JOURNAL OF BACTERIOLOGY, Aug. 1985, p. 648–653
0021-9193/85/080648-06$02.00/0

Vol. 163, No. 2

Bacillus subtilis Gene Involved in Cell Division, Sporulation, and Exoenzyme Secretion†

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Received 26 February 1985/Accepted 20 May 1985

Bacillus subtilis **strains carrying *div-341* or *sacU* mutations, or both, have been characterized to reveal the roles of both genes in the initiation of sporulation, as well as in cell division and exoenzyme secretion. Both mutations were closely linked by transformation and caused the pleiotropic effects on sporulation and sporulation-associated events. Some *sacU* mutations (*sacU^h*) resulted in hyperproduction of exoenzymes, reduced autolysis, and an ability to sporulate in the presence of excess nutrients. The *div-341* mutation, on the other hand, resulted in filamentous growth at a higher temperature (45°C) and showed *spo0* properties at an intermediate permissive temperature (37°C) in the usual sporulation medium. However, the *div-341* strain sporulated better than wild-type strain at 37°C in the presence of excess nutrients. Exoenzyme production and autolysis were reduced at 37°C in the *div-341* strain. A double mutant with *sacU^{h}32* and *div-341* showed the complex phenotypes. It showed the *sacU^{h}32* property of autolysis and exoenyzme secretion. It showed the *sacU^{h}32* property of sporulation at 30°C and the *div-341* property at 37°C. Slow growth and defective spore outgrowth of the *div-341* strain at 37°C were not observed in the double-mutant strain. Based on pleiotropic phenotypes and close linkages of both mutations, we discuss the relationship between the *sacU* and *div-341* genes and their roles in sporulation, exoenzyme secretion, and cell division.**

The initiation of sporulation in *Bacillus subtilis* seems to be repressed by a nitrogen-containing metabolite(s) (20). There are two types of mutations, repressed or derepressed, for sporulation (16). Some mutations (*sacU^h*) in the *sacU* locus belong to the latter type of mutation. The strain with the *sacU^h* mutation can sporulate better than the wild-type strain even in the presence of excess nutrients (5). The *sacU* locus was first identified as a gene which specifies secretion of levansucrase (6). The *sacU^h* mutations, including *pap-9*, which was found in *sacU* locus, result in pleiotropic phenotypes: hyperproduction of some exoenzymes, including levansucrase, inability to develop competence for transformation, depletion of flagella, reduced activity of autolytic enzymes, and formation of a long chain of cells (1, 5, 6, 22). Therefore, the *sacU* gene may be related to the construction of surface layers of *B. subtilis* cells. However, the causal relationship between the presumed surface alteration and the high sporulation incidence still remains unknown.

During the course of our study on the effect of septum initiation mutations on sporulation and competent cell formation in *B. subtilis* (19), we found that the mutation *div-341* resulted in the reduced production of exoenzymes, as well as spores and competent cells, at an intermediate permissive temperature (37°C), at which vegetative growth was largely intact. The *div-341* mutant was isolated as a temperature-sensitive septum-initiation mutant. It grows filamentously at 45°C (10). Thus, the intact *div-341* function is required for sporulation and sporulation-associated events, as well as for vegetative cell division. The opposing properties of the *sacU^h* and *div-341* strains prompted us to seek the linkage relationship between *div-341* and *sacU^h* mutations, as both mutations were found linked to *hisA* by PBS1 transduction (6, 10), which is not adequate for the evaluation of the linkage of closely located markers. In this communication, we describe the establishment of a linkage of *sacU^h* and *div-341* by transformation and the construction of a double mutant with *div-341* and *sacU^{h}32* used in examining the interaction of both genes. We compared the properties of the *div-341* or *sacU^{h}32* mutants, or both, and discuss the role of both genes in the initiation of sporulation, as well as in exoenzyme production and cell division, of *B. subtilis*.

MATERIALS AND METHODS

Bacterial strains. The bacterial strains used are described in Table 1.

Transformation procedures. The extraction of transforming DNA and procedures for transformation are described elsewhere (18). Transformants or recombinants were selected on broth-supplemented minimal salts agar (18).

