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Krebs cycle function is required for activation of the Spo0A transcription factor in *Bacillus subtilis*

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ABSTRACT **Expression of genes early during sporulation in *Bacillus subtilis* requires the activity of the transcription factor encoded by *spo0A*. The active, phosphorylated form of Spo0A is produced through the action of a multicomponent pathway, the phosphorelay. A mutant defective in the first three enzymes of the Krebs citric acid cycle was unable to express early sporulation genes, apparently because of a failure to activate the phosphorelay. Cells that produce an altered Spo0A protein that can be phosphorylated by an alternative pathway were not dependent on Krebs cycle function for early sporulation gene expression. These findings suggest that Krebs cycle enzymes transmit a signal to activate the phosphorelay and that *B. subtilis* monitors its metabolic potential before committing itself to spore formation.**

Developmental processes are often coupled to the nutritional state of the cell. For example, spore formation in the bacterium *Bacillus subtilis* initiates when the environment becomes depleted of rapidly metabolizable sources of carbon, nitrogen, or phosphorus (1, 2). In addition, excess glucose inhibits sporulation by overriding the effects of nitrogen or phosphorus depletion (3).

The end product of sporulation is a metabolically dormant spore that is unusually resistant to heat, strong chemicals, desiccation, and radiation. Thus, spore formation can be viewed as a protective response to unfavorable growth conditions. However, to form the spore, the cell needs to expend energy and synthesize specialized structures, and the ability to sporulate depends on both nutrient depletion and the availability of sufficient metabolic reserves to complete the developmental process.

Enzymes of the Krebs citric acid cycle are particularly important in spore formation. Transcription of the genes for these enzymes is repressed by excess glucose and induced as cells exit the exponential growth phase (4). The activities of these enzymes are required for sporulation (5–8), and it is likely that at least one of their roles is to provide energy-yielding compounds and metabolic intermediates essential for sporulation.

One of the key regulatory proteins controlling the initiation of sporulation is the transcription factor encoded by *spo0A*. Spo0A represses synthesis of AbrB (9), itself a repressor of several genes involved in sporulation (10–13), and activates transcription of genes (e.g., *spoIIA*, *spoIIE*, and *spoIIG*) whose products are required for sporulation and cell type-specific gene expression (14–20). To be active as a transcription factor, Spo0A must be phosphorylated, and a considerable amount of evidence indicates that a small amount of Spo0A~*P* is needed to repress transcription of *abrB* and a greater amount is needed to activate transcription of *spoIIA*, *spoIIE*, and *spoIIG* (21–26). Spo0A~*P* is produced by a pathway (the phosphorelay) that involves one or more histidine protein kinases (KinA, KinB, KinC) (23–25, 27–29), an intermediate phosphoacceptor (Spo0F), and a phosphotransferase (Spo0B) (30, 31). The multiple steps of the phosphorelay provide an opportunity for the cell to regulate Spo0A activity in response to multiple signals that reflect the status of the cell with respect to culture density (32), nutrition (32), DNA replication (33, 34), chromosome partition (35), and DNA damage (34). Integration of these signals determines whether the cell will initiate spore formation (26, 32).

In this paper we show that the activity of the Krebs cycle also regulates the phosphorelay. Mutant cells lacking the Krebs cycle enzymes citrate synthase, aconitase, and isocitrate dehydrogenase are defective in the initiation of sporulation and in expression of genes controlled by Spo0A~*P*. Mutations in *spo0A* that bypass the need for the phosphorelay, allowing Spo0A to be phosphorylated by an alternative pathway, reverse the effect of the Krebs cycle defect on Spo0A-controlled genes. We also show that cells in which Krebs cycle genes have been engineered to be expressed from a glucose-insensitive promoter sporulate more efficiently in glucose-containing medium than do wild-type cells. These results imply that the inhibitory effect of glucose on spore formation is due, at least in part, to its repressive effect on Krebs cycle genes. The Krebs cycle appears to provide a signal for Spo0A phosphorylation, acting either as a sensor of nutritional conditions or as a metabolic checkpoint to assure that the cell does not initiate sporulation unless the metabolic requirements to complete the process have been met.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions. All *B. subtilis* strains were derived from strain JH642 (36) and contain the *trpC2* and *pheA1* mutations.

The *citA citZ citC* mutant strains carry deletion–insertion mutations, Δ*citA*::*neo* Δ*citZ*::*spc*, in both genes for citrate synthase (8) and a 1-bp insertion mutation at nucleotide 14 of the *citC* coding sequence (S.J. and A.L.S., unpublished data). This frameshift mutation creates a chain-termination codon that would cause translation to abort after synthesis of a decapeptide. In addition to lacking citrate synthase and isocitrate dehydrogenase enzyme activities, the triple mutant is also devoid of aconitase activity (data not shown), apparently because the strain is unable to synthesize citrate, the inducer of aconitase gene expression (37).

