Glutamate Metabolism in *Bacillus subtilis*: Gene Expression and Enzyme Activities Evolved To Avoid Futile Cycles and To Allow Rapid Responses to Perturbations of the System†

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Glutamate is a central metabolite in all organisms since it provides the link between carbon and nitrogen metabolism. In *Bacillus subtilis*, glutamate is synthesized exclusively by the glutamate synthase, and it can be degraded by the glutamate dehydrogenase. In *B. subtilis*, the major glutamate dehydrogenase RocG is expressed only in the presence of arginine, and the bacteria are unable to utilize glutamate as the only carbon source. In addition to rocG, a second cryptic gene (*gudB*) encodes an inactive glutamate dehydrogenase. Mutations in *rocG* result in the rapid accumulation of *gudB*1 suppressor mutations that code for an active enzyme. In this work, we analyzed the physiological significance of this constellation of genes and enzymes involved in glutamate metabolism. We found that the weak expression of *rocG* in the absence of the inducer arginine is limiting for glutamate utilization. Moreover, we addressed the potential ability of the active glutamate dehydrogenases of *B. subtilis* to synthesize glutamate. Both RocG and GudB1 were unable to catalyze the anabolic reaction, most probably because of their very high *K_m* values for ammonium. In contrast, the *Escherichia coli* glutamate dehydrogenase is able to produce glutamate even in the background of a *B. subtilis* cell. *B. subtilis* responds to any mutation that interferes with glutamate metabolism with the rapid accumulation of extragenic or intragenic suppressor mutations, bringing the glutamate supply into balance. Similarly, with the presence of a cryptic gene, the system can flexibly respond to changes in the external glutamate supply by the selection of mutations.

Among the metabolites of a bacterial cell, glutamate is of central importance. It is this amino acid that stands at the intersection between catabolism and anabolism, i.e., between carbon and nitrogen metabolism. Glutamate is synthesized from α-ketoglutarate, an intermediate of the tricarboxylic acid (TCA) cycle, and serves as the amino group donor for nearly all nitrogen-containing metabolites of the cell, besides being one of the proteinogenic amino acids. In agreement with this important aspect of glutamate is the fact that it is one of most abundant metabolites in bacterial cells and that its concentration is high under all conditions of nutrient supply (10, 18, 36). Moreover, its metabolism must be tightly controlled to guarantee a constant, sufficient supply of this essential intermediate for all anabolic reactions.

In the gram-positive soil bacterium *Bacillus subtilis*, glutamate is synthesized exclusively by the reductive amination of α-ketoglutarate by the enzyme glutamate synthase (also called glutamate-oxoglutarate amidotransferase [GOGAT]; encoded by the *gltAB* operon) (Fig. 1) (3). This enzyme produces two molecules of glutamate from α-ketoglutarate and glutamine, the primary product of ammonium assimilation. Of these two molecules, one remains in the cycle, whereas the second can be used for protein biosynthesis or transamination reactions to provide the cell with nitrogen-containing compounds. The second enzyme of glutamate metabolism, glutamate dehydrogenase (GDH; encoded by the *rocG* gene), is required for the utilization of arginine as a carbon source (4). This enzyme is devoted to glutamate degradation and is not involved in its biosynthesis, since *B. subtilis* *glt* mutants unable to produce a functional GOGAT are auxotrophic for glutamate (3). This is an important difference from the situation in *Escherichia coli* and many other bacteria. In those bacteria, the GDH has an anabolic activity and is the main contributor to ammonium assimilation and glutamate biosynthesis at high ammonium concentrations (27). In addition to the GDH encoded by *rocG*, there is a cryptic gene, *gudB*, encoding an inactive GDH. The corresponding protein contains a duplication of three amino acids at the active center, resulting in a loss of catalytic activity (4).