Exoenzyme activity. The activity of levansucrase and protease was measured as described elsewhere (2, 6). Production of exoenzyme by the washed cells was measured as described elsewhere (17). Cells grown at 30°C for 2 days (Klett units, ca. 100) were washed and resuspended in minimal salts medium at half the volume of the original culture. The samples were incubated at 30 and 37°C. Cells were sedimented, and the supernatant fractions were assayed for α-amylase and protease. The required amino acids were added to the culture or resuspension medium at the concentration of 50 μg/ml.

Autolysis of whole cells. Cells were collected by filtration and resuspended in TK buffer (1), and the decrease in turbidity was followed.

Antibiotics and chemicals. Decoyinine was a gift of Y. Fujita, Hamamatsu Medical College, Hamamatsu, Japan.

RESULTS

Linkage relationships of *div-341* and *sacU* loci. As the *div-341* and *sacU* mutations are found linked to *uvrA* in the order of *hisA*, *uvrA*, and *div-341* or *sacU* (6, 10), a strain with

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† Contribution no. 1633 from the National Institute of Genetics, Mishima, Japan.

TABLE 1. Bacterial strains[a]

Strain	Genotype	Source
YN9	*purB6 metB5 trpB3 pap-9*	K. Yamane (1)
QB136	*leuA8 trpC2 sacU^{h}32*	K. Yamane (6)
NIG1121	*met his*	(18)
NIG1152	*met his div-341*	(19)
ts341	*thyA thyB hisB31 div-341*	T. Komano (10)
UVS1	*uvrA10 thy trpC2 met-14 sul*	N. Munakata (12)
NIG1142[b]	*met his sacU^{h}32*	
NIG1143[c]	*met his pap-9*	
NIG1155[d]	*his div-341 uvrA10*	
NIG1156[e]	*his div-341 sacU^{h}32*	
NIG1158[f]	*met his div-341 sacU^{h}32*	

[a] Selection of transformants are described in Table 2, footnote *a*.
[b] Div$^+$ SacU^h 32 transformant of NIG1152 with DNA from QB136.
[c] Div$^+$ Pap-9 transformant of NIG1152 with DNA from YN9.
[d] Met$^+$ UvrA10 transformant (transformation by congression) of NIG1152 with DNA from UVS1.
[e] Uvr$^+$ SacU^{h}32 transformant of NIG1155 with DNA from QB136.
[f] Leu$^+$ Div$^-$ SacU^h transformant of HA101 (*met his leu*) (15) with DNA from NIG1156 (transformation by congression).

TABLE 2. Three-point transformational cross involving *sacU* (*sacU^{h}32* and *pap-9*), *div-341*, and *uvrA* markers

Donor genotype	Recipient genotype	Primary selection	Class	No.	Order implied by the results
sacU^{h}12	*div-341*	Div$^+$[a]	Div$^+$ Uvr$^-$ SacU$^+$	25	*sacU^{h}32 div-341 uvrA10*
	uvrA10		Div$^+$ Uvr$^-$ SacU^h	19	
			Div$^+$ Uvr$^+$ SacU$^+$	16	
			Div$^+$ Uvr$^+$ SacU^h	9	
pap-9	*div-341*	Div$^+$[a]	Div$^+$ Uvr$^-$ Pap$^+$	62	*pap-9 div-341 uvrA10*
	uvrA10		Div$^+$ Uvr$^-$ Pap$^-$	57	
			Div$^+$ Uvr$^+$ Pap$^+$	47	
			Div$^+$ Uvr$^+$ Pap$^-$	48	

[a] Div$^+$ recombinants were selected as temperature-resistant (42°C) colonies. Uvr$^+$ recombinants were selected as UV-resistant (40 J/m^2) colonies. SacU^h or Pap$^-$ recombinants were selected as described elsewhere (6). To detect exolevansucrase activity, Wako glucose B-test reagent (Wako, Tokyo, Japan) was used. After sucrose treatment, the reagent was overlaid, and *sacU^h* or *pap* colonies developed a red halo.

two mutations, *div-341* and *uvrA10*, was constructed to carry out a three-point transformational cross involving *div*, *uvrA*, and *sacU* markers to observe their relative locations on the chromosome. The *div-341* mutation was found to be between *sacU^{h}32* (or *pap-9*) and *uvr10* (Table 2). The distance between *div-341* and *sacU^{h}32* or *pap-9* obtained by two-factor transformational crosses was ca. 60 (100 − the percent cotransformation).

