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Expression of Competence Genes in *Bacillus subtilis*

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A set of competence (*com*) mutants of *Bacillus subtilis* was constructed by using Tn*917lacZ* as a mutagen. In about half of the mutants, the promoterless *lacZ* element on the transposon was placed under control of putative *com* promoters. Expression of the mutant *com* genes was studied by using the β-galactosidase tag. Two of the mutant genes (those represented by *com-124* and *com-138*) were expressed early in the growth cycle in all of the media tested and were not dependent on the *spo0A* or *spo0H* product for expression. The remaining mutants, which represented a minimum of four additional genes, expressed β-galactosidase in stationary phase during the period in which competence developed. We conclude that expression of *com* genes is probably regulated transcriptionally and in a growth stage-specific manner. Expression of these genes was also dependent on growth in competence medium and, like competence development, required the presence of glucose and was dependent on the *spo0H* products. The dependence on the *spo0A* gene product was partially bypassed by the *abrB703* mutation. These effects were qualitatively equivalent to those on competence development. The latter was dependent on *spo0A* and *spo0H*, and the *spo0A* dependency was partially suppressed by *abrB703*. Several of the mutants were still capable of resolution into light and heavy buoyant density cell fractions when grown in competence medium. All of these expressed β-galactosidase to a greater extent in the light fraction, showing that expression of these *com* genes was cell type specific. Development of competence was not markedly affected by mutations in *spo0B*, *spo0E*, *spo0F*, *spo0J*, or *sigB*, the structural gene for σ^{37}.

Development of genetic competence in *Bacillus subtilis* is a complex process that involves profound alterations in cell physiology (5) accompanied by elaboration of gene products necessary for binding and uptake of transforming DNA. During this process, the competent culture differentiates into two cell types that can be resolved by isopycnic centrifugation on Renografin gradients (3, 12) or by sedimentation through sucrose gradients (22). The light buoyant density, slow-sedimenting fraction makes 10 to 20% of the culture and consists of uninucleate, small cells, a percentage of which are competent for transformation. The remaining material exhibits low competence and high buoyant density in Renografin and consists of larger multinucleate cells.

We isolated 28 mutants deficient in competence (*com*), obtained by mutagenesis with transposon Tn*917lacZ* (29). These mutants have been genetically mapped and classified into seven groups based on map position and their behavior with respect to several physiological criteria (13). Mutants in two groups (III and V) failed to resolve in Renografin gradients, exhibiting a single band with the buoyant density of noncompetent cells. Fifteen of the mutants produced β-galactosidase, suggesting that in each case the promoterless *lacZ* determinant on the transposon was probably under the control of a *com* promoter.

We examined expression of β-galactosidase under the control of *com* gene promoters as a function of growth stage, medium, and genetic background. Several of the *com* genes were shown to be expressed in a manner that suggests stage-specific expression of competence genes under the control of nutritional signals. Most of the *com* genes were also expressed in a cell type-specific manner since β-galactosidase specific activity was higher in the Renografin light buoyant density fraction than in the heavy fraction.

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MATERIALS AND METHODS

Strains and strain construction. The strains used are listed in Table 1. The *com* mutations were moved into different genetic backgrounds by selection for resistance to erythromycin (5 μg/ml) or erythromycin (5 μg/ml) plus lincomycin (25 μg/ml). *spo0* markers were moved by congression. *spo0A* mutants were routinely checked for protease and antibiotic production to confirm the presence or absence of *abr* partial suppressor mutations. The *sigB* mutation (2), kindly provided by R. Losick, was moved into different genetic backgrounds by selection for chloramphenicol (5 μg/ml) resistance since the *sigB* gene had been disrupted by insertion of a chloramphenicol resistance fragment. In general, strain construction in *com* backgrounds was accomplished by transduction with phage PBS1 or AR9.

Competence regimen and transformation. For this study we used a one-step competence regimen to facilitate the correlation of enzyme production in the mutant strains with the development of competence in the wild type. The competence medium used in this procedure was that used for the second step of our usual two-step procedure, with the omission of $CaCl_2$ (8). Overnight cultures were grown at 30 to 32°C in this medium and diluted 10-fold to initiate growth to competence. Cultures were incubated at 37°C with vigorous shaking. Maximum competence usually appeared at 1.5 to 2.5 h after the end of exponential growth (t_{15} to t_{25}). In some instances, an earlier wave of competence appeared soon after dilution of the overnight culture (see Fig. 1). The maximum levels of competence achieved with this procedure were no more than two- to fivefold lower than those obtained with the conventional method. Transformability was determined by incubation of cells with DNA carrying

