A NAD-dependent glutamate dehydrogenase coordinates metabolism with cell division in Caulobacter crescentus

François Beaufay¹, Jérôme Coppine¹, Aurélie Mayard¹, Géraldine Laloux², Xavier De Bolle¹ & Régis Hallez¹,*

Abstract

Coupling cell cycle with nutrient availability is a crucial process for all living cells. But how bacteria control cell division according to metabolic supplies remains poorly understood. Here, we describe a molecular mechanism that coordinates central metabolism with cell division in the α-proteobacterium Caulobacter crescentus. This mechanism involves the NAD-dependent glutamate dehydrogenase GdhZ and the oxidoreductase-like KidO. While enzymatically active GdhZ directly interferes with FtsZ polymerization by stimulating its GTPase activity, KidO bound to NADH destabilizes lateral interactions between FtsZ protofilaments. Both GdhZ and KidO share the same regulatory network to concomitantly stimulate the rapid disassembly of the Z-ring, necessary for the subsequent release of progeny cells. Thus, this mechanism illustrates how proteins initially dedicated to metabolism coordinate cell cycle progression with nutrient availability.

Keywords cell division; cytokinesis; FtsZ; GdhZ; glutamate dehydrogenase

Subject Categories Metabolism; Cell Cycle; Microbiology; Virology & Host Pathogen Interaction

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Introduction

Cell division is a key process to ensure survival, development, and spreading of all living beings. At the heart of this mechanism, self-assembly proteins play a crucial role (Margolin, 2000; Osteryoung, 2001). In eukaryotes, actin and myosin drive cell constriction, whereas microtubules form the mitotic spindle. In prokaryotes, the actin-like ATPase MreB and the tubulin-like GTPase FtsZ control cell growth and cytokinesis (Karsenti & Vernos, 2001; Margolin, 2009; Laporte et al., 2010). Accordingly, self-assembly proteins are targeted by checkpoints all along the cell cycle. For instance, cyclin-dependent kinases trigger the assembly of the mitotic spindle upon completion of DNA replication (Li & Zheng, 2004; Yokoyama & Gruss, 2013), while the nucleoid occlusion system inhibits FtsZ polymerization over the replicating chromosome and thereby prevents genome bisection (Wu & Errington, 2004; Bernhardt & de Boer, 2005). In vitro, FtsZ self-assembles in a GTP-dependent manner into protofilaments, which in turn associate through lateral interactions into structures of higher complexity (Mukherjee & Dutkenhaus, 1994; Erickson & Stoffler, 1996; Gonzalez et al., 2003). In vivo, FtsZ assemblies at the division site into a dynamic ring-like structure, called the Z-ring, where it acts as a scaffold for the recruitment of cell division proteins and plays an active role in cytokinesis (Goehring & Beckwith, 2005; Li et al., 2007; Goley et al., 2011). Although the exact molecular mechanism by which the Z-ring disassembly drives cell constriction in vivo is still under intense debate, GTPase activity and lateral interactions play a crucial role in this process (Monahan et al., 2009; Dajkovic et al., 2010; Erickson et al., 2010; Osawa & Erickson, 2011). A recent study showed that the Z-ring is very likely continuous, composed of either overlapping shorter filaments or a single filament rolled up on itself (Szwedziak et al., 2014). Most importantly, the authors proposed that cell constriction requires the filaments to slide along each other (Szwedziak et al., 2014). Although lateral interactions between FtsZ filaments would play a direct role in this process, the GTPase activity might be crucial in vivo to initiate Z-ring disassembly as well as to maintain the dynamic state of the Z-ring, essentially by stimulating filament shrinkage (Stricker et al., 2002; Osawa & Erickson, 2011; Szwedziak et al., 2014).

The α-proteobacterium Caulobacter crescentus is a powerful model to study cell division control since it divides asymmetrically to give rise to two different daughter cells, a small swarmer cell and a large stalked cell. The sessile stalked cell initiates DNA replication (S phase) shortly after the previous cytokinesis, whereas the motile swarmer cell first enters in a non-replicative G1 phase (Fig 1A). The swarmer cell then differentiates into a stalked cell by ejecting the polar flagellum, retracting the polar pili, and synthesizing a stalk at the same pole. This swarmer-to-stalked cell transition coincides with the initiation of DNA replication (G1-to-S transition). Although

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1 Bacterial Cell Cycle & Development (BCcD), URBM, University of Namur, Namur, Belgium
2 de Duve Institute, Université catholique de Louvain, Brussels, Belgium
*Corresponding author. Tel: +32 81 724 244; E-mail: regis.hallez@unamur.be
The Z-ring is built up at the onset of the S phase, cell constriction only starts at the early predivisional stage (late S phase) and is followed by a rapid contraction of the Z-ring in late predivisional stage (G2 phase) (Degnen & Newton, 1972; Osley & Newton, 1980; Holden et al, 2014).

Among the regulators of FtsZ identified so far, only few have been described to coordinate cell division with metabolism (Kirkpatrick & Viollier, 2011). The glucosyltransferases, UgtP in Bacillus subtilis and OpgH in Escherichia coli, interfere with FtsZ dynamics to delay cell division, allowing cells to adapt their size according to nutrient availability (Weart et al, 2007; Hill et al, 2013). In contrast to E. coli or B. subtilis, C. crescentus does not vary its cell length in response to changes in nutrient availability (Campos et al, 2014). Nevertheless, the small NAD(H)-binding protein KidO has been proposed to coordinate cell division with the metabolic state of the cell (Radhakrishnan et al, 2010). KidO prevents premature Z-ring formation during G1 phase and promotes Z-ring disassembly in G2 phase, but cannot interfere with FtsZ during S phase since it is degraded by the ClpXP protease at the G1-to-S transition (Radhakrishnan et al, 2010). However, the exact mechanism used by KidO to modulate FtsZ dynamics remains unknown.

Here, we identified a conserved bifunctional NAD-dependent glutamate dehydrogenase (GDH) that controls Z-ring disassembly. A GDH catalyzes the interconversion of glutamate into α-ketoglutarate and ammonia by using NAD(P)H as a cofactor, thereby bridging Krebs and nitrogen cycles (Fig 1B) (Minambres et al, 2000). We show that GdhZ (for glutamate dehydrogenase interacting with FtsZ) stimulates cell constriction during cytokinesis by directly interfering with Z-ring stability and that GDH activity is essential to mediate this effect. In addition, we found that GdhZ and KidO use different molecular mechanisms to stimulate depolymerization of FtsZ, but share the same regulatory network to synergistically prevent premature Z-ring formation in G1 swarmer cells and to trigger Z-ring disassembly in G2 predivisional cells. This mechanism illustrates how bacteria can adapt their cell cycle according to nutrient availability.

