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Growth Medium-Independent Genetic Competence Mutants of *Bacillus subtilis*

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The development of competence in *Bacillus subtilis* is normally dependent on the growth medium. Expression of late competence genes occurs in glucose-minimal salts-based media but not in complex media. Expression is also inhibited when glutamine is added to competence medium and when glycerol is substituted for glucose. Mutations have been identified in two regulatory loci, *mecA* and *mecB*, which render competence development independent of these variables. Although in *mec* mutants the expression of late competence genes, as well as of competence itself, occurred in all media tested, this expression was still growth stage regulated. Thus at least some forms of medium-dependent and growth stage-specific regulation are genetically separable. One of the *mecB* mutations (*mecB31*) conferred oligosporogenicity. The *mecB* mutations were tightly linked by transformation to *rif*, *lpm*, and *std* markers and were located between *rif-2103* and *cysA14*. The *mecA42* mutant was linked by transduction to *argC4*.

The development of competence for transformation in *Bacillus subtilis* is subject to several controls (reviewed in reference 7). First, competence develops only in glucose-minimal salts medium and not in complex media. Second, competence appears after the time of transition from the exponential to the stationary phase of growth (T_0). Finally, maximally competent cultures can be resolved on density gradients into noncompetent and competent cell fractions (5, 15). Since these two populations of cells are physiologically distinct, it appears that the development of competence involves the nonterminal differentiation of the culture into two cell types.

Genetic competence in *Bacillus subtilis* depends on the expression of several gene products that mediate the binding, processing, and uptake of transforming DNA. The synthesis of these late products is dependent on other, regulatory proteins that are encoded by early competence (*com*) genes. The early genes are expressed throughout growth and in all growth media. The expression of late genes accurately mirrors the regulation of competence described above. These genes are not expressed in complex media and are turned on after T_0 and only in the competent cell fraction. The signals involved in these regulatory phenomena are poorly understood. Also not clear is the extent to which the three modes of regulation are interrelated and the roles of the known early gene products in each of these regulatory processes.

Among the known early *com* genes are several that are considered to be components of signal transduction pathways. The amino acid sequences of their gene products are similar to those of other members of the bacterial two-component regulatory systems (reviewed in reference 34). These genes include *comA* (14, 37), *degU* (19, 23, 36), and *spo0A* (22), which resemble so-called effector or response regulator proteins. The activities of effector proteins are generally regulated by interaction with a second class of proteins, known as sensors, or signal transducers. The transfer of phosphate groups between the members of each sensor-effector pair is thought to mediate this regulatory event, in response to some pertinent signal detected by the sensor. *comP* is an essential competence gene that appears to encode the cognate sensor for *comA* (38), while *degS* (19, 23) encodes a sensor that probably works together with the product of *degU*. Other regulatory genes that are required for the development of competence include *spo0H* (3, 11), *sin* (12), and *abrB* (16, 27). These specify known DNA-binding proteins and may be involved in the transcriptional control of competence.

To investigate the nature of the nutritional control of competence and to explore its relationship to growth stage-dependent control, we have isolated several mutations that render the development of competence independent of the growth medium. We have genetically mapped some of these mutations and have used them to demonstrate that the growth stage and nutritional controls can be genetically separated under at least some conditions.

MATERIALS AND METHODS

Strains. All strains used in this study were derivatives of *B. subtilis* 168. The strain used for mutagenesis was constructed by transducing DB48 with PBS1 grown on a donor carrying the Tn*917lacZ* fusion mutation *comG12* (1, 2). DB48 was obtained from C. Price and carries the mutations *acf-2*, *aroD120*, and *dnaE20*. Selection was for the erythromycin resistance (Em^r) marker carried by Tn*917lacZ*. An Em^r $AroD^-$ transductant that had lost the *comG*-linked temperature-sensitive *dnaE20* mutation was selected and named BD1444. In addition, some experiments were carried out with strains constructed in the BD630 (*hisA1 leu-8 metB5*) background, as described in Results. Strains DR1010 (*rif-2103 std-10*) and LS1051 (*rif-2103 lpm-105 purB6 metB5 leuA8*) were obtained from A. L. Sonenshein.

Media. The liquid media were competence medium (CM) (3), VY broth (25 g of veal infusion [Difco Laboratories], 5 g of yeast extract [Difco], 1,000 ml of water), or L broth. The solid media were either tryptose blood agar base (TBAB; Difco) or minimal medium (4). When required, nutritional supplements were added to 50 μg/ml. Erythromycin and chloramphenicol were added to 5 μg/ml as required. Lipiarmycin, streptolydigin, and rifampin were added to 12, 100, and 2 μg/ml, respectively, for the scoring and selection of

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recombinants. Rifampin was used at 5 μg/ml for estimating mutation frequencies.

