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Signal Transduction Pathway Controlling Synthesis of a Class of Degradative Enzymes in *Bacillus subtilis*: Expression of the Regulatory Genes and Analysis of Mutations in *degS* and *degU*

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The rates of synthesis of a class of both secreted and intracellular degradative enzymes in *Bacillus subtilis* are controlled by a signal transduction pathway defined by at least four regulatory genes: *degS*, *degU*, *degQ* (formerly *sacQ*), and *degR* (formerly *prtR*). The DegS-DegU proteins show amino acid similarities with two-component procaryotic modulator-effector pairs such as NtrB-NtrC, CheA-CheY, and EnvZ-OmpR. By analogy with these systems, it is possible that DegS is a protein kinase which could catalyze the transfer of a phosphoryl moiety to DegU, which acts as a positive regulator. DegR and DegQ correspond to polypeptides of 60 and 46 amino acids, respectively, which also activate the synthesis of degradative enzymes. We show that the *degS* and *degU* genes are organized in an operon. The putative σ^A promoter of the operon was mapped upstream from *degS*. Mutations in *degS* and *degU* were characterized at the molecular level, and their effects on transformability and cell motility were studied. The expression of *degQ* was shown to be subject both to catabolite repression and DegS-DegU-mediated control, allowing an increase in the rate of synthesis of *degQ* under conditions of nitrogen starvation. These results are consistent with the hypothesis that this control system responds to an environmental signal such as limitations of nitrogen, carbon, or phosphate sources.

To adapt to changes in the environment, procaryotes possess a wide variety of signal transduction pathways mediating gene expression. Most of these signaling systems have been described as two-component systems, since they depend upon the interaction of two regulatory proteins (42, 46). One acts as a transcriptional regulator, called an effector, and the second modifies the activity of the effector and is therefore called a modulator. Typically, the modulator has two different functional domains (25). One of these, a transmembrane segment located at its N-terminal part, may be involved in the reception of an extracellular signal. This signal is then transduced via a second domain located at the C-terminal part of the modulator, the transmitter.

The transmitter domains of different modulators present amino acid sequence similarities. This domain interacts with the receiver of the cognate effector, defined as a conserved amino acid sequence domain located at the N-terminal part of the effector. The transmitter-receiver interaction may involve phosphorylation of the effector. Indeed, four modulators, CheA, NtrB, EnvZ, and PhoR, have been identified as protein kinases which are autophosphorylated at a histidine residue and, in turn, phosphorylate their cognate effectors at an aspartate residue (20, 21, 23, 41, 43, 55, 60, 61). These His and Asp residues are conserved in all members of these modulator-effector systems, including DegS and DegU of *Bacillus subtilis* (19, 26, 43, 53), which suggests that the DegS protein may modify the DegU protein through a similar mechanism (GenBank DNA sequence accession no. M23649). Two of these well-studied signaling pairs, CheA-CheY of *Salmonella typhimurium* and *Escherichia coli* and NtrB-NtrC of *E. coli*, are somewhat unusual. The CheA and NtrB modulators do not have transmembrane N-terminal domains allowing reception of extracellular signals, but, instead, have receiver domains allowing interaction with additional signal transduction proteins. In this way, the NtrB protein kinase receives information about ammonia availability via two signal transduction proteins from the sensor glutamine synthetase (31).

The DegS-DegU signaling system in *Bacillus subtilis* is involved in the control of the rates of synthesis of degradative enzymes, including an intracellular protease and several secreted enzymes: levansucrase, proteases, α-amylase, β-glucanase(s), and xylanase (2, 5, 17, 27, 47). It also affects transformation by exogenous DNA, presence of flagella and sporulation efficiency in the presence of glucose (5, 27). Some features of DegS-DegU are reminiscent of NtrB-NtrC. First, the DegS and DegU proteins contain, respectively, transmitter and receiver domains (19, 26, 43, 53), but the DegS modulator does not present any obvious transmembrane segment (26). Second, the DegS and DegU genes are organized in an operon, as will be shown in this paper. Additional genes also seem to be part of this signal transduction pathway. The presence of the *degQ* (*sacQ*) or *degR* (*prtR*) gene on high-copy-number plasmids also leads to an increased production of the degradative enzymes mentioned above (2, 39, 58, 62, 63). Since this process requires functional *degS* and *degU* genes (2, 57; data not shown), it is likely that the small polypeptides encoded by *degQ* and *degR* belong to the same signal transduction pathway as the DegS and DegU proteins.

The *degR* and *degQ* genes are dispensable to degradative enzyme production, since their deletion does not lead to any recognizable phenotype (62, 63). On the contrary, the *degS* and *degU* genes are essential, since mutations in both these genes have been identified which lead to deficiency of

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degradative enzyme synthesis. Another class of *degS* and *degU* mutations, designated *degS*(Hy) and *degU*(Hy), was identified by the overproduction of degradative enzymes (Hy phenotype) compared with the reference strain *Bacillus subtilis* 168. Several *degS*(Hy) and *degU*(Hy) mutations have been characterized at the molecular level (19), but none of the *degS*- or *degU*-deficient mutations have been characterized. In this paper, we present the identification and analysis at the molecular level of additional *degU*(Hy) mutations as well as that of *degS*- and *degU*-deficient mutations. We also characterized the following features of this control system: (i) the *degS-degU* operon is transcribed from a σ^A promoter; (ii) the DegS-DegU regulatory pair affects the expression of the *degQ* gene; and (iii) this regulatory system is probably involved in the response of the cell to limitations in nitrogen, carbon, or phosphate sources.

MATERIALS AND METHODS

Strains. *B. subtilis* strains used in this study are listed in Table 1. *E. coli* K-12 strain TG1 [(Δ*lac-proAB*) *supE thi hsdD5* F′ *traD36 proA*$^+$ *proB*$^+$ *lacI*^{q} *lacZ* ΔM15] (T. J. Gibson, Ph.D. thesis, University of Cambridge, Cambridge, 1984) or SR101, a *tonA* derivative of strain JM101 (64), was used for plasmid constructions and as the host for M13 bacteriophages. *E. coli* was transformed as previously described (7) with selection on LB plates supplemented with ampicillin plus chloramphenicol (50 and 2.5 μg/ml, respectively) or ampicillin plus kanamycin (100 and 5 μg/ml, respectively). Transformation of *B. subtilis* was as previously described (3, 26), and selection was carried out on SP or tryptose blood agar base containing chloramphenicol (5 μg/ml), kanamycin (5 μg/ml), or erythromycin plus lincomycin (1 and 25 μg/ml, respectively) (26).

Media. *E. coli* was grown in LB broth, and *B. subtilis* was grown in Penassay antibiotic medium 3 (Difco Laboratories, Detroit, Mich.) or C medium (70 mM K_2HPO_4, 30 mM KH_2PO_4, 25 mM $(NH_4)_2SO_4$, 0.5 mM $MgSO_4$, 0.01 mM $MnSO_4$, 22 mg of ferric ammonium citrate per liter, 100 mg of auxotrophic requirements per liter) supplemented with the following nutrients: 20 mM potassium succinate and 50 mM potassium glutamate (CSE medium), 2% glucose and 50 mM potassium glutamate (CGE medium), or 2% glucose and 0.5% casein hydrolysate (CGCH medium). Nitrogen starvation was achieved by suspending exponentially growing *B. subtilis* cells in glucose phosphate medium (GP) (2% glucose, 30 mM KH_2PO_4, 70 mM K_2HPO_4, 0.5 mM $MgSO_4$, 0.01 mM $MnSO_4$, 22 mg of ferric ammonium citrate per liter, 100 mg of auxotrophic requirements per liter). Levansucrase production was detected on sucrose-tryptone plates (26). Overproduction of proteases by *degS*(Hy) and *degU*(Hy) mutants was detected on plates containing 2% skim milk, 60 mM K_2HPO_4, 44 mM KH_2PO_4, 3 mM trisodium citrate, 2 mM $MgSO_4$, 0.01 mM $MnCl_2$, 0.5 mM $CaCl_2$, 22 mg of ferric ammonium citrate per liter, and 100 mg of auxotrophic requirements per liter.