Exoenzyme production in the *div-341* or *sacU^h* strains, or both. As described elsewhere (19), sporulation and competence development were severely reduced in the strain with the *div-341* mutation when the temperature was raised to an intermediate permissive level (37°C), whereas vegetative growth was largely intact. Therefore, the *div-341* mutant can be classified as a conditional *spo0* mutant strain. The early *spo0* mutants have a reduced ability to produce exoenzymes (16). α-Amylase production from the washed cells grown in the maltose medium was temperature-sensitive in the *div-341* strain (Fig. 1A). α-Amylase production was reduced at 37°C, whereas protease production was lost under the same con-

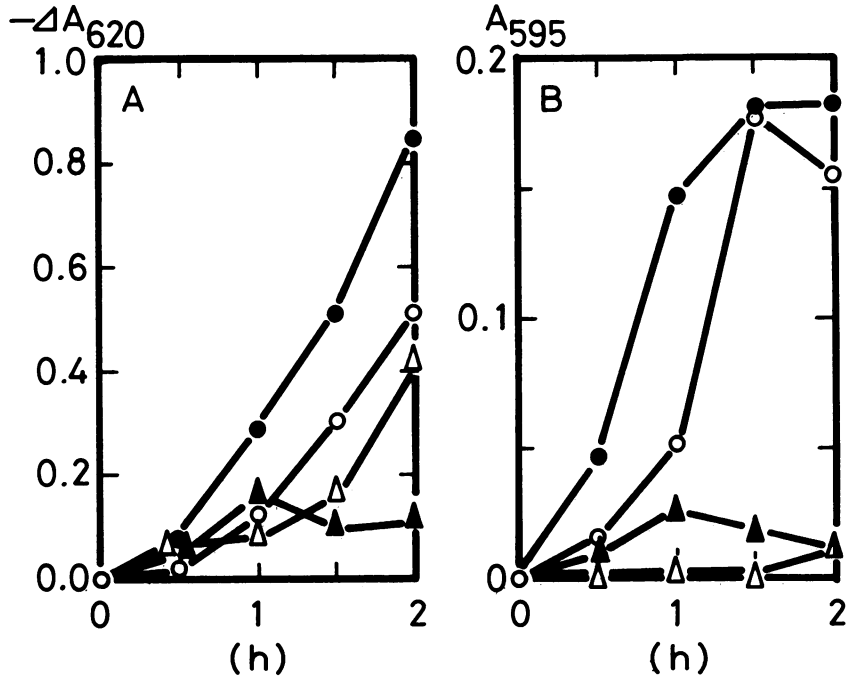

FIG. 1. Exoenzyme production by the washed cells of the *B. subtilis div-341* strain. (A) α-Amylase; (B) protease. The reaction mixture for protease contained 0.8 ml of sample and 5 mg of hide powder in 0.5 ml of imidazol buffer. It was incubated at 37°C for 16 h. The extinction at 595 nm was measured. For α-amylase activity, 0.4 ml of the sample was incubated with 1 ml of starch solution (0.05% starch, 45 mM NaCl, 45 mM phosphate buffer [pH 6.0]) at 37°C for 30 min. Iode solution (0.5 ml) was added, and the extinction at 620 nm was measured. The activity was expressed as a decrease in the A_{620}. Symbols: ●, ○, NIG1121 (wild type); ▲, △, NIG1152 (*div-341*). Washed cells were incubated at 30°C (open symbols) and 37°C (closed symbols).