As observed for many enzymes of central metabolism, the regulation of the enzymes of glutamate metabolism involves signals from carbon and nitrogen metabolism (10, 31). The expression of the *rocG* gene depends on the alternative sigma factor, σ^54^; and the transcription factor RocR that activates the *rocG* promoter in the presence of arginine (1, 5). In addition, *rocG* transcription is strongly repressed by glucose and other highly metabolizable carbon sources. This regulation is mediated by the pleiotropic transcription repressor CcpA (8). The cryptic *gudB* gene is constitutively expressed (4, 12). The expression of the biosynthetic *gltAB* operon is controlled by several metabolites: ammonium and glucose or other sugars are required for the induction of the operon, whereas arginine and, to a lesser extent, glutamate repress its expression. These regulatory events involve two transcription factors that are under the control of trigger enzymes with both enzymatic and...
regulatory activities (13). The master regulator of nitrogen metabolism, TnRA, represses the expression of the operon in the absence of one of the substrates of the enzymatic reaction, i.e., ammonium (6). This regulation is mediated by the regulatory interaction and concomitant inactivation of TnRA with the glutamine synthetase in the presence of ammonium (37). In contrast, the GltC protein activates the gltA promoter in the presence of sugars and in the absence of arginine. In the presence of arginine or in the absence of glucose, RocG is synthesized and binds GltC, thus inactivating it (7, 11). In addition, GltC activity is modulated by the direct binding of low-molecular-weight effectors; α-ketoglutarate stimulates its activity, whereas glutamate inhibits it (26). Thus, the two major players of glutamate metabolism are expressed under mutually exclusive conditions, and the control of GltC activity by the trigger enzyme RocG is an additional intrinsic control to ensure this mutual exclusivity of the two biochemical activities.

In previous studies, it was observed that mutations that interfere with glutamate metabolism are readily suppressed by secondary mutations. Strains carrying a defective rocG gene are unable to catabolize arginine, ornithine, or citrulline. During growth on complex media, suppressor mutations in the gudB gene (designated gudB1) restore the GDH activity. In all cases studied so far, the duplicated nine bases are deleted in such suppressor mutants (4, 12). Similarly, ccpA mutants are unable to express the gldxB operon and thus are auxotrophic for glutamate due to the lack of repression of the negative effector protein RocG (11, 35); mutations of ccpA that restore glutamate synthesis were found in the rocG gene, thus preventing the inactivation of Gldx by RocG (12, 15).

The aim of the present study was to explore the rationale for the adjustment of the genes and enzymes involved in glutamate metabolism. For this purpose, we expanded the existing system beyond its natural boundaries by overexpressing the genes and by isolating mutants with altered enzymatic characteristics. Our work revealed that both the regulation of the genes and the enzymatic properties of the relevant enzymes have evolved to satisfy the following two demands: (i) there has to be sufficient glutamate under all conditions of nutrient supply, and (ii) the system has to be sufficiently flexible to allow the adaptation to specific, unusual conditions. The cryptic gudB gene serves as a buffer that may compensate for mutations in the rocG gene and that can also be decrypified for the utilization of glutamate as a single carbon source.

**MATERIALS AND METHODS**

**Bacterial strains and growth conditions.** The *B. subtilis* strains used in this study are shown in Table 1. *B. subtilis* was grown in C minimal medium [70 mM K2HPO4, 30 mM KH2PO4, 25 mM (NH4)2SO4, 0.5 mM MgSO4, 10 μM MnSO4, 22 mg ferric ammonium citrate/liter] supplemented with tryptophan (at 50 mg liter⁻¹) (15). CSE medium is C minimal medium supplemented with sodium succinate (6 g liter⁻¹) and potassium glutamate (8 g liter⁻¹) (15). C-Glc is C minimal medium supplemented with glucose (1 g liter⁻¹), and CE-Glc is C minimal medium supplemented with potassium glutamate and glucose (8 g liter⁻¹ and 1 g liter⁻¹, respectively). *E. coli* DH5α (29) was used for the cloning experiments. *E. coli* was grown in LB medium, and transformants were selected on plates containing ampicillin (100 μg ml⁻¹), LB medium, SP complex medium (23), and CG minimal medium plates were prepared by the addition of 17 g liter⁻¹ Bacto agar (Difco) to the medium.