Plasmids and plasmid construction. Plasmids pBQ1, pBU100, and pBU101 are briefly described in Table 1. Plasmid pIS112 was a gift from I. Smith and is a vector allowing the construction of translational fusions with codon 8 of β-galactosidase (29). Plasmid pAF1, a derivative of ptrpBG1 (51), was a gift from A. Fouet and carries the pC194 chloramphenicol resistance determinant and a promoterless *lacZ* gene between two fragments of the *B. subtilis amyE* gene, allowing direct selection of single-copy integration of β-galactosidase fusions at the *B. subtilis amyE* locus.

TABLE 1. *B. subtilis* strains and parental plasmids used in this study

Strain or plasmid	Genotype or description[a]	Source or reference
Strains		
168	*trpC2*	Laboratory stock
BG4065	*trpC2* Δ*degQ*::*cat*	62
QB127	*trpC2 leuA8 degS200*(Hy)	27
QB136	*trpC2 leuA8 degU32*(Hy)	27
QB152	*trpC2 leuA8 degU31*(Hy)	Laboratory stock
QB157	*trpC2 leuA8 degS100*(Hy)	Laboratory stock
QB254	*trpC2 hisA1 sacA321 degS42*	28
QB256	*trpC2 hisA1 sacA321 degU122*	28
QB257	*trpC2 hisA1 sacA321 degS39*	Laboratory stock
QB260	*sacA331 degU32*(Hy) *degU143*	27
QB261	*sacA331 degU32*(Hy) *degU146*	Laboratory stock
QB264	*sacA331 degU32*(Hy) *degU193*	Laboratory stock
QB266	*sacA331 degU32*(Hy) *degU200*	27
QB269	*sacA331 degU32*(Hy) *degS220*	27
QB315	*trpC2 leuA8 degU24*(Hy)	28
QB323	*trpC2 leuA8 degU500*(Hy)	28
QB4210	*trpC2 degU*::*lacZ erm*	26
QB4222	*trpC2 degS*::*aphA3*	26
QB4238	*trpC2* Δ(*degS degU*)::*aphA3*	pBU106[b]→168
QB4255	*trpC2 amyE*::(*degQ*′-′*lacZ cat*)	pBQ106[b]→168
QB4256	*trpC2 amyE*::(*degS degU*′-′*lacZ cat*)	pBU114[b]→168
QB4257	*trpC2 hisA1 sacA321 degS42 amyE*::(*degS degU*′-′*lacZ cat*)	pBU114[b]→QB254
QB4258	*trpC2 amyE*::(*degSΔA degU*′-′*lacZ cat*)	pBU116[b]→168
QB4260	*trpC2 amyE*::(*degQ*′-′*lacZ cat*) Δ(*degS degU*)::*aphA3*	QB4238→QB4255
QB4261	*trpC2 leuA8 amyE*::(*degQ*′-′*lacZ cat*) *degU32*(Hy)	QB4255→QB136
QB4262	*trpC2 amyE*::(*degSΔC degU*′-′*lacZ cat*)	pBU118[b]→168
QB4263	*trpC2 amyE*::(*degSΔB degU*′-′*lacZ cat*)	pBU119[b]→168
QB4267	*trpC2 amyE*::(*degSΔD degU*′-′*lacZ cat*)	pBU122[b]→168
QB4268	*sacA331 degU32*(Hy) *degS220 amyE*::(*degS degU*′-′*lacZ cat*)	QB4256→QB269
QB4274	*trpC2* Δ*degQ*::*cat amyE*::(*degS degU*′-′*lacZ cat*::*aphA3*)	pBU124[b]→BG4065
Plasmids		
pBQ1	3-kb fragment[c] carrying the *degQ* gene	2
pBU100	2.4-kb *Eco*RI fragment carrying the *degS* gene and the first 139 codons of the *degU* gene	26
pBU101	1.2-kb *Bam*HI-*Sal*I fragment composed of two subfragments from the *degS-degU* region: a 510-bp *Bam*HI-*Eco*RI fragment upstream from *degS-degU* and a 690-bp *Eco*RI-*Sal*I fragment containing the last 90 codons of the *degU* gene	26

[a] *cat* indicates the pC194 chloramphenicol acetyltransferase gene; *erm* indicates the Tn*917* erythromycin resistance gene; *aphA3* indicates the *Streptococcus faecalis* kanamycin resistance gene. Plasmid descriptions show the *B. subtilis* chromosomal DNA insert.

[b] Plasmids described in the text. Arrows (→) indicate construction by transformation.

[c] Obtained from a chromosomal DNA *Sau*3AI partial digest.

Translational gene fusions of the amino-terminal regions of *degU* or *degQ* to codon 8 of *lacZ* were constructed by using the pIS112 plasmid vector and integrated as single copies at the *amyE* locus in the following way. The fusions were transferred from the pIS112 derivatives to plasmid pAF1 by using unique *Eco*RI and *Sac*I restriction sites, located, respectively, upstream of and within the *lacZ* gene, replacing the promoterless *lacZ* gene with the translational fusions. The constructed plasmids were linearized by using unique *Pst*I or *Sca*I sites, and the translational fusions were integrated into the chromosome by homologous recombination at the *amyE* locus by using chloramphenicol selection.

A *degU'-'lacZ* translational gene fusion was constructed by cloning a 2,137-base-pair (bp) *Eco*RI-*Eco*RV fragment from pBU100, carrying regions upstream of *degS*, the *degS* gene, and the first 56 codons of *degU*, between the *Eco*RI and *Sma*I sites of pIS112, thus fusing codon 56 of *degU* to codon 8 of *lacZ* to produce plasmid pBU113. Transfer of this fusion to plasmid pAF1 gave plasmid pBU114. Plasmids pBU116, pBU118, pBU119, and pBU122 were derivatives of pBU114, deleted for different regions upstream of the *degU'-'lacZ* fusion, and are described below.

A *degQ'-'lacZ* fusion was constructed by using plasmid pBQ1 as previously described for plasmid pNPRS30 (62), fusing codon 33 of *degQ* to codon 8 of the *lacZ* gene. As above, the *lacZ* fusion was transferred to the pAF1 vector to give plasmid pBQ106.

Plasmid pBU106 was constructed by replacing the 544-bp *Eco*RI-*Sac*I fragment of pBU101 with a 1.5-kbp *Cla*I fragment carrying the kanamycin resistance determinant *aphA3* from *Streptococcus faecalis* (59) and used for the deletion of the *degS-degU* region of the chromosome. After transformation of *B. subtilis* 168, spontaneous Km^r Cm^s integrants arose through a double-crossover event, replacing the *degS-degU* region from the *Eco*RI site upstream of *degS* to the *Sac*I site downstream of *degU* with the kanamycin resistance determinant in the constructed strain, QB4238.

Plasmid pBU124 is a derivative of plasmid pBU114, carrying a *degU'-'lacZ* translational fusion between two fragments of the *amyE* gene, and was constructed by disrupting the pC194 *cat* gene through insertion of the *aphA3* kanamycin resistance determinant at the *Stu*I unique restriction site.