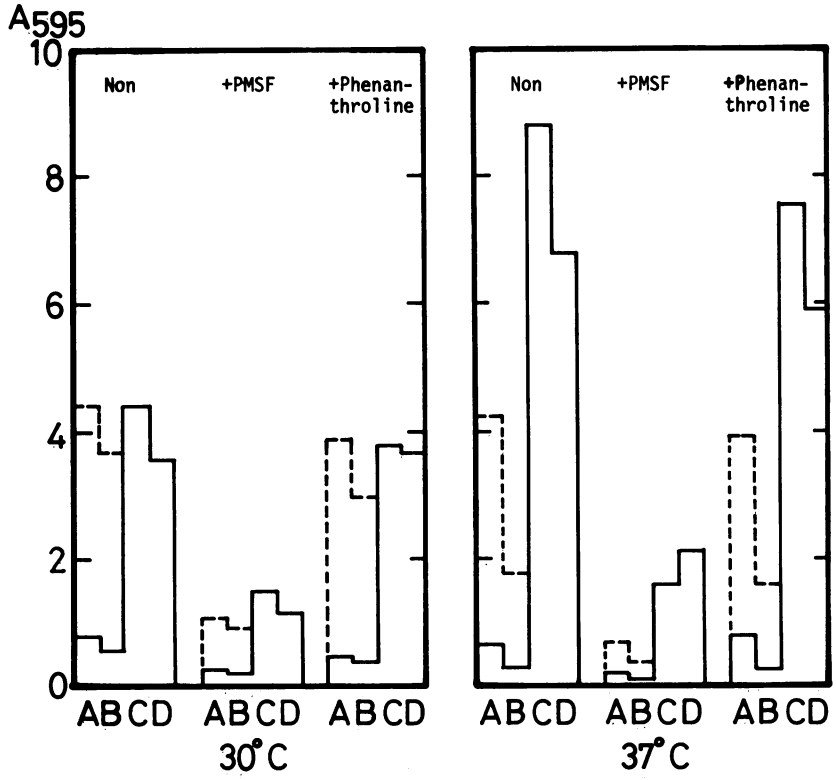

FIG. 2. Extracellular protease production by *B. subtilis sacU^{h}32* or *div-341* strains, or both. Cells were grown to the T_3 stage in SPI medium containing 50 μg each of histidine and methionine per ml and 10 μM $MnCl_2$ (19). They were sedimented by centrifugation at 5,000 rpm for 15 min, and the resulting supernatant was stored at 4°C overnight. The reaction mixture (2 ml) contained 0.5 ml of sample, 1.5 ml of 0.1 M imidazol-hydrochloride buffer (pH 7.2), and 50 mg of hide powder (Sigma). The sample was incubated at 37°C for 2.5 h (solid line) and for 17 h (broken line), and the release of blue dye was measured. Inhibitors were added at 2 mM (phenylmethylsulfonyl fluoride [PMSF], an inhibitor of serine-protease) and at 1 mM (1,10-phenamthroline, an inhibitor of metallo-protease). Bars: A, NIG1121 (wild type); B, NIG1152 (*div-341*); C, NIG1142 (*sacU^{h}32*); D, NIG1158 (*div-341 sacU^{h}32*).

dition even at 30°C (Fig. 1B). We further examined the production of exoenzymes by the T_3 stage cells of the *div-341* strain grown in an exhaustion medium. The results are shown in Fig. 2. Production of extracellular protease (mostly serine-protease) was inhibited by 50% at 37°C in the *div-341* mutant, whereas it was reduced only by 15% at a lower temperature (30°C). Therefore, the *div-341* mutant can be considered as a temperature-sensitive *spo0* mutant with a defect in the early sporulation process.

On the other hand the *sacU^{h}32* mutation in the *sacU* locus makes the cell a hyperproducer of exoenzymes (6). The extracellular protease (mostly serine-protease) activity was enhanced in the *sacU^{h}32* mutant (Fig. 2). A strain with two mutations, *sacU^{h}32* and *div-341*, was constructed (Table 1) to determine the hierarchy of both genes. Exoenzyme production (serine-protease) was elevated in the double mutant at 30°C, and it was not reduced at the higher temperature, unlike that of the *div-341* strain (Fig. 2).

Sporulation of the *div-341* or *sacU^h* strains, or both, in the presence of excess nutrients. The *sacU^h* mutations result in derepression for sporulation in the presence of excess nutrients (5), whereas the *div-341* mutation results in asporogeny at a higher intermediate temperature (37°C) in an exhaustion medium (19). We next compared sporulation with the same medium and with isogenic *sacU^h* or *div-341* strains, or both.