Mutagenesis. Strain BD1444 was grown to stationary phase in VY broth, washed once in minimal salts (4), and then suspended to the original volume in minimal salts. A 2-ml portion of this suspension was mixed with 30 μg of ethyl methanesulfonate per ml and incubated with shaking at 32°C for 30 min. The treated cells were washed twice by centrifugation and resuspended to 2 ml in VY broth. Samples of 0.05 ml were inoculated into 10 tubes, each containing 5 ml of VY broth, and incubated overnight at 32°C. To each culture was added 0.5 ml of glycerol, and the cultures were frozen in 1.0-ml volumes. Testing one such sample revealed the presence of 7.3×10^3 CFU/ml, capable of growth on 5 μg of rifampin per ml. An identically grown control, untreated with mutagen, yielded 5 Rif^r mutants per ml. The total CFU per milliliter in the two cultures were 4×10^8 and 5×10^8, respectively.

Transformation and transduction. Competent cells were prepared by the one-step protocol using CM and transformed as described previously (3). When competence development in VY or L broth was tested, samples were washed and suspended in CM immediately before the addition of transforming DNA. Transduction with bacteriophage PBS1 was carried out as described elsewhere (8).

Test for sporulation. Strains were grown in nutrient sporulation medium (30) with and without 2% glucose for 24 h at 37°C. Dilutions were made for viable counts, and the samples were plated on TBAB before and after being heated at 80°C for 20 min.

Determination of β-galactosidase activity. Cultures were grown by the one-step competence protocol, and samples were withdrawn at various times. The samples were assayed for β-galactosidase activity as described previously (13) and for protein with the Bio-Rad reagent as specified by the manufacturer. The results were expressed as units of β-galactosidase per milligram of protein. β-Galactosidase was detected on plates either by including 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) at 80 μg/ml in the solid medium or by spraying colonies with a solution containing 10 mg of 4-methylumbelliferyl-β-D-galactoside (MUG) per ml in dimethyl sulfoxide (39).

RESULTS

Isolation of medium-independent mutants. To identify new loci involved in the regulation of competence, it was decided to isolate mutants capable of expressing a late competence gene in complex medium. For this purpose, the Tn*917lacZ* fusion mutation *comG12* (3, 16) was introduced by transduction with PBS1 into a strain carrying *aroD120* to yield BD1444. The *aroD120* marker was included to facilitate subsequent genetic manipulations (see below). BD1444 was mutagenized as described in Materials and Methods and plated on TBAB after growth in liquid medium to permit segregation. Strains carrying the *comG-lacZ* fusion will ordinarily express β-galactosidase only on glucose-minimal salts-based media. The TBAB plates were sprayed with MUG and examined for the presence of fluorescent colonies. The frequency of these mutants, able to express β-galactosidase from the *comG* fusion on TBAB, was about 5×10^{-4}. Fifteen fluorescent colonies, each derived from a culture grown separately after mutagenesis or exhibiting clearly different levels of fluorescence, were selected for further study.

Although it was desired to isolate mutants with lesions in new regulatory loci, two less interesting types of mutations could in principle give rise to the phenotype observed. First, mutations may have occurred that increased the activity of the weak *B. subtilis* endogenous β-galactosidase (3). Second, *cis*-acting mutations may have augmented *comG* expression. To exclude the first category, the mutant clones were transduced by using PBS1 with selection for aro^+. The *comG* operon is about 30% linked by transduction to *aroD* (16). This selection should therefore result in the loss of the *comG12* Tn*917lacZ* element and consequently result in the conversion of about a third of the transductants to Em^s. The latter transductant class should continue to fluoresce on MUG-sprayed TBAB plates only if the mutation has resulted in an increase of the endogenous β-galactosidase activity. By this criterion, 2 of the 15 mutants tested exhibited increased endogenous activity. To determine whether any of the remaining 13 mutant strains possessed *cis*-acting *comG* mutations, the *comG12* fusion was reintroduced into the Em^s transductant strains by PBS1 transduction, and the fluorescent behavior of the transductants on TBAB-MUG was examined. Twelve of the strains expressed β-galactosidase on TBAB and thus appeared to carry mutations that were not closely linked to *comG12*. The 13th strain was difficult to score consistently because the level of fluorescence was low and variable. On the basis of these tests, it was tentatively concluded that at least 12 independent mutations had been isolated that acted to elevate the level of *comG* transcription on TBAB plates and which were not closely linked to the *comG* locus. This conclusion was supported by subsequent experiments, described below. The mutations were designated *mec* (for medium-independent expression of competence).