DNA manipulations. Standard procedures were used for extracting plasmids from *E. coli* and *B. subtilis* (2, 32). Restriction enzymes, T4 DNA polymerase, avian myeloblastosis virus reverse transcriptase, the Klenow fragment of DNA polymerase I, and T4 DNA ligase were used as recommended by the manufacturers. When necessary, 5′ and 3′ protruding ends of DNA fragments were repaired to flush ends by using Klenow DNA polymerase, T4 DNA polymerase, and deoxynucleoside triphosphates. DNA fragments were purified either from agarose gels by using a Gene Clean kit (Bio101, La Jolla, Calif.) or from polyacrylamide gels by electroelution.

Single-stranded M13 phages (34) were used to generate templates for DNA sequencing, which was carried out by using the dideoxy-chain termination method (50) and modified T7 DNA polymerase (56) (U.S. Biochemical Corp., Cleveland, Ohio).

For chromosomal DNA isolation, 50 ml of exponentially growing cells was centrifuged, suspended in 2 ml of TEN buffer (0.1 M Tris hydrochloride [pH 8], 0.1 M EDTA, 0.15 M NaCl), and treated with lysozyme (2 mg/ml) for 10 min at 37°C and RNase (50 μg/ml) for 15 min at 50°C, and then sodium dodecyl sulfate (1%) and proteinase K (100 μg/ml) were added and the mixture was incubated at 65°C for 3 h. Chromosomal DNA was then prepared as previously described (49).

Polymerase chain reactions (38, 48) were carried out by using thermostable *Thermus aquaticus* DNA polymerase as recommended by New England BioLabs, Inc., Beverly, Mass. Oligonucleotide primers were 25 to 33 nucleotides in length and included mismatches from the native sequence, allowing the creation of *Bam*HI or *Hin*dIII restriction sites.

After an initial denaturation step of 15 min at 95°C, amplification was carried out for 25 rounds. The DNA was denatured at 95°C for 1 min, annealed at 50°C for 1 min, and extended at 72°C. The extension period was 4 min for the first 10 cycles and 6 min for the last 15.

Samples were successively extracted with phenol and chloroform, ethanol precipitated, digested with appropriate restriction enzymes, and gel purified before being cloned into M13. Since DNA amplification by this method consistently led to misincorporation errors (0.1% under our conditions), sequencing was carried out by using either pooled templates or two separate templates for each mutant strain to differentiate nucleotide changes in the input DNA from errors during amplification.

β-Galactosidase assays. *B. subtilis* cells containing *lacZ* fusions were grown in the indicated media, harvested by centrifugation for 2 min in an Eppendorf microcentrifuge, suspended in Z buffer (35), and treated with lysozyme and DNase. Cell debris were eliminated by centrifugation, and β-galactosidase specific activity was determined as previously described (35, 44) and expressed in Miller units per milligram of protein. Protein concentrations were determined by using a protein assay (Bio-Rad Laboratories, Richmond, Calif.). Assays for each culture were carried out in triplicate.

B. subtilis colonies containing *lacZ* fusions were detected by overlaying colonies with 8 ml of soft agar (7.5 mg/ml) containing lysozyme (2 mg/ml) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (250 μg/ml).

Reverse transcriptase mapping of the mRNA start point. Total RNA was isolated from exponentially growing *B. subtilis* cells as previously described (15, 33). A recombinant M13mp9 bacteriophage, containing the 343-bp *Sph*I-*Pvu*II fragment of pBU100, including the amino-terminal region of *degS*, was used for the synthesis of a ^{32}P-labeled DNA primer. Single-stranded DNA (1 μg) was hybridized with the 17-mer universal sequencing primer and incubated for 20 min with 1.48 MBq of [α-^{32}P]dATP (110 TBq/mmol) in the presence of dGTP, dCTP, and dTTP (2 mM each) and 5 U of the Klenow fragment of DNA polymerase. Cold dATP (2 mM) was then added, and the reaction was incubated for another 20 min. The DNA was then digested with *Bst*XI, and the radioactive single-stranded *Bst*XI-*Pvu*II primer was purified by denaturing polyacrylamide gel electrophoresis (6% acrylamide). Primer extension and product analysis were performed as previously described (9).

RESULTS

Nature of the *degS*(Hy), *degU*(Hy), *degS*, and *degU* mutations. The experimental approach to the identification of mutations was similar to that previously described (19). Total chromosomal DNA from *B. subtilis* containing a mutation in the *degS-degU* DNA region was used as a template for amplification by using the polymerase chain reaction (see Materials and Methods). Restriction sites introduced during amplification were used to clone fragments into phage M13 replicative form for sequence analysis. All of

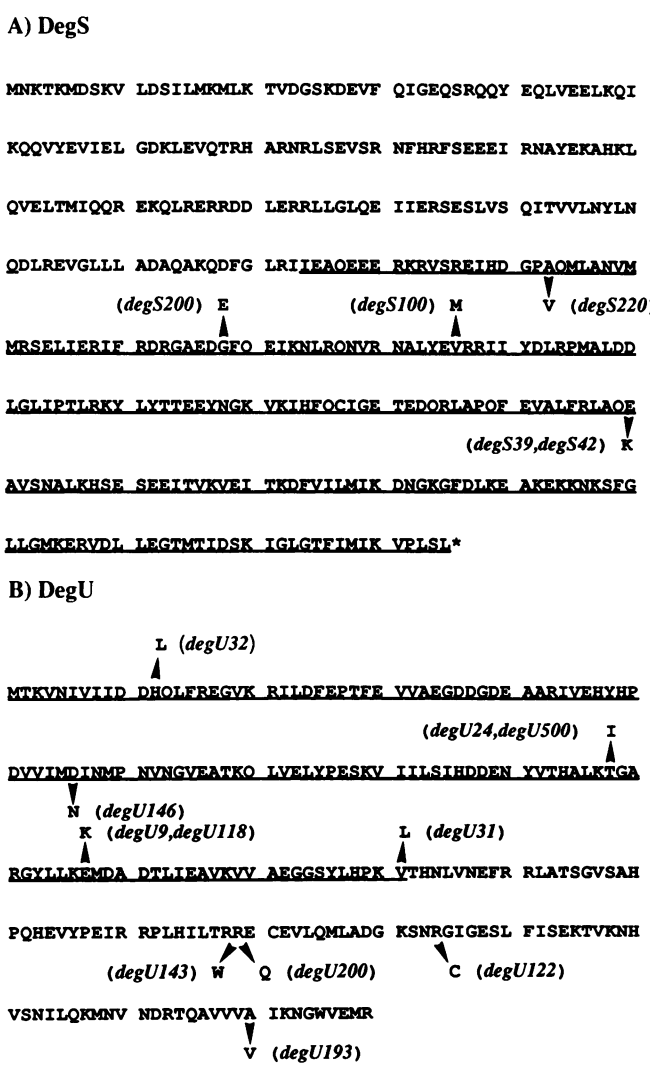

FIG. 1. Primary structure modifications and associated phenotypes of mutational changes in the DegS modulator (A) and the DegU effector (B). Mutant alleles and corresponding phenotypes, i.e., hyperproduction (▲) or deficiency (▼) of degradative enzyme synthesis, are indicated. The *degS100*, *degS200*, *degU9*, *degU32*, and *degU118* mutations are from reference 19. The transmitter domain of the DegS modulator and the receiver domain of the DegU effector are underlined.

the mutations identified were missense mutations. Nucleotide and amino acid residue changes are indicated in Fig. 1 and Table 2. The sequence of the entire DNA region spanning the *degS* and *degU* coding sequences was determined in each case, and no other nucleotide changes were found.