TABLE 1. Strains

Strain	Characters	Source or reference
BD630	*leu metB5 hisA1*	
BD1197	*spo0AΔ204 leu hisA1*	10; this work
BD1198	*spo0HΔHind leu hisA1*	27; this work[a]
BD1201	*spo0AΔ204 abrB703 trpC2 pheA1*	P. Zuber (ZB369)
BD1202	*abrB703 trpC2 pheA1*	P. Zuber (ZB449)
BD1236	*com-38 leu metB5 hisA1*	13
BD1237	*com-530 leu metB5 hisA1*	13
BD1238	*com-524 leu metB5 hisA1*	13
BD1241	*com-138 leu metB5 hisA1*	13
BD1243	*com-124 leu metB5 hisA1*	13
BD1245	*com-413 leu metB5 hisA1*	13
BD1247	*com-518 leu metB5 hisA1*	13
BD1248	*com-12 leu metB5 hisA1*	13
BD1252	*com-210 leu metB5 hisA1*	13
BD1254	*com-39 leu metB5 hisA1*	13
BD1256	*com-412 leu metB5 hisA1*	13
BD1258	*com-56 leu metB5 hisA1*	13
BD1260	*com-107 leu metB5 hisA1*	13
BD1264	*com-530 spo0AΔ204 leu hisA1*	This work
BD1265	*com-138 spo0AΔ204 leu hisA1*	This work
BD1266	*com-124 spo0AΔ204 leu hisA1*	This work
BD1267	*com-413 spo0AΔ204 leu hisA1*	This work
BD1268	*com-518 spo0AΔ204 leu hisA1*	This work
BD1269	*com-12 spo0AΔ204 leu hisA1*	This work
BD1270	*com-210 spo0AΔ204 leu hisA1*	This work
BD1271	*com-39 spo0AΔ204 leu hisA1*	This work
BD1272	*com-412 spo0AΔ204 leu hisA1*	This work
BD1273	*com-56 spo0AΔ204 leu hisA1*	This work
BD1274	*com-107 spo0AΔ204 leu hisA1*	This work
BD1275	*com-530 spo0HΔHind leu hisA1*	This work
BD1276	*com-138 spo0HΔHind leu hisA1*	This work
BD1277	*com-124 spo0HΔHind leu hisA1*	This work
BD1278	*com-413 spo0HΔHind leu hisA1*	This work
BD1279	*com-518 spo0HΔHind leu hisA1*	This work
BD1280	*com-12 spo0HΔHind leu hisA1*	This work
BD1281	*com-210 spo0HΔHind leu hisA1*	This work
BD1282	*com-39 spo0HΔHind leu hisA1*	This work
BD1283	*com-412 spo0HΔHind leu hisA1*	This work
BD1284	*com-56 spo0HΔHind leu hisA1*	This work
BD1285	*com-107 spo0HΔHind leu hisA1*	This work
BD1286	*com-412 spo0AΔ204 abrB703 trpC2 pheA1*	This work
BD1287	*com-413 spo0AΔ204 abrB703 trpC2 pheA1*	This work
BD1288	*com-518 spo0AΔ204 abrB703 trpC2 pheA1*	This work

[a] The *spo0HΔHind*, *spo0BΔPst*, and *spo0FΔPst* deletion mutations were introduced by cleaving appropriate cloned fragments with *Hin*dIII or *Pst*I, trimming with S1 nuclease, and ligating. These constructions were carried out in the laboratory of I. Smith.

the wild-type allele of auxotrophic markers (1 μg/ml) for 30 min at 37°C, followed by plating on suitable selective media.

Media. Solid media were tryptose blood agar base (TBAB; Difco Laboratories) and minimal medium (1) with appropriate growth supplements. Liquid media were VY (25 g of veal infusion [Difco], 5 g of yeast extract [Difco], 1,000 ml of water), LB broth (10 g of tryptone [Difco], 5 g of yeast extract [Difco], 5 g of NaCl, 1,000 ml of water), and competence medium (7) for growth of phage and recipient cultures in transduction experiments or growth to competence.

β-Galactosidase determination. Samples were taken at the indicated times during growth, and β-galactosidase was determined as previously described (11). β-Galactosidase activities are expressed as units per milligram of protein. Protein was determined from standard curves relating turbidity (Klett units) and protein concentration in the bacterial cultures. To construct these curves, protein was measured with Bio-Rad reagent by following manufacturer instructions.

Resolution in Renografin gradients. Competent cultures were resolved in Renografin gradients as described by Cahn and Fox (3) and modified by Joenje et al. (15).

RESULTS

Competence and β-galactosidase activity in the *com*⁺ strain. The kinetics of competence development in IS75, a *com*$^+$ strain, is shown in Fig. 1. Competence developed late in growth and reached a maximum during the stationary phase, about 2 h after the end of exponential growth. In many experiments an earlier and weaker wave of competence was detected (Fig. 1). *B. subtilis* 168 strains exhibited weak β-galactosidase activity that increased during the stationary phase (Fig. 1). This activity was repressed by growth in glucose-containing media.

***com* gene-related β-galactosidase expression in competence medium.** Figure 2 shows the β-galactosidase specific activities of a representative set of *com* mutants as a function of growth. These mutants were isolated (13) after mutagenesis with Tn*917lacZ* (29). This transposon carries a promoterless copy of *lacZ* situated so that *lacZ* expression can be placed under the control of a promoter extrinsic to the transposon. The sole group II mutant does not produce β-galactosidase above the wild-type level and was omitted from this study.