Genetic mapping of *mec-42*. To determine the map positions of several of the *mec* mutations, the mapping kit described by Dedonder et al. (6) was used. To permit the scoring of the Mec^- phenotype, the *comG12-lacZ* fusion was introduced into each member of the mapping kit by PBS1 transduction, with selection for Em^r. These strains were then used as recipients for transduction by PBS1 lysates grown on *mec* strains, with selection for the various markers carried by the recipient strains. Transductants were then picked and scored for their Mec^- phenotypes on L broth plates containing X-Gal.

mec-42 was found to be 22% linked to the *glyB133* marker and 15% to *metC3*. This indicated that *mec-42* was roughly equidistant between *glyB* and *metC* at about 105° on the *B. subtilis* map (28). To confirm this location, the transductional linkage of *mec-42* to *argC* was measured, using as a recipient BD1548 (*argC4 hisA1 pyr-1 comG12*:Cm^r). This strain carried an intact *comG* operon, as well as a disrupted copy of *comG*, and had been constructed by Campbell-like integration of a plasmid carrying *comG12* (2). Selection for $argC^+$ revealed 81% linkage to *mec-42*. None of four other *mec* mutants (*mec-13*, *-23*, *-31*, and *-52*) exhibited linkage when tested for cotransduction with *glyB*, *metC*, or *argC*. *mec-42* defines a new competence-regulatory locus, which was named *mecA*.

Genetic mapping of *mec-23*, *mec-31*, and *mec-52*. *mec-23* and *mec-31* were found to be closely linked (>90%) by transduction to *cysA14*. In a single experiment, *mec-52* was also found to be 86% linked to *cysA14* and 6% to *purA16*. A series of three-, four-, five-, and six-point transformation crosses were carried out to map *mec-23* and *mec-31* with respect to *cysA*, *rif-2103*, *lpm-105*, *std-10*, *strA1*, and the temperature-sensitive marker (*ts-1*). The latter is a lesion in Ef-G (9). Results of a typical experiment are shown in Table

TABLE 1. Mapping *mec-23* and *mec-31*

Cross (selected gene) and proposed gene order[a]	No. of recombinants requiring multiple crossovers	
	mec-23	*mec-31*
Four-point (*std* selection)[b]		
mec rif std str	2	2
rif mec std str	26	39
rif std mec str	20	17
rif std str mec	50	34
Five-point (*rif* selection)[c]		
mec rif std str ts-1	2	8
rif mec std str ts-1	10	51
rif std mec str ts-1	12	49
rif std str mec ts-1	12	19
rif std str ts-1 mec	42	13

[a] The orders of all the markers other than *mec* were those given in the literature (32) and were consistent with the data generated in our crosses.
[b] In this cross, DNA from a *rif std* donor was used to transform a *mec str* recipient.
[c] In this cross, DNA from a *rif std* donor was used to transform a *mec str ts-1* recipient.

1, and a map of the region summarizing all the crosses is presented in Fig. 1. Both *mec* markers were located between *cysA* and *rif-2103* and were close to the latter marker. The *rif-2103* and *lpm-105* lesions are probably located in the β subunit of RNA polymerase (17, 25, 32). The *std-10* mutation was reported to be in the gene coding for the β′ subunit (18). *mec-23*, *mec-31*, and *mec-52* are provisionally regarded to lie in the same gene, named *mecB*. The possible identity of this gene is discussed below.

Growth stage-specific regulation in *mec* mutants. We wished to determine whether the *mec* mutants were altered in growth stage-specific regulation, as well as in nutritional control. This possibility has been tested by using the *comG12-lacZ* β-galactosidase activities of the *mecB23*, *mecB31*, and *mecA42* strains in both the original (*aroD120*) and in the *hisA1 leuA8 metB5* backgrounds and in CM, L broth, and VY broth. Similar experiments were also carried out in Com$^+$ strains which carried an intact as well as a disrupted copy of *comG*. All of the results were consistent with those shown in Fig. 2 for *mecB23* and *mecA42* in CM and in VY broth. The *mec*$^+$ strain expressed β-galactosidase in CM, but not in VY or L broth, as expected. The two *mec* mutants expressed β-galactosidase in all three media and to levels severalfold higher than that of the *mec*$^+$ strain. However, the *mec* mutants still exhibited late-growth control in all media. These results, and those of many similar experiments, suggested that the nutritional and growth stage controls were separable by mutation.

