detectably different in the *aroK ftsZ84* double mutant strain, and that
154 the mecillinam resistance was not dependent on AroK's characterized enzymatic activity or *relA*,
155 the authors concluded that AroK likely possesses a second, moonlighting function related to cell
156 division regulation (31). Another possibility is that *aroK* inactivation causes a change in carbon
157 flux. We can envision two ways that this could occur, both of which involve enhancing the
158 availability of PG precursor pools to the elongasome and divisome machineries. First, the next
159 step in the shikimate pathway utilizes PEP, so the *aroK* mutant could possess elevated levels of
160 PEP. PEP is both a critical intermediate in gluconeogenesis and required for the first dedicated
161 step of PG synthesis, the MurA-dependent conversion of UDP-N-acetylglucosamine (UDP-
162 GlcNAc) to UDP-GlcNAc-enolpyruvate (42, 43). In addition, the levels of (p)ppGpp, although
163 doubled in the *aroK* mutant, were lower than wildtype in the mecillinam resistant *relA aroK*
164 double mutant, leading the authors to conclude that enhanced (p)ppGpp could not account for the
165 resistance. However, the quantification of (p)ppGpp was performed in a minimal medium
166 supplemented which glucose, amino acids, and shikimate, whereas the mecillinam resistance was
167 assayed for on LB plates (31). Therefore the possibility that the resistance was conferred by

(p)ppGpp accumulation on LB was not directly ruled out. If there were elevated (p)ppGpp levels, this could lead to enhanced PG precursor synthesis through the mechanism discussed above.

Gluconeogenic factor YvcK

Another example of a connection between carbon metabolism and cell elongation is demonstrated by studies on YvcK. Homologs exist in a variety of bacteria (44-46), but YvcK is most extensively studied in *B. subtilis* (44, 47, 48). Although *yvcK* is not essential during growth in LB, cells with a *yvcK* deletion are round and lyse under gluconeogenic growth regimes, indicating a deficiency in elongasome function (44). Consistent with this hypothesis, *yvcK*'s conditional essentiality is bypassed by overexpressing MreB (47) or by providing a growth condition that suppresses problems with elongasome function (extra magnesium)(44, 49-52). YvcK is phosphorylated by the highly conserved PrkC Ser/Thr kinase (53-55) which also regulates the essential WalRK two-component system involved in regulating PG metabolism (56). PrkC also phosphorylates several proteins involved in metabolic regulation (Hpr, Icd, and GlnA)(55), protein synthesis and cell shape determination (EF-Tu, EF-G, and CpgA)(57-59), and one protein implicated in mediating the switch between cell elongation and cell division, GpsB (60-62). Although phosphorylation of YvcK is not required for its role in gluconeogenesis, only the phosphorylated form can bypass *mreB* essentiality (48) and restore the septal localization of the major transglycosylase/transpeptidase PBP1 (47, 48).

YvcK's role in promoting gluconeogenic growth in *B. subtilis* is unknown, but its essentiality is bypassed by transposon insertions in several genes related to carbon and nitrogen metabolism (*zwf*, *cggR*, and *mfd*) and cell envelope synthesis (*yfnI*, *dgkA*, *yqfF*) (44). For

example, the *yvcK* mutant could be rescued by inactivation of Zwf, which feeds glucose-6-phosphate into the pentose phosphate pathway, or CggR, which inhibits synthesis of glucose-6-phosphate by repressing genes in the bottom half of gluconeogenesis (44). The transposon insertion in *mfd*, which results in decreased synthesis of an extracellular polymer of glutamate called poly-γ-glutamate (PGA), would be expected to result in enhanced flux through other glutamate utilizing pathways (63). The insertions in *yfnI* and the first gene in the *yqfF* operon likely perturb different processes in cell envelope synthesis. YfnI is important for generation of the polyglycerolphosphate and GroP-Glc_2-DAG glycolipid components of lipoteichoic acid during cell stress (64), whereas the last gene in the *yqfF* operon, *dgkA*, may be involved in phosphorylating undecaprenol to generate Und-P (65). *yqfF* itself encodes a protein involved in cyclic-di-AMP turnover, an essential molecule involved in cell envelope homeostasis in Gram positive organisms (66). Intriguingly, *whiA* (a cotranscribed gene downstream of *yvcK*)(67) exhibits a synthetic cell division defect when deleted in combination with *zapA (68).* The cell division defect is also rescued by mutations in genes involved in carbon transformations, specifically *gtaB* and *pgcA* (68), the latter of which is discussed in more detail below. These results hint that YvcK and WhiA take part in related processes (68).