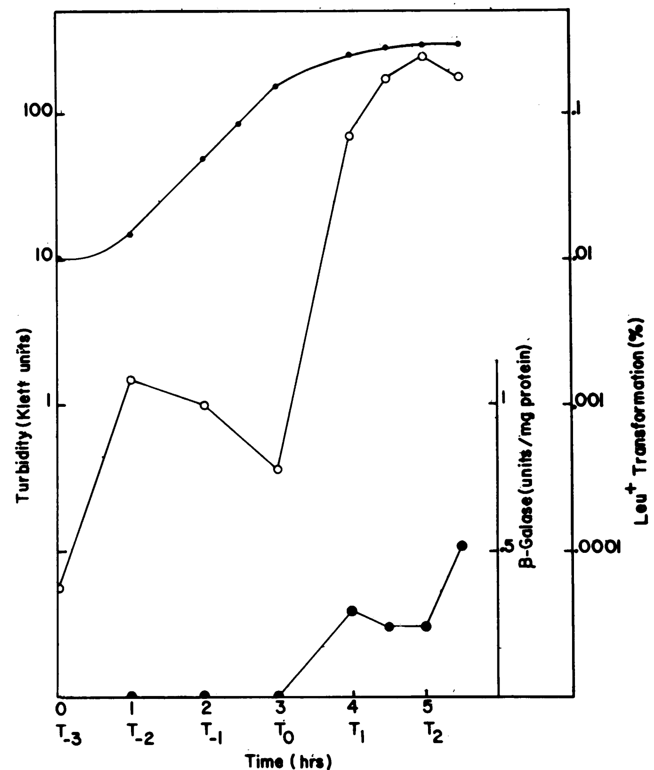

FIG. 1. Development of competence and expression of β-galactosidase activity in a *com*$^+$ strain. IS75 was grown in competence medium. Turbidity readings (•) and measurements of competence (○) and β-galactosidase activity (●) were carried out on samples withdrawn at the indicated times. t_0 is defined as the time of departure from exponential growth. Turbidity and competence (expressed as percent Leu$^+$ transformation) are plotted on logarithmic scales, and enzyme activity is on a linear scale.

Group VII mutants display abberrant colony properties and are difficult to collect by centrifugation; they were also omitted. At least one representative of each of the remaining six groups is shown in Fig. 2. Several members of groups IV and VI were included in this study since we suspected that each of these groups represents more than one gene.

The sole group I representative that expressed β-galactosidase (*com-530*) displayed low enzyme activity until after t_0, at which point the activity increased. Of the five group III mutants, only two (*com-124* and *com*-138) expressed enzyme activities above the wild-type level. In repeated experiments, the *com-138* mutant enzyme activity was seen to rise severalfold after t_{-2} and to remain constant after t_{-1} to t_0. The *com-124* mutant exhibited a gradual increase in β-galactosidase activity, beginning at about t_{-1} and reaching a constant level at t_1. The four group IV mutants produced little enzyme activity before t_1. Following this time, these mutants exhibited a burst of enzyme synthesis, with *com-518* apparently increasing earliest. The group V mutant (*com-12*) exhibited a dramatic increase in activity, beginning between t_1 and t_2. Finally, the various group VI mutants (*com-412*, *com-107*, *com-39*, and *com-56*) behaved similarly, increasing in activity from a low basal level beginning at t_1 to $t_{1.5}$. The relative extents of enzyme synthesis in these various group VI strains were characteristic and reproducible. In several experiments, *com-39*, *com-12*, and *com-412*, which reached the highest β-galactosidase levels, displayed a transient burst of enzyme synthesis, peaking at about t_{-1} to t_0. This may correspond to the earlier minor wave of competence shown by the wild-type strain (IS75) (Fig. 1). We conclude that expression of β-galactosidase in these mutants is likely under the control of *com* promoters, that the *com* genes are probably controlled transcriptionally in a growth stage-specific manner, and that some are expressed to a greater extent than others. The genes represented by *com-138* and *com-124* are expressed early in the growth cycle but exhibit characteristically different levels of expression. Most of the remaining genes appear to turn on at about t_0 to t_1. The gene represented by *com-56* seems to be expressed late (about $t_{1.5}$). Based on these differences in time of expression and on the various extents of expression, it seems likely that groups III, IV, and VI each include several *com* genes.

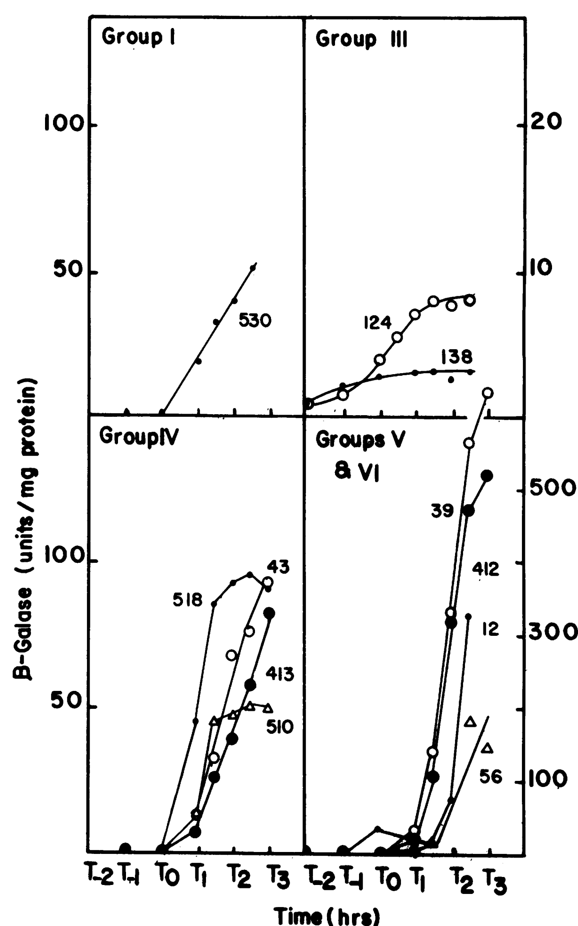

FIG. 2. Expression of β-galactosidase activity in *com* mutants. The strains were grown in competence medium, and samples were withdrawn at the indicated times for determination of β-galactosidase specific activity. Results for group I and IV strains are plotted on the left-hand scale. Those for group V and VI strains are plotted on the right-hand scale. The *com-124* and *com-138* data are plotted on the right and left scales, respectively.