Strain SJB93 carries a version of the *citZCH* operon (8, 38) in which the promoter upstream of *citZ* has been replaced by a mutant version of the *dpp* promoter. This mutant promoter, *dppP129* (*dcs-129*) is active in medium containing excess sources of carbon, nitrogen, and amino acids (39). A 267-bp restriction fragment containing the *dppP129* promoter was cloned along with a 1082-bp *Hin*dIII fragment that contains

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the N-terminal coding sequence of *citZ* into the integrative vector pJPM1 (21) to create pCS58. Strain SJB93 was made by integrating pCS58 into the chromosome of JH642, creating the *dppP129-citZCH* fusion.

Other strains and genotypes are described in the text, tables, and figures.

Unless stated otherwise, *B. subtilis* cultures were grown in nutrient broth sporulation (DS) medium (1) supplemented with appropriate antibiotics; samples removed at indicated times were assayed for β-galactosidase activity as described (38, 40). In some cases, DS medium was supplemented with 2% (wt/vol) glucose. Cultures incubated for 12–16 hr after the end of exponential growth were tested for spore formation by assaying survival to incubation at 80°C for 10–20 min.

***lacZ* Fusions.** Fusions of various *B. subtilis* promoters to the *lacZ* reporter gene from *Escherichia coli* were used to measure gene expression. Fusions were introduced in single copy into the chromosome of *B. subtilis* strains by transformation or specialized transduction. The fusions, their sites of integration in the chromosome, and the antibiotic resistance(s) markers linked to them were as follows: *spoVG-lacZ*, SPβ locus, MLS^r, Cam^r (41); *spoIIG-lacZ*, SPβ locus, MLS^r, Cam^r (42); *spoIIA-lacZ*, SPβ, MLS^r, Neo^r (43); *cotA-lacZ*, SPβ, MLS^r, Tet^r (44); *gsiA-lacZ, amyE* locus, MLS^r (21); *dpp-lacZ, dpp* locus, Cam^r (45), where Tet is tetracycline, Neo is neomycin, Cam is chloramphenicol, and MLS is erythromycin and lincomycin.

RESULTS

Expression of Stationary-Phase Genes in a *citA citZ citC* Mutant Strain. Strains with null mutations in *citA, citZ*, and *citC* have no detectable activity of citrate synthase, aconitase, or isocitrate dehydrogenase (8) (S.J. and A.L.S., unpublished results) and sporulate poorly (8). To determine the stage at which sporulation is blocked in the multiply mutant strain SJB36 (*citA::neo citZ::spc citC7*), we measured expression of several genes that are normally induced early during sporulation. Expression of these genes is a reflection of the production of Spo0A~*P* and a good indication of the ability of cells to initiate sporulation. *lacZ* fusions to the regulatory regions of interest were introduced into the multiple *cit* mutant, and accumulation of β-galactosidase specific activity was used as an indication of gene expression.

Transcription of *spoIIG* is normally induced within 1 hr after cells enter stationary phase. This induction requires the direct binding of Spo0A~*P* to sequences in the *spoIIG* promoter region (14, 15, 42). In the *citA citZ citC* triple mutant, expression of *spoIIG-lacZ* was delayed by several hours, and accumulation of β-galactosidase activity was at a slower rate than in wild-type cells (Fig. 1*A*). A similar result was obtained for expression of *spoIIA* (see Fig. 2), another gene that is directly activated by Spo0A~*P* (18). These effects on expression of *spoIIG* and *spoIIA* are similar to the effects caused by other conditions or mutations that decrease activity of the phosphorelay (23, 33, 35).

Transcription of *gsiA* is normally induced at the onset of stationary phase. After about 1 hr, expression is shut off by a process that depends on Spo0A~*P* (46). In the *citA citZ citC* triple mutant, transcription of *gsiA* was not shut off (Fig. 1*B*), and β-galactosidase specific activity accumulated to a high