**Results**

A NAD-dependent glutamate dehydrogenase interacts with FtsZ

To find additional regulators of FtsZ, a yeast two-hybrid screen was performed using a prey library of random genomic DNA fragments of C. crescentus (see details in Supplementary Materials and Methods). We fished out a fragment encompassing the uncharacterized CCNA_00086 gene (here referred to as gdhZ), coding for a NAD-dependent GDH. To provide biochemical evidence that GdhZ and FtsZ are part of the same complex, lysates from strains in which gdhZ was replaced by either 3FLAG-gdhZ or gdhZ-3FLAG were subjected to immunoprecipitation with α-FLAG antibodies. These experiments showed that FtsZ was co-purified with both GdhZ fusions, further supporting the interaction between GdhZ and FtsZ (Fig 1C).

**gdhZ deletion leads to a severe cell division defect**

By interacting with FtsZ, GdhZ might regulate cell division. To address this question, we first generated an in-frame deletion of gdhZ (ΔgdhZ) and found that ΔgdhZ cells displayed a large cell size heterogeneity with a high proportion of tiny and filamentous cells (Fig 2A and B). The ΔgdhZ mutant also exhibited a serious growth defect, with a doubling time of ~165 min in complex media (PYE) compared
Figure 2. Inactivation of gdhZ leads to a severe cell division defect.

A Cell size distribution of wild-type (RH50) and ΔgdhZ (RH534) strains grown in complex PYE media. The cell length was measured by using MicrobeTracker software (Sliusarenko et al, 2011). The mean cell size ± standard deviation (in µm) of the different strains is indicated in brackets.

B Phase contrast imaging of wild-type (RH50), ΔgdhZ (RH534), ftsZ79K (NR6102), and ftsZ79KΔgdhZ (RH1329) strains, showing that combination of ftsZ79K with ΔgdhZ leads to a conspicuous filamentation. Scale bar, 5 µm.

C Expression of ftsZ-yfp suppresses ΔgdhZ cell division defect over time. Wild-type (RH53) and ΔgdhZ (RH853) cells bearing an additional copy of ftsZ fused to yfp at the vanA locus (PvanA::ftsZ-yfp) were grown in PYE and imaged at different timepoints following induction of ftsZ-yfp expression with 0.5 mM vanillate. Scale bar, 5 µm.

D Immunoblot of FtsZ and MreB steady-state levels in wild-type and ΔgdhZ cell lysates showing that both strains have similar amount of FtsZ proteins. Protein extracts were prepared from wild-type (RH50) and ΔgdhZ (RH534) strains, and twofold serial dilutions of cell lysates were separated on SDS–PAGE. The relative abundance of FtsZ and MreB intensities was quantified using ImageJ software, normalized according to the dilution factor. FtsZ levels were finally normalized according to MreB (FtsZ/MreB 100% in wild-type versus 103% in ΔgdhZ).

E Overexpression of gdhZ leads to cell filamentation. ΔgdhZ cells expressing a venus-gdhZ fusion from PxylX at the xylX locus (RH740) were grown in PYE and imaged at different timepoints following induction of venus-gdhZ expression with 0.1% xylose. Scale bar, 5 µm.

F Immunoblot of GdhZ and MreB showing levels of Venus-GdhZ increased over time in cells overexpressing venus-gdhZ. Proteins extracts were prepared from ΔgdhZ PvanA::venus-gdhZ (RH740) strain at the indicated timepoints after xylose induction and separated on SDS–PAGE. The level of GdhZ-GFP in strain RH602 was used as a control.

Source data are available online for this figure.
to ~85 min for the wild-type strain (Supplementary Fig S1). In addition, the proportion of late predivisional (constricting) cells, that is, predivisional cells with a visible ongoing constriction, was significantly (P < 0.001) higher in ΔgdhZ (~25%) than in wild-type (~10%). Surprisingly, ΔgdhZ phenotypes were rescued when glucose, xylose, or alanine was added to PYE or used as the sole carbon source in synthetic media (Supplementary Fig S1 and data not shown). None of these carbon sources does require GDH activity to be catabolized in vivo. In contrast, amino acids feeding the Krebs cycle thanks to GdhZ activity (i.e. glutamate, histidine, proline, arginine, and glutamine; Fig 1B) did suppress neither growth nor cell division defects of ΔgdhZ (data not shown). These results indicate that GdhZ might regulate cell division according to the carbon source used and that GDH activity might be essential for this regulation.

As the proportion of late predivisional cells was abnormally high in the ΔgdhZ strain, we asked whether the deletion of gdhZ could specifically affect the G2 phase of the cell cycle. Flow cytometry analyses of the DNA content over the cell cycle showed that G1 and S phases were similar in both wild-type and ΔgdhZ strains (Supplementary Fig S2). Likewise, the timing of localization of the G1-to-S transition markers MipZ-CFP (Thanbichler & Shapiro, 2006) and StpX-GFP (Hughes et al., 2010, 2013) was not affected in the ΔgdhZ cells, supporting that GdhZ does regulate neither the G1 phase nor S phases. We also observed that the ΔgdhZ mutant that tolerates xylose (Fig 2E and F), without seriously affecting the distribution of late predivisional cells, that is, small G1 swarmer cells (~70%) and occasionally at midcell (7.1%, n = 70) of wild-type cells (~2 μm). In ΔgdhZ cells (~2 μm), FtsZ-YFP localized more often at a midcell position (24.7%, P < 0.001, n = 121), and less frequently at a pole (38.8%, n = 121). Thus, FtsZ is prematurely assembled as a Z-ring in ΔgdhZ G1 swarmer cells. As the expression of ftsZ:yfp suppresses ΔgdhZ cell division defects (Fig 2C), we sought to use a strain expressing gfp-fzlC from the inducible xylX promoter (P_{xylX};gfp-fzlC) to monitor FtsZ localization throughout the cell cycle. Indeed, GFP-FzlC not only mirrors FtsZ localization (Goley et al., 2011), but most of all, its expression does not mitigate ΔgdhZ phenotypes (data not shown). Time-lapse experiments revealed that GFP-FzlC failed to disappear from the contracting Z-ring in late predivisional cells, but also relocated at the midcell of future daughter cells prior to the completion of cell division in ΔgdhZ cells (Fig 3B).