Modifications could be identified in the DegU protein at the N-terminal receiver domain, which is common to all effectors. The *degU*(Hy) mutations led to a pleiotropic phenotype including hyperproduction of degradative enzymes, deficiency in transformation, absence of flagella and capacity to sporulate in the presence of glucose. All the identified *degU*(Hy) mutations corresponded to modifications in the N-terminal receiver domain (Fig. 1; Table 2). Four of these mutations, *degU24*, *degU500*, *degU9*, and *degU118*, led to modifications in a subdomain which was proposed by Tanaka and Kawata (57) to be involved in DNA binding. Mutagenesis of a *degU32*(Hy) strain led to the isolation of mutants deficient in degradative enzyme synthesis (27, 28). Deficient mutations affecting either *degS* or *degU* were identified in these strains, which all retained the previously characterized *degU32*(Hy) mutation (Fig. 1) as verified by sequence analysis. One of these strains (QB261), containing two mutations in *degU*, *degU32*(Hy), and *degU146*, presented a complete reversion of the pleiotropic phenotype of *degU32*(Hy): degradative enzyme production was deficient, transformability was restored to the wild-type level (Table 3), and the strain was normally motile. The *degU146* mutation, an Asp-56-to-Asn change, was the only deficient mutation identified modifying the N-terminal receiver domain. We propose (see the Discussion) that this mutation inactivates the potential site of phosphorylation, locking the DegU effector in its nonphosphorylated state.

It has been pointed out that a subfamily of effectors exists, which includes DegU, FixJ, NarL, UhpA, PhoB, OmpR, and SfrA (8, 43, 53). In this subfamily, which we shall call the FixJ subfamily, amino acid similarities are not restricted to the N-terminal receiver domain, but extend instead over the entire lengths of the effectors. We also found mutations modifying the C-terminal domain characteristic of the FixJ subfamily (Fig. 1; Table 2). The *degU143*, *degU200*,

TABLE 2. Mutational changes in DegS and DegU

Strain	Relevant genotype	Codon change and nucleotide sequence position[a]	Amino acid change
QB315	*degU24*(Hy)	ACA→ATA (1752)	Thr-98→Ile
QB323	*degU500*(Hy)	ACA→ATA (1752)	Thr-98→Ile
QB152	*degU31*(Hy)	GTT→CTT (1850)	Val-131→Leu
QB254	*degS42*	GAA→AAA (1117)	Glu-300→Lys
QB257	*degS39*	GAA→AAA (1117)	Glu-300→Lys
QB256	*degU122*	CGC→TGC (2009)	Arg-184→Cys
QB260[b]	*degU143*	CGG→TGG (1964)	Arg-169→Trp
QB261[b]	*degU146*	GAT→AAT (1625)	Asp-56→Asn
QB264[b]	*degU193*	GCC→GTC (2118)	Ala-220→Val
QB266[b]	*degU200*	CGG→CAG (1965)	Arg-169→Gln
QB269[b]	*degS220*	GCT→GTT (797)	Ala-193→Val

[a] Nucleotide sequence positions are from reference 26.
[b] Strains QB260, QB261, QB264, QB266, and QB269 each carry the *degU32* (Hy) mutation, a His-to-Leu change at position 12 of DegU (19).

TABLE 3. Characterization of mutations in *degS* and *degU*

Strain	Relevant genotype	Relevant phenotype	
		Degradative enzyme production	Transformation frequency[a]
168		Wild type	1.0
QB127	*degS200*(Hy)	Hy	$<10^{-3}$
QB136	*degU32*(Hy)	Hy	1.4×10^{-3}
QB152	*degU31*(Hy)	Hy	1.6×10^{-3}
QB157	*degS100*(Hy)	Hy	8×10^{-2}
QB254	*degS42*	Deficient	1.0
QB256	*degU122*	Deficient	1.6
QB260	*degU32*(Hy) *degU143*	Deficient	$<10^{-3}$
QB261	*degU32*(Hy) *degU146*	Deficient	1.3
QB264	*degU32*(Hy) *degU193*	Deficient	$<10^{-3}$
QB266	*degU32*(Hy) *degU200*	Deficient	3×10^{-3}
QB269	*degU32*(Hy) *degS220*	Deficient	3.3
QB315	*degU24*(Hy)	Hy	5×10^{-3}

[a] The transformation frequency is expressed relative to that of the wild-type strain 168, which gave 4×10^{-6} transformants per recipient cell, and was determined with chromosomal DNA from strain QB4238 at 2 μg/ml, with selection for kanamycin resistance.

degU122, and *degU193* mutations led to a deficiency of degradative enzyme synthesis. The *degU143* and *degU200* mutations led to the replacement of an arginine residue, which is also found at equivalent positions in three other effectors (NarL, UhpA, FixJ), by either tryptophan or glutamine, which are not found at these positions in any effector of the FixJ subfamily (43, 53). The *degU122* mutation led to the change of an arginine residue to a cysteine residue at a position where three other effectors, NarL, UhpA, and FixJ, also possess a basic residue (lysine). This modification is located in a subdomain which may be an alternative possibility for DNA binding (see the Discussion). The *degU193* mutation led to the modification of a position which does not seem to be strongly conserved in the effector family.

The DegU effector is not only required for degradative enzyme synthesis, but is also essential to transformability (57). The *degU* mutations modifying the C-terminal portion did not alter the transformation frequency (Table 3). Indeed, the *degU122* mutant, deficient in degradative enzyme production, retained wild-type transformability. When a second mutation was present in *degU32*(Hy)-bearing strains, competence was still low if the suppressor mutation was in the C-terminal part of DegU (Table 3, strains QB260, QB264, and QB266). In the one case in which the second mutation was in the N-terminal region (*degU146*), competence was restored.

As was previously shown for the effectors, it may be possible that the modulators are also distributed into subfamilies. We found, for instance, that the FixL and DegS modulators present amino acid sequence similarities extending well beyond the characteristic C-terminal transmitter domain (Fig. 2). In the DegS modulator, however, we identified only mutations leading to modifications in the C-terminal transmitter domain. The identification of the *degS220* mutation in strain QB269, which suppressed the hyperproduction (Hy) phenotype of *degU32* and restored transformability (Table 3) and motility (data not shown), seems to indicate that the *degU32*-encoded effector still requires a functional DegS protein for expression of the Hy phenotype. This was confirmed by introducing a wild-type copy of the *degS* gene at the *amyE* locus of the QB269 chromosome, which restored the *degU32*-encoded Hy phenotype in the constructed strain, QB4268 (data not shown). Introduction of a wild-type *degS* allele at the *amyE* locus of the *degS42* strain QB254 also restored levansucrase production in the constructed strain, QB4257 (data not shown), indicating that both the *degS42* and *degS220* mutant alleles are recessive to the wild-type *degS* allele.

We propose that the *degS* gene may encode a protein kinase, whose activity could be lost in the *degS220*, *degS39*, and *degS42* mutants, which are deficient for degradative enzyme production. It is interesting that *degS220* modifies the amino acid Ala-193, in the immediate vicinity of His-189, which is conserved in all modulators and may be a candidate for the autophosphorylation site. The position modified by *degS39* or *degS42*, a Glu-300-to-Lys change, leads to the replacement of a negative by a positive charge in the amino acid sequence.