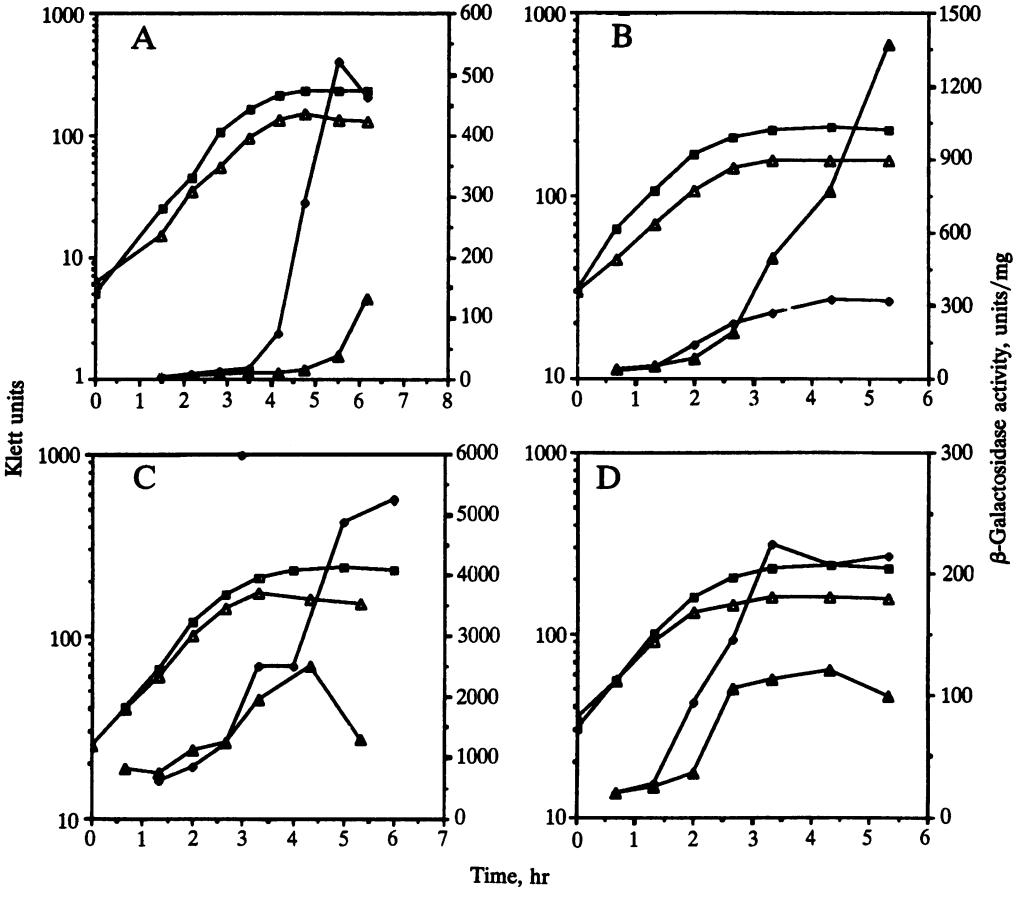

FIG. 1. Expression of genes early in stationary phase in wild type and the *citA citZ citC* mutant. Wild type (squares and diamonds) and the *citA citZ citC* mutant (triangles) were grown in DS medium, and samples were taken at indicated times to monitor cell growth (Klett units, squares and open triangles), and β-galactosidase specific activity (closed triangles and diamonds). (*A*) *spoIIG-lacZ*. Strains: wild-type SJB83 (*cit$^+$ spoIIG-lacZ*); mutant SJB845 (*citA citZ citC spoIIG-lacZ*). (*B*) *gsiA-lacZ*. Strains: wild-type SJB77 (*cit$^+$ gsiA-lacZ*); mutant SJB75 (*citA citZ citC gsiA-lacZ*). (*C*) *spoVG-lacZ*. Strains: wild-type SJB78 (*cit$^+$ spoVG-lacZ*); mutant SJB76 (*citA citZ citC spoVG-lacZ*). (*D*) *dpp-lacZ*. Strains: wild-type SJB79 (*cit$^+$ dpp-lacZ*); mutant SJB74 (*citA citZ citC dpp-lacZ*).