In an exhaustion medium, where gross sporulation of the wild-type strain occurs, no apparent difference in sporulation between the wild-type strain and the *sacU^h* strain was observed. In this medium, the *div-341* strain showed temperature-sensitive poor sporulation (Table 3). This indicates that the *div-341* gene is required for the full induction of sporulation. Introduction of *sacU^{h}32* into the *div-341* strain could not restore the ability to sporulate at 37°C. On the other hand, in an enriched medium, where gross sporulation of the wild-type strain was suppressed, sporulation of the *sacU^h* strain was elevated strongly at 30 and 37°C compared with the wild-type strain at the same temperatures (5; Table 4). These four strains with same genetic background sporulated poorly at a higher temperature (37°C) for unknown reasons. The *div-341* strain, to our surprise, showed elevated sporulation at 37°C compared with the wild-type strain at the same temperature. Therefore, slight inactivation

TABLE 3. Sporulation of *sacU^{h}32* or *div-341* strains, or both, in an exhaustion medium[a]

Strain	No. of cells (10^6) at:			
	30°C		37°C	
	Spores/ml	CFU/ml	Spores/ml	CFU/ml
NIG1121 (wild type)	30.0	241	18.5	575
NIG1152 (*div-341*)	0.16	17.5	0.00170	263
NIG1142 (*sacU^{h}32*)	46.3	90.0	4.08	275
NIG1158 (*div-341 sacU^{h}32*)	21.5	88.8	0.00480	5.75

[a] An overnight culture of each strain grown in Penassay broth (Difco Laboratories, Detroit, Mich.) at 30°C was added as a 2.5% (vol/vol) inoculum into SPI medium containing 10 μM $MnCl_2$ and 50 μg each of histidine and methionine per ml (19). Cells were grown at 30°C for 64 h and at 37°C for 24 h.

TABLE 4. Sporulation of *sacU^{h}32* or *div-341* strains, or both, in the presence of excess nutrients[a]

Strain	No. of cells (10^6) at:			
	30°C		37°C	
	Spores/ml	CFU/ml	Spores/ml	CFU/ml
NIG1121 (wild type)	0.00060	0.0011	0.000005	0.000070
NIG1152 (*div-341*)	0.00011	0.00087	0.00032	0.00039
NIG1142 (*sacU^{h}32*)	825	780	0.0012	0.0025
NIG1158 (*div-341 sacU^{h}32*)	850	1000	0.00021	36.0

[a] Cells were grown in SRG medium (5) containing 100 μg each of histidine and methionine per ml and 100 μg of tryptophan per ml at 30°C for 4 days and at 37°C for 45 h. Heat resistant (75°C for 20 min) spores were scored on minimal salts agar (18).

of the *div-341*$^+$ gene might derepress sporulation in the presence of excess nutrients. The double mutant also showed stimulated sporulation at both temperatures (30 and 37°C) compared with the wild-type strain at the same temperatures. Thus, the high incidence of sporulation caused by *div-341* and *sacU^{h}32* mutations independently was also maintained in the double-mutant strain. However, their effects were not additive. The number of the viable cells of each strain to stage zero was ca. 5×10^8/ml at 30 and 37°C, although that number declined in some strains after prolonged incubation.

Induction of sporulation by guanine nucleotide deprivation in *div-341* or *sacU^h* strains, or both. Sporulation of *B. subtilis* can be induced by guanine nucleotide deprivation, which is accomplished either by a limited supply of guanine to a guanine auxotroph or by the addition of decoyinine, which blocks GMP synthetase (8, 9, 14). We next examined whether or not the addition of decoyinine restored sporulation of the *div-341* strain at 37°C in an exhaustion medium. Decoyinine did not restore sporulation of the *div-341* strain at 37°C in an exhaustion medium (data not shown), indicating that the *div-341* gene product is obligatory for sporulation and that the decrease in GTP concentration as a result of the addition of decoyinine (7) occurs before the onset of the *div-341* gene. Decoyinine was also not effective on the double-mutant strain (*sacU^h div-341*) at 37°C (data not shown).

Autolytic activities of the *div-341* or *sacU^h* strains, or both. As the *pap-9* mutation makes cells resistant to autolysis (1), we compared the autolysis of the *div-341* or *sacU^h* strains, or both. The *sacU^{h}32* strain was resistant to autolysis, and the *div-341* strain showed temperature-sensitive autolysis (Fig. 3). The lysis of the *div-341* strain seemed to depend on the growth temperature of the cells before they were resuspended in a lysis buffer. The mutant cells grown at 30°C lysed in a manner similar to that of the wild-type cells at 30 and 37°C, whereas the mutant cells grown at 37°C did not lyse at either incubation temperature. Cells grown at 30°C were transferred to 37°C and resuspended in the lysis buffer. Heat (37°C) treatment for only 15 min blocked lysis of the mutant cells (data not shown).