**DNA manipulation.** The transformation of *E. coli* and plasmid DNA extraction were performed using standard procedures (29). Restriction enzymes, T4 DNA ligase, and DNA polymerases were used as recommended by the manufacturers. DNA fragments were purified from agarose gels using the NucleoSpin DNA extraction kit (Macherey and Nagel). DNA sequences were determined by the manufacturer. DNA sequences were determined by using the dye deoxy chain termination method (29). The chromosomal DNA of *B. subtilis* was isolated as previously described (23).

**Transformation and characterization of the phenotype.** *B. subtilis* was transformed with plasmid and chromosomal DNA according to a previously described two-step protocol (23). Transformants were selected on SP plates containing chloramphenicol (5 μg ml⁻¹), spectinomycin (100 μg ml⁻¹), kanamycin (5 μg ml⁻¹), or erythromycin and lincomycin (1 and 10 μg ml⁻¹, respectively). Quan-

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**TABLE 1. *B. subtilis* strains used in this study**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>168</td>
<td>trpC2</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>GP27</td>
<td>trpC2 ΔgudB:zat amyE::(gldxA-lacZ apbA3)</td>
<td>12</td>
</tr>
<tr>
<td>GP28</td>
<td>trpC2 ΔgudB:zat rocG::Tn10 spc amyE::(gldxA-lacZ apbA3)</td>
<td>12</td>
</tr>
<tr>
<td>GP342</td>
<td>trpC2 amyE::(gldxA-lacZ apbA3)</td>
<td>35</td>
</tr>
<tr>
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<td>trpC2 gltC::Tn10 spc amyE::(gldxA-lacZ apbA3)</td>
<td>11</td>
</tr>
<tr>
<td>GP675</td>
<td>trpC2 ΔgudB::zat rocG::Tn10 spc amyE::(gldxA-lacZ apbA3) gldB2</td>
<td>11</td>
</tr>
<tr>
<td>GP717</td>
<td>trpC2 ΔgudB::zat rocG::Tn10 spc amyE::(gldxA-lacZ apbA3) gltB1</td>
<td>11</td>
</tr>
<tr>
<td>GP754</td>
<td>trpC2 rocG::amyl::(gldxA-lacZ apbA3)</td>
<td>11</td>
</tr>
<tr>
<td>GP755</td>
<td>trpC2 rocG::amyl::(gldxA-lacZ apbA3) gldB1</td>
<td>11</td>
</tr>
<tr>
<td>GP801</td>
<td>trpC2 rocG::amyl::(gldxA-lacZ apbA3)</td>
<td>11</td>
</tr>
<tr>
<td>GP802</td>
<td>trpC2 rocG::amyl::(gldxA-lacZ apbA3) gudB1</td>
<td>11</td>
</tr>
<tr>
<td>GP804</td>
<td>trpC2 amyl::(gldxA-lacZ apbA3) gudB1</td>
<td>11</td>
</tr>
<tr>
<td>GP805</td>
<td>trpC2 ΔgudB::zat rocG::Tn10 spc amyE::(gldxA-lacZ apbA3) ΔgltB::ermC</td>
<td>This work (see Materials and Methods)</td>
</tr>
</tbody>
</table>

*ΔgltB2, ΔC994 C996G ΔT1010 ΔG1011.*
titative assays of lacZ expression in B. subtilis were performed with cell extracts by using o-nitrophenyl galactopyranoside as the substrate (23). One unit of o-nitrophenolase per min at 28°C.

**Construction of a gdhB mutant strain.** To construct a gdhB mutant strain, a PCR technique using long flanking homology regions was used (34). Briefly, a cassette carrying the ermC resistance gene was amplified from plasmid pDG647 (16). DNA fragments of about 1,000 bp flanking the gdhB region at its 5′ and 3′ ends were amplified using the primer pairs FC73 (5′ AGCACTGGAAGACCA GCCACGCTTG)/FC74 (5′ CCTATACCTCTAAATTGCGCCAGATCG). With FC124, the ribosomal binding site of the B. subtilis gdhA gene was placed in an appropriate position upstream of the gdhA gene. The PCR products were digested by BamHI and PstI (the sites were introduced with the PCR primers; they are underlined in the sequences) and ligated with pBG200 linearized with the same enzymes. The identities of the cloned inserts were verified by sequencing, and the resulting plasmids were pGP529 (rocG) and pGP934 (gdhA).