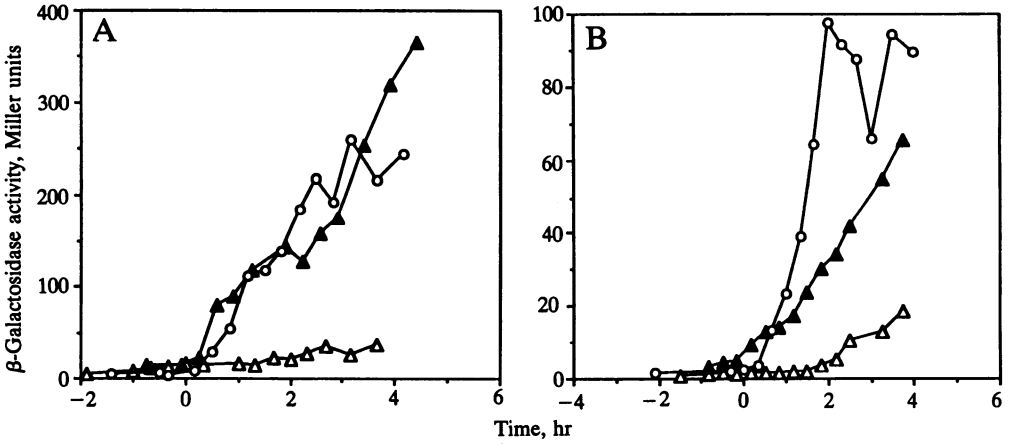

FIG. 2. The *sof-1* mutation in *spo0A* restores expression of early sporulation genes in the *citA citZ citC* mutant. Cells were grown in DS medium, and samples were taken at the indicated times for determination of β-galactosidase specific activity. Zero time is defined as the time the culture leaves exponential growth. (*A*) *spoIIA-lacZ* expression in strains SIK86 (*cit*$^+$ *spo0A*$^+$ *spoIIA-lacZ*) (○), KI1671 (*citA citZ citC spo0A*$^+$ *spoIIA-lacZ*) (△), and KI1672 (*citA citZ citC sof-1 spoIIA-lacZ*) (▲). (*B*) *spoIIG-lacZ* expression in strains AG1349 (*cit*$^+$ *spo0A*$^+$ *spoIIG-lacZ*) (○), KI1664 (*citA citZ citC spo0A*$^+$ *spoIIG-lacZ*) (△), and KI1670 (*citA citZ citC sof-1 spoIIG-lacZ*) (▲).

level, similar to the pattern seen in *spo0A* or *kinA* mutants (46). Based on these results, it appeared that there was a defect in the production of high levels of Spo0A~*P* in the *citA citZ citC* mutant.

Transcription of *spoVG* and *dpp* is normally induced within the first few minutes after nutrient deprivation. Their induction is dependent on the ability of Spo0A~*P* to interfere with repression by AbrB (9, 11, 12, 45). Expression of *spoVG* and *dpp* was only slightly reduced in the *citA citZ citC* triple mutant (Fig. 1 *C* and *D*). These results indicate that in the *cit* triple mutant, enough Spo0A~*P* is produced to cause at least partial repression of *abrB*, but not enough is produced to activate expression of *spoII* genes.

Suppression of the *cit* Triple Mutant by the *sof-1* Mutation in *spo0A*. Certain mutations in *spo0A* (e.g. *sof-1, rvtA11*) allow the gene product to be phosphorylated by a mechanism that is independent of KinA, Spo0F, and Spo0B (27, 29, 47–51). If the defect in early sporulation gene expression in the *citA citZ citC* triple mutant strain were due to a failure to activate the phosphorelay, this defect would be bypassed in a *sof-1* mutant strain. In fact, the *citA citZ citC sof-1* strain produced 10–50 times more spores than did the otherwise isogenic *citA citZ citC* (*spo0A*$^+$) strain (Table 1). In addition, the *sof-1* mutation restored expression of *spoIIG-lacZ* and *spoIIA-lacZ* in the *citA citZ citC* mutant to or near wild-type levels (Fig. 2). A similar suppressing effect was seen with the *rvtA11* mutation (data not shown). These results indicate that the *citA citZ citC* mutant is unable to express the *spoIIA* and *spoIIG* operons because it cannot activate Spo0A by the normal phosphorylation pathway.

Table 1. The *sof-1* mutation in *spo0A* partially suppresses the sporulation defect of the *citA citZ citC* mutant

Strain	Relevant genotype	Spores per ml
JH642	*cit*$^+$ *spo0A*$^+$	7.3×10^7
AG1470	*citA citZ citC*	1.9×10^5
KI1655	*citA citZ citC spo0A*$^+$ *cat*	2.3×10^5
KI1656	*citA citZ citC sof-1 cat*	3.2×10^6

Cultures were grown in DS medium at 37°C and assayed for spore formation approximately 18 hr after the end of exponential growth. Spores per ml were determined as the total number of colony-forming units after heating samples to 80°C for 10 min. The results of a representative experiment are shown. In multiple trials, strain AG1470 gave 5×10^4 to 3×10^5 spores per ml and an average sporulation frequency of 0.1%; strain KI1656 gave 1×10^6 to 1×10^7 spores per ml and an average sporulation frequency of 15%. All strains are derived from JH642 (*trp phe*; also known as AG174) and contain the *trp* and *phe* mutations. AG1470 (Δ*citA*::*neo* Δ*citZ*::*spc citC7*); KI1655 (Δ*citA*::*neo* Δ*citZ*::*spc citC7 spo0A*$^+$*cat*); KI1656 (Δ*citA*::*neo* Δ*citZ*::*spc citC7 sof-1 cat*). The *sof-1* mutation in *spo0A* was introduced into AG1470 by cotransformation with the chloramphenicol acetyltransferase (*cat*) marker that had been inserted downstream of *spo0A* (33, 52).