Phosphomannose isomerase (ManA)(B. subtilis)

ManA is another protein in *B. subtilis* implicated in both carbon transformations and cell shape. In *B. subtilis*, ManA is required for rod shape and viability in LB, but not in minimal media containing glucose as a carbon source (69). Characterized ManA homologs catalyze the reversible conversion between mannose-6-phosphate and fructose-6-phosphate. In some archaea and bacteria, the enzyme responsible for phosphomannose isomerase activity is also a

phosphoglucose isomerase, catalyzing the isomerization between glucose-6-phosphate and fructose-6-phosphate (70). In most bacteria, including *B. subtilis*, the characterized phosphoglucose isomerase is encoded by *pgi*. However, *B. subtilis* ManA can carry out isomerization of several sugars in *in vitro*, suggesting there may be substrate promiscuity in the active site (71); therefore, one speculation is that it can also act as a phosphoglucose isomerase.

In *B. subtilis*, a Δ*manA* mutant becomes rounded and stops growing shortly after shifting from minimal media to LB. The requirement for ManA in cell shape is likely attributable to its enzymatic activity, as several predicted catalytic mutants are unable to complement the Δ*manA* mutant (69). Since LB does not contain mannose, these results are somewhat perplexing. The cell envelope of the *manA* mutant shows reduced levels of galactose and GalNac (N-acetylglucosamine, a component of teichoic acid), so it was proposed that the defect in the *manA* mutant was due to problems with teichoic acid synthesis (71). However, since the UDP-GalNac used to synthesize teichoic acids is primarily generated from epimerization of UDP-GlcNac (72), the result hints that the *manA* mutant is also likely compromised in its ability to synthesize UDP-GlcNac. Consistent with this possibility, the *manA* mutant phenocopied cells inhibited for synthesis of Lipid I by tunicamycin (71). No difference in GlcNac levels were observed between the *manA* mutant and wildtype (71); however, the mutant also stopped growing shortly after the shift to LB, and neither intracellular GlcNac levels, nor PG synthesis were measured directly, thus the possibility that PG synthesis was essentially paused in the *manA* mutant growing in LB was not ruled out.

The widened cells and altered chromosome structures of the *manA* mutant are remarkably similar to those observed when the pools of pyrimidine precursors are limited in *E. coli* (73). Since DNA and PG synthesis can share a common precursor, UTP (used to generate UDP-

GlcNac and dCTP/dTTP respectively), these results suggest that cell growth is particularly sensitive to perturbations in UTP, and possibly provide a link for the correlation observed between cell growth and DNA replication during steady state (74). One possibility that could explain all of the observed results is that in LB not supplemented with glucose, ManA contributes significantly to the conversion of fructose-6-phosphate to glucose-6-phosphate to feed pathways for PG, techoic acid, and DNA synthesis. In principal, this would be an easy hypothesis to test since the addition of glucose to the LB medium would be predicted to alleviate the cell shape defect. Alternatively, ManA could possess a moonlighting role, regulating some aspect of cell envelope biogenesis directly, although its diffuse localization provides no clues as to how such a mechanism would occur (69).

Metabolism and Cell Division

Evidence for genetic interactions between carbon and nitrogen metabolism and cell division are also abundant, and several examples are summarized in more depth in recent reviews (7, 75, 76). In most cases, discussed in more detail below, a metabolic enzyme is implicated in inhibiting FtsZ activity either directly or indirectly. In some cases, the loss of the corresponding gene leads to more active FtsZ (*pgm*, *pgcA*, *ugtP*, *opgH*, and *pykA* in an FtsZ temperature sensitive background) causing cells to divide more often, while in other cases (*gdhZ*, *kidO*, and *pycK* in a wildtype background), the loss of the gene inhibits cell division.

