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Metabolic control of cell division in α-proteobacteria by a NAD-dependent glutamate dehydrogenase

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ABSTRACT

Prior to initiate energy-consuming processes, such as DNA replication or cell division, cells need to evaluate their metabolic status. We have recently identified and characterized a new connection between metabolism and cell division in the α-proteobacterium *Caulobacter crescentus*. We showed that an NAD-dependent glutamate dehydrogenase (GdhZ) coordinates growth with cell division according to its enzymatic activity. Here we report the conserved role of GdhZ in controlling cell division in another α-proteobacterium, the facultative intracellular pathogen *Brucella abortus*. We also discuss the importance of amino acids as a main carbon source for α-proteobacteria.

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Undoubtedly, a cell needs to coordinate growth and cell division with nutrient availability. This metabolic control of cell division is particularly important to ensure cell size homeostasis of a growing population.[1–4] Although this concept is widely accepted, little is known about how the nutritional status is communicated to the cell cycle. Our recent discovery that the glutamate dehydrogenase GdhZ coordinates metabolism with cell division in *C. crescentus* (Figure 1) highlights the central role of metabolic enzymes in signaling nutrient fluctuations to the cell cycle.[5] Here we discuss the conserved role of GdhZ in controlling cell division in α-proteobacteria, and debate the importance of amino acids catabolism among those bacteria.

GdhZ coordinates growth with cell division in the intracellular pathogen *brucella abortus*

The α-subdivision of proteobacteria includes gram-negative bacteria with diverse and contrasting lifestyles, ranging from free-living, symbiotic to pathogenic bacteria.[6] Despite this apparent heterogeneity, α-proteobacteria share some unexpected common features such as an asymmetric cell division followed by a differentiation process.[7–9] As the large NAD-dependent glutamate dehydrogenases, to which GdhZ belongs, are particularly well conserved among α-proteobacteria,[10] we postulated that the cell division control found in *C. crescentus* might be conserved as well. To address this question we created an in-frame deletion of *gdhZ* homolog in the facultative intracellular pathogen *Brucella abortus* 2308 ($gdhZ_{Ba}$). Similarly to *gdhZ* loss-of-function mutants in *C. crescentus*,[5] the $\Delta gdhZ_{Ba}$ strain had a strong growth defect when grown in complex medium (Figure 2A). Interestingly, $\Delta gdhZ_{Ba}$ cells were slightly elongated and branched (Figure 2B), which typically indicates a cell division defect in bacteria growing from one pole.[11,12] Furthermore growth and cell division defects were suppressed when glucose or xylose was added to the complex media or when xylose was used as the sole carbon source in a synthetic medium (Figure 2A and data not shown). Altogether these observations suggest that the role of GdhZ in controlling cell division might be conserved in *B. abortus*, and likely in other α-proteobacteria.

GdhZ is required for efficient intracellular replication of *Brucella abortus*

As an intracellular pathogen, *Brucellae* reside preferentially within trophoblasts and macrophages,[13,14] in which it can survive and proliferate to produce chronic infections.[15,16] Intracellular *Brucellae* hijack the intracellular vesicular trafficking to finally reach and replicate into the endoplasmic reticulum.[17] Althougth widely accepted to be a nutrient poor environment, the exact composition of the *Brucella* containing vacuole (BCV) remains