Cell type-specific expression of *com* genes. The mutant strains that produced a level of β-galactosidase in excess of the endogenous activity of *B. subtilis* were grown through the competence regimen and tested for their resolution into two density fractions of Renografin gradients. As reported elsewhere (13), all of the strains but *com-162*, *com-194*, *com-540*, *com-543*, *com-124*, *com-138*, *com-145*, and *com-12* were observed visually to resolve into two density fractions in a manner indistinguishable from that of the wild-type strain. The material from the group VII strains (*com-162* and *com-194*) was distributed throughout Renografin gradient and failed to resolve into distinct bands. The other strains that failed to resolve in Renografin gradients (groups III and V) exhibited a single sharp band indistinguishable in location and appearance from the heavy (noncompetent) band of the wild-type strain. The light and heavy fractions were recovered from those strains that appeared to resolve normally in Renografin, and the amounts of total protein in each fraction were determined together with the β-galactosidase activities (Table 2). In each of these mutants, as in the wild-type strain, there was roughly 10-fold more protein in the heavy cell fraction, confirming the visual impression that the distribution of cellular material was normal in these strains. In addition, the specific activities of β-galactosidase in these mutant strains were about 10-fold higher in the light fraction than in the heavy fraction. Thus, most of the mutants not only resolved into light and heavy buoyant density fractions but expressed β-galactosidase in a cell type-dependent manner. It is likely that at least some of the enzyme activity detected in the bottom fractions represented trapping of competent (light fraction) cells, so the differences noted in Table 2 are probably underestimates.

Expression of *com* genes during growth in various media. The results presented so far demonstrated the cell type and growth stage-specific expression of most of the *com* genes. These experiments were carried out with the mutant strains grown through the one-step competence regimen. To determine whether expression of β-galactosidase in the *com* strains is growth medium dependent, they were grown in LB broth with or without addition of 0.5% glucose, and samples were withdrawn during exponential growth and the stationary phase. Expression of *com-530* (group I), *com-518* (group IV), *com-12* (group V), and *com-107* and *com-39* (group VI) in these media was approximately equal to that of the endogenous *B. subtilis* β-galactosidase expressed by the isogenic *com*$^+$ strain IS75 (data not shown). The latter was

TABLE 2. Resolution of mutant strains in Renografin gradients and expression of *com* genes

Strain	Fraction[a]	Protein (mg)	β-Galactosidase (U/mg of protein)
Com⁺	T	0.007	ND[b]
	B	0.108	1.0
com-39	T	0.012	2,190
	B	0.115	203
com-210	T	0.007	2,560
	B	0.096	245
com-412	T	0.016	1,589
	B	0.108	221
com-56	T	0.016	977
	B	0.100	208
com-43	T	0.018	413
	B	0.164	45
com-413	T	0.025	297
	B	0.152	48
com-510	T	0.020	227
	B	0.147	48
com-518	T	0.018	517
	B	0.120	96
com-530	T	0.012	132
	B	0.110	14
com-107	T	0.009	1,844
	B	0.108	226

[a] T and B refer to the top (light) and bottom (heavy) fractions in a Renografin gradient.
[b] ND, Not detectable.

elevated to 1 to 3 U/mg of protein in LB broth without glucose and remained at about 0.2 to 0.5 U/mg of protein in medium with glucose. However, expression of *com-124* and *com-138* (group III) was the same in LB broth as it was in competence medium and remained at a constant specific activity after t_1. No obvious effect of glucose was noted. In LB broth without added carbohydrate, the specific activity reached appeared to be about half of that achieved in the presence of glucose. Thus, expression of all of the *com* genes but those in group III appeared to be minimal in LB broth. The genes represented by the latter group were expressed in either medium. They were also distinguished by their earlier turn on during growth when compared with the other genes (Fig. 2). The level of competence achieved by the wild-type strain grown in LB broth with or without glucose was about 10^6-fold lower than that reached in competence medium.

The influence of growth medium on the expression of *com* genes was examined further by varying the carbohydrate present in competence medium. The wild-type strain (IS75) and the mutant strains were grown in competence medium containing glucose (0.028 M), glycerol (0.028 M), or no added carbohydrate. These media were tested for their effects on β-galactosidase production and on the level of competence reached by the wild-type strain. The growth curves in glucose and in glycerol were essentially indistinguishable. The cultures grown in the absence of added carbohydrate grew somewhat slower and reached stationary phase at a final density about three- to four-fold lower than when glucose or glycerol was added. The maximum transformation frequencies for the *leu* marker were 0.07, 0.006, and 0.003% in glucose, glycerol, and no carbohydrate, respectively. The results of the enzyme determinations are shown in Fig. 3. Growth in glycerol depressed the enzyme levels of *com-530*, *com-518*, *com-107*, and *com-39* compared with expression in glucose-competence medium. The effects on *com-12* and *com-530* were less marked and were detected as a delay in expression. Expression of *com-138* and *com-124* was unaffected by the presence of glycerol instead of glucose. When carbohydrate was omitted from the growth medium, expression of β-galactosidase by *com-107*, *com-530*, *com-518*, *com-39*, and *com-12* was markedly reduced, while that by *com-138* and *com-124* was somewhat less affected.