UDP-Glucose and UgtP (B. subtilis)

The first study to demonstrate a direct link between FtsZ regulation and a metabolic enzyme was published in 2007 (77). The authors identified a gene disruption in *pgcA* (encoding

phosphoglucomutase) that resulted in cells that were ~1/3 shorter than wildtype yet maintained a wildtype growth rate in LB. PgcA catalyzes the reversible conversion between glucose-6-phosphate to glucose-1-phosphate in the production of UDP-glucose (78). These investigations revealed that UgtP, an enzyme that can transfer glucose from UDP-glucose to diacylglycerol-containing sugar acceptors *in vitro* (79), also exhibited a short cell phenotype. UgtP interacts directly with FtsZ and inhibits its polymerization in a manner that depends upon UDP-glucose both *in vivo* and *in vitro* (77). According to their model, nutrient rich conditions result in excess glucose-6-P, resulting in an increased flux towards UDP-glucose synthesis. In the presence of its substrate, UgtP shows preference for interaction with FtsZ over itself, resulting in inhibition of FtsZ and longer cells (80).

UDP-Glucose and OpgH (E. coli)

Regulation of FtsZ assembly by another enzyme utilizing UDP-glucose has also been observed in *E. coli*. In this study, the authors observed that a knockout of *pgm*, the functional equivalent of *pgcA*, also produced small cells (81). The authors eventually implicated OpgH (formerly MdoA) in the small cell size. OpgH is a glucosyltransferase involved in the synthesis of osmoregulated periplasmic glucans. OpgH is not homologous to UgtP and is involved in the synthesis of a distinct macromolecule, yet both proteins appear to inhibit FtsZ polymerization in a UDP-glucose dependent manner (77, 80, 81). Deletion of *opgH* results in more frequent divisions, while overexpression results in filamentation. OpgH also colocalizes with FtsZ in an FtsZ-dependent manner during fast growth. A truncation of OpgH consisting of amino acids 1-138 (and devoid of the membrane spanning domains) strongly inhibits FtsZ assembly *in vitro*, indicating that OpgH interacts directly with FtsZ to inhibit cell division (81). *In vitro* assays

283 suggest that OpgH inhibits FtsZ polymerization by binding FtsZ monomers, thereby increasing
284 the apparent concentration of FtsZ required for GTP hydrolysis. How this mechanism would
285 suffice *in vivo* given that the native copy number of OpgH is likely to be low (82) is less clear.
286 Unlike UgtP in *B. subtilis,* OpgH appears to localize to FtsZ in a UDP-glucose independent
287 manner. Curiously, OpgH still localizes to FtsZ during fast growth, but becomes more diffuse in
288 the membrane as growth slows (81).

289 The data presented above strongly indicate that UgtP and OpgH interact directly with
290 FtsZ, although it is less clear if these enzymes possess an express role in regulating FtsZ
291 dynamics *in vivo*. As with any data, it is worth considering alternative models. Another
292 possibility is that UgtP and/or OpgH utilize interactions with FtsZ to localize to regions in the
293 cell where UDP-glucose is initially produced and metabolized. To that end, there are numerous
294 protein-protein interaction and substrate channeling studies that certainly argue bacteria are too
295 clever to depend on diffusion alone to control metabolite flow (83-90). Although the *in vitro*
296 data argue for possible FtsZ-regulating mechanisms, especially for UgtP, any protein that
297 interacts with an FtsZ surface could conceivably act as an inhibitor of FtsZ activity *in vitro*.
298 How then, could one account for the UDP-glucose levels having such a profound impact on two
299 entirely different organisms? Assuming the pathways outlined in Fig. 1 are accurate, mutations
300 in several of the genes discussed above would likely result in increased availability of glucose-6-
301 phosphate to other pathways. Such a shift might be expected to increase the availability of Lipid
302 II to support FtsZ-dependent cell division and MreB-dependent cell elongation. The net result
303 one might expect, assuming FtsZ is more sensitive to substrate availability than MreB, would be
304 more active FtsZ and shorter cells. Some of the mutations (in *opgH* and *ugtP*) might also result
305 in enhanced Und-P availability (discussed more below). Synthesis of teichoic acids requires