These data indicate that GdhZ inhibits the formation and/or stability of the Z-ring, presumably in G2 phase to stimulate its disassembly and in G1 phase to prevent the assembly of a new Z-ring before the constricting one is disassembled.

GdhZ colocalizes with FtsZ at midcell

To further characterize the role of GdhZ in cell division control, we determined its subcellular localization in strains expressing either gdhZ::gfp at the native gdhZ locus or venus-gdhZ from the inducible P_{xylX} Promoter. Both GdhZ-GFP and Venus-GdhZ fusions accumulated at a medial position (Fig 3C). Interestingly, GdhZ-GFP no longer localized at midcell when glucose or xylose was added to the culture medium (data not shown). Let’s remind that these two carbon sources suppressed cell growth and division defects of ΔgdhZ cells (Supplementary Fig S1 and data not shown). Coexpressing gdhZ::gfp and ftsZ::mcherry from the inducible P_{xylX} promoter confirmed that GdhZ-GFP fluorescence signal was enriched at or near the Z-ring (i.e. where FtsZ-mCherry localized) in more than 70% (n = 437) of cells displaying a GdhZ-GFP signal (Fig 3D). Similar results were obtained with GdhZ-GFP and mCherry-FzlC, since both proteins colocalized in ~65% of cells (Supplementary Fig S4A). In addition, demographic representations of GdhZ-GFP and FtsZ-mCherry signals allowed us to monitor the dynamic localization of both proteins along the cell cycle from a heterogeneous population. As expected, FtsZ-mCherry was found at one pole of small G1 swarmer cells before being relocated at a medial position in larger cells (Figs 1A and 3E). In contrast, GdhZ-GFP was not localized at one pole in small G1 swarmer cells (Fig 3E), but started to accumulate at midcell in larger cells, slightly

GdhZ regulates FtsZ dynamics in vivo

Since gdhZ is required for efficient cell division, we tested whether GdhZ controls FtsZ dynamics in vivo by monitoring the distribution of FtsZ-YFP in wild-type and ΔgdhZ cells (Fig 3A). By plotting FtsZ-YFP intensity profile along the cell length, we did not detect any FtsZ misplacement in ΔgdhZ cells (Fig 3A). In contrast, we found that the proportion of cells with a detectable medial Z-ring was significantly (P < 0.001) higher in ΔgdhZ (87.5 ± 1.8%; n = 672) than in the wild-type strain (70.4 ± 5.4%; n = 716). Similar observations were made with MreB, which is recruited very early at the Z-ring (Goley et al., 2011), since 90.0 ± 2.6% (n = 937) of ΔgdhZ cells displayed a midcell localization for GFP-MreB versus 76.3 ± 3.5% (n = 635) for wild-type cells (P < 0.001). These results suggest that FtsZ could be prematurely recruited at the midcell in small G1 swarmer cells in the absence of GdhZ. To test that, we determined the localization of FtsZ-YFP in wild-type and ΔgdhZ small G1 swarmer cells (~2 μm). As expected, FtsZ-YFP was mostly observed at a pole (75.7%, n = 70) and occasionally at midcell (71.7%, n = 70) of wild-type cells (~2 μm). In ΔgdhZ cells (~2 μm), FtsZ-YFP localized more often at a midcell position (24.7%, P < 0.001, n = 121), and less frequently at a pole (38.8%, n = 121). Thus, FtsZ is prematurely assembled as a Z-ring in ΔgdhZ G1 swarmer cells. As the expression of ftsZ::gfp suppresses ΔgdhZ cell division defects (Fig 2C), we sought to use a strain expressing gfp-fzlC from the inducible xylX promoter (P_{xylX};gfp-fzlC) to monitor FtsZ localization throughout the cell cycle. Indeed, GFP-FzlC not only mirrors FtsZ localization (Goley et al., 2011), but most of all, its expression does not mitigate ΔgdhZ phenotypes (data not shown). Time-lapse experiments revealed that GFP-FzlC failed to disappear from the contracting Z-ring in late predivisional cells, but also relocated at the midcell of future daughter cells prior to the completion of cell division in ΔgdhZ cells (Fig 3B).

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Since gdhZ is required for efficient cell division, we tested whether GdhZ controls FtsZ dynamics in vivo by monitoring the distribution
after the Z-ring was assembled at the division site (Fig 3E). This result suggests that GdhZ could be recruited at midcell directly by FtsZ. To test that, the localization of GdhZ-Venus and mCherry-FzlC was assessed in a strain where the only copy of \textit{ftsZ} was under the control of the inducible P\textsubscript{xylX} promoter. After 3 h of xylose depletion, GdhZ-Venus still displayed a discrete localization (≤ 1 focus per cell), while mCherry-FzlC was mostly diffuse in the cytoplasm (Supplementary Fig S4B and C). However, GdhZ-Venus no longer concentrated at midcell (Supplementary Fig S4D), suggesting that GdhZ midcell recruitment relies on FtsZ.
These observations suggest that GdhZ accumulates at the division site in a FtsZ-dependent manner very likely to stimulate Z-ring disassembly in late predivisional cells.

GdhZ modulates FtsZ dynamics in vitro

Our observations so far implicate GdhZ as a negative regulator of the Z-ring. We thus tested the ability of GdhZ to modulate FtsZ dynamics in vitro. For this purpose, we purified native GdhZ and monitored its activity (oxidative deamination) by following NADH production at 340 nm. The reaction was defined as a function of the concentration of NAD⁺ or glutamate (Supplementary Fig S5). The NAD⁺ saturation curve followed a Michaelis–Menten kinetics, yielding a Kₘ value of 0.038 mM (Supplementary Fig S5A). In contrast, the glutamate saturation curve was sigmoidal, and a Hill plot established a S₀.₅ of 42.5 mM with an interaction coefficient (n) of 1.6, indicating a positive cooperativity in glutamate binding (Supplementary Fig S5B).