Igo and Losick (13a) have reported that expression of the σ^{37}-dependent *B. subtilis* gene *ctc* was stimulated by growth in glucose, although in the case of *ctc* the effect was manifested in LB broth. These authors suggested that this behavior is due to inhibition of expression of *ctc* by a tricarboxylic acid cycle intermediate. In support of this, the stimulatory effect of glucose is augmented by the presence of glutamine (13a), since these two compounds work synergistically to repress tricarboxylic acid cycle enzymes (23). Also, several mutants with lesions in tricarboxylic acid cycle enzymes express *ctc* at an elevated level in the absence of glucose. We tested the effects of both of these conditions on the expression of *com* genes. The mutants were grown in competence medium containing glucose (0.028 M) plus glu-

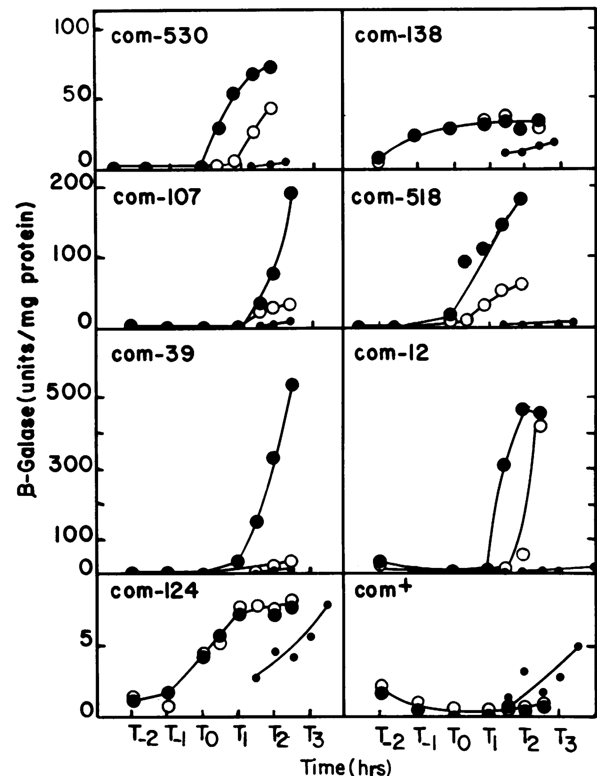

FIG. 3. Effect of carbohydrate supplement on expression of β-galactosidase activity by *com* mutant strains. The mutant and isogenic *com*⁺ strains were grown in competence medium containing 0.028 M glucose (●) or 0.028 M glycerol (○) or with no added carbohydrate (•). Samples were withdrawn at the indicated times to determine β-galactosidase specific activity.

tamine (0.014 M). No effect of glutamine on growth was noted. However, expression of β-galactosidase was inhibited by glutamine in all of the mutants but *com-138* and *com-124* to about the same extent as that effected by growth in competence medium plus glycerol (data not shown). We have no explanation for this effect. In addition, we constructed strains carrying *citB75* as well as the various *com* mutations. Strains carrying *citB75* are deficient in aconitase (20). Expression of β-galactosidase in these strains was the same as in the isogenic *cit*$^+$ strains (data not shown). Thus, regulation of our *com* genes in response to the nutritional environment was different from that of *ctc*.

Transformation efficiency in *spo0A*, *abrB*, and *sigB*::Cm^r backgrounds. It has been reported that certain *spo0* mutants exhibit diminished competence (19, 21, 24). To investigate this further, we constructed an isogenic set of *spo0* mutants in the YB886 background. These were tested for transformability (Table 3). Only the *spo0A*, *spo0G*, and *spo0H* mutations exhibited greater than a fivefold decrease in transformation frequency. However, the *spo0G14* mutation has been shown to be an allele of *spo0A* (cited in reference 16). Table 3 shows that the *spo0A*Δ*204* mutation reduced the transformation frequency about 1,000-fold compared with isogenic Spo$^+$ parent and about 28-fold more than did the *spo0A12* mutation. The *spo0H* deletion had a lesser effect, reducing transformation about 16-fold. We tested the resolution in Renografin of *spo0A*Δ*204* and *spo0H*Δ*Hin*d cells grown through the competence regimen. Neither strain yielded material with the density of light (competent) cells. Both exhibited bands with the buoyant density of heavy (noncompetent) cells.

abrB mutations are partial suppressors of *spo0A* mutants. *abrB spo0A* strains regain the ability to produce protease and antibiotic and exhibit restored competence but fail to sporulate (25, 26). The effect of *abrB* on competence is shown in Table 3. The *abrB703* mutation increased the transformability of a strain carrying *spo0A*Δ*204* about 30-fold. Interestingly, a strain carrying *abrB703* alone exhibited this same level of competence, which was about 30-fold lower than that of the isogenic wild-type strain.