306 Und-PP-sugar intermediates (91, 92), so defects in the pathway prior to Und-P commitment
307 would be expected to increase overall Und-P pools. Similarly, the glycosyltransferase activity of
308 OpgH uses polyprenyl phosphates (including decaprenyl phosphate) as a substrate (93).
309 Although Und-P has not been formally tested as a substrate, an *opgH* mutant exhibits increased
310 resistance to bacitracin (which inhibits recycling of Und-PP to Und-P)(94) and enhanced EPS
311 production (95, 96); each of these phenotypes is consistent with enhanced availability of Und-P.
312 The idea that elevated pools of cell envelope precursors (especially PG precursors) could lead to
313 enhanced cell division is also supported by additional studies discussed below.
314
315 *Pyruvate, PykA, and PdhA (B. subtilis)*
316 In *B. subtilis*, deletion of *pykA* (pyruvate kinase), which catalyzes the conversion of PEP
317 to pyruvate, rescues a temperature sensitive allele of *ftsZ* (97), possibly suggesting that enhanced
318 PEP levels (or enhanced gluconeogenic flux) rescues defects in cell division. However, in
319 wildtype, deletion of *pykA* also disrupts normal FtsZ regulation, resulting in ~40% of cells
320 possessing either subpolar or multiple FtsZ rings. Although no direct interaction between PykA
321 and FtsZ could be detected, subsequent experiments demonstrated that inactivation of an enzyme
322 that feeds pyruvate into the TCA cycle, PdhA, could restore normal FtsZ ring formation in the
323 temperature sensitive FtsZ background (97). PdhA also shows an unusual localization pattern
324 reminiscent of nucleoid staining in wildtype, which undergoes redistribution to nucleoid-free
325 regions in the *pykA* mutant (97). Notably, this latter pattern is very similar to what has been
326 observed for several components of the *B. subtilis* RNA degradosome (98). Colocalization with
327 the nucleoid is restored in the *pykA* mutant by the addition of exogenous pyruvate, suggesting
328 that PdhA depends on the product of PykA (pyruvate) and not PykA itself for wildtype

329 localization and normal FtsZ assembly. The authors propose that in the presence of pyruvate,
330 PdhA acts as positive regulator of FtsZ assembly, and that PdhA's subpolar positioning in the
331 *pykA* mutant promotes subpolar FtsZ-ring formation. More studies will be required to assess if
332 PdhA's role in controlling FtsZ dynamics is direct or indirect.
333
334 *Nitrogen metabolism, GdhZ, and KidO (Caulobacter crescentus)*
335 Glutamate is a key central metabolite, connecting pathways for cellular anabolism and
336 catabolism; glutamate is also the central donor for nearly all anaplerotic metabolites containing
337 nitrogen, and is maintained at high intracellular concentrations under all growth conditions (99).
338 In *E. coli*, mutations in several genes important for nitrogen uptake and metabolism (*glnD*, *glnG*,
339 and *glnL*) restore growth of an *ftsZ84* mutant at non-permissive temperatures by promoting
340 (p)ppGpp accumulation (100). Studies in *C. crescentus* have also implicated nitrogen
341 metabolism, and in particular glutamate conversions, in the direct regulation of FtsZ assembly.
342 The enzyme responsible for the catalytic conversion of glutamate and NAD+ to α-ketoglutarate,
343 ammonia, and NADH in *C. crescentus* is called GdhZ (101). GdhZ was identified in a screen for
344 FtsZ interacting proteins and comes down as an FtsZ partner in coimmunoprecipitation
345 experiments. Deletion of *gdhZ* results in abnormal cell divisions, leading to a pleiomorphic
346 mixture of short, normal, and filamented cells in mixed population cultures. GdhZ colocalizes
347 with FtsZ *in vivo* and stimulates FtsZ's GTPase activity in a glutamate-dependent manner *in*
348 *vitro*. GdhZ's enzymatic activity is required for its effect on FtsZ *in vivo*, as a catalytically dead
349 mutant phenocopies the *gdhZ* deletion strain; the same variant does not stimulate GTPase *in vitro*
350 (101), suggesting that a conformational cycling of GdhZ may be required to stimulate hydrolysis

of GTP. Alternatively, pH effects on FtsZ's GTPase activity resulting from the conversion of glutamate to α-ketogluturate and ammonia in the reaction were not ruled out.