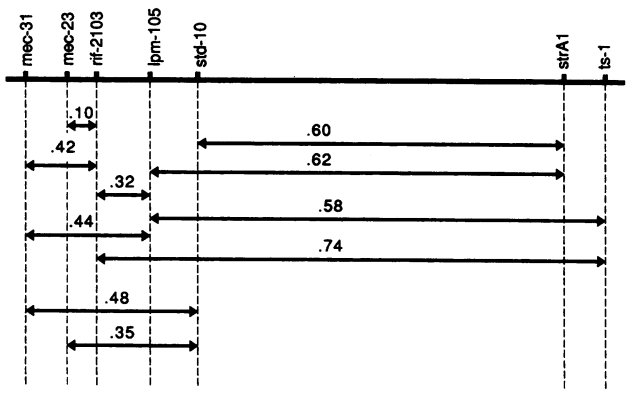

FIG. 1. Genetic map of *mecB* mutations. The map order was derived from several multifactor transformation crosses. The distances represent average values of 1 − the cotransformation frequency. The *cysA* locus is to the left and not shown.

Effect of *mec* mutations on expression of late competence genes. It was next determined whether the *mec* mutations affected other *com* genes in addition to *comG*. For this purpose, some of the strains described above, which had been cured of their *comG12* markers by transduction to *aroD* prototrophy, were used as recipients for transduction by PBS1 grown on several other *com* mutants which carried *com-lacZ* fusions. In this way the *mecB23* and *mecA42* mutations were combined with *comC530*, *comD413*, *comE510*, and *comG107*. All of the strains, when streaked on TBAB plates, were found to express β-galactosidase by the MUG test. Isogenic *mec*$^+$ control strains failed to express β-galactosidase on TBAB but did express it as expected on minimal salts plates. These results demonstrated that the two *mec* mutations tested were not specific for *comG12* but affected all the late competence genes in our collection. In addition, two other strains, carrying *mec-13* and *mec-53*, were tested only with *comC530* and were found to allow expression of the *lacZ* fusion on TBAB plates. Further tests with all these strains demonstrated that *mec*-induced expression also occurred on L broth plates.

Effect of *mec* mutations on expression of competence. Although the results described above were consistent with the simple hypothesis that *mec* mutations affect all essential late competence genes, we tested this directly by examining the expression of competence in *mec* mutants. To do this, we first constructed a set of isogenic strains in the BD630 (*hisA1 leuA8 metB5*) background, since this strain is reasonably competent and is the standard strain used in our laboratory. Chromosomal DNA was isolated from the *mecB23*, *mecB31*, and *mecA42* strains which also carried the Tn*917lacZ*-induced *comG12* mutation. The transposon conferred Em^r. The chromosomal DNA was used to transform BD630, with selection for Em^r. The transformants were then scored for expression of β-galactosidase on L broth plates containing X-Gal. In this way, the *comG12* mutation was introduced by selection and the unlinked *mec* mutations were introduced by congression. To test the competence of these strains, it was also necessary to replace the Em^r *comG12* mutation with a Cm^r version that carried both an intact copy of the *comG* operon and a transcriptional fusion of *comG* with *lacZ* (2). The latter was accomplished by PBS1 transduction. The competence of the resulting *mecB23* and *mecA42* strains, as well as of the isogenic *mec*$^+$ strain, was tested in CM and in L broth containing chloramphenicol to maintain selection for the Campbell-like *comG12* integrational construct. These experiments demonstrated that *mecB23* and *mecA42* permitted the enhanced expression of competence, and therefore of all essential competence genes, in complex medium, consistent with the effects noted on expression of the known late competence genes. Results for the *mec*$^+$ and *mecB23* strains are shown in Fig. 3. The *mec*$^+$ strain exhibited 200-fold lower competence in L broth than in CM. In contrast, the *mecB23* strain was only 2.7-fold less competent in L broth than in CM. Similar results for *mecA42* revealed 2.2-fold less competence in L broth than in CM (not shown). The absolute levels of competence measured in these experiments were 10- to 100-fold lower than usual, even for the *mec*$^+$ strain, because growth was carried out in the presence of chloramphenicol to ensure against loss of the intact copy