Since purified GdhZ was active in vitro, we then measured the intrinsic GTPase activity of FtsZ and visualized the formation of FtsZ ordered structures (protofilaments and bundles) by negative-stain electron microscopy (EM), in the presence or absence of GdhZ. First, the addition of increasing concentration of GdhZ did not substantially affect the GTPase activity of FtsZ, with only a slight increase (~30%) in GTP hydrolysis at a 2:1 GdhZ:FtsZ molar ratio (Fig 4A). In contrast, the addition of GdhZ together with its substrates (NAD⁺ and glutamate) strongly stimulated the GTPase activity of FtsZ in a dose-dependent manner (Fig 4B) (~4.5-fold increase at 2:1 GdhZ:FtsZ ratios). Interestingly, the presence of only one of the substrates (NAD⁺ or glutamate) was sufficient for GdhZ to stimulate the GTPase activity of FtsZ (Supplementary Fig S6A), suggesting that a conformational change of GdhZ induced by substrate binding allows GdhZ to interfere with FtsZ dynamics in vitro. Then, we examined the effect of GdhZ on FtsZ ultrastructures by EM. In agreement with earlier studies, in the presence of GTP, FtsZ assembled into straight protofilaments that can associate into bundles (Fig 4C). Consistent with the above results, FtsZ structures were still visible in the presence of GdhZ alone (Fig 4D), but barely detectable when GdhZ and its substrates were included in the reactions, with only a few short protofilaments observed at a 1:1 GdhZ:FtsZ ratio (Fig 4E). Note that GdhZ also self-assembles into higher ordered structures in a concentration-dependent manner (F. Beaufay and R. Hallez, unpublished results), which affected the negative-staining contrast. Importantly, neither substrates nor products of GDH reaction (NADH, glutamate, ammonium, or α-ketoglutarate) did, on themselves, neither stimulate the GTPase activity nor influence the polymeric structures of FtsZ in the tested conditions (Fig 4B and data not shown). Likewise, the dynamics of FtsZ was not affected by the unrelated bovine L-glutamic dehydrogenase GdhA with its substrates (Supplementary Fig S6B and E). Finally, we found that FtsZ₆₇₉K, which displays a twofold reduced GTPase activity compared to wild-type FtsZ, was still fully sensitive to active GdhZ (Supplementary Fig S6C and F). The GTPase activity of FtsZ₆₇₉K was indeed strongly stimulated by active GdhZ (~ninefold at 2:1 GdhZ:FtsZ₆₇₉K ratio).

On the basis of these in vitro data, we propose that catalytically active GdhZ very likely disassembles FtsZ polymers by triggering the GTPase activity of FtsZ.

GdhZ activity is required to control the cell division process

The fact that GdhZ substrates are required in vitro for GdhZ to stimulate the GTPase activity of FtsZ prompted us to investigate the effect of a catalytically inactive mutant of GdhZ (GdhZ₆₈₇₆K) on cell division. GdhZ₆₈₇₆K was stable in vitro and purified GdhZ₆₈₇₆K displayed no detectable GDH activity in vitro in contrast to wild-type GdhZ (Supplementary Fig S7A). We found that gdhZ₆₈₇₆K phenocopied ΔgdhZ in terms of (i) growth and cell division defects (Supplementary Figs S1, S7B and S7C), (ii) synthetic interaction with ftsZ₇₉₉E (Supplementary Fig S7C), and (iii) premature GFP-FzlC recruitment at midcell (Supplementary Fig S7D and data not shown). In addition, GdhZ₆₈₇₆K did not affect FtsZ dynamics (Supplementary Fig S6D and E), even if it could still pull down FtsZ (Supplementary Fig S7E).

These results further support the idea that GdhZ activity is strictly required for GdhZ to control cell division but not to mediate interaction with FtsZ.

GdhZ abundance oscillates over the cell cycle

The abundance of FtsZ and several of its regulators (FtsA, FzIα, FtsQ, or KidO) was previously shown to oscillate during the cell cycle in order to coordinate cell division events (Kelly et al., 1998; Martin et al., 2004; Goley et al., 2010; Radhakrishnan et al., 2010). To determine whether GdhZ is also cell-cycle-regulated, we monitored GdhZ abundance over the cell cycle in a synchronized population of Caulobacter cells in which the native copy of gdhZ was replaced by a functional 3FLAG-gdhZ fusion. As illustrated in Fig 5A, 3FLAG-GdhZ oscillated throughout the cell cycle, reaching the highest level in swarmer and late predivisional cells and the lowest level in stalked cells. This cell cycle fluctuation was also observed in the wild-type strain using polyclonal anti-GdhZ antibodies (Fig 5B). Thus, GdhZ starts to disappear in differentiating stalked cells (i.e. when the Z-ring is built up) and reappears in late predivisional cells (i.e. when the Z-ring starts to be disassembled) (Fig 5A and Supplementary Fig S8A).

Several evidence support that GdhZ might be subjected to a cell cycle-dependent proteolysis. First, adding a 3FLAG tag or GFP at GdhZ C-terminus (GdhZ-3FLAG or GdhZ-GFP) or substituting the last two alanine residues by two aspartate residues (GdhZAA::DD) completely abrogated oscillation (Fig 5C and Supplementary Fig S8B and data not shown). This is reminiscent of that seen for other cell-cycle-regulated proteins degraded by the ClpXP protease (Domin et al., 1997; Radhakrishnan et al., 2010; Abel et al., 2011). Indeed, GdhZ has a C-terminal end homologous to the proteolytic SsrA peptide tag (Fig 5D) known to deliver substrates to ClpXP. Masking or mutating this conserved motif could block proteolysis as it is the case for CtrA or KidO (Domian et al., 1997; Radhakrishnan et al., 2010; Abel et al., 2011). Second, the steady-state level of GdhZ increased in the absence of the ClpP peptidase subunits (Fig 5E). Third, 3FLAG-GdhZ did not oscillate anymore in mutants (acpdR, acroD, and AropA) unable to degrade ClpXP-dependent substrates (Supplementary Fig S8C) (Iniesta et al., 2006; McGrath et al., 2006; Duerig et al., 2009). Together these results suggest that, similar to KidO, GdhZ undergoes a ClpXP-dependent degradation throughout the cell cycle.

The reappearance of GdhZ in late predivisional cells could be due to the transcriptional activation of gdhZ by the response
regulator CtrA, as described for KidO (Radhakrishnan et al., 2010). Indeed, CtrA, known to modulate the transcription of cell-cycle-regulated genes (Laub et al., 2000), has recently been shown to tightly bind the promoter region of gdhZ (P_{gdhZ}) (Fumeaux et al., 2014). To test this hypothesis, the transcription of gdhZ was monitored with a P_{gdhZ}:lacZ transcriptional fusion in strains in which CtrA is either underactivated (ΔpleC) or overactivated (ΔdivJ or ΔdivK) (Supplementary Fig S9A). We found that P_{gdhZ} activity was reduced by ~25% in ΔpleC cells and increased by ~25% in ΔdivJ or ΔdivK cells in comparison with wild-type cells (Supplementary Fig S9B). Accordingly, the steady-state level of GdhZ was increased in ΔdivK cells and decreased in a strain expressing a thermosensitive loss-of-function mutant of ctrA (Supplementary Fig S9C). These results strongly suggest that CtrA-P directly activates the transcription of gdhZ.