***citA citZ citC sof-1* Strain Is Blocked at a Late Stage of Sporulation.** The *citA citZ citC sof-1* strain sporulated more efficiently than did the otherwise isogenic *citA citZ citC* strain but still only formed about 5% as many spores as did the *cit*$^+$ *spo0A*$^+$ wild-type strain (Table 1). To determine the stage at which development is blocked in the majority nonsporulating fraction of the population, we tested expression of a late gene. Expression of *cotA* typically begins approximately 3–4 hr after the initiation of sporulation and depends on stage III and most stage IV genes (44, 53). Accumulation of β-galactosidase specific activity from a *cotA-lacZ* fusion, which was greatly reduced in the *citA citZ citC* triple mutant, was restored to wild-type levels, albeit with a delay in the time of induction, in the otherwise isogenic *citA citZ citC sof-1* mutant (Fig. 3). These results indicate that the block in sporulation is at a late stage, perhaps stage V or later, after expression of *cotA*.

Mechanism of Inhibition of Sporulation by Glucose. The addition of high concentrations of glucose to nutrient broth sporulation medium inhibits spore formation (3). The specific mechanism by which glucose interferes with sporulation is

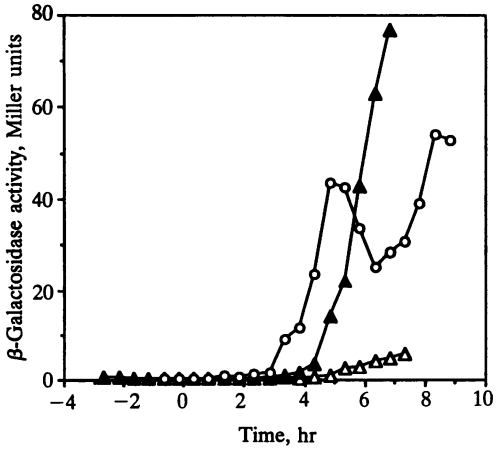

FIG. 3. The *sof-1* mutation in *spo0A* restores expression of *cotA* in the *citA citZ citC* mutant. Cells were grown as described for Fig. 2. ○, KI2055 (*cit*$^+$ *spo0A*$^+$ *cotA-lacZ*); △, KI2053 (*citA citZ citC spo0A*$^+$ *cotA-lacZ*); ▲, KI2054 (*citA citZ citC sof-1 cotA-lacZ*).

unknown, but it involves the phosphorelay since *sof-1, rvtA, coi*, and *sad* mutations in *spo0A* allow cells to sporulate relatively efficiently in the presence of excess glucose (refs. 54 and 55; K.I. and A.D.G., unpublished results). Since transcription from the *citA* and *citZ* promoters is repressed in glucose-containing media (38), it seemed possible that repression of the citrate synthase operons might be the ultimate sporulation-inhibiting activity of glucose. To test this notion, the *citZ* operon was placed under the control of the glucose-insensitive promoter *dppP129* (see *Materials and Methods*). In nutrient broth medium containing 2% glucose, sporulation of the strain containing the *dppP129-citZ* fusion was about 100-fold more efficient than in the otherwise isogenic strain without the fusion (Table 2). These results suggest that the *citZ* promoter is a major target of glucose repression and that an important role of this operon in sporulation is to activate the pathway for phosphorylation of Spo0A.

The *citZ* promoter does not appear to be the only target of the glucose inhibition of sporulation. The sporulation frequency of the strain with *citZ* expressed from the *dppP129* promoter was consistently lower in the presence of glucose (Table 2). One of the other targets of glucose might be the *citC* promoter. *citC* has two modes of transcription; about half of the *citC* transcripts originate at the *citZ* promoter and half at a second promoter located near the 3′ end of *citZ* (38), and both promoters are repressed by rapidly metabolizable carbon sources (38) (S.J., unpublished data). The *dppP129-citZ* fusion used here probably only partly relieves the effect of glucose on transcription of *citC*. In addition, there is probably a target of glucose repression that is independent of the *cit* genes, since addition of glucose to the *citA citZ citC* triple mutant caused a further decrease in sporulation (K.I. and A.D.G., unpublished results).

DISCUSSION

Our results suggest that components of the Krebs citric acid cycle serve two functions during spore formation. One function is to provide energy, reducing power, and intermediates for biosynthesis; the second is to regulate the initiation of sporulation by influencing the production of Spo0A~*P*, the key regulatory protein for sporulation gene expression. Since the *sof-1* and *rvtA11* alleles of *spo0A* allow Spo0A to be phosphorylated in the absence of either the normal phosphorelay or the first three genes of the Krebs cycle, it seems likely that Krebs cycle function determines in some way the activity of the phosphorelay. This conclusion implies that stationary-phase cells respond to a signal (accumulation of an activating metabolite or removal of an inhibitory metabolite or the presence of the enzymes themselves) generated by the Krebs cycle. This signal might indicate nutritional depletion or serve as a checkpoint for development, allowing activation of the Spo0A phosphorelay and transcription of Spo0A-dependent genes only when the cell has the metabolic potential to complete the sporulation cycle. The signal could stimulate autophosphorylation of histidine kinases or phosphotransfer to Spo0F, Spo0B, and Spo0A or could inhibit dephosphorylation of a phosphorelay component.