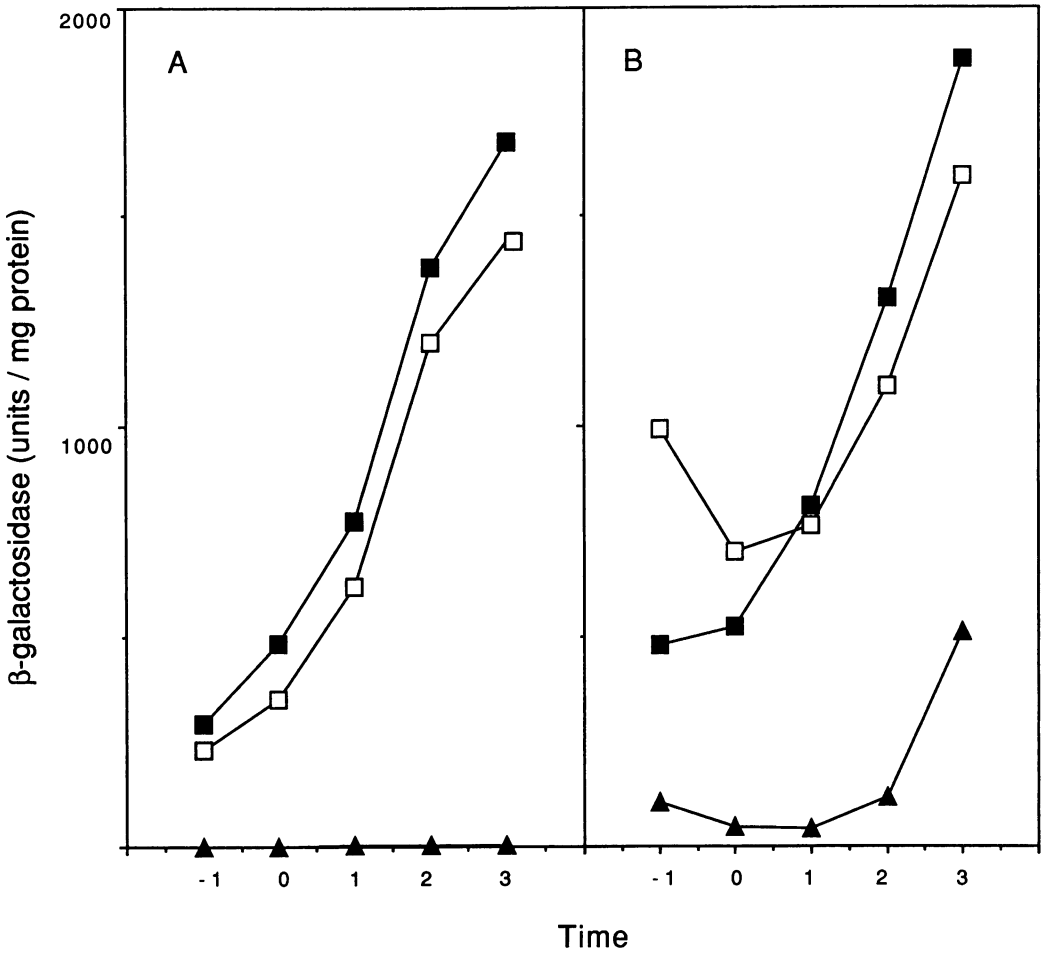

FIG. 2. Expression of *comG12-lacZ* β-galactosidase activity in VY broth (A) and in CM (B). Data are shown for the *mec*$^+$ (▲), *mecB23* (□), and *mecA42* (■) strains. The time scale refers to hours before or after T_0, the time of transition from the exponential to the stationary growth phase.

of *comG* by intrachromosomal recombination. We have consistently noted this effect of chloramphenicol. In all cases, the development of competence in the *mec* strains, in both CM and L broth, proceeded with growth stage-dependent kinetics similar to that of the *mec*$^+$ strain in CM. We have observed previously that in the one-step competence protocol, a relatively minor initial wave of competence is followed by a major wave which begins at about T_0 (3). This pattern is evident in Fig. 3, confirming the results shown in Fig. 2 but extending them to the complete ensemble of essential competence genes.

Response of *mec* mutants to glutamine and to the absence of glucose. The expression of late competence genes is subject to inhibition in CM containing glutamine or in CM in which glycerol is substituted for glucose (3). The effects of *mec* mutations on expression of *comG* in glutamine- and in glycerol-containing CM was tested. The *mecB23*, *mecB31*, and *mecA42* mutations rendered *comG* expression independent of the presence of glutamine and the substitution of glycerol for glucose (Fig. 4). Again, the *mec* mutations did not appear to alter growth stage-specific regulation, and as in Fig. 2, higher levels of β-galactosidase expression were obtained with the *mec* than with the *mec*$^+$ strains. Similar experiments were carried out with the strains possessing intact and disrupted copies of *comG*, measuring competence rather than β-galactosidase expression in CM containing glycerol in place of glucose. Again, the *mec* mutations permitted the full expression of competence in the modified CM (not shown).