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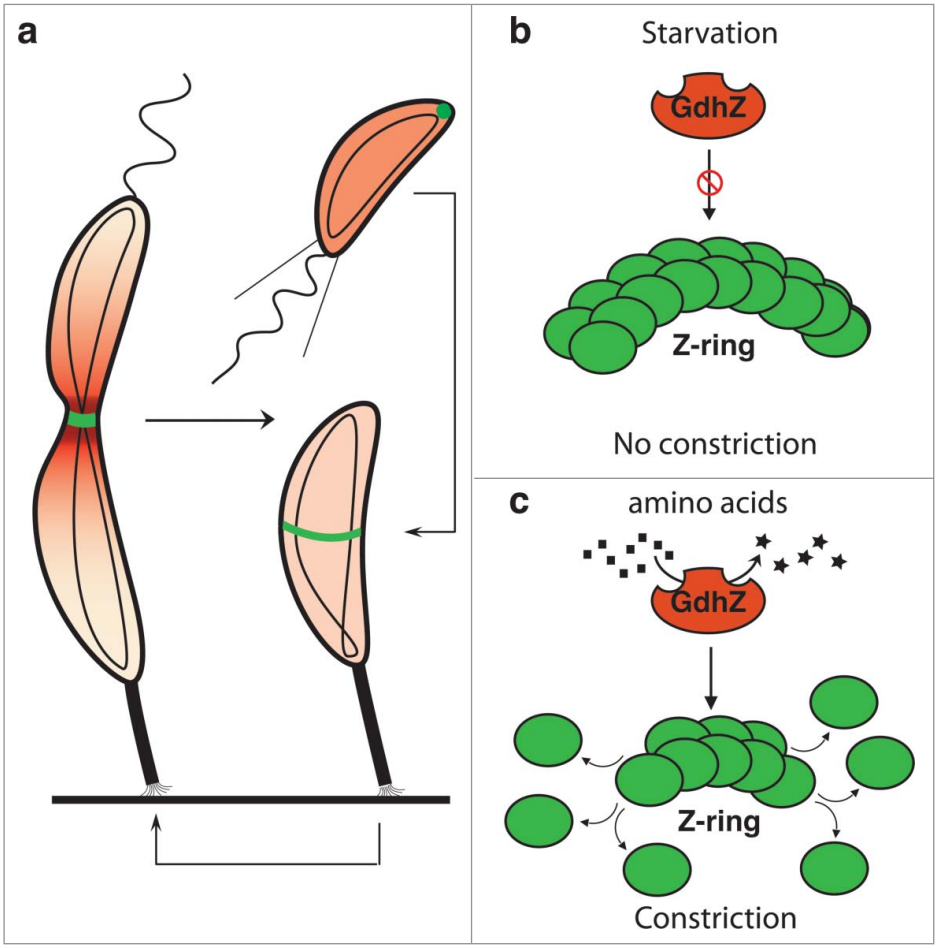

Figure 1. Metabolic control of cell division by GdhZ in *Caulobacter crescentus C. crescentus* has developed a complex asymmetric cell cycle to optimize its survival in oligotrophic environments. (a) Each cell division produces a small swarmer cell and a large stalked cell. The swarmer cell is proposed to be a settler looking for new environments whereas the stalked cell is responsible for colonizing these environments by giving birth to progeny cells. When a swarmer cell finds favorable conditions, it differentiates into a stalk cell to enter into a replicative cycle. (b-c) *C. crescentus* has evolved a system in which the NAD-dependent glutamate dehydrogenase GdhZ acts as a proxy, signaling nutrient availability to the division apparatus, thereby coordinating growth with cell division. FtsZ and GdhZ are respectively represented in green and red.[5]