Table 2. Constitutive expression of *citZCH* from *dppP129* partially relieves the glucose inhibition of sporulation

Strain	Relevant genotype	2% glucose	Spores per ml	Relative spore frequency
JH642	Parental	−	3.3×10^8	1.0
		+	6.0×10^4	2×10^{-4}
SJB93	*dppP129-citZCH*	−	2.9×10^8	0.88
		+	6.2×10^6	2×10^{-2}

Cell were grown in DS medium with (+) or without (−) the addition of glucose to 2% (wt/vol) final concentration. Samples were tested for spore formation approximately 16 hr after the end of exponential growth. The results shown are from a single experiment for each strain. In multiple trials, the presence of the *dppP129-citZCH* fusion increased spore formation by 50- to 3000-fold.

It seems likely that increased transcription of genes for the Krebs cycle enzymes early during sporulation plays an important role in the activation of Spo0A. During rapid exponential growth in a medium rich in carbon-energy sources and biosynthetic precursors, genes for Krebs cycle enzymes are repressed, and by-products of glycolysis are excreted into the medium rather than being fully metabolized. As rapidly metabolizable nutrients become exhausted, the genes for the Krebs cycle enzymes are induced and previously excreted compounds are taken up and metabolized. Almost simultaneously, transcription of several sporulation genes is induced, but mutations that inactivate the Krebs cycle enzymes block induction of early sporulation genes.

Inhibition of transcription of some of the Krebs cycle genes appears to play an important role in glucose repression of sporulation. Glucose is a potent inhibitor of the initiation of sporulation and seems to inhibit production or accumulation of Spo0A~*P* (54, 55) (K.I. and A.D.G., unpublished results). Our results indicate that at least part of the glucose inhibition of sporulation is due to glucose repression of transcription of Krebs cycle genes. Expression of *citZ* and *citC* from a glucose-insensitive promoter allowed significant relief of glucose repression of sporulation. However, the relief was not complete, indicating that the Krebs cycle is probably not the only target of glucose repression. Moreover, the sporulation defect of a *citA citZ citC* mutant is more severe in the presence of glucose (K.I. and A.D.G., unpublished results), again consistent with the notion that there is at least one additional target of glucose repression.

Many mutations have been isolated that cause a catabolite-resistant sporulation (Crs) phenotype (3). Among these are mutations in *pai* (56), *hpr* (57), and *rpoD* (58). While it is not known how these mutations cause the Crs phenotype, we suspect that it is by somehow increasing the amount of Spo0A~*P* produced or perhaps by affecting expression of the *cit* genes or affecting other regulators of the phosphorelay. It has been suggested that the sporulation promoter upstream of *spo0A* might be a target of catabolite repression (59, 60). It is clear that the σ^H-controlled promoter upstream of *spo0A* is necessary for sporulation (22) and is repressed in the presence of glucose (59, 60). However, since this promoter, P_s, requires Spo0A~*P* for expression (61), it seems most likely that P_s is not a direct target of glucose repression. Rather, expression from P_s is a reflection of the phosphorelay and the amount of Spo0A~*P*.

The *sof-1* mutation restored early sporulation gene expression to a *citA citZ citC* mutant, but the majority of the cells still failed to form spores and were blocked late in development. This late block probably reflects the metabolic role of the Krebs cycle in sporulation and is similar to the effects of a mutation in *ald* (*spoVN*), the gene for alanine dehydrogenase (62). Alanine dehydrogenase also seems to play a role in providing energy for late sporulation events, and *ald* mutants are blocked at a late stage in sporulation (62).

Phosphorylation of Spo0A is coupled not only to Krebs cycle function and availability of nutrients but also to DNA replication (33), the absence of DNA damage (34), high cell density (32), and the presence of an intact chromosome partitioning machinery (35). Integration of these multiple conditions or signals through the phosphorelay may ensure that sporulation initiates only when food sources are limited and the entire process of spore formation can be completed successfully. It would not be surprising if other developmental systems that require energy production and that are subject to regulation by nutrient availability also have regulatory mechanisms for coupling differentiation to metabolic function. For instance,

normal embryonic development of *Caenorhabditis elegans* requires the presence of nutrients. Starvation and crowding can cause arrest of normal development and activation of an alternative regulatory pathway that leads to the development of the less active, more resistant dauer larva. Addition of nutrients to the dauer larvae allows resumption and completion of normal development (63). It is likely that many developmental events are controlled by switches and checkpoint mechanisms that couple gene expression to metabolic sufficiency.