Sporulation phenotypes of *mec* mutants. *mecA42*, *mecB23*, and *mecB31* strains were tested for sporulation. For this, the *hisA1 leuA8 metB5* background was used, carrying the Em^r *comG12-lacZ* fusion (Table 2). The *mecB23* strain appeared to sporulate about as well as the *mec*$^+$ wild type. In two additional experiments, the sporulation efficiencies of this strain were 0.75 and 0.20. The *mecB31* strain was oligosporogenic, exhibiting sporulation frequencies of 0.09 in the experiment whose results are shown and of 0.04 and 0.07 in two other trials. The total CFU attained by *mecB31* in this medium was consistently lower than that reached by the *mec*$^+$ strain. The *mecA42* strain appeared to sporulate normally, with frequencies of 0.69 in the experiment whose results are shown and 1.3 in an additional experiment. Also, the sporulation of the *mecB* mutants responded as did the wild type to the presence of glucose (Table 2). The *mecA42* strain, on the other hand, appeared to sporulate better than the wild type in glucose-containing medium, thus exhibiting a so-called CRS (catabolite-resistant sporulation) phenotype (35). However, the data are subject to another interpretation, since the total viable count reached in the presence of glucose was 7.4-fold lower than that reached in the absence of glucose. This effect was reproducible (not shown). It is

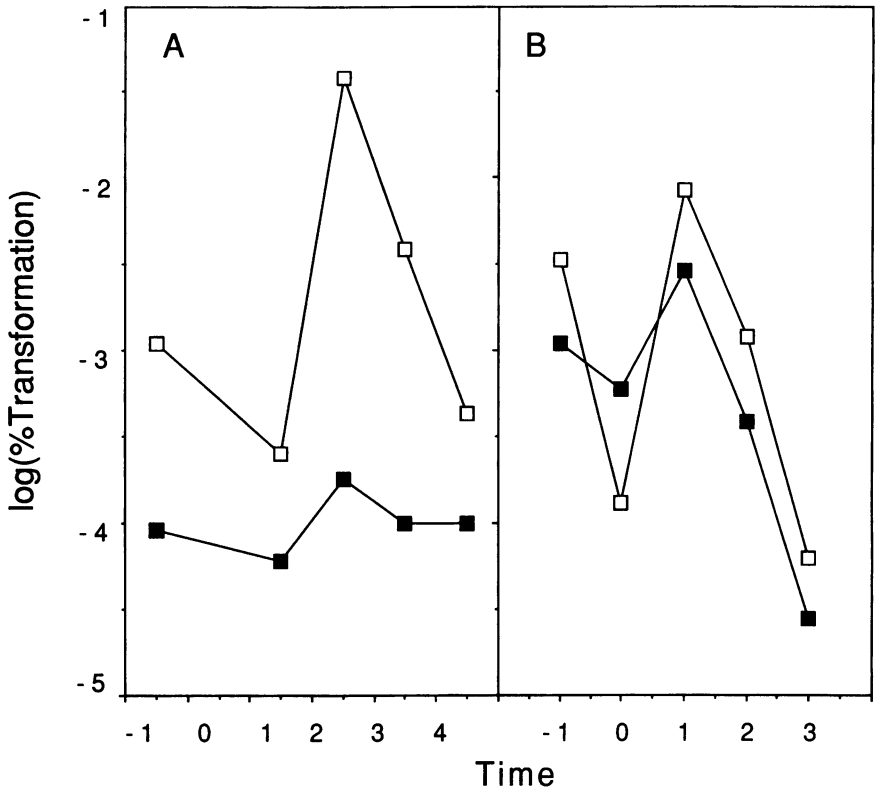

FIG. 3. Expression of competence in L broth (A) and CM (B). Results are shown for *mec*$^+$ (■) and *mecB23* (□) strains. Transformation frequency was determined for Leu$^+$. The time scale refers to hours before or after T_0.

possible that growth in the glucose-containing sporulation medium resulted in reduced viability, while those cells which formed spores before dying were able to survive.