The putative DegS protein kinase activity is not required for transformation, since a deletion of the *degS* coding sequence did not abolish transformability (data not shown). The *degS42* mutation did not lead to loss of transformability (Table 3). The low transformation frequencies of the *degS*(Hy) mutants and strain QB4222 containing an insertionally inactivated *degS* gene may be the consequence of

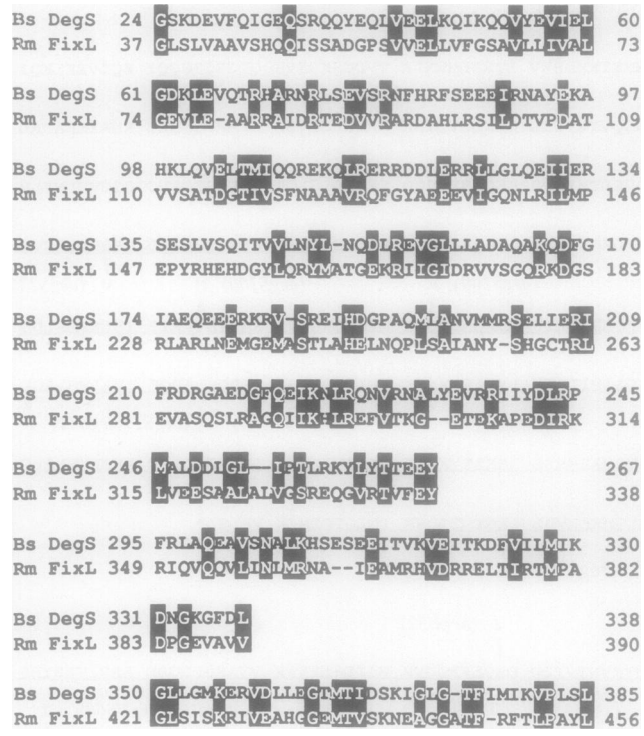

FIG. 2. Alignment of the *B. subtilis* DegS and *Rhizobium meliloti* FixL modulators. Homologous residues are indicated by black boxes. Accepted conservative substitutions are as follows: I, L, V, and M; K and R; S and T; D and E; F and Y; N and Q; and G and A. Numbers indicate the positions in the amino acid sequences of the respective proteins.

indirect effects on *degU* gene expression. Indeed, we suppose that the *degS*(Hy) mutants have increased rates of phosphorylation of the DegU effector, whereas the phenotype of the disrupted gene may be ascribed to a polar effect on *degU* gene expression. After alignment of the DegS amino acid sequence with those of other modulators (43, 53), no particular remarks can be made concerning the positions modified by *degS100*(Hy) or *degS200*(Hy), since the *degS100*(Hy) mutation changes a hydrophobic residue (valine) to another residue of the same class and the *degS200*(Hy) mutation leads to a change in a domain which is not strongly conserved (19) (Fig. 1).

Several *degS*(Hy) and *degU*(Hy) mutants do not possess flagella and acquire the capacity to sporulate in the presence of glucose. However, disruption of the *degS* and *degU* genes in strains QB4222 and QB4210 neither abolished motility nor affected sporulation (data not shown). This indicates that these cellular functions do not require the presence of these regulatory genes, but are affected by mutations in either *degS* or *degU*.

Since the presence of both these regulatory genes is required for degradative enzyme production and since genes encoding modulator-effector pairs are often organized in operons, it was tempting to examine this possibility for *degS-degU*.

Organization and expression of the *degS* and *degU* genes. The *degS* and *degU* genes are adjacent. The direction of transcription is from *degS* to *degU*, and no obvious transcriptional terminator sequence is present between the two genes, suggesting that they are organized in an operon (19, 26). To test this possibility, we constructed a *lacZ* fusion in

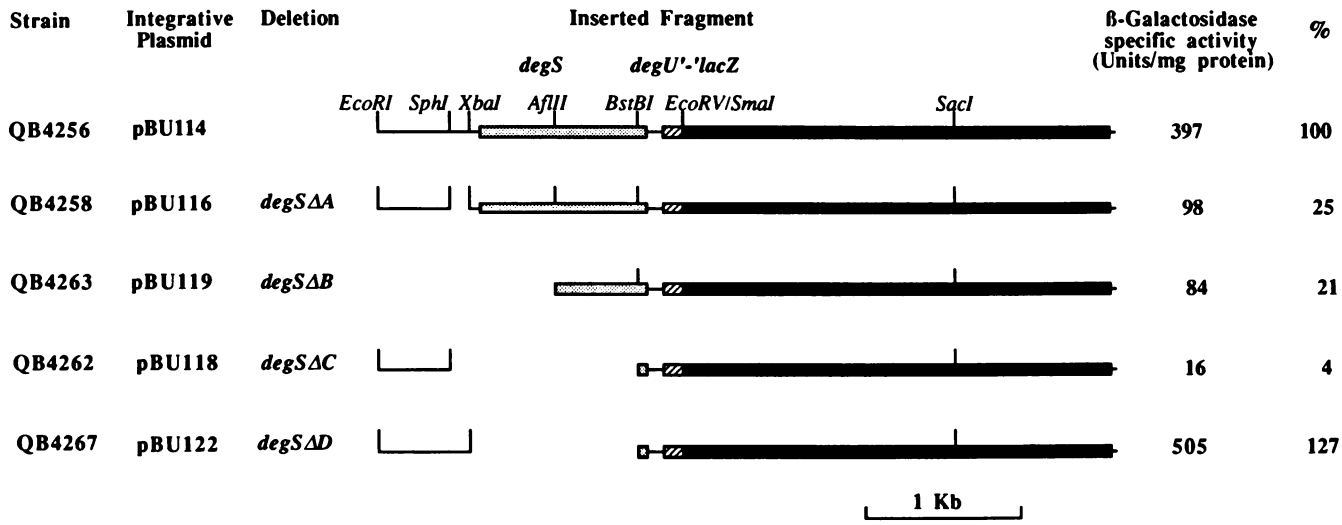

FIG. 3. Simplified restriction map of DNA fragments carrying *degU'-'lacZ* fusions integrated at the *B. subtilis amyE* locus. Regions upstream of the gene fusion were progressively deleted by using unique restriction sites, as follows: *degS* ΔA: Δ*Sph*I-*Xba*I; *degS*ΔB: Δ*Eco*RI-*Afl*II; *degS*ΔC: Δ*Sph*I-*Bst*BI; *degS*ΔD: Δ*Xba*I-*Bst*BI. β-Galactosidase specific activities (expressed in Miller units per milligram of protein) were determined for each strain and expressed as a percentage of the activity of strain QB4256, which carries the intact fragment. Symbols: —, chromosomal regions; ▨, ▨, ■, *degS*, *degU*, and *lacZ* coding regions, respectively.

which the regulatory region upstream of *degS-degU* controls the expression of a *degU'-'lacZ* translational fusion. In the constructed plasmid, pBU114, a DNA fragment containing the putative regulatory region, the *degU* ribosome-binding site, and the first 56 amino acids of DegU was fused in the correct reading frame to the eighth amino acid of LacZ. These sequences were flanked by a chloramphenicol resistance determinant and by portions of the *B. subtilis amyE* gene, allowing integration at the *amyE* locus of the *B. subtilis* chromosome (51) (see Materials and Methods).

In a similar way, deleted subfragments of the *Eco*RI-*Sac*I fragment were cloned in pAF1, giving plasmids pBU116, pBU118, pBU119, and pBU122, and integrated into the chromosomal *amyE* gene (Fig. 3). In this set of isogenic strains, which differ only in the DNA regions upstream from the *degU'-'lacZ* fusion, β-galactosidase activities were measured. The activities of strains QB4258 and QB4263, in which the *Sph*I-*Xba*I and *Eco*RI-*Afl*II DNA fragments were deleted, respectively, were lowered to about 20% of that of strain QB4256 containing the entire fragment (Fig. 3). A larger deletion extending from *Sph*I to *Bst*BI in strain QB4262 abolished most of this residual β-galactosidase activity, indicating that no promoter is present in the intergenic region between *degS* and *degU*. A deletion from the *Xba*I to the *Bst*BI sites gave the same activity as the entire fragment, indicating that the main promoter is located in a 121-bp fragment between the *Sph*I and *Xba*I sites (Fig. 3). A residual activity of 20% might be due to minor promoter activity within the *degS* coding sequence between the *Afl*II and *Bst*BI sites (see the Discussion). We conclude that *degS* and *degU* are organized in an operon, as is the case for many two-component systems.