Deletion mutants of *spo0F* and *spo0B* were hardly affected

TABLE 3. Transformation of *spo0*, *abrB*, and *sigB* mutants

Genotype	Transformation[a]
hisA1 leu metB5	1.0 (0.2%)
hisA1 leu metB5 sigB::Cm^r	1.0
hisA1 leu metB5 spo0F Δ*Pst*	4×10^{-1}
trpC2	1.0 (2.5×10^{-2}%)
trpC2 sigB::Cm^r	1.0
trpC2 pheA1	1.0 (0.1%)
*trpC2 pheA1 spo0A*Δ*204 abrB703*	3×10^{-2}
trpC2 pheA1 abrB703	3×10^{-2}
*trpC2 pheA1 spo0A*Δ*204*	1×10^{-3}
trpC2 metB5 xin-1 SPβ$^-$	1.0 (0.48%)
trpC2 xin-1 SPβ$^-$ *spo0A12*	2.8×10^{-2}
trpC2 xin-1 SPβ$^-$ *spo0B*Δ*Pst*	4.2×10^{-1}
trpC2 xin-1 SPβ$^-$ *spo0D8*	2.1×10^{-1}
trpC2 xin-1 SPβ$^-$ *spo0E11*	3.8×10^{-1}
trpC2 xin-1 SPβ$^-$ *spo0F221*	3.5×10^{-1}
trpC2 xin-1 SPβ$^-$ *spo0G14*	8×10^{-3}
trpC2 xin-1 SPβ$^-$ *spo0H*Δ*Hin*d	6.4×10^{-2}
trpC2 xin-1 SPβ$^-$ *spo0J87*	4.8×10^{-1}

[a] Transformation is expressed relative to isogenic and wild-type strains. Transformation was with prototrophic chromosomal DNA at a concentration of 1 μg/ml. Selection was for Leu$^+$, Met$^+$, or Trp$^+$. The number in parentheses gives the absolute transformation frequency of the wild-type member of each isogenic set.

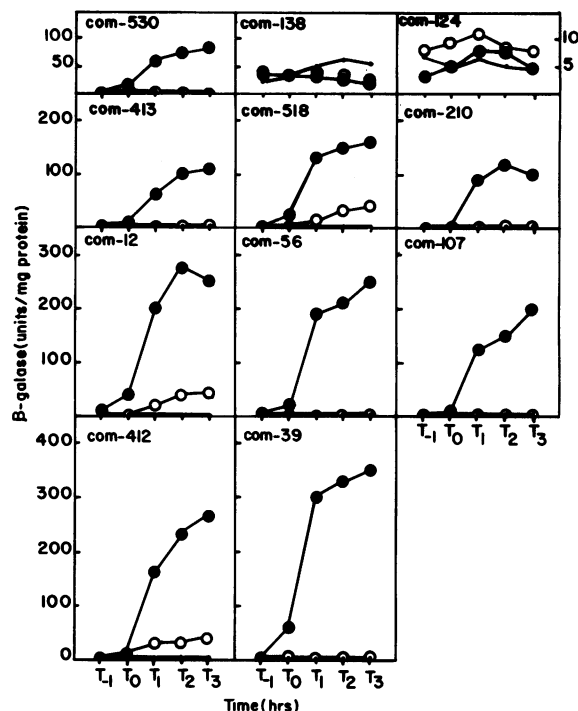

FIG. 4. Expression of *com* mutant genes in *spo0A* and *spo0H* backgrounds. The strains were grown in competence medium, and samples were withdrawn at the indicated times for determination of β-galactosidase specific activity. The various *com* mutants were assayed in the *spo0*$^+$ (●), *spo0A*Δ*204* (•), and *spo0H*Δ*Hin*d (○) backgrounds. The values for *com-124* are presented on the right-hand scale.

in their competence. *spo0D* has been found to be an allele of *spo0B* (cited in reference 16), and we appropriately observed that competence is only slightly affected in the *spo0D* background. *spo0E* and *spo0J* mutants were also affected only slightly, but null mutants in these loci were not available.

Finally, we examined the effect of a null mutation in *sigB*, the structural gene for σ^{37} (2). This mutation was constructed by inserting a chloramphenicol resistance element into *sigB* (R. Losick, personal communication). Because transformability in the *trpC2* background was relatively poor, we transferred the *sigB*::Cm^r mutation into the BD630 background and again assayed for competence. In both cases, the *sigB* mutation had no detectable effect on competence.