The double mutant (*sacU^{h}32 div-341*) showed resistance to autolysis when grown at 30 and 37°C, as did the single *sacU^{h}32* strain.

Effect of the *sacU^{h}32* mutation on the growth and spore outgrowth of the *div-341* strain. The *div-341* strain shows

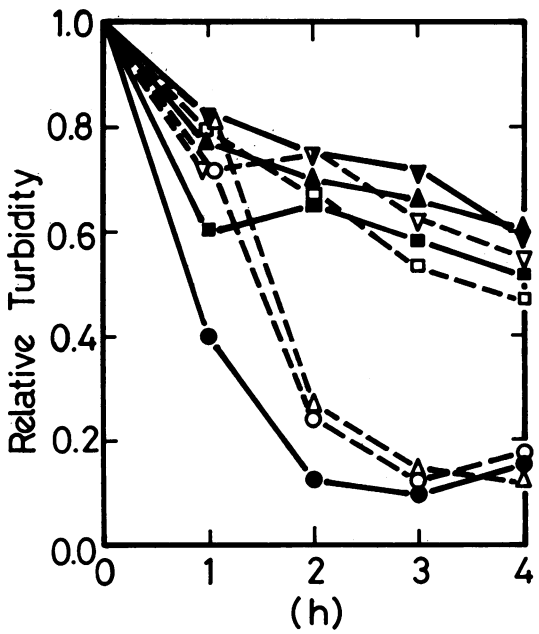

FIG. 3. Autolysis at pH 8.0 of whole bacterial cells from exponential-phase cultures of *B. subtilis sacU^{h}32* or *div-341* strains, or both. Symbols: ●, ○, NIG1121 (wild type); ▲, △, NIG1152 (*div-341*); ▼, ▽, NIG1142 (*sacU^{h}32*); ■, □, NIG1158 (*div-341 sacU^{h}32*). Cells were grown at 30°C (open symbols) and 37°C (closed symbols). Autolysis was measured at 37°C. Turbidity was measured by a Klett-Summerson colorimeter with a red filter. Cells were grown in SPI medium containing $MnCl_2$ and amino acids as described in the legend to Fig. 2.

filamentous growth at 45°C (10), and the *sacU^{h}32* strain has no apparent defect in cell growth. Viability of the double-mutant strain (*div-341 sacU^{h}32*) was examined to see whether the *sacU^{h}32* mutation restores the viability of the *div-341* strain at the nonpermissive temperature. The *sacU^{h}32* mutation could not restore the number of CFU of the *div-341* strain at 42°C on Difco nutrient agar or on minimal glucose agar (data not shown). However, the slow growth shown by the *div-341* strain at 37°C was recovered with the *sacU^{h}32 div-341* strain. Both strains were grown overnight at 30°C, transferred to the fresh medium, and incubated at 37°C (Table 5). In similar experiments we observed a prolonged lag time only for the *div-341* strain only at 37°C, whereas the double-mutant strain showed a lag time similar to that of the wild-type strain at 37°C.

Slow growth after transfer to the fresh medium at 37°C in the *div-341* strain may suggest a defective outgrowth of

TABLE 5. Doubling time of *sacU^{h}32* or *div-341* strains, or both, at 30 or 37°C[a]

Strain	Doubling time (min) at:	
	30°C	37°C
NIG1121 (wild type)	50	26
NIG1152 (*div-341*)	55	65
NIG1142 (*sacU^{h}32*)	52	28
NIG1158 (*div-341 sacU^{h}32*)	59	32

[a] Cells grown on Difco nutrient agar at 30°C overnight were suspended at ca. 5 Klett units in SPI medium containing $MnCl_2$ and amino acids as described in the legend to Fig. 3. They were then incubated at 30 or 37°C with aeration. The time required for the doubling of the turbidity was measured.

spores. Spores of the *div-341* or *sacU*^{h}*32* strains, or both, were prepared at 30°C and germinated at 30 and 37°C. Only the *div-341* strain showed slow outgrowth at 37°C. Outgrowing spores of the *div-341* strain became a twisted chain of cells, whereas those of the double-mutant strain showed a straight chain of cells (data not shown).

DISCUSSION

The close linkage by transformation and the pleiotropic effect on exoenzyme secretion, sporulation, competence development, and autolysis described in the present communication suggest that the *div-341* and *sacU* genes are related to each other. They seem to play an essential role not only in cell division but also in the physiology of *B. subtilis* cells.