**Western blot analysis.** For Western blot analysis, proteins were separated on 12.5% sodium dodecyl sulfate-polyacrylamide gels. After electrophoresis and blotting onto a polyvinylidene difluoride membrane was performed, the proteins were transferred to a polyvinylidene difluoride membrane. To test the effect of RocG overexpression on glutamate utilization, derivatives of the B. subtilis strain GP342, either carrying the empty vector pBG200 (Δ) or expressing RocG (pGP529) (▲), respectively, were grown in CE minimal medium containing 0.8% (wt/vol) glutamate in the presence (B) and in the absence (C) of 0.5% (wt/vol) glucose. OD 600, optical density at 600 nm.

**RESULTS**

Constitutive expression of a GDH allows B. subtilis to utilize glutamate as the single carbon source. B. subtilis possesses the complete genetic equipment for the transport of glutamate, its conversion to the TCA cycle intermediate α-ketoglutarate, and its subsequent metabolism. However, B. subtilis 168 is unable to grow with glutamate as the only source of carbon and energy, suggesting that other factors are growth limiting. Since the expression of the rocG gene encoding GDH is induced by arginine and is low in its absence, we considered the possibility

![FIG. 2. Effect of RocG overexpression on glutamate utilization. (A) Western blot analysis of RocG expression in the B. subtilis strain GP342 (wild type) either carrying the empty vector pBG200 or expressing RocG (pGP529). Cells were grown in C minimal medium containing either 0.5% (wt/vol) glucose and 0.8% (wt/vol) glutamate (CE-Glc) or 0.5% (wt/vol) arginine (CR), respectively. A total of 20 μg crude extract of each culture was loaded on a 12.5% sodium dodecyl sulfate-polyacrylamide gel. After electrophoresis and blotting onto a polyvinylidene difluoride membrane was performed, RocG was detected using rabbit polyclonal antibodies raised against RocG. Purified RocG (100 ng) served as a control. To test the effect of RocG overexpression on glutamate utilization, derivatives of the B. subtilis strain GP342, either carrying the empty vector pBG200 (Δ) or expressing RocG (pGP529) (▲), respectively, were grown in CE minimal medium containing 0.8% (wt/vol) glutamate in the presence (B) and in the absence (C) of 0.5% (wt/vol) glucose. OD 600, optical density at 600 nm.](http://jb.asm.org/ Downloaded from on October 23, 2017 by guest)
that the overexpression of rocG might confer to B. subtilis the ability to utilize glutamate. To test this hypothesis, we constructed plasmid pGP529 to express the rocG gene under the control of a strong promoter. The functionality of this system was verified by Western blot analysis (Fig. 2A). In the absence of the inducer arginine (i.e., in medium containing glucose and glutamate), B. subtilis GP342 carrying the empty vector pBQ200 produced very small amounts of RocG, whereas large amounts of the protein were detected in the strain harboring plasmid pGP529. In medium containing arginine, high levels of RocG protein were detected, irrespective of the plasmid present. This induction is in good agreement with a previous quantitative analysis of RocG regulation (11). To assess the role of rocG expression levels in glutamate utilization, the B. subtilis strain GP342 carrying the empty vector pBQ200 or pGP529 was grown in minimal medium containing either glucose and glutamate or glutamate as the only carbon source. As shown in Fig. 2B, the expression of rocG did not affect growth in the presence of glucose and glutamate. In contrast, B. subtilis carrying the vector pBQ200 grew very slowly with glutamate as the only substrate (less than one doubling in 8 hours). However, the expression of the GDH driven by plasmid pGP529 allowed the bacteria to utilize glutamate (Fig. 2C). This observation confirms that the low expression of rocG in the absence of the inducer arginine is the growth-limiting factor when glutamate is the only carbon source.