Dependence of *com* gene expression on *spo0A* and *spo0H*. Since *spo0A* and *spo0H* mutants exhibited striking competence deficiencies, we tested the effects of the *spo0A* and *spo0H* deletion mutations on the expression of β-galactosidase in representative *com* mutants which express β-galactosidase as a result of insertion downstream from presumptive *com* gene promoters. Several *com* mutations were moved by transduction into the *spo0A*Δ*204* and *spo0H*Δ*Hin*d strains. The resulting double mutants were grown through the one-step competence regimen, and β-galactosidase activity was measured at selected times (Fig. 4). Expression of β-galactosidase activity in group III (*com-124* and *com-138*) mutants was not significantly affected by the *spo0* mutations. Expression of all of the other *com* genes was strongly decreased in both the *spo0A* and *spo0H* mutant backgrounds. The genes represented by *com-518* (group IV), *com-12* (group V), and *com-412* (group

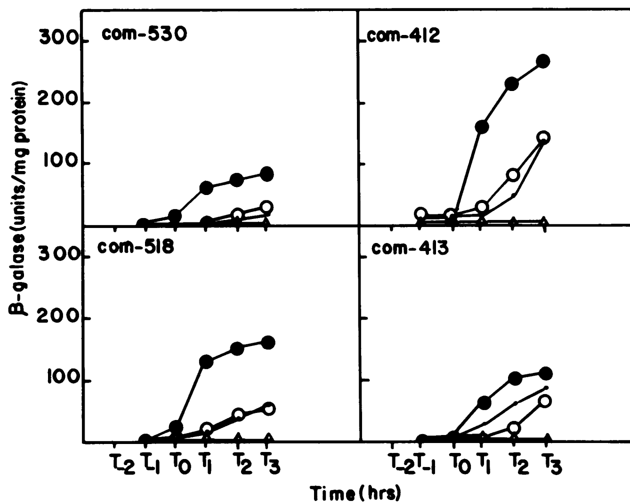

FIG. 5. Effect of the *abrB703* mutation on expression of *com* mutant genes. Several *com* mutations were placed in the *spo0A*$^+$ *abrB*$^+$ (●), *spo0A*$^+$ *abrB703* (○), *spo0A*Δ*204* *abrB*$^+$ (Δ), and *spo0A*Δ*204* *abrB703* (•) backgrounds. The strains were grown in competence medium, and samples were withdrawn at the indicated times for determination of β-galactosidase specific activities.

VI) were less strikingly affected by *spo0H*Δ*Hin*d than by *spo0A*Δ*204*. The residual levels of β-galactosidase activity in these *spo0H* mutant backgrounds seemed to increase concomitantly with growth, as in the isogenic *spo0*$^+$ strains. The difference in the effects of *spo0H*Δ*Hin*d on the two group IV strains (*com-413* and *com-518*) suggests that these mutations may reside in different genes. This is consistent with the results of DNA binding and uptake measurements, which also suggest that these represent different genes (13). The *sigB*::Cm^r mutation was introduced by transduction into the strains carrying *com-518*, *com-12*, and *com-412* plus *spo0H*Δ*Hin*d. *sigB*::Cm^r had no detectable effect on the residual β-galactosidase expressed by these strains (data not shown). We conclude from these experiments and those described above that σ^{37} plays no important role in the development of competence.

As noted above, *abrB703* partially suppressed the decreased competence noted in the *spo0A*Δ*204* background. We moved selected *com* mutations into *abrB703* and *abrB703 spoOA*Δ*204* background and measured the effect of this suppressor mutation on expression of *com*-associated β-galactosidase. *abrB703* raised β-galactosidase expression in *spo0A* mutant strain to the level observed in the strain carrying *abrB703* alone (Fig. 5). This level is about fivefold lower than that observed in the *spo0A*$^+$ *abrB*$^+$ strain. Thus, the effects of *abrB703* on β-galactosidase expression qualitatively parallel its effects on competence. β-Galactosidase expression in the *abrB703* strains showed the same growth stage dependence noted in the *abrB*$^+$ backgrounds.

DISCUSSION

Competence in *B. subtilis* is developmentally regulated and is dependent on specific nutritional signals. Thus, as shown in Fig. 1, competence develops late in growth, reaching a maximum at about t_2. In addition, competence fails to develop in complex media such as LB broth, and glucose is required for maximal development of competence in competence medium. The use of Tn*917lacZ* as a mutagen to generate *com* mutants provided us with a useful tool to monitor the expression of *com* genes as a function of growth stage, medium, and genetic background. In general, we have regarded β-galactosidase as an appropriate marker for the expression of our *com* genes. In those cases in which expression is stage, cell type, and competence medium specific, this appears to be a strong assumption. However, particularly in the cases of *com-124* and *com-138*, our conclusions must be regarded as tentative. For instance, the low-level expression throughout growth noted in these mutants might be explained by readthrough transcription into a Tn*917lac* element inserted in the antisense orientation and might be unrelated to competence.

β-Galactosidase expression by each of the *com* mutants occurred at a characteristic time in competence medium. For most of the mutants, this was at t_0 to $t_{1.5}$, corresponding to the time during which competence was developing. The group III mutants *com-124* and *com-138* expressed β-galactosidase activity earlier (t_{-2} to t_{-1}). It is interesting in this regard that the rates of DNA and stable RNA synthesis decrease in cells destined for competence several hours before the peak of competence occurs (5). Also consistent with early expression of the gene(s) corresponding to *com-124* and *com-138* is the observation that these mutations prevent the development of two cell types during growth in competence medium. The latter is a relatively early event in competence development, occurring shortly after precompetence physiological changes alluded to above (5). It is clear that the various *com* genes are turned on in a reproducible and characteristic temporal sequence, preceding the appearance of the wave of maximal competence. Since Tn*917lacZ* results in the formation of operon fusions, this temporal control is probably exerted transcriptionally.

The correspondence of *com* gene expression and competence development was also revealed by the cell type-specific expression of most of the strains tested. Of the mutants that resolve into two cell types, expression was markedly greater in the light than in the heavy buoyant density cell fractions. Again, we conclude that the factors resulting in this cell-specific expression probably exert their controlling effects on the level of transcription.