The constitutive mutations (*sacT* and *sacS*) for sucrase secretion fall within a distance of ca. 50 (100 − the percent cotransformation) from the structural gene for sucrase (*sacA*), which also covers a chromosomal segment with a distance of ca. 50 by transformation (6). The *div-341* and *sacU* genes, separated by a distance of ca. 60 by transformation, might be the same gene, the structural gene and its regulatory gene, or two structural genes. The *div-341* gene is essential for vegetative cell division (10, 11) and for sporulation and sporulation-associated events (19). The latter properties are more temperature-sensitive than vegetative growth in the mutant. This may suggest that the *div-341* gene product is required for early sporulation events in a larger amount than for vegetative growth. We assume that the *div-341* gene is a structural gene whose product is required for vegetative cell division in a small amount and for sporulation events in a large amount. Therefore, upon starvation for nutrients, the *div-341* gene might be derepressed to carry out early sporulation events. We further assume that the *sacU* gene is a regulatory gene of the *div-341* structural gene. The *sacU*^{h} mutation might result in the derepression of the *div-341* gene even in the presence of excess nutrients and make the cell initiate sporulation and sporulation-associated events. This might be the reason why exoenzyme production is elevated in the *sacU*^{h} mutant, as exoenzyme secretion is generally observed in early-sporulating cells. The *div-341* gene product seems to be involved in a step of secretion of some membrane proteins, as well as extracellular enzymes. Vegetative cell division might be affected in the *div-341* strain because of a defect in the transportation of proteins required for cell division to the surface layer of *B. subtilis* cells. Presumed derepression of the *div-341* gene explains the recovery of slow growth or defective spore outgrowth by the *div-341* strain at 37°C as a result of the *sacU*^{h}*32* mutation, although lethality at a higher temperature (42°C) could not be recovered. Derepressed synthesis of the slightly defective product of the *div-341* gene at 37°C may restore its defective function.

Modification of transcription during early sporulation (3) may be directly related to the presumed derepression. To confirm these possibilities, we are carrying out the cloning and sequencing of *div-341* gene with the temperature phage ρ_{11}, which was used for the molecular analysis of the *spo0F* gene (21).

Apparent derepression for sporulation in the *sacU*^{h}*32* strain might be different from that of the *spd* strains (4, 13), which can sporulate throughout exponential growth. The strains with *div-341* or *sacU*^{h}*32* mutations, or both, were grown exponentially for ca. 12 h by repeated dilution of the cultures with fresh medium. We could not observe derepressed sporulation in the mutant strains (data not shown). Reduced autolytic activities of the *div-341* strain at 37°C and the *sacU*^{h}*32* strain may correlate with high sporulation after the end of growth in the presence of excess nutrients.

The effect of decoyinine is very similar to that of the *sacU*^{h} mutation in that the cells sporulate in the presence of excess nutrients and become refractory to autolysis (23). Addition of guanine counteracts the effect of decoyinine (9). Guanine (up to 100 μg/ml), however, could not alter the effects of the *sacU*^{h}*32* or *div-341* mutations (data not shown).

ACKNOWLEDGMENTS

We thank R. H. Doi for reading the manuscript critically and K. Yamane and N. Munakata for bacterial strains. We are grateful to Y. Fujita for decoyinine.

This work was supported by a grant from the Ministry of Education of Japan.