KidO is another protein shown to promote FtsZ disassembly in *C. crescentus*. KidO is believed to act in conjunction with GdhZ to regulate FtsZ disassembly both during the cell cycle and under conditions of nitrogen limitation (101). The phenotypes associated with a *kidO* deletion are not as pronounced as the *gdhZ* deletion; however, the proteins follow similar cell cycle regulation, colocalize with FtsZ rings, and co-purify (101, 102). *In vitro*, KidO inhibits lateral bundling of FtsZ filaments in an NADH-dependent manner. KidO is proposed to act in cooperation with GdhZ, utilizing the NADH produced from GdhZ's enzymatic activity to inhibit FtsZ bundling (101).

Although much of the data points to the direct regulation of FtsZ by GdhZ and KidO, as with UgtP and OpgH, other (metabolically-based) models can be invoked. In a subsequent study by the same group, the authors found that when intracellular pools of glutamine drop, the (p)ppGpp hydrolysis function of SpoT becomes inhibited by the phosphorylated form of EIIA (part of the PTS^{NTR} system responsible for nitrogen uptake). This leads to (p)ppGpp accumulation, slowed growth, and extended G1 phase in a proportion of synchronized cells (103). In the *gdhZ* mutant, the glutamate unable to feed into the TCA cycle could conceivably be converted to glutamine by GlnA (glutamine synthetase), also generating *C. crescentus*' preferred carbon source, ammonium as a byproduct (103). However, the *glnA* gene is also repressed by high levels of glutamine (103), so the balance between glutamate and glutamine may be disrupted in the *gdhZ* mutant, leading to elevated (p)ppGpp levels. This scenario would also be consistent with the authors' observation that exogenous addition of glutamine did not

rescue the observed cell division defects in the *gdhZ* mutant. This alternative possibility could presumably be tested by determining if the *gdhZ* phenotype depended on SpoT.

Und-P availability as a regulator of growth and division

The lipid carrier Und-P is critical for inner membrane export of a highly diverse array of sugar-containing molecules including various exopolysaccharides, capsule, PG, teichoic acids, periplasmic glucans, O-antigen, and even glycosylated proteins, such as those associated with the flagella of several pathogens (91); as such, Und-P is an excellent target candidate for the development of novel antimicrobials. A growing body of research also indicates that Und-P is a limited resource that each of the aforementioned pathways must compete for, and disruptions in both cell division and cell elongation are associated with Und-P perturbations. How Und-P's distribution among these pathways is regulated remains an open question, but one might assume that the most essential processes (eg. PG synthesis) are preferentially fed before others. Und-P is generated from Und-PP de novo by UppS, and Und-P can subsequently act as an acceptor of sugars/glycans linked to a nucleotide diphosphate donor. Once the sugars/glycans are flipped across the membrane, they can be acted upon by additional enzymes, released, or transferred to another acceptor such as PG or lipoteichoic acid. Following the termination reaction, Und-PP is recycled to Und-P and reutilized (91). Mutations that block steps following the commitment of Und-P to a pathway (transfer of the first sugar/glycan to the lipid carrier) trap the Und-PP-conjugated intermediates in a dead-end pathway that effectively depletes the cellular pool of Und-P, resulting in cell division and cell shape defects (104-107). The phenotypic consequences of Und-P depletion likely depend on the drain imposed by the broken pathway pulling from the remaining Und-P pool, the metabolic demand for Und-P in other active and competing pathways,

396 and the level of coordination required for these pathways (which could become disrupted as
397 Und-P becomes limiting).