Overall, we propose that GdhZ uses the same regulatory network as KidO to oscillate throughout the cell cycle, very likely to limit its abundance during the S phase of the cell cycle, that is, when the Z-ring has to be built up. Taken this into consideration, the localization of GdhZ-GFP at the midcell during S phase (stalked and early predivisional cells), depicted on Fig 3E, very likely resulted from the stabilization of the fusion protein. Therefore, we can postulate that GdhZ is recruited to the division site in late predivisional cells, very likely to trigger Z-ring disassembly.

**KidO inhibits lateral interactions between FtsZ protofilaments**

By inhibiting Z-ring formation in swarmer cells (G1) and stimulating Z-ring disassembly in late predivisional cells (G2), GdhZ would play a role similar to the one already proposed for KidO (Radhakrishnan et al., 2010). However, the exact mechanism by which KidO, once bound to NAD(H), interferes with FtsZ remains unknown. To address this question, KidO was purified and its effect on the polymerization of FtsZ was measured in vitro. In contrast to GdhZ with its substrates, KidO alone (Fig 6A) or with its cofactor (NAD+ or NADH, Fig 6B) did not substantially affect the GTPase activity of FtsZ. At a 2:1 KidO:FtsZ ratio, KidO slightly increases the GTP hydrolysis rate of FtsZ (~30%) in the presence of either NADH or NAD+ (compare Figs 6B and 4B). However, EM analyses revealed that, in the presence of NADH but not NAD+, KidO drastically

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**Figure 4.** *Active GdhZ promotes FtsZ’s GTPase activity in vitro leading to filaments disassembly.*

A Inorganic phosphate (P) release over time by FtsZ (0.5 μM) incubated with 1 mM GTP in the presence of 0, 0.25, 0.5, or 1 μM GdhZ, respectively. The rates of GTPase (P released per FtsZ molecule per min) are indicated for each condition.

B Stimulation of FtsZ’s GTPase activity with active GdhZ. Inorganic phosphate (P) release over time by FtsZ (0.5 μM) incubated with 1 mM GTP, 100 mM glutamate, 5 mM NAD+ in the presence of 0, 0.25, 0.5, or 1 μM GdhZ, respectively. The rates of GTPase (P, molecules released per FtsZ per min) are indicated for each condition. Note that GdhZ with its substrates (100 mM glutamate, 5 mM NAD+) does not display any detectable GTPase activity on itself.

C–E Negative-stain electron microscopy of FtsZ (0.5 μM) incubated with 1 mM GTP in the absence (c) or presence of 0.5 μM GdhZ alone (d) or together with 100 mM glutamate and 5 mM NAD+ (e). Scale bar, 100 nm.

Source data are available online for this figure.
reduced FtsZ bundling without interfering with single protofilaments (Fig 6D). These results suggest that KidO bound to NADH hampers lateral interactions between FtsZ protofilaments rather than stimulating FtsZ’s GTPase activity. This observation is consistent with the drastic increase of bundling capacity that we observed in comparison with wild-type (RH50) cells to follow GdhZ and CtrA abundance throughout the cell cycle.

As both GdhZ and KidO (i) inhibit Z-ring assembly/stability in swarmer and late predivisional cells, (ii) share the same regulatory network to oscillate throughout the cell cycle, and (iii) use different molecular mechanisms to control cell division explains that both proteins are part of the same complex (Supplementary Fig S10C).

These results suggest that GdhZ and KidO act in synergy to trigger Z-ring disassembly.
Here, we found that the NAD-dependent glutamate dehydrogenase GdhZ coordinates metabolism with cell division in *C. crescentus*. GdhZ directly stimulates the GTPase activity of FtsZ, and this regulation requires GdhZ catalytic activity. The central position of GDH reaction between the TCA and nitrogen cycles makes GdhZ an excellent candidate and as a metabolic regulator of the cell cycle (Figs 1B and 7). So far, the best characterized bacterial metabolic regulators of cell division are UgtP in *Bacillus subtilis* and OpgH in *Escherichia coli*; both are glucosyltransferases using UDP-glucose as a substrate (Weart *et al.*, 2007; Hill *et al.*, 2013). These proteins coordinate growth with cell division to adapt cell size according to nutrient availability. In contrast to *C. crescentus*, *E. coli* and
**B. subtilis** can indeed adjust their cell size depending on their growth rate (Sargent, 1975; Campos et al., 2014). Similar to UgtP and OpgH, GdhZ directly interferes with FtsZ. However, in contrast to GdhZ, UgtP and OpgH do not require their substrates to inhibit FtsZ polymerization *in vitro*. The cell division control by UgtP or OpgH is rather driven by their steady-state levels, which, in turn, are determined by nutrient availability. In addition, GdhZ mainly stimulates the GTPase activity of FtsZ, while UgtP inhibits FtsZ single-filament formation and OpgH sequesters FtsZ monomers preventing them from assembly (Weart et al., 2007; Hill et al., 2013).

KidO is another metabolic regulator of cell division in *C. crescentus*, described to stimulate the disassembly of the Z-ring in a NAD (H)-dependent manner (Radhakrishnan et al., 2010), during the G1 and G2 phases of the cell cycle. We showed that GdhZ and KidO share the same regulatory network. First, ClpXP degrades both proteins at the G1-to-S transition, to ensure that they mainly disappear when the Z-ring needs to be assembled at the division site (early S phase). Second, the master regulator CtrA positively regulates the transcription of *gdhZ* and *kidO*, and this coregulation allows reaccumulation of both proteins in G2 phase to stimulate the rapid depolymerization of the Z-ring during cytokinesis (Fig 7).