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1. Schaeffer, P., Millet, J. & Aubert, J. (1965) *Proc. Natl. Acad. Sci. USA* **54,** 704–711.
2. Dawes, I. W. & Mandelstam, J. (1970) *J. Bacteriol.* **103,** 529–535.
3. Takahashi, I. (1979) *Can. J. Microbiol.* **25,** 1283–1287.
4. Hederstedt, L. (1993) in *Bacillus subtilis and Other Gram-Positive Bacteria: Biochemistry, Physiology and Molecular Genetics*, eds. Sonenshein, A. L., Hoch, J. A. & Losick, R. (Am. Soc. Microbiol., Washington, DC), pp. 181–197.
5. Fortnagel, P. & Freese, E. (1968) *J. Bacteriol.* **95,** 1431–1438.
6. Freese, E. B., Vasantha, N. & Freese, E. (1979) *Mol. Gen. Genet.* **170,** 67–74.
7. Yousten, A. A. & Hanson, R. S. (1972) *J. Bacteriol.* **109,** 886–894.
8. Jin, S. & Sonenshein, A. L. (1994) *J. Bacteriol.* **176,** 4669–4679.
9. Strauch, M. A., Webb, V., Spiegelman, G. & Hoch, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1801–1805.
10. Weir, J., Predich, M., Dubnau, E., Nair, G. & Smith, I. (1991) *J. Bacteriol.* **173,** 521–529.
11. Robertson, J. B., Gocht, M., Marahiel, M. A. & Zuber, P. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8457–8461.
12. Zuber, P. & Losick, R. (1987) *J. Bacteriol.* **169,** 2223–2230.
13. Strauch, M. A., Spiegelman, G. B., Perego, M., Johnson, W. C., Burbulys, D. & Hoch, J. A. (1989) *EMBO J.* **8,** 1615–1621.
14. Baldus, J. M., Green, B. D., Youngman, P. & Moran, C. P., Jr. (1994) *J. Bacteriol.* **176,** 296–306.
15. Bird, T. H., Grimsley, J. K., Hoch, J. A. & Spiegelman, G. B. (1993) *Mol. Microbiol.* **9,** 741–749.
16. Satola, S., Kirchman, P. A. & Moran, C. P., Jr. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4533–4537.
17. Satola, S. W., Baldus, J. M. & Moran, C. P., Jr. (1992) *J. Bacteriol.* **174,** 1448–1453.
18. Trach, K., Burbulys, D., Strauch, M., Wu, J.-J., Dhillon, N., Jonas, R., Hanstein, C., Kallio, C., Perego, M., Bird, T., Spiegelman, G., Fogher, C. & Hoch, J. A. (1991) *Res. Microbiol.* **142,** 815–823.
19. York, K., Kenney, T. J., Satola, S., Moran, C. P., Jr., Poth, H. & Youngman, P. (1992) *J. Bacteriol.* **174,** 2648–2658.
20. Losick, R. & Stragier, P. (1992) *Nature (London)* **355,** 601–604.
21. Mueller, J. P., Bukusoglu, G. & Sonenshein, A. L. (1992) *J. Bacteriol.* **174,** 4361–4373.
22. Siranosian, K. J. & Grossman, A. D. (1994) *J. Bacteriol.* **176,** 3812–3815.
23. Antoniewski, C., Savelli, B. & Stragier, P. (1990) *J. Bacteriol.* **172,** 86–93.
24. Trach, K. A. & Hoch, J. A. (1993) *Mol. Microbiol.* **8,** 69–79.
25. Perego, M., Cole, S. P., Burbulys, D., Trach, K. & Hoch, J. A. (1989) *J. Bacteriol.* **171,** 6187–6196.
26. Chung, J. D., Stephanopoulos, G., Ireton, K. & Grossman, A. D. (1994) *J. Bacteriol.* **176,** 1977–1984.
27. Kobayashi, K., Shoji, K., Shimizu, T., Nakano, K., Sato, T. & Kobayashi, Y. (1995) *J. Bacteriol.* **177,** 176–182.
28. LeDeaux, J., Yu, N. & Grossman, A. D. (1995) *J. Bacteriol.* **177,** 861–863.
29. LeDeaux, J. R. & Grossman, A. D. (1995) *J. Bacteriol.* **177,** 166–175.
30. Burbulys, D., Trach, K. A. & Hoch, J. A. (1991) *Cell* **64,** 545–552.
31. Hoch, J. A. (1993) *Annu. Rev. Microbiol.* **47,** 441–465.