398 Many Gram-positive organisms utilize Und-P for synthesis of wall teichoic acids
399 (WTAs) and PG, and synthesis in the two pathways is likely coordinated (108). Consistent with
400 this idea, *B. subtilis* WTAs are essential for normal rod cell shape. However, WTAs are not
401 required for viability and lethality only occurs in mutants defective in the stages following
402 commitment of Und-P (via TagO) to the pathway (109). Similarly, *Staphylococcus aureus* WTA
403 mutants are lethal in most cases except when the gene encoding the Und-P dedicating enzyme
404 (*tarO*) is deleted (110). Moreover, epistasis experiments demonstrate that the lethality associated
405 with deletions in latter stages of the WTA synthesis pathway can be rescued by the *tarO* deletion
406 (110). These results suggest that the sequestration of Und-P, not the essentiality of the pathway
407 products, leads to lethality. Similar results were found in pathways for *Streptococcus*
408 *pneumoniae* capsule production (111) and *E. coli* O-antigen and enterobacterial common antigen
409 (ECA) synthesis (104, 105), implying that any mutation that significantly affects Und-P levels
410 has the potential to have an adverse consequences for any other pathway utilizing Und-P.

411 In the case of ECA or O-antigen synthesis, the deleterious effects of Und-P sequestration
412 (manifested as cell division and cell shape defects) can be overcome by increasing expression of
413 UppS or MurA, the latter of which makes the PG synthesis pathway more competitive for the
414 available Und-P (104, 105). These results strongly argue that the phenotypes observed in at least
415 some mutants disrupted at intermediate steps in Und-P utilizing pathways do not result from
416 toxic build-up of intermediates, rather dysregulation of Und-P utilizing reactions. In contrast,
417 UppS overexpression does not rescue mutants halted at intermediate steps in colonic acid
418 biosynthesis, leaving open the possibility that least some intermediate products may be toxic

(112). Although little is understood about how Und-P availability is regulated, Und-P recycling is a likely candidate. Given the large number of Und-P recycling enzymes already discovered, it is even possible that certain pathways possess their own dedicated recycler. Of course, this would suggest that Und-P production or utilization is controlled spatially, as multiple enzymes are still competing for an identical substrate. There is at least some evidence for this possibility in *B. subtilis*, where MreB localization was shown to depend on the synthesis of Und-P linked precursors (113). This extraordinary finding suggests that the availability of precursors at specific sites in the cell envelope is likely to act upstream of MreB (and likely FtsZ) in determining where and when cells grow or divide.

CONCLUSIONS

It is likely that many readers have either carried out, or are at least familiar with results from an impeccably designed screen related to their favorite cellular process that implicated one or more seemingly boring, if not completely incomprehensible metabolic genes. For many of us, metabolism is a white elephant in the room. We know it is lurking there, but ignoring it is easier than facing Figure 1. With metabolism, everything is connected and one can quickly become overwhelmed. However, even though cells are rather complex amalgamations of nucleic acid, protein, lipid, and carbohydrate, a surprisingly small number of key metabolites weave these macromolecules neatly together.

One of the themes we encountered while assembling this minireview is that growth is profoundly impacted by changes in carbon flux between glucose-6-phosphate and pyruvate. In particular, PG synthesis seems to be finely tuned to the relative levels of PEP, pyruvate, and glucose-6-phosphate present within cells. Often mutations that enhance glucose-6-phosphate

availability or promote carbon flow through gluconeogenesis rescue mutants with elongasome and/or divisome defects. The activity of both the divisome and elongasome is highest when there is an abundance of glucose-6-phosphate available the PEP/pyruvate ratios are high. When cells are grown in minimal medium utilizing TCA intermediates or non-PTS sugars, sugars such as glucose-6-phosphate must be synthesized, and PEP/pyruvate ratios are higher. Not surprisingly, this slows down PG synthesis and cell growth in general. It is striking that many of the mutations we highlighted either enhance production of glucose-6-phosphate or result in higher PEP/pyruvate ratios. Either of these conditions would be expected to increase the pool of both Und-P and UDP-GlcNac to the PG synthesis machinery. Conversely, decreasing the PEP/pyruvate ratio artificially (by depleting PdhA) delays cell division (97). Notably, knocking down *gapA* in LB (which should decrease the PEP/pyruvate and the precursors available for synthesis of Und-P and UDP-GlcNac) results in cell bulging in *B. subtilis* (114). Could these results hint that the final arbitrator of cell size (the decision of whether to grow or divide) is not a protein, but the availability of the metabolites themselves?