Change in NAD(H) levels during the cell cycle has been initially proposed to drive KidO-dependent cell division control (Radhakrishnan et al., 2010). Alternatively, GdhZ might directly deliver NADH to KidO in the vicinity of the Z-ring, through its catabolic activity (Fig 7). This “substrate channeling-like” process (Schendel et al., 1988; Arentson et al., 2012; Dunn, 2012) would coordinate GdhZ and KidO activities on the Z-ring during the G2 phase of the cell cycle, in order to facilitate Z-ring disassembly and cytokinesis. Similar to KidO, GdhZ also prevents premature Z-ring formation during the G1 phase. Indeed, time-lapse experiments showed that ΔgdhZ predivisional cells could assemble a new Z-ring at the division site of future daughter cells prior to the completion of cell division (Fig 3B). In addition, a ΔgdhZ mutant gives birth to small swarmer progeny cells that have already built up a Z-ring (Fig 3A).

On the basis of these observations, we propose a model (Fig 7) in which GdhZ and KidO coordinately prevent premature assembly of the Z-ring in G1 swarmer cells. Then, the ClpXP-dependent proteolysis of GdhZ and KidO at the G1-S transition allows the building of the Z-ring. In the cell cycle (G2 phase), both proteins reaccumulate to reach their maximal levels at the late predivisional stage of the cell cycle. At that time, KidO destabilizes lateral interactions between FtsZ protofilaments, thereby increasing filament sliding, while active GdhZ stimulates the GTPase activity of FtsZ to ensure that the Z-ring does not reach a too condensed state that could block cytokinesis (Szwedziak et al., 2014). The concomitant recruitment of two negative regulators of the Z-ring at the onset of the G2 phase could therefore be responsible for the 2-step motion of cytokinesis (first slow, then rapid) described for *C. crescentus* (Holden et al., 2014).

**Figure 7.** Model for the coordination of cell division with metabolism through the concomitant and synergic action of GdhZ and KidO. GdhZ and KidO abundance oscillate throughout the cell cycle to reach maximal levels during G1 and G2 phases. Localization of GdhZ and KidO to the Z-ring allows both proteins to accumulate in the vicinity of the division site, at the constriction step. Each protein can, independently of each other, avoid premature Z-ring assembly in G1 swarmer cell and promote Z-ring disassembly in G2 predivisional cells. As being part of the same complex, we propose that GdhZ could locally provide KidO with NADH thanks to glutamate catabolism. This "substrate channeling-like" mechanism could enhance Z-ring disassembly. According to nutrient availability, the synergic action of both GdhZ and KidO could be responsible for the rapid constriction observed during G2 phase (Holden et al., 2014). In starving conditions, GdhZ and KidO would not stimulate constriction, therefore delaying cell division and preventing the release of the swarmer progeny cells without enough energy and nutrients to colonize new environments.
Although ΔgdhZ cells spend significantly more time to proceed to cytokinesis, a ΔgdhZ cell population contains many tiny cells smaller than the smallest wild-type G1 swarmer cells, a phenotype usually attributed to polar cell division events (Lutkenhaus, 2007). Surprisingly, it is unlikely to be the case for ΔgdhZ cells as the Z-ring still assembles at midcell in the vast majority of cells (Fig 3A). Instead, it probably arises from a miscoordination between metabolism/growth and cell division. Indeed, the premature Z-ring assembly in G1 swarmer cells coupled to a slow growth rate could lead to smaller predivisional cells, which could in fine generate smaller progeny cells.

The kinetic parameters determined for GdhZ (high $K_m$ for glutamate and low $K_m$ for NAD$^+$) suggest that the catabolic activity of GdhZ would be mainly controlled in vivo by glutamate availability rather than by NAD$^+$ levels. In oligotrophic environments, amino acids could serve as a major carbon source for bacteria such as C. crescentus. High GDH activity could therefore be sensed as a signal of plenty for the cell division apparatus, allowing the release of daughter cells with sufficient nutrient/energy supplies. Such a mechanism would favor the survival of swarmer progeny cells in poor nutrient environments. The conservation of GdhZ among α-proteobacteria also suggests that amino acids catabolism could be another common feature for these bacteria. In support of this idea, an in-frame deletion of gdhZ homolog in Brucella abortus (ΔgdhZ$_{Ba}$), an intracellular pathogen of cattle, led also to growth and cell division defects (data not shown). Moreover, ΔgdhZ$_{Ba}$ cells failed to sustain efficient intracellular replication in murine macrophages (F. Beaufay and R. Hallez, unpublished results), strongly suggesting that amino acids could be a major carbon source encountered by Brucella during infection, as it is the case for other intracellular pathogens (Zhang & Rubin, 2013). Altogether these data suggest that cell division control mediated by GdhZ could be conserved among α-proteobacteria. The suppression of ΔgdhZ defects by the addition of a GdhZ-independent carbon source (e.g. glucose) raises the possibility that (an)other enzyme(s) regulate(s) cell division according to the main carbon source encountered in the environment. It is worth to note that a connection between glycolysis and cell division has been recently highlighted in B. subtilis (Monahan & Hajduk, 2014). The authors proposed that pyruvate levels control Z-ring assembly thanks to the pyruvate dehydrogenase (PDH) activity (Monahan & Hajduk, 2014). As the final product of hexose (e.g. glucose) catabolism, it would be interesting to check whether pyruvate and/or PDH controls cell division in C. crescentus grown in the presence of glucose or other complex hexoses.

As a metabolic enzyme, GDHs are subjected to various post-translational regulations (Minambres et al., 2000; Commichau et al., 2007; Oliveira & Sauer, 2012). Our in vitro analysis also revealed that GDH activity of GdhZ is inhibited by TCA intermediates (e.g. citrate) and stimulated by some amino acids such as arginine (data not shown). We also observed that GdhZ auto-assembles into ordered structures (F. Beaufay and R. Hallez, unpublished results), even we do not know whether this self-assembly has any effect on GDH activity or cell division control. Self-assembly of glutamate dehydrogenase has been described for a long time in various organisms (Josephs & Borisy, 1972; O’Connell et al., 2012). It is worth noting that UgpP’s oligomerization state was shown to play an important role in the control of cell division in B. subtilis (Chien et al., 2012).

A single protein harboring multiple functions is referred to as a “moonlighting protein” (reviewed in Huberts & van der Klei, 2010). Moonlighting proteins are commonly found among metabolic enzymes; for example, 7 out of the 10 proteins in the glycolytic pathway and 7 out of the 8 proteins in the TCA cycle display a moonlighting function. Because of their interaction with cell metabolites and the subsequent conformational change induced upon substrate binding, metabolic enzymes constitute good candidates for signal transduction (Huberts & van der Klei, 2010).

How cells coordinate growth with cell cycle according to nutrient availability and energy supplies remains an exciting open question. Although we have now uncovered a new bifunctional enzyme that couples cell division with amino acids catabolism, further work is needed to highlight other links between metabolism and cell cycle.