unclear.[16] To test whether GdhZ is important for intracellular replication, murine raw macrophages were infected with either wild-type or Δ$gdhZ_{Ba}$ strain, and the number of intracellular bacteria was determined at 2 hrs, 24 hrs and 48 hrs post-infection (PI). As GdhZ constitutes a key entry point into the tricarboxylic acid (TCA) cycle for several amino acids, the Δ$gdhZ_{Ba}$ mutant would be unable to efficiently replicate inside host cells if amino acids were a main carbon source during infection. As illustrated in Figure 3A, the Δ$gdhZ_{Ba}$ mutant was already impaired 2 hrs PI and intracellular *Brucellae* failed to replicate efficiently at 24 hrs and 48 hrs PI. During the first 6–8 hrs following entry into the cell, intracellular *Brucellae* are in a G1 state and do not proliferate.[18] Therefore, the difference in the number of intracellular bacteria between the strains at 2 hrs post-infection might be due to a defect in the internalization process. Since bacteria were cultivated in complex media prior to infect, the proper entry of Δ$gdhZ_{Ba}$ cells inside macrophages might be affected by their morphological defect (Figure 2). To circumvent this problem, *B. abortus* wild-type and Δ$gdhZ_{Ba}$ strains were grown in complex medium supplemented with glucose prior to infect murine macrophages. Interestingly, when glucose was added to the preculture medium, both wild-type and Δ$gdhZ_{Ba}$ intracellular bacteria were present at similar levels at 2 hrs PI, indicating that the entry impairment observed with Δ*gdhZBa* cells was very likely a secondary effect (Figure 3B). Nevertheless, at 24 hrs and 48 hours PI, Δ$gdhZ_{Ba}$ cells failed to efficiently replicate compared to wild-type cells, whatever the medium used for cultivating *Brucella* before infecting macrophages. Altogether these results strongly suggest that GdhZ is required for optimal intracellular replication of *Brucella*, and

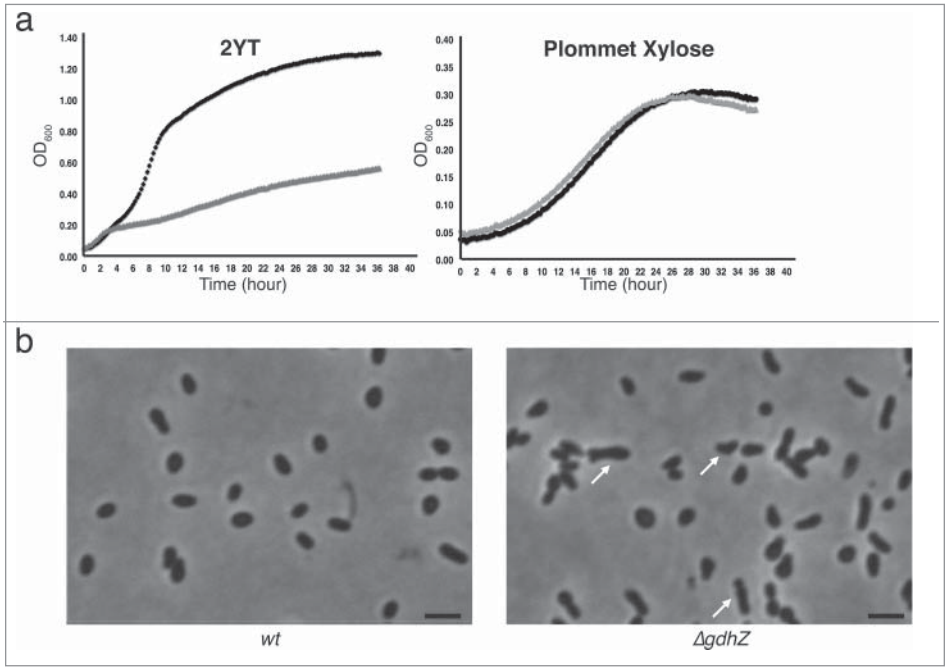

Figure 2. GdhZ coordinates growth with cell division in *Brucella abortus*. (a) Growth of *B. abortus* wild-type (black) and Δ$gdhZ_{Ba}$ (gray) cells in complex medium (2YT) or in synthetic medium with xylose as the only carbon source (Plommet Xylose), showing GdhZ is required for optimal growth in complex mediium. (b) Phase contrast imaging of *B. abortus* wild-type and Δ$gdhZ_{Ba}$ cells grown in complex medium (2YT) illustrating the morphological defects with elongated and branched cells (arrows) developed by Δ$gdhZ_{Ba}$ cells. Cells were imaged in mid-exponential phase of growth. Scale bar, 2 μm.

emphasize the importance of amino acids as a main carbon source for *Brucellae* inside host cells.