Further correspondence is shown by the parallel dependencies of competence and β-galactosidase expression on the growth medium and particularly on the carbohydrate supplement. Both competence and β-galactosidase synthesis are maximal in glucose-containing media. Glucose is not a sufficient signal since addition of this sugar to LB broth failed to elicit either competence or increased β-galactosidase synthesis. Regulation of the competence genes that respond in this fashion is distinct from that of *ctc* since the latter is expressed in *cit* mutants in the absence of glucose (13a), while the *com* genes are not. The only *com* mutants that failed to respond to the nutritional environment were *com-124* and *com-138*, both of which were turned on early during growth in all of the media tested. Since the genes corresponding to these two mutations are expressed early and irrespective of the medium, it is possible that they are required for events in competence development that precede the transduction of environmental signals into an appropriate competence-specific intracellular response or are involved in mediating that response. Thus, these genes may play a role in competence development analogous to that of certain *spo0* genes in sporulation.

We tested the dependencies of competence development on several *spo0* genes and *sigB*. From results obtained with the *sigB*::Cm^r mutant, we conclude that σ^{37} is not required for competence development. Our results concerning the

effects of *spo0* mutants on competence differ from those reported by other workers. Ionesco et al. (14) and Sadaie and Kada (21) have reported that *spo0G14* strains exhibited normal competence. Our finding that the *spo0G* strain is transformation deficient seems to be consistent with the conclusion that this mutation is an allele of *spo0A* (cited in reference 16). It is possible that certain *spo0G* strains have accumulated *abr* mutations, leading to suppression of the competence deficiency. In addition, Sadaie and Kada (21) have reported that mutants with lesions in *spo0B*, *spo0D*, *spo0E*, and *spo0F* are markedly competence deficient. In the case of *spo0D*, we and Sadaie and Kada are using what is nominally the same mutation. *spo0D* mutations are apparently alleles of *spo0B* (cited in reference 16), so our respective laboratories are at least consistent in the results obtained. In the case of *spo0E*, our laboratories are using different mutations, and this may account for the discrepancy. This explanation is unlikely in the cases of *spo0B* and *spo0F* since in the present study we used deletion mutations in each of these loci. We agree with Sadaie and Kado in the cases of *spo0A* and *spo0H*. Competence was depressed about 10^3-fold and 16-fold, respectively, in these two mutant backgrounds. We noted above that the *spo0A* and *spo0H* mutant strains failed to resolve into two buoyant density fractions on Renografin gradients. If even a single *com* gene that is required for Renografin resolution is dependent for its expression on *spo0A* and *spo0H*, we would have a sufficient explanation for this finding. The gene represented by *com-12* fits this description (13).

spo0A is involved in the control of sporulation and competence and in the synthesis of several products that are elaborated during the stationary phase. It has been suggested that the *spo0A* product must interact with the products of *spo0B*, *spo0F*, and *spo0E* to fulfill its necessary function during sporulation (10). Since the latter three genes do not appear to be absolutely required for competence development, the *spo0A* product can apparently function in this process without such interaction. All of the *com* genes tested, except those represented by *com-124* and *com-138*, require *spo0A* for expression. This is consistent with the dependence of competence development on *spo0A*.

The nonsporulation functions of *spo0A* (including competence) can be suppressed by *abrB* mutations (25). We have shown here that this suppression of competence is partial, that similar partial suppression of the expression of several *com* genes occurs, and that the *abrB703* mutation alone depresses competence markedly. It is also worth noting that the temporal dependencies of *com* gene expression are not altered in the *abrB* background, unlike expression of the *B. brevis* antibiotic gene in *B. subtilis* (18). Marahiel et al. (18) have proposed that *abrB* acts as a repressor of several late-growth-associated functions and that the *spo0A* product is required to reverse this repression. The observations just quoted suggest that, in this simple form, the repressor model is not directly applicable to the case of competence.

spo0H has been shown to be the probable structural gene for a sigma factor (σ^{30}) (4, 6; J. Weir, I. Smith, H. L. Carter, and C. P. Moran, personal communication). σ^{30} is required for sporulation and full expression of serine protease (9; I. Smith, personal communication). In this study we have demonstrated that it is also required for development of competence and expression of several *com* genes. Our results do not demonstrate that σ^{30} is directly involved in the transcription of any of our *com* genes. The work of Carter and Moran (4) and Dubnau et al. (6) has demonstrated that a functional *spo0A* copy is required for expression of *spo0H*. It is not likely that the entire dependence of competence development on *spo0A* is due to this requirement since the effect of a *spo0A* null mutant on competence is greater than that of a *spo0H* null mutant.

The results presented in this study justify the assertion that development of competence involves a genetically programmed and temporally orchestrated series of events that involve specific responses to environmental signals and are subject to controls that intersect with those for sporulation and the synthesis of certain late-growth-associated products. In addition, some overlap may be present between regulation of competence and that of the SOS system since expression of *B. subtilis din* genes is activated during the development of competence (17). We found, however, using β-galactosidase indicator plates, that mitomycin C, an inducer of *din* functions, did not turn on any of our *com* genes (data not shown). Thus, the interaction between these systems is apparently not symmetric.

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