The transcription start site for the *degS-degU* operon was determined by primer extension analysis with mRNA isolated from exponentially growing cells of the wild-type strain 168 (see Materials and Methods). The position of the transcription start site is shown in Fig. 4 and 5 and is located, as expected, between the *Sph*I and *Xba*I sites. The −35 and −10 regions are indicated in Fig. 5 and share homology with the consensus sequence of vegetative σ^A promoters (37).

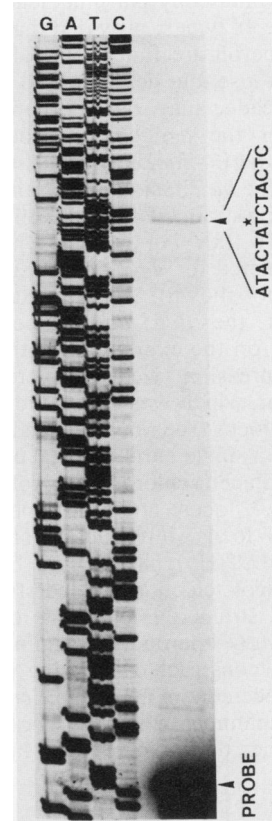

FIG. 4. Reverse transcriptase mapping of the transcription start point for the *degS-degU* operon. Total RNA was isolated from exponentially growing *B. subtilis* 168 cells. The probe (bottom of the gel) was elongated, and the position of the cDNA extended fragment was compared with those obtained by sequencing the M13mp9 recombinant phage used to synthesize the probe (noncoding strand).

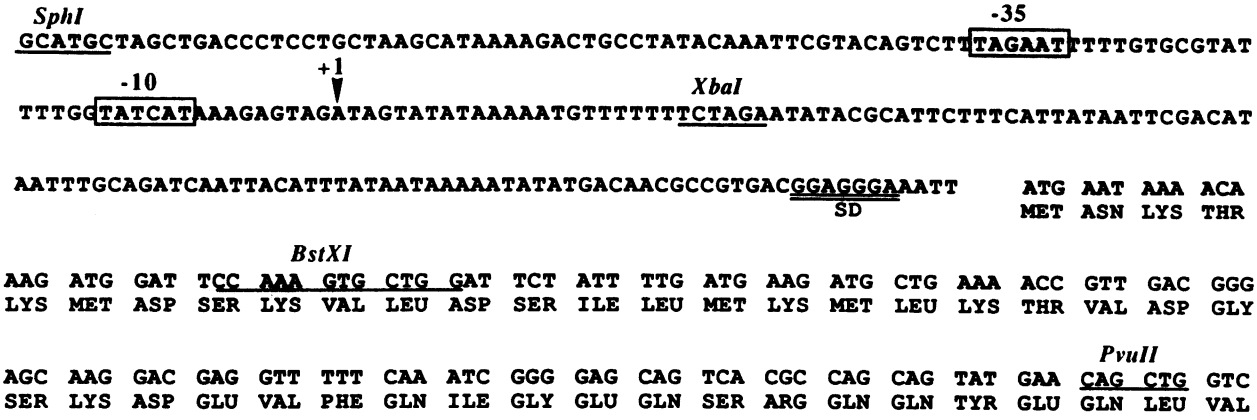

FIG. 5. *degS-degU* promoter region. The sequence of a 351-bp fragment is presented (19, 26), including the beginning of the *degS* coding sequence. The potential ribosome-binding site (SD) and relevant restriction sites used for deletions and probe synthesis are underlined. The transcription start point (+1) is indicated by a vertical arrow. The −10 and −35 regions, corresponding to the transcription start point, are boxed.

Primer extension analysis was carried out in the same way with RNA isolated from a strain deleted for *degS-degU* and gave no primer extension product, as expected (data not shown).

***degS-degU* mediated control of *degQ* expression.** In addition to the *degS-degU* operon, two other genes (*degQ* and *degR*) encoding small polypeptides are involved in this signal transduction pathway. As mentioned above, the presence of *degQ* or *degR* on multicopy plasmids leads to overproduction of degradative enzymes, provided that the two genes of the *degS-degU* operon are functional. This suggested that the expression of these four genes, which apparently belong to the same cascade, may be interdependent. We first examined whether the *degQ* gene product affected the expression of the *degU'-'lacZ* fusion. Plasmid pBU124 was used to introduce the *lacZ* fusion into strain BG4065, deleted for *degQ* (62), giving strain QB4274. No effect was observed, since strain QB4256, carrying the wild-type *degQ* gene, and strain QB4274, deleted for *degQ*, gave similar β-galactosidase levels (data not shown). We then examined the effects of a deletion of the *degS-degU* operon and of the *degU32*(Hy) allele on the expression of the *degQ* gene. To monitor *degQ* expression, we constructed a *degQ'-'lacZ* translational fusion, which was integrated into the chromosome at the *amyE* locus (see Materials and Methods). Strain QB4255 contained a single chromosomal copy of the *degQ'-'lacZ* fusion, an adjacent chloramphenicol resistance determinant at the *amyE* locus, and wild-type *degS* and *degU* genes at the *sacU* locus. Introduction of the *degQ'-'lacZ* fusion into a *degU32*(Hy) background by transformation with chloramphenicol selection yielded strain QB4261. Another *degQ'-'lacZ* strain, QB4260, was constructed, from which the *degS-degU* operon was eliminated by deletion. This deletion was constructed by using plasmid pBU106, leading to the replacement of a 2.9-kbp *Eco*RI-*Sac*I chromosomal fragment spanning the *degS-degU* region with a kanamycin resistance determinant (see Materials and Methods).

The expression of *degQ'-'lacZ* in strains QB4255, QB4261, and QB4260, containing, respectively, wild-type *degS* and *degU* genes, a wild-type *degS* and a *degU32*(Hy) allele, and a *degS-degU* deletion, were compared. When using a *degQ'-'lacZ* fusion to study *degQ* expression, it is important to consider that this expression is subject to growth phase regulation (62). The rate of *degQ'-'lacZ*-encoded β-galactosidase synthesis in minimal medium containing 0.5% glucose and 0.05% casein hydrolysate was low during the exponential growth phase and increased substantially in the stationary phase. We reasoned that this phenomenon may be due to nutrient limitation in the stationary growth phase, for instance, after depletion of carbon or nitrogen sources (phosphate is not a limiting nutrient as minimal medium contains an excess [50 mM] of potassium phosphate).

β-Galactosidase activities in strains QB4255, QB4261, and QB4260 were assayed under two sets of conditions, mimicking either carbon or nitrogen limitation. In the first case, poor carbon sources (glutamate plus succinate) were used, corresponding to conditions of catabolite derepression, and activities were measured during the exponential growth phase. In the second case, exponentially growing cells were suspended in nitrogen-free medium and the time course of *degQ'-'lacZ* expression was monitored during nitrogen starvation.