We observed that cultures of GP342 carrying plasmid pBQ200 started to grow with glutamate as the only carbon source after a long incubation of about four generations in this medium. This resumed growth might have resulted from an adaptation of the bacteria or from the acquisition of suppressor mutations. To distinguish between these two possibilities, we tested the ability of cells present in such a culture to utilize glutamate. Indeed, these cells were able to grow on agar plates with glutamate as the only substrate, suggesting that they had acquired suppressor mutations. An obvious candidate for such mutations is the cryptic gudB gene, which encodes a nonfunctional GDH. As expected, sequence analysis of the gudB alleles of three independent suppressor mutants revealed the presence of the gudB1 allele encoding a functional GDH. One of these mutants was designated GP804. Thus, the “decryptification” of the gudB gene that is expressed even in the absence of arginine (4, 12) is a second mechanism that allows glutamate utilization.

The ability of B. subtilis to synthesize glutamate depends on the carbon source and the rocG allele. We have previously reported that B. subtilis 168 is unable to grow with succinate and ammonium as the single sources of carbon and nitrogen, respectively. However, the bacteria are capable of growing in minimal media with glucose or citrate/malate as the carbon sources and ammonium as the nitrogen source or in the presence of glutamate (12, 15, 33). In contrast, rocG mutants affected in the catabolic GDH are prototrophic for glutamate even in the presence of succinate as the single carbon source (12). To study in more detail the effect of the carbon source on the biosynthesis of glutamate, we plated the B. subtilis gudB mutant strain GP27 and its isogenic rocG derivative GP28 on medium with glucose or with different intermediates of the TCA cycle as the only carbon source and ammonium as the only source of nitrogen. As shown in Table 2, both strains grew in the presence of glucose, citrate, fumarate, or malate as the carbon source. In contrast, B. subtilis GP27 was unable to grow with succinate and ammonium, but growth was possible if glutamate was added to the medium (data not shown), thus confirming that this strain is auxotrophic for glutamate if succinate is the only carbon source. The isogenic rocG mutant GP28 was able to grow with succinate and ammonium. These findings confirm previous observations and suggest a specific negative role for GDH if succinate is the only carbon source (see Discussion).

### Table 2. Growth of B. subtilis with different intermediates of the TCA cycle

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>GP27 ΔgudB</th>
<th>GP28 ΔgudB rocG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Citrate</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Succinate</td>
<td>−</td>
<td>+E</td>
</tr>
<tr>
<td>Fumarate</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Malate</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

*The bacteria were plated on C minimal medium supplemented with the indicated carbon sources (0.5% [wt/vol]). ++++, very good growth; ++, good growth; −, no growth.*

The GDHs of B. subtilis are unable to catalyze glutamate biosynthesis in vivo. In E. coli, the GDH GdhA is the major contributor to glutamate synthesis under conditions of ammonium excess (27). We have already shown that the low expression of RocG in the absence of arginine is the bottleneck that prevents glutamate utilization in B. subtilis. Next, we wanted to test whether the overexpression of RocG would also allow ammonium assimilation by this enzyme. For this purpose, we introduced the vector pBQ200 and the expression plasmid pGP529 into the gldC mutant strain GP650. As observed for GP342, the GDH RocG was strongly expressed in GP650 carrying pGP529 (data not shown). In GP650, the gldAB operon encoding GOGAT was not expressed, due to the lack of the transcription activator GltC (9, 11). To analyze the potential glutamate biosynthesis by RocG, GP650 carrying either of the plasmids and the isogenic wild-type strain GP342 carrying pBQ200 were grown in minimal medium with ammonium and glutamate or with ammonium as the only source of nitrogen. As a control, we used GP650 carrying pGP934 that expressed the E. coli gdhA gene encoding GDH. As shown in Fig. 3A, all four strains were able to grow in the presence of glutamate. Similarly, the wild-type strain GP342 was able to assimilate ammonium. As expected, the inactivation of the gldC gene (GP650/pBQ200) resulted in the inability to grow when ammonium was the only nitrogen source. This could not be suppressed by the overexpression of RocG when pGP529 was used (Fig. 3B). In contrast, the expression of the E. coli GDH allowed GP650 to grow in the absence of glutamate (Fig. 3B), suggesting that the E. coli enzyme is able to synthesize glutamate in the biological context of a B. subtilis cell. Thus, we have to assume that RocG is unable to catalyze glutamate biosynthesis in vivo.