32. Ireton, K., Rudner, D. Z., Siranosian, K. J. & Grossman, A. D. (1993) *Genes Dev.* **7,** 283–294.
33. Ireton, K. & Grossman, A. D. (1994) *EMBO J.* **13,** 1566–1573.
34. Ireton, K. & Grossman, A. D. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8808–8812.
35. Ireton, K., Gunther, N. W., IV, & Grossman, A. D. (1994) *J. Bacteriol.* **176,** 5320–5329.
36. Perego, M., Spiegelman, G. B. & Hoch, J. A. (1988) *Mol. Microbiol.* **2,** 689–699.
37. Fouet, A. & Sonenshein, A. L. (1990) *J. Bacteriol.* **172,** 835–844.
38. Jin, S. & Sonenshein, A. L. (1994) *J. Bacteriol.* **176,** 4680–4690.
39. Slack, F. J., Mueller, J. P. & Sonenshein, A. L. (1993) *J. Bacteriol.* **175,** 4605–4614.
40. Jaacks, K. J., Healy, J., Losick, R. & Grossman, A. D. (1989) *J. Bacteriol.* **171,** 4121–4129.
41. Zuber, P. & Losick, R. (1983) *Cell* **35,** 275–283.
42. Kenney, T. J., York, K., Youngman, P. & Moran, C. P., Jr. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9109–9113.
43. Wu, J. J., Piggot, P. J., Tatti, K. M. & Moran, C. P., Jr. (1991) *Gene* **101,** 113–116.
44. Cutting, S., Oke, V., Driks, A., Losick, R., Lu, S. & Kroos, L. (1990) *Cell* **62,** 239–250.
45. Slack, F. J., Mueller, J. P., Strauch, M. A., Mathiopoulos, C. & Sonenshein, A. L. (1991) *Mol. Microbiol.* **5,** 1915–1925.
46. Mueller, J. P. & Sonenshein, A. L. (1992) *J. Bacteriol.* **174,** 4374–4383.
47. Hoch, J. A., Trach, K., Kawamura, F. & Saito, H. (1985) *J. Bacteriol.* **161,** 552–555.
48. Kawamura, F. & Saito, H. (1983) *Mol. Gen. Genet.* **192,** 330–334.
49. Sharrock, R. A., Rubenstein, S., Chan, M. & Leighton, T. (1984) *Mol. Gen. Genet.* **194,** 260–264.
50. Shoji, K., Hiratsuka, S., Kawamura, F. & Kobayashi, Y. (1988) *J. Gen. Microbiol.* **134,** 3249–3257.
51. Spiegelman, G., Van Hoy, B., Perego, M., Day, J., Trach, K. & Hoch, J. A. (1990) *J. Bacteriol.* **172,** 5011–5019.
52. Grossman, A. D., Lewis, T., Levin, N. & DeVivo, R. (1992) *Biochimie* **74,** 679–688.
53. Sandman, K., Kroos, L., Cutting, S., Youngman, P. & Losick, R. (1988) *J. Mol. Biol.* **200,** 461–473.
54. Olmedo, G., Ninfa, E. G., Stock, J. & Youngman, P. (1990) *J. Mol. Biol.* **215,** 359–372.
55. Doi, R. H., Gitt, M., Wang, L., Price, C. W. & Kawamura, F. (1985) in *Molecular Biology of Microbial Differentiation*, eds. Hoch, J. A. & Setlow, P. (Am. Soc. Microbiol., Washington, DC), pp. 157–161.
56. Honjo, M., Nakayama, A., Fukazawa, K., Kawamura, K., Ando, K., Hori, M. & Furutani, Y. (1990) *J. Bacteriol.* **172,** 1783–1790.
57. Perego, M. & Hoch, J. A. (1988) *J. Bacteriol.* **170,** 2560–2567.
58. Kawamura, F., Wang, L. & Doi, R. H. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8124–8128.
59. Chibazakura, T., Kawamura, F. & Takahashi, H. (1991) *J. Bacteriol.* **173,** 2625–2632.
60. Yamashita, S., Kawamura, F., Yoshikawa, H., Takahashi, H., Kobayashi, Y. & Saito, H. (1989) *J. Gen. Microbiol.* **135,** 1335–1345.
61. Strauch, M. A., Trach, K. A., Day, J. & Hoch, J. A. (1992) *Biochimie* **74,** 619–626.
62. Siranosian, K. J., Ireton, K. & Grossman, A. D. (1993) *J. Bacteriol.* **175,** 6789–6796.
63. Riddle, D. (1988) in *The Nematode Caenorhabditis elegans*, eds. Wood, W. B. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 393–412.