The expression of *degQ'-'lacZ* turned out to be subject to catabolite repression, since the β-galactosidase activity of strain QB4255 was about 10-fold higher in succinate-glutamate (CSE) minimal medium than in glucose-glutamate (CGE) medium. A comparable catabolite repression was observed for *degQ'-'lacZ* expression in the *degU32*(Hy) strain QB4261 and in strain QB4260 deleted for *degS-degU* (Table 4). However, the rates of β-galactosidase synthesis determined in the absence or in the presence of glucose were consistently lower in these two strains than in the isogenic reference strain QB4255, carrying wild-type *degS* and *degU* genes (Table 4). This seems to indicate that the wild-type alleles of *degS* and *degU* somehow contribute to the expression of the *degQ* gene. More obvious was the effect of *degS-degU* on *degQ'-'lacZ* expression under conditions of nitrogen starvation. Exponentially growing cells of strain QB4255, in which the rate of *degQ'-'lacZ* expression was repressed to a low level by the simultaneous presence of 2% glucose and 0.5% casein hydrolysate, were suspended in nitrogen-free medium. A 10-fold increase of *degQ'-'lacZ* expression was observed 3 to 4 h after resuspension (Fig. 6). Since no significant change in optical density was observed (data not shown), it is possible that this synthesis occurs at the expense of intracellular proteins, which may be degraded. Interestingly, both in the *degU32*(Hy) strain QB4261

TABLE 4. Effect of catabolite repression on *degQ'-'lacZ* expression[a]

Strain	Relevant genotype	β-Galactosidase sp act (U/mg of protein) in[b]:	
		CGE medium	CSE medium
QB4255	*amyE*::(*degQ'-'lacZ cat*)	68	890
QB4260	*amyE*::(*degQ'-'lacZ cat*) Δ(*degS degU*)::*aphA3*	19	240
QB4261	*amyE*::(*degQ'-'lacZ cat*) *degU32*(Hy)	33	520

[a] Cultures were grown at 37°C in C medium supplemented with either glucose plus potassium glutamate (CGE) or potassium succinate plus potassium glutamate (CSE) and chloramphenicol (5 μg/ml).

[b] β-Galactosidase specific activities were determined in extracts prepared from exponentially growing cells.

and in strain QB4260, deleted for *degS-degU*, this increase did not occur (Fig. 6).

Recently, we showed that limitation of phosphate also led to an increase of *degQ* expression (the results will be published elsewhere). These results suggest that this sensory transduction pathway may respond to a nutritional signal, possibly limitation of carbon, nitrogen, or phosphate sources, leading to an increased expression of the target genes.

DISCUSSION

The adjacent *degS* and *degU* genes controlling the rates of synthesis of a class of degradative enzymes constitute an operon transcribed from a putative σ^A promoter upstream from the *degS* gene, although some minor promoter activity may be present within the *degS* coding sequence (see Results), as has also been observed for the *E. coli* NarX-NarL two-component system (43, 53). The use of *lacZ* fusions indicated that the *degS-degU* operon was expressed at a low level, which did not vary noticeably under the different culture conditions we have used.

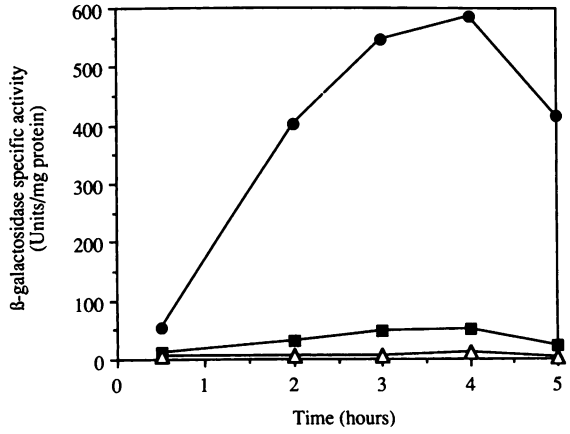

FIG. 6. Time course of *degQ* expression as measured by a *degQ'-'lacZ* translational fusion after resuspension of cells in nitrogen-free medium. Cells were grown to the mid-log phase in CGCH medium and suspended in GP medium. β-Galactosidase specific activities were determined as a function of time (hours) after resuspension. Symbols: ●, strain QB4255; ■, strain QB4260; △, strain QB4261.

FIG. 7. Alignment of possible catalytic domains of the putative *B. subtilis* protein kinase DegS and of eucaryotic protein kinases, which may be implicated in the phosphorylation of their substrates. The common pair of aspartate residues may allow binding through Mg^{2+} of the ATP phosphate groups (6). Amino acid sequences are as reviewed by Hanks et al. (16). Abbreviations: Src, human fetal liver cell homolog of oncogene product from Rous avian sarcoma virus; Yes, human fibroblast homolog of oncogene product from Yamaguchi avian sarcoma virus; SRA3, cyclic AMP-dependent protein kinase from *Saccharomyces cerevisiae*; cAPK, cyclic AMP-dependent protein kinase from bovine cardiac muscle; cGPK, cyclic GMP-dependent protein kinase from bovine lung; CDC28, cell-division-cycle gene product from *S. cerevisiae*; SNF1, protein kinase involved in catabolite repression of *S. cerevisiae* invertase. Numbers correspond to positions in the respective amino acid sequences. Comparisons were made as indicated in the legend to Fig. 2.

Two independent regulatory mechanisms, the *degS-degU* system and induction by sucrose, affect the expression of *sacB*, encoding the secreted sucrose-hydrolyzing enzyme levansucrase. Two targets of these regulatory systems have been identified in the DNA region upstream from *sacB*: a putative target of the DegU effector upstream from the *sacB* promoter (18, 24) and a palindromic sequence, downstream from this putative σ^A promoter, which acts as a transcriptional terminator. This latter sequence is the target of the SacY antiterminator, allowing the expression of *sacB* in the presence of inducer (4, 65).

It is probable that the DegS protein of *B. subtilis* is a protein kinase, since it shows strong amino acid sequence similarities with the procaryotic protein kinases NtrB, CheA, EnvZ, and PhoR (20, 21, 23, 26, 43, 53, 60, 61). Amino acid similarities between DegS and eucaryotic protein kinases are not obvious, except for a short domain (Fig. 7) which was found in eucaryotic protein kinases and a number of bacterial phosphotransferases that use ATP as a phosphate donor (6, 16). It contains two aspartate residues, corresponding to Asp-152 and Asp-168 in DegS, which may interact with the phosphate group of ATP through Mg^{2+} salt bridges. It may be worth mentioning that this domain is located in the vicinity of the histidine residue which is conserved in seven modulators (43, 53) and which may therefore be a candidate for an autophosphorylation site of DegS. The mechanism established in chemotaxis (Che), phosphate (Pho), and nitrogen (Ntr) regulation involves transfer of a phosphoryl moiety from a histidine residue of the protein kinase (CheA, PhoR, and NtrB) to an aspartate residue in the effector (CheY, PhoB, and NtrC) (20, 21, 43, 55, 60). The CheY effector contains three aspartate residues which are clustered to form an acidic binding pocket, as determined from the three-dimensional structure (54, 55). These three residues are also present at the corresponding positions in DegU: Asp-10, Asp-11, and Asp-56. Moreover, the replacement of Asp-56 by Asn in the *degU146* mutant abolishes the capacity of the DegU protein to activate degradative enzyme synthesis. An aspartate residue at the corresponding position of PhoB, Asp-53, was shown to be phosphorylated by PhoR (K. Makino et al., 11th Annual

Meeting of the Molecular Biology Society of Japan, 1988; cited by Nohno et al. [43]). These data support the hypothesis that the DegU effector may also be phosphorylated at an aspartate residue and that the *degU146* mutation inactivates this site of phosphorylation. The *degU32*(Hy) mutation modifies position 12 of DegU, which is close to the aspartate residues which could contribute to the structure of the possible phosphorylation site. This mutation not only leads to hyperproduction of degradative enzymes, but also allows sporulation in the presence of glucose. Interestingly, a similar glucose-insensitive sporulation phenotype results from the *sof-1* mutation in Spo0A, which also represents a modification close to the putative phosphorylation site of this effector, which belongs to the same family as DegU (22, 52).