Alternatively, the decryptified GDH GudB1 might be capable of synthesizing glutamate. To address this idea, we isolated gudB1 suppressor mutants by streaking the rocG mutant GP754 and the rocG gldC double mutant GP755 on SP plates.
These plates contain arginine that cannot be catabolized in rocG mutants, probably resulting in the accumulation of toxic intermediates. On a background of translucent rocG mutants, the gudB1 suppressors were isolated as well-growing colonies (12), and the gudB1 alleles were verified by sequencing. To analyze the ability of the GudB1 proteins to synthesize glutamate, the resulting strains GP801 and GP802 (gltC) and their isogenic parents were grown in C-Glc minimal medium in the presence and absence of glutamate. As expected, all four strains grew equally well if both glutamate and glucose were available (Fig. 3C). In the absence of glutamate, the wild-type strain GP754 grew at a rate comparable to that on medium containing glucose and glutamate, whereas the gltC mutant strain GP755 was auxotrophic for glutamate (Fig. 3D). The “decryptification” of gudB resulted in a severe reduction of the growth rate of the wild-type strain on C-glucose medium (Fig. 3D, GP801). This may have been caused by the catabolic activity of GudB1 (see above) and the resulting reduced glutamate pool. Strain GP802 carries a gltC mutation and the gudB1 allele. As shown in Fig. 3D, the active GDH GudB1 is not able to suppress the glutamate auxotrophy of the gltC mutant. In conclusion, the decryptified GudB1 protein is capable of catalyzing the catabolic reaction but is unable to act in ammonium assimilation.

Isolation and characterization of gltB mutants. It is well established that rocG mutants accumulate gudB1 suppressor mutations, resulting in a functional GDH upon growth on complex medium containing arginine (4, 12). Similarly, we observed the occurrence of colonies with an appearance that is indistinguishable from the wild type when we plated the rocG gudB double mutant strain GP28 on SP medium, an arginine-containing complex medium. Such a suppressor mutant strain was designated GP717 and used for further analyses. Growth experiments using liquid media revealed that GP717 was, unlike its parent, auxotrophic for glutamate, suggesting the presence of a mutation in either the gltAB operon or the gltC gene. Nucleotide sequencing of these loci revealed a deletion of two consecutive base pairs in the gltB gene (T1010, G1011), resulting in a frameshift and the expression of a truncated protein and, thus, an inactive GOGAT. The inactivation of gltB in strain GP717 might serve to avoid a futile cycle (see Discussion); glutamate derived from arginine accumulates in any strain that is devoid of a functional GDH and, in addition, the expression of the gltAB operon is constitutive in such mutants, resulting in
TABLE 3. Effect of mutations in genes involved in glutamate metabolism on the activity of the gltA promoter

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>β-Galactosidase activitya</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CSE</td>
</tr>
<tr>
<td>GP27</td>
<td>ΔgudB</td>
<td>10</td>
</tr>
<tr>
<td>GP28</td>
<td>ΔgudB rocG</td>
<td>207</td>
</tr>
<tr>
<td>GP717</td>
<td>ΔgudB rocG gltB1</td>
<td>1,008</td>
</tr>
<tr>
<td>GP675</td>
<td>ΔgudB rocG gltB2</td>
<td>527</td>
</tr>
<tr>
<td>GP805</td>
<td>ΔgudB rocG ΔgltB</td>
<td>1,430</td>
</tr>
</tbody>
</table>

a Cultures of the B. subtilis strains were grown on CSE minimal medium (succinate, glutamate, and ammonium). β-Galactosidase activity was measured in extract prepared from exponentially growing cells (optical density at 600 nm, 0.6 to 0.8).

b β-Galactosidase activity is expressed in units per mg of protein. Representative results from two or three independent experiments are shown. The variance of the different sets of experiments did not exceed 20%.