Many factors outlined in this review are proposed to have moonlighting functions, acting as both enzymes and regulators of FtsZ. In the space allotted, we were barely able to scratch the surface of proteins predicted to act in similar ways to coordinate metabolism with regulation of cytoskeletal function or DNA replication (115-119). Based on the growing number of factors that appear to interact with MreB and/or FtsZ and have a seemingly unrelated enzymatic function, we would like to end with a speculation: that at least some of the proteins proposed to have moonlighting functions may actually be utilizing interactions with FtsZ and MreB to localize their enzymatic activities to sites in the cell where their substrate is actively metabolized. For example, at least some evidence suggests that the Lipid I and II generated for cell elongation

is synthesized at non-random, punctate-helical locations in the cell where it then attracts components of the elongasome, including MreB (113). Since many of the components of the cell envelope share precursors like Und-P, UDP-glucose, and UDP-GlcNac, it is conceivable that at least some of the enzymes involved in making these components utilize interactions with the elongasome and divisome to coordinate synthesis and/or to compete for substrate. From this perspective, MreB and FtsZ may not be "cytoskeletal" factors so much as they are middlemen, mediating interactions between metabolites and enzymes. Regardless of the ultimate mechanism, understanding the regulatory crosstalk that must occur to coordinate cell growth, chromosome replication, and cell division requires delving into metabolism, and there is likely considerable insight to be gained by analyzing the cell biology from this additional perspective. Perhaps it's time we all rummaged through our freezers and gave those perplexing mutants another chance.

ACKNOWLEDGEMENTS

Thank you to members of the Herman lab for helpful comments on this review.

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Figure 1. Metabolic pathways implicated in the regulation of cell shape and size

Enzymes are indicated by green, red, or blue text. Enzymes discussed in the text are indicated in red or blue. Enzymes shown in blue denote steps dedicating Und-P (also in blue) to one or more pathways. Enzymes predicted to coincide with or precede Und-P dedication to one or more pathways are followed by blue question marks. In most cases, the relevant enzyme(s) for both *E. coli* and *B. subtilis* are given; however, not all organisms possess every enzyme shown. Enzymes that are less studied or have not been tested experimentally are generally excluded. Enzymes shown to interact directly with FtsZ are denoted with an asterisk. Only regulators of glycolysis and gluconeogenesis discussed in the text are shown in the left-hand block.

BIOGRAPHICAL INFORMATION

Jennifer K. Herman

Dr. Jen Herman is an Assistant Professor in the Department of Biochemistry and Biophysics at Texas A&M University. She completed her PhD at Indiana University in 2005, working on *Caulobacter crescentus* stalk synthesis in the laboratory of Dr. Yves Brun. This was followed by postdoctoral training at Harvard Medical School, where she studied *Bacillus subtilis* sporulation under the mentorship of Dr. David Rudner. Dr. Herman's interest in bacterial metabolism dates back to creating her first Winogradsky column in an undergraduate bacterial physiology course. Current projects in her laboratory focus on understanding the mechanistic relationship between bacterial morphogenesis and gluconeogenic carbon flux.

Anthony M. Sperber

Anthony Sperber obtained his B.S. in Biochemistry at the University of Missouri where he worked on the *E. coli* secretory system as an undergraduate researcher in the laboratory of Dr. Linda Randall. He is currently a PhD student in the Biochemistry and Biophysics Department at Texas A&M University, where he works on projects related to both bacteriophage biology and characterizing genes of unknown function in *Bacillus subtilis* in the laboratories of Dr. Jennifer Herman and Dr. Ryland Young. Anthony became interested in bacterial physiology when one of his PhD projects collided head-on with carbon metabolism.