Materials and Methods

Bacterial strains and growth conditions

Escherichia coli Top10 was used for cloning purpose and grown aerobically in Luria–Bertani (LB) broth (Sigma) (Casadaban & Cohen, 1980). Electrocomptent cells were used for transformation of E. coli. All Caulobacter crescentus strains used in this study are derived from the synchronize wild-type strain NA1000 and were grown in peptone–yeast extract (PYE,) or synthetic M2 glucose (M2G) or xylose (M2X) media at 28–30°C. Growth was monitored by following the OD (600 nm) during 24 h in an automated plate reader (Bioscreen C; Lab Systems) with continuous shaking at 30°C. When indicated, media were supplemented with glucose (G), xylose (X), vanillate (Van), and alanine (A) at a final concentration of 0.2%. Genes expressed from the inducible vanA promoter (P$_{vanA}$) were induced with 0.5 mM vanillate; genes expressed from the inducible xylX promoter (P$_{xylX}$) were induced with ≤ 0.05% xylose in a ΔxylX background or with 0.1% xylose in xylX$^+$ background. For FtsZ deletion experiments, cells grew in the presence of 0.05% xylose for induction, then washed and resuspended in PYE liquid media without xylose. Generalized transduction was performed with phage ΦCrf30 according to the procedure described in Ely (1991).

For E. coli, antibiotics were used at the following concentrations (µg/ml; in liquid/solid medium): ampicillin (50/100), kanamycin (30/50), oxytetracycline (12.5/12.5), spectinomycin (100/100), streptomycin (50/50), and hygromycin (100/100). For C. crescentus, media were supplemented with kanamycin (5/20), oxytetracycline (1/2.5), spectinomycin (25/50), or streptomycin (5/5), where appropriate.

Escherichia coli S17-1 and E. coli MT607 helper strains were used for transferring plasmids to C. crescentus by respectively bi- and tri-parental mating. In-frame deletions were created by using the pPNTS138-derivative plasmids, by following the procedure described in Thanbichler and Shapiro (2006).

Strains and plasmids used in this study are listed in Supplementary Tables S2 and S3, together with construction details provided in the Supplementary Materials and Methods.

Y2H screen

Y2H screen was performed using a N-terminal fragment of C. crescentus ftsZ (ftsZ$_{1-324}$) fused to Gal4BD as bait, and a library of
random genomic DNA (gDNA) fragments of *C. crescentus* fused to Gal4AD as prey (Davis et al., 2013). The library was transformed into *Saccharomyces cerevisiae* strain Y187 (Clontech) and selected onto synthetic medium without leucine (SD-L). The pGBK7-5dB-GAL4AD (pHR284) plasmid was transformed into *S. cerevisiae* strain Y2H Gold (Clontech) and selected onto synthetic medium without tryptophan (SD-W). Y2H screen was performed essentially as proposed by the manufacturer (Clontech). Both yeast strains were grown separately either in SD-L or in SD-W, washed, and allowed to grow together in complex rich medium supplemented with adenine for 24 h at 30°C with slow shaking. More than 1 × 10^7 diploids were screened either on SD-LW supplemented with 5 mM 3-Amino-1,2,4-triazole (3-AT) or on SD-LWA. Isolated prey plasmids were transformed into *E. coli* strain Top10 and the inserts identified by DNA sequencing.

**Immunoprecipitation**

*Caulobacter crescentus* strains were grown in PYE liquid media (OD_600_~0.7), harvested by centrifugation for 15 min at 6,000 × g, 4°C and resuspended in 5 ml ice-cold phosphate buffer saline (PBS) containing 0.05% Triton X-100, complete EDTA-free anti-proteases, 20 mg/ml lysozyme, 10 U/ml DNase I. Cells were first lysed by sonication, and then, zirconium beads were added and cells were disrupted by FastPrep cycles (5 × 20 s) and harvested by centrifugation for 30 min at 14,000 × g, 4°C. Cell pellets were discarded, and 1 mg of protein lysates was mixed to 50 μl of anti-FLAG M2 magnetic beads (Sigma) resuspended in PBS 20 mg/ml BSA and incubated at 4°C for 1 h. Beads were pelleted by using magnets and washed six times in PBS containing 0.05% Triton X-100. Beads were then resuspended in 100 μl SDS-loading containing 0.5 mg/ml of 3×Flag peptides (Sigma). Lysates and eluates were analyzed by Western blot. For Co-IP analysis with KidO, 0.05% xylose was added to the culture 1 h before harvesting cells, to allow mCherry expression.

**Immunoblot analysis**

Proteins crude extracts were prepared by harvesting cells from exponential growth phase (OD_600_0.1–0.4). Cells were resuspended in SDS–PAGE loading buffer and lysed by incubation at 90°C for 10 min. Proteins were then subjected to electrophoresis in a 4–15% SDS–polyacrylamide gel, transferred onto a nitrocellulose membrane, and immunoblotted for ≥ 1 h with primary antibodies: α-FtsZ (1:20,000), α-FLAG (1:5,000) (Stratagene), α-CtrA (1:5,000), α-MreB (1:5,000), α-KidO (1:2,000) (Radhakrishnan et al., 2010), α-GdhZ (1:10,000), and α-DsRed (Clontech) (1:1,000). Membranes were then immunoblotted for ≤ 1 h with secondary antibodies: 1:10,000 anti-mouse (for α-FLAG) or 1:7,500 anti-rabbit (for all the others) linked to peroxidase (GE Healthcare), and visualized thanks to Western Lightning Plus-ECL chemiluminescence reagent (PerkinElmer) and ImageQuant LAS400 (GE Healthcare).

**Synchronization of cells**

For synchrony, cells were grown in 200 ml of PYE to OD_600_0.6, harvested by centrifugation for 15 min at 6,000 × g, 4°C, resuspended in 60 ml of ice-cold phosphate (PO_4^-_2-) buffer, and combined with 30 ml of Ludox LS Colloidal Silica (30%) (Sigma-Aldrich) (Jenal & Shapiro, 1996). Cells resuspended in Ludox were centrifuged for 40 min at 9,000 × g, 4°C. Swarmer cells, corresponding to the bottom band, were isolated, washed twice in ice-cold PO_4^-_2- buffer, and finally resuspended in prewarmed PYE media for growth at 30°C. Samples were collected every 15 min for Western blot, microscopy, and FACS analyses.