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However, glutamate utilization is possible only upon either high-level expression of RocG or mutational activation of the cryptic GDH GudB. The following question arises from this observation: why is GudB inactive in B. subtilis 168? Since the gudB gene is only weakly regulated by the carbon and nitrogen sources (4, 12), it is tempting to speculate that the presence of only one GDH (i.e., RocG) allows B. subtilis 168 to achieve higher growth rates, due to the reduced glutamate degradation. This assumption is in good agreement with the observation that B. subtilis 168 is able to grow with succinate and ammonium as carbon and nitrogen sources, respectively, in the absence of an active GDH (12) (Table 2). Moreover, the expression of an active GDH in B. subtilis cells growing in medium without glutamate results in an ATP-consuming futile cycle of glutamate production and degradation with a concomitant growth defect (Fig. 3D, compare results for GP754 and GP801). The inactivation of the gudB gene in B. subtilis 168 is, under certain growth conditions, disadvantageous for the bacteria. As a consequence of the “cryptification” of the gudB gene, the spectrum of potential carbon sources is reduced; i.e., the bacteria are unable to utilize glutamate. This problem can be circumvented by the “decryptification” of the gudB gene (4; see also this work). Interestingly, the gudB gene is not cryptic in some wild-type isolates of B. subtilis, such as NCIB3610 (our unpublished results).

Cryptic genes also exist in other bacteria. The ilv/GEDA operon of E. coli K-12 contains a frameshift in the ilvG gene. The inactivation of ilvG encoding an acetolactate synthase isozyme that is insensitive to feedback inhibition prevents the waste of resources due to the excessive synthesis of branched-chain amino acids. Similarly to B. subtilis gudB, the ilvG gene is decrypted under certain growth conditions. The growth of E. coli K-12 is inhibited in the presence of valine, whereas a mutant strain bearing the decrypted ilvG gene is insensitive to valine (17). Thus, the growth conditions determine whether the cryptic or activated ilvG allele specifies the advantageous phenotype. It is tempting to speculate that the accumulation of cryptic genes is a phenomenon of the cultivation of “wild-type” strains under rather constant laboratory conditions (32).

What is the physiological significance of the adjustment of expression and activities of the enzymes involved in glutamate metabolism? The avoidance of a futile cycle between glutamate-synthesizing and -degrading enzymes is certainly a major driving force in the evolution of glutamate metabolism. This idea is supported by the observation that the two enzymatic systems are never active at the same time (12). Even more so, active GDH acts as a trigger enzyme and prevents glutamate synthesis by inhibiting the transcription factor GltC (11). The regulatory embedding of RocG in the arginine catabolic pathway followed by the resulting inability to utilize glutamate as a single carbon source may be another way to prevent the loss of glutamate resources but may also reflect the availability of different amino acids in natural ecosystems of B. subtilis. There, the availability of glutamate in the absence of arginine may be an exception. Similarly, B. subtilis is unable to induce the genes for xylose utilization in the absence of arabinose, a common cosubstrate of xylose (22). However, the system has immanent reserves and is sufficiently flexible to acquire mutations affecting the expression or activity of the components of glutamate metabolism whenever a selective pressure occurs. As shown in this work, a wide variety of such mutations can easily be obtained, depending on the selection exerted on the bacteria. Interestingly, similar observations have recently been reported for glutamate metabolism in enteric bacteria (38).

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We acknowledge the help of Ingrid Wacker and Christina Herberz with some experiments. We are grateful to Thorsten Mascher for the gift of some oligonucleotides.

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  ers MaoN (YufR) and YfS, and is essential for utilization of malate in
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