DISCUSSION

Two loci (*mecA* and *mecB*) governing the medium-dependent expression of competence have been identified. Mutations in each of these loci were capable of altering the normal regulation of competence development so that this process occurred in complex media as well as in glucose-minimal salts-based medium. In both types of mutants, the expression of all essential late competence genes was affected. Although the expression of late competence genes was normally inhibited by the presence of glutamine as well as by the substitution of glycerol for glucose in the growth medium, *mec* mutants failed to exhibit these effects.

In contrast to the medium-independent response of competence development, growth stage-dependent regulation was not noticeably altered by *mec* mutations. The two modes of regulation therefore appeared to be genetically separable and must proceed at least in part along independent pathways. We cannot conclude from these observations that growth stage regulation is not ultimately dependent on nutritional signalling. There may be a form of such signalling that occurs in VY and L broth and is independent of *mecA* and *mecB*.

The only other phenotypes associated with the *mec* mutations concerned sporulation. The *mecA42* mutation did not interfere with sporulation but may have permitted this process to proceed to some extent in the presence of a concentration of glucose that markedly inhibited sporulation of the *mec*$^+$ strain. However, we must be cautious in interpreting these experiments, since growth of the *mecA42* mutant in sporulation medium containing glucose may have resulted in reduced viability. At least one *mecB* mutation (*mecB31*) conferred an oligosporogenic phenotype. It is possible that *mecB31* consisted of more than one mutation. Nevertheless, these observations suggested that the regulation of sporulation and competence involves overlapping pathways and shares regulatory products. In fact, few, if any, of the proteins that regulate early events in the activation of growth stage-specific systems in *B. subtilis* play roles unique to only one of those systems. For instance, the products of *mecA*, *mecB*, *spo0A* (3, 21), *spo0H* (3, 21), *sin* (12), *degS* (26, 29), *degU* (26, 29, 36), *comA* (37), *comP* (38), and *spo0F* (24) can all affect both sporulation and competence when in mutant form or when overexpressed. Other stationary-phase response systems, including motility, degradative enzyme synthesis, and surfactin production, are mutually dependent on similar regulatory products. This reinforces the notion that a common control network communicates environmental and intracellular signals to later-acting, system-specific response elements that transcriptionally activate the various systems.

We can only speculate as to the identities and specific roles of *mecA* and *mecB*. The products of these genes may play positive or negative regulatory roles in competence. The apparent ability of *mecA42* to render both competence and sporulation independent of glucose, in one case relieving dependence on this sugar and in the other relieving glucose repression, suggests that the *mecA* product may be a component of a signalling system that responds to the availability of C source.