LITERATURE CITED

1. **Ayusawa, D., Y. Yoneda, K. Yamane, and B. Maruo.** 1975. Pleiotropic phenomena in autolytic enzyme(s) content, flagellation, and simultaneous hyperproduction of extracellular α-amylase and protease in a *Bacillus subtilis* mutant. J. Bacteriol. **124**:459–469.
2. **Dancer, B. N., and J. Mandelstam.** 1975. Criteria for categorizing early biochemical events occurring during sporulation of *Bacillus subtilis*. J. Bacteriol. **121**:411–415.
3. **Doi, R. H.** 1977. Genetic control of sporulation. Annu. Rev. Genet. **11**:29–48.
4. **Endo, T., H. Ishihara, and E. Freese.** 1983. Properties of a *Bacillus subtilis* mutant able to sporulate continually during growth in synthetic medium. J. Gen. Microbiol. **129**:17–30.
5. **Kunst, F., M. Pascal, J. Lepesant-Kejzlarova, J. A. Lepesant, A. Billaut, and R. Dedonder.** 1974. Pleiotropic mutations affecting sporulation conditions and synthesis of extracellular enzymes in *Bacillus subtilis* 168. Biochimie **56**:1481–1489.
6. **Lepesant, J. A., F. Kunst, J. Lepesant-Kejzlarova, and R. Dedonder.** 1972. Chromosomal location of mutations affecting sucrose metabolism in *Bacillus subtilis* Marburg. Mol. Gen. Genet. **118**:135–160.
7. **Lopez, J. M., A. Dromerick, and E. Freese.** 1981. Response of guanosine 5′-triphosphate concentration to nutritional changes and its significance for *Bacillus subtilis* sporulation. J. Bacteriol. **146**:605–613.
8. **Lopez, J. M., C. L. Marks, and E. Freese.** 1979. The decrease of guanine nucleotides initiates sporulation of *Bacillus subtilis*. Biochim. Biophys. Acta **587**:238–252.
9. **Mitani, T., J. E. Heinze, and E. Freese.** 1977. Induction of sporulation in *Bacillus subtilis* by decoyinine or hadacidin. Biochem. Biophys. Res. Commun. **77**:1118–1125.
10. **Miyakawa, Y., and T. Komano.** 1980. Study of the cell cycle of *Bacillus subtilis* using temperature-sensitive mutants. I. Isolation and genetic analysis of the mutants defective in septum formation. Mol. Gen. Genet. **181**:207–214.
11. **Miyakawa, Y., T. Komano, and Y. Maruyama.** 1982. Timed action of the gene products required for septum formation in the cell cycle of *Bacillus subtilis*. J. Bacteriol. **149**:673–680.
12. **Munakata, N.** 1977. Mapping of the genes controlling excision repair of pyrimidine photoproducts in *Bacillus subtilis*. Mol. Gen. Genet. **156**:49–54.
13. **Ochi, K., and E. Freese.** 1982. A decrease in *S*-adenosylmethionine synthetase activity increases the probability of spontaneous sporulation. J. Bacteriol. **152**:400–410.
14. **Ochi, K., J. Kandala, and E. Freese.** 1982. Evidence that *Bacillus subtilis* sporulation induced by the stringent response is caused by the decrease in GTP or GDP. J. Bacteriol. **151**:1062–1065.
15. **Okubo, S., and T. Yanagida.** 1968. Isolation of a suppressor mutant in *Bacillus subtilis*. J. Bacteriol. **95**:1187–1188.
16. **Piggot, P. J., and J. G. Coote.** 1976. Genetic aspects of bacterial endospore formation. Bacteriol. Rev. **40**:908–962.
17. **Priest, F. G.** 1975. Effect of glucose and cyclic nucleotides on

the transcription of α-amylase mRNA in *Bacillus subtilis*. Biochem. Biophys. Res. Commun. **63**:606–610.
18. **Sadaie, Y., and T. Kada.** 1983. Formation of competent *Bacillus subtilis* cells. J. Bacteriol. **153**:813–821.
19. **Sadaie, Y., and T. Kada.** 1983. Effect of septum-initiation mutations on sporulation and competent cell formation in *Bacillus subtilis*. Mol. Gen. Genet. **190**:176–178.
20. **Schaeffer, P., J. Millet, and J. P. Aubert.** 1965. Catabolite repression of bacterial sporulation. Proc. Natl. Acad. Sci. U.S.A. **54**:707–711.
21. **Shimotsu, H., F. Kawamura, Y. Kobayashi, and H. Saito.** 1983. Early sporulation gene *spo0F*: nucleotide sequence and analysis of gene product. Proc. Natl. Acad. Sci. U.S.A. **80**:658–662.
22. **Steinmetz, M., F. Kunst, and R. Dedonder.** 1976. Mapping of mutations affecting synthesis of exocellular enzymes in *Bacillus subtilis*. Identification of the *sacU*^{h}, *amyB* and *pap* mutations. Mol. Gen. Genet. **148**:281–285.
23. **Uratani, B., J. M. Lopez, and E. Freese.** 1983. Effect of decoyinine on peptidoglycan synthesis and turnover in *Bacillus subtilis*. J. Bacteriol. **154**:261–268.