**Light and fluorescent microscopy**

All strains were imaged during exponential growth phase after immobilization on 1% agarose pads (Ely, 1991). For time-lapse experiments, cells were grown in PYE, transferred onto freshly prepared PYE 1.5% agarose pads, covered, and sealed with 1:1 mixture of paraffin, lanolin, and vaseline. Microscopy was performed using Axioskop microscope (Zeiss), Orca Flash 4.0 camera (Hamamatsu), and Zen 2012 software (Zeiss). Images were processed with ImageJ.

**Microbetracker and matlab analysis**

Cell identification and analysis were obtained using the Matlab-based open-source software MicrobTracker. Further quantitative analyses from cell meshes were performed with Matlab software (The MathWorks Inc., Natick, MA). Demographic representations were created using scripts derived from Hocking et al. (2012). Briefly, each cell was sorted by length and segmented, and fluorescence in each segment was integrated, normalized to the brightest segment, and plotted as a heat map from 0 (no fluorescence) to 1 (maximum fluorescence). Polar and midcell signals were considered when a maximal fluorescence (≥ 1.5-fold of the mean fluorescence) was localized up to 0.1 from each pole (0–0.1; 0.9–1) or from 0.4 to 0.6, respectively.

**Proteins purification**

FtsZ purification was performed as described in Thanbichler and Shapiro (2006).

GdhZ purification was performed essentially as described in Minambres et al. (2000) with the exception of GdhZ overproduction. *Escherichia coli* BL21 (DE3) was transformed with plasmid pH354 (pET-21b-gdhZb) or pH573 (pET-21b-gdhZbK837A) and grown to an OD_600_ of 0.6 in LB medium (2 L) at 37°C. Isopropyl-β-D-thiogalactoside (IPTG) was added to a final concentration of 1 mM and incubated for 4 h. Cells were harvested by centrifugation for 30 min at 5,000 × g, 4°C. Following purification, samples were dialyzed in 50 mM HEPES, pH 7.2, 50 mM KCl, 10 mM MgCl₂, 10% glycerol, 5 mM β-mercaptoethanol, and aliquoted, snap-frozen in liquid N₂, and stored at −80°C. Purified GdhZ was also used to immunize a rabbit in order to produce α-GdhZ polyclonal antibodies.

To purify KidO, *E. coli* BL21 (DE3) was transformed with pH624 (pET-21b-kidO) and grown to an OD_600_ of 0.6 in LB medium (2 L) at 37°C. IPTG was added to a final concentration of 1 mM and incubated for 4 h. Cells were harvested by centrifugation for 30 min at 5,000 × g, 4°C. Cells were resuspended in 20 ml of buffer A (50 mM Tris, pH 8.5, 1 mM ethylenediaminetetraacetic acid (EDTA), 10% glycerol, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF) containing 10 U/ml DNase I and complete EDTA-free anti-proteases (Roche)). Resuspended cells were then disrupted by...
sonication and cell debris pelleted by centrifugation for 30 min at 37,000 × g, 4°C. The supernatant was then passed through HiTrap DEAE Fast Flow columns (Sigma, 2 × 5 ml) equilibrated with buffer A. Columns were washed with 100 ml of buffer A, and elution was carried out with buffer A2 (buffer A + 80 mM KCl). Aliquots of 3 ml were collected at a flow rate of 3 ml/min. Fractions containing the KdoO were detected by SDS-PAGE and Coomassie blue staining. These fractions were pooled, dialyzed for 24 h in 50 mM HEPES, pH 7.5, 50 mM KCl, 10 mM MgCl₂, 10% glycerol, 5 mM β-mercaptoethanol, and aliquoted, snap-frozen in liquid N₂, and stored at –80°C.

In order to immunize rabbits for production of specific polyclonal antibodies, His6-MreB142-347, FtsZ-His6, and His6-CtrA were purified according to published works (Quon et al., 1996; Quardokus et al., 2001; Figge et al., 2004).

GTPase assay

The GTPase activity of FtsZ was assayed with the GTPase Assay Kit (Innova Biosciences, high fidelity) following the manufacturer’s protocol. GTPase assays were performed with 0.5 μM FtsZ or FtsZE79K in polymerization buffer (50 mM HEPES pH 7.2, 50 mM KCl, 0.1 mM EDTA, 1 mM β-mercaptoethanol, 10 mM MgCl₂). Reactions were started by the addition of 1 mM GTP before adding KidO and/or GdhZ or GdhZK837A. When present, NAD⁺ and glutamate were used at a final concentration of 5 mM and 100 mM, respectively. Hydrolysis rates were calculated from the slope of the Pi values plotted according to time. Pi values were normalized according to corresponding blank conditions containing all reagents except GTP.

Electron microscopy

As for GTPase assays, reactions were performed with 0.5 μM FtsZ in polymerization buffer and initiated by addition of 1 mM GTP. Polymerization mixtures were incubated for 5 min at RT. Samples were then applied to glow-discharged carbon-coated grids, stained with 2% uranyl acetate for 1 min, washed with a drop of distilled water, blotted, and air-dried. Images were taken at 80 kV on a TECNAI 10 transmission electron microscope with a Gatan 967 slow-scan, cooled CCD camera.

β-Galactosidase assay

Cells resuspended in 800 μl of Z buffer (60 mM Na₂HPO₄, 40 mM NaH₂PO₄, 10 mM KCl, 1 mM MgSO₄) were lysed with chloroform. After the addition of 200 μl ONPG (4 mg/ml), reactions were incubated at 30°C until color turned yellowish. Reactions were then stopped by the addition of 50 μl of 1 M Na₂CO₃, and absorbance at 420 nm was measured. Miller units are defined as (OD₄₂₀ × 1,000)/(OD₆₆₀ × t × v), where “OD₆₆₀” is the absorbance at 660 nm of the cultures before the β-galactosidase assays, “t” is the time of the reaction (min), and “v” is the volume of cultures used in the assays (ml). All the experiments were performed with three biological replicates and were normalized according to the wild-type strain harboring the P$_{gdhZ-lacZ}$ fusion cultivated at 30°C.

Supplementary information for this article is available online: http://emboj.embopress.org

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Author contributions

FB and RH conceived and designed the experiments. FB performed all the experiments except otherwise stated. JC did the Y2H screen and constructed the Aghdh2 mutant. AM purified all the native proteins. GL performed the quantitative analysis on Matlab software. FB, XDB, and RH analyzed the data and contributed reagents/materials/analysis tools. FB and RH wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

References