Three hypotheses have been proposed concerning the mode of action of the *degU32*-encoded protein (19). The first is that this change alleviates the need for phosphorylation and causes this protein to become a constitutive transcriptional activator. The second is that the change makes the protein a better substrate for a related modulator protein, which activates the modified effector by "cross-talk." The third is that this mutation leads to an increase in the rate of phosphorylation or a decrease in the rate of dephosphorylation of the DegU protein. In view of one of the results presented here, i.e., the suppression of the Hy phenotype of *degU32* by the *degS220*-deficient mutation, the first two hypotheses become unlikely, leaving only the third hypothesis as a plausible one.

In addition to mutations modifying the N-terminal receiver domain of DegU, leading either to hyperproduction (*degU32*, *degU9*, *degU24*) or to deficiency of degradative enzyme production (*degU146*), we identified deficient mutations modifying its C-terminal portion. The DegU protein belongs to a subfamily of effectors (8, 43, 53), in which similarities extend well beyond the N-terminal receiver domain. In another member of this subfamily, OmpR, mutations could also be identified that altered its C-terminal domain (40). This indicates that these regions of the DegU and OmpR regulatory proteins are required for their function.

The control of the target genes by the DegU effector, which is hypothesized to be a transcriptional activator containing a DNA-binding domain, may be the result of direct interaction between the effector and target sites, such as the DNA regions upstream from the structural genes of levansucrase and proteases. Two possible helix-turn-helix DNA-binding motifs can be proposed for the DegU protein. One was located by Tanaka and Kawata (57) in the N-terminal receiver part, which gave a negative score (−333) in the Dodd-Egan method for predicting lambda Cro-like DNA-binding regions (10). A second domain in the C-terminal region of DegU can be deduced from amino acid sequence similarities with putative DNA-binding domains of UhpA, FixJ, and ComA (8, 11, 14) and is shown in Fig. 8. The low positive score for this second domain is still not sufficient for it to be considered a likely candidate involved in DNA binding. It remains to be determined whether DegU directly binds to DNA.

Two other genes, *degQ* and *degR*, which encode small polypeptides, are involved in the *degS-degU*-mediated control of the target genes. Although the presence of multiple copies of the *degQ* or *degR* genes led to an increase of degradative enzyme production, elimination of both genes from the *B. subtilis* genome did not lead to any recognizable phenotype or prevent the expression of the *degU32*(Hy)

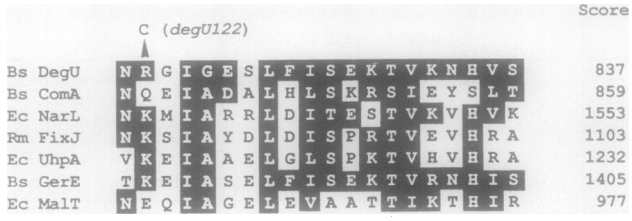

FIG. 8. Possible helix-turn-helix domain in the DegU protein. Amino acid sequence similarities were found with DNA-binding domains proposed for the FixJ, ComA, and UhpA effectors (8, 11, 14). Alignments between DegU and the GerE and MalT regulatory proteins were as described previously (19). Indicated scores correspond to predictions of λ Cro-like DNA-binding regions by using the weight matrix of Dodd and Egan (10). Comparisons were made as indicated in the legend to Fig. 2.

phenotype (62, 63). The role of the *degQ* and *degR* genes as regulators of the expression of the target genes is therefore not clear. However, we have evidence that the expression of the *degQ* gene is regulated by the DegS-DegU pair. Indeed, we observed that the synthesis of the DegQ polypeptide is subject to catabolite repression by glucose, but that the level of synthesis, whether or not it is repressed by glucose, is always higher in a strain carrying *degS-degU* than in a strain deleted for the operon. This observation seems to indicate that the *degS-degU* operon somehow contributes to the expression of *degQ*. An additional effect was observed under conditions of nitrogen starvation, since these led to a strong increase in the level of *degQ* expression in strain QB4255 carrying wild-type *degS* and *degU* alleles. However, no increase was observed in a strain from which *degS* and *degU* were deleted or in a *degU32*(Hy) strain. Since the increase of the rate of DegQ synthesis in strain QB4255 occurred in the presence of an excess of glucose, it apparently overcame catabolite repression of DegQ synthesis. This situation is reminiscent of histidase synthesis in *Klebsiella aerogenes*, in which activation by NtrB-NtrC under conditions of nitrogen limitation occurred independently from catabolite repression (30). A similar increase of DegQ synthesis was observed under conditions of phosphate limitation (data not shown), leading to the hypothesis that this signal transduction pathway may be involved in sensing limitations of carbon, nitrogen, or phosphate sources, which usually occur at the end of the exponential growth phase. This could trigger an adaptive response of the cell, which may react to such a limitation by raising the level of enzymes providing alternative nutrients. It would explain the increase in the rates of synthesis of proteases in rich media after the end of the exponential growth phase (12, 36). As expected, such an increase is not observed in *degS*- or *degU*-deficient mutants (27). In minimal medium, however, activation by DegS-DegU is not restricted to the late growth phase. Indeed, activation of levansucrase synthesis occurs during the exponential growth phase in minimal C medium containing 1% glucose and 0.05% casein hydrolysate. Under these conditions, strain QB4093 synthesizes levansucrase at a high constitutive level, since the transcriptional terminator downstream from the *sacB* promoter was deleted in this strain (24). Introduction of a *degS*-deficient mutation into this strain or elimination of *degS-degU* by deletion lowers this level about 10-fold (24; data not shown). In the same way, a *degS-degU* deletion abolishes the *degQ36*(Hy) phenotype (data not shown).

The data presently available are not sufficient to present a detailed model for the complex regulatory system defined by

the *degS*, *degU*, *degR*, and *degQ* genes. However, we would like to propose a hypothetical model for the function of the DegU effector which may guide further work. We postulated earlier that the *degU32*(Hy) mutation leads to an increase in the rate of phosphorylation of the DegU effector, which, in turn, leads to hyperproduction of degradative enzymes and low transformation frequency. The presence of both the *degU32*(Hy) and *degU146* mutations in the *degU* gene led to a loss of degradative enzyme production, but restored transformability. Apparently, transformation frequency is high when degradative enzyme production is low, and vice versa. Since the DegU effector is thought to exist in two different forms, a phosphorylated and a nonphosphorylated form, we propose that the DegU effector is an ambivalent gene regulator, as was shown previously for the *abrB* regulatory protein of *B. subtilis* (45). The phosphorylated form of DegU would be necessary for degradative enzyme production, and the nonphosphorylated form would be required for transformation and for the increase of DegQ synthesis under conditions of nitrogen starvation. Both forms would act as positive regulators of these processes, since either deletion of *degS-degU* or disruption of *degU* abolished degradative enzyme production, transformability, and the increase of DegQ during nitrogen starvation. This model is also consistent with the suppression of the *degU32*(Hy)-associated phenotypes by the *degS220* mutation, which abolished degradative enzyme production and restored transformability. This phenotype is as expected if the *degS220* mutation leads to loss of DegS protein kinase activity, leaving the DegU effector in the nonphosphorylated state. Moreover, the phosphorylated form of DegU appears to be a negative regulator of flagellar synthesis, since the *degU32*(Hy) mutant is devoid of flagella, in contrast with the strain deleted for *degS-degU*, which is normally motile.

A comparable model has been presented for the OmpR effector, in which the phosphorylated and nonphosphorylated forms of the protein are considered to be two alternative structures, activating either the *ompF* or *ompC* target genes (1, 13).

Further work is needed to gather biochemical data to test the hypotheses presented in this paper.

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