The *rif-2103* and *lpm-105* lesions are located in *rpoB*,

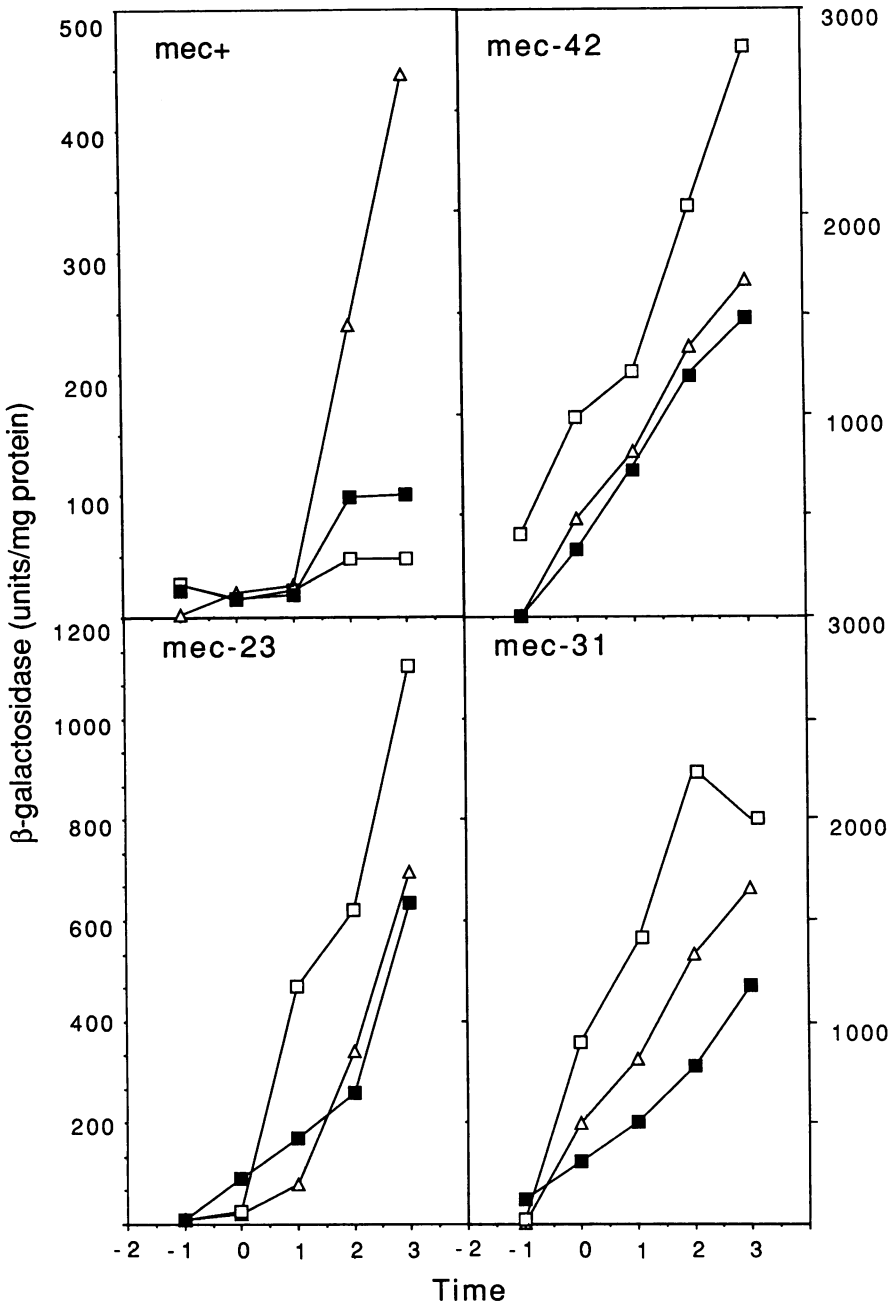

FIG. 4. Expression of *comG12-lacZ* β-galactosidase activity in CM (△), CM plus glutamine (□), and CM with glycerol substituted for glucose (■). Results are shown for the *mec*$^+$ strain and for several *mec* mutants. The time scale refers to hours before or after T_0.

TABLE 2. Sporulation of *mec* strains

Strain genotype	Sporulation on medium without glucose			Sporulation on medium with glucose		
	CFU/ml		Frequency[a]	CFU/ml		Frequency[a]
	Total	Heat-resistant		Total	Heat-resistant	
mec$^+$	4.1×10^8	4.3×10^8	1.29	8.3×10^8	1.0×10^6	1.24×10^{-3}
mecB23	2.8×10^8	1.9×10^8	0.69	1.3×10^8	2.1×10^4	1.6×10^{-4}
mecB31	3.3×10^7	3.1×10^6	0.09	9.2×10^7	2.2×10^4	2.3×10^{-4}
mecA42	2.0×10^8	1.4×10^8	0.69	2.7×10^7	1.4×10^6	0.05

[a] Total divided by heat-resistant CFU.

which probably encodes the β subunit of RNA polymerase (17, 25, 32). The close transformational linkage of the *mecB* mutations to *rif-2103* and the oligosporogenicity of *mecB31* suggest that the *mecB* mutations may be alleles of *rpoB*, encoding the β subunit of RNA polymerase (33). The *std-10* mutation was reported to be in the gene coding for the β′ subunit (18). If the *mecB* mutations are in *rpoB*, then the phenotypes they confer may be due to altered interaction of RNA polymerase with regulatory cofactors or with competence- and sporulation-specific promoters. On the other hand, the *mecB* mutations might be located in another gene situated between *rpoB* and *cysA*. C. Price, M. Duncan, and S. Thomas (personal communication) have discovered an open reading frame of unknown identity, located just upstream of *rpoB* and between it and *cysA*. Alternatively, *mecB* mutations may be alleles of *relC*, which has been mapped to the interval between *spo0H* and *rpoB* (31). Another possibility is that the *mecB* mutations are alleles of *spo0H*, which is located between *cysA* and *rpoB* (20). However, two arguments tentatively suggest that this may not be so. First, the linkage of *spo0H75* to *rif-29* has been reported to be only about 30% by transformation (31). Second, we have transformed a *mecB23* strain with a multicopy plasmid carrying the *Bacillus licheniformis spo0H* gene, which is known to express in *B. subtilis* and to complement *spo0H* mutations for their inability to sporulate (10). The resultant transformants were not complemented for *mec* (unpublished results). A clearer understanding of these genes and mutations will hopefully emerge following their characterization by cloning and sequencing.

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