Amino acids as a main carbon source for α-proteobacteria

Other metabolic regulators of cell division have been described in *Escherichia coli* and *Bacillus subtilis*.[19–21] Interestingly, all these regulators link glycolysis to cell division and both species use glucose and hexoses as major carbon sources. On the other hand, the deletion of *gdhA* in *E. coli*, coding for an anabolic NADP-dependent glutamate dehydrogenase, did neither slow down growth in complex (LB) or synthetic (M9G) media, nor interfere with cell division (data not shown). In contrast, *C. crescentus* and *B. abortus* use GdhZ, catabolizing amino acids, to coordinate metabolism with cell division. Based on these observations we postulate that bacteria have

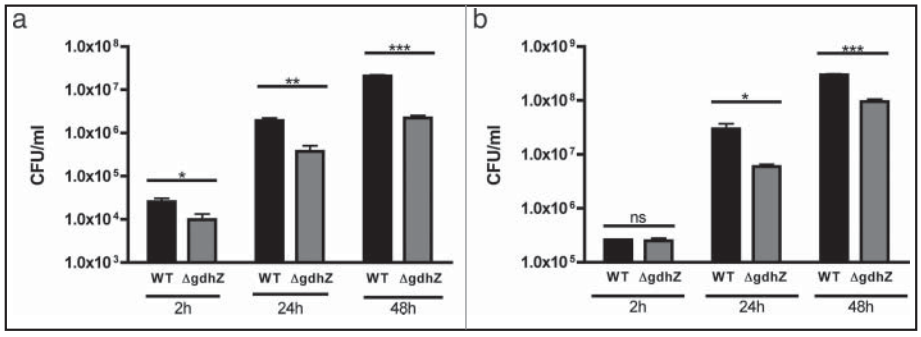

Figure 3. GdhZ is required for efficient replication of *Brucella abortus* inside murine raw macrophages. Internalization and intracellular replication of *B. abortus* wild-type and Δ$gdhZ_{Ba}$ cells into murine raw macrophages. RAW 264.7 macrophages were cultured at 37 °C (5 % CO_2 atmosphere) in DMEM (Invitrogen) supplemented with 10% fetal bovine serum (Gibco), 4.5 g/L glucose, 1.5 g/L $NaHCO_3$ and 4 mM glutamine. Cultures of *Brucella* were prepared in DMEM at a multiplicity of infection of 50. Bacteria were centrifuged at 400 x *g* for 10 min at 4 °C and then incubated for 1 hr at 37 °C (5 % CO_2 atmosphere). Cells were washed twice with fresh medium and then incubated in medium supplemented with 50 μg/ml gentamicin to kill extracellular bacteria. Prior to infections, bacteria were grown in (a) 2YT or (b) 2YT + 0.2 % glucose. The significant pairwise comparisons are indicated for $p < 0.5$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) in Student t-tests.

evolved by connecting the cell division machinery with the catabolic routes whose activity reflects nutrient fluctuations in the environment. In other words, such regulations would underline the main carbon sources used by bacteria, indicating that amino acids would constitute a favorite carbon source for *C. crescentus, B. abortus*, and likely other α-proteobacteria. This hypothesis is further supported by the fact that amino acids transporters and catabolic enzymes are induced in BCV.[22]

It is noteworthy that, whatever the main carbon source is, metabolic enzymes constitute perfect candidates to fulfill additional regulatory functions. Indeed, the conformational change induced upon substrate(s) binding or product(s) release, can serve as a proxy for the cell to monitor nutrient availability. This is thereby not surprising that most of the proteins in the glycolytic and the TCA pathways are moonlighting enzymes, *i.e.* enzymes with secondary (regulatory) functions.[23] In this perspective and knowing that a NAD-dependent glutamate dehydrogenase connects the nitrogen cycle to the TCA cycle, GdhZ was very likely selected to coordinate growth with cell division in α-proteobacteria.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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