Suppression of Early Competence Mutations in *Bacillus subtilis* by *mec* Mutations

MANUELA ROGGIANI, JEANETTE HAHN, AND DAVID DUBNAU*

Department of Microbiology, The Public Health Research Institute, 455 First Avenue, New York, New York 10016

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Although competence normally develops only in glucose-minimal salts media, *mecA* and *mecB* mutations permit the expression of competence and of late competence genes in complex media as well (D. Dubnau and M. Roggiani, J. Bacteriol. 172:4048–4055, 1990). The expression of late competence genes is dependent on the products of the regulatory genes *comA*, *comB*, *comP*, *sin*, *abrB*, *spo0H*, and *spo0A*. We show here that this list must be extended to include *degU*, *csh-293*, and *spo0K*. *mecA* and *-B* mutations bypass most of these requirements, making the expression of late competence genes and of competence itself independent of all of these regulatory genes, with the exceptions of *spo0A* and *spo0K* (in the case of *mecB*). The expression of late competence genes in *mec* mutants that are deficient for each of the bypassed regulatory functions is still under growth stage-specific regulation. The implications of these findings are discussed, and a provisional scheme for the flow of information during the development of competence is proposed.

The development of competence in *Bacillus subtilis* is a genetically programmed global response to a variety of signals that are poorly understood. These signals appear to include nutritional information, since competence develops only in minimal salts-based media, requires the presence of glucose, and is inhibited by the addition of glutamine. In addition, the development of competence occurs postexponentially. These two types of control are genetically separable under at least some conditions, since mutations exist in the *mec* loci that permit the development of competence in any growth medium tested but do not detectably alter growth stage-specific control (7).

Mutations in several loci other than *mecA* and *-B* have been shown to affect the development of competence, playing regulatory roles (reviewed in reference 5). These regulatory genes are components of a control network that is responsible for the appropriate expression of a set of late competence genes. In general, inactivation of any one of the regulatory genes prevents the normal post-exponential-phase expression of the late competence genes, which specify products directly involved in the binding, processing, and transport of transforming DNA. It has become evident that the competence control network is at least partly congruent with the regulatory systems that determine the onset of sporulation, degradative enzyme synthesis, motility, surfactin production, and the late-growth-phase SOS response. In fact, few, if any, of the known regulatory genes affect only one of these post-exponential-phase response, when mutated or overexpressed.

The DNA sequences of the competence-regulatory genes *comP* (28) and *comA* (27) suggest that these genes specify sensor and effector members of the bacterial two-component signal transduction systems (24). Genetic evidence has indicated that they work together, possibly signalling the availability of C and N sources. *degS* and *degU* also appear to specify a sensor and an effector, respectively (13, 16, 26), and mutations in either of these genes can affect competence and the expression of late competence genes (19, 26). In addition, *spo0A* encodes a protein with similarity to the

effector class (14), and mutations in this gene have long been known to adversely affect competence (23).

In addition to these genes, several others have been implicated as regulators of competence. *spo0H* encodes a minor sigma factor, σ^H (8), and is required for the full expression of competence (3). Presumably at least one essential competence gene is read by σ^H -RNA polymerase. A candidate for a gene that may be read directly by σ^H -RNA polymerase is defined by the *csh-293-lacZ* fusion mutation (15). This mutation confers competence deficiency, and full expression of β -galactosidase from the fusion requires the *spo0H* product. The *sin* gene is also required for the development of competence (9) and for the expression of late competence genes (11). *Sin* is a DNA-binding protein, and it may play a role in competence-specific transcriptional control. *AbrB* is another DNA-binding protein and a transcriptional regulator (20) and is required for the expression of late competence genes (3). One role for the *Spo0A* protein is to antagonize the synthesis or activity of *AbrB*, which can also play a negative role (18). *spo0A* mutants are competence deficient, and it has been suggested that the sole task of *Spo0A* during the development of competence is to antagonize *AbrB* (3).

Clearly this multiplicity of factors required for the appropriate triggering of competence gene expression poses a daunting challenge. Genetic approaches offer a powerful although indirect tool for examining the relationships among these genes and factors and for determining their orders of action along a hypothetical competence regulation pathway. In this study, we have used *mecA* and *-B* mutations to bypass the dependencies of late competence gene expression on certain of the regulatory genes.

MATERIALS AND METHODS

Strains. All the strains used were derivatives of *B. subtilis* 168. The primary strains from which the various mutations were derived and their sources are listed in Table 1. Since the designations of several of the mutations are cumbersome, an abbreviated symbolism has been adopted. The *degU::pDH55* and *degS::pDH64* null mutations are referred to as *degUΔ55* and *degSΔ64*, respectively. *comG12* will be used to refer to the original erythromycin-resistant (*Em*^r)

* Corresponding author.

TABLE 1. Strains

Strain	Genotype ^a	Source or reference
	<i>degU</i> ::pDH55	13
	<i>degS</i> ::pDH64	13
1A95	<i>degU32</i> (Hy) <i>trpC2 leuA8</i>	13
1A340	<i>degU118</i> (Hy) <i>amyE1 amyR1</i>	13
	<i>aroI116 metB5 str pro</i>	
1A199	<i>degS200</i> (Hy) <i>trpC2 leuA8</i>	13
AG475	<i>spo0A</i> ::Cm(Δ HpaI-BglII) <i>trpC2 pheA1</i>	A. D. Grossman
AG881	Tn917 <i>spo0K141</i>	A. D. Grossman
BD630	<i>hisA1 leuA8 metB5</i>	
BD1241	<i>comB138</i> ::Tn917 <i>lacZ</i> ⁺ <i>hisA1 leuA8 metB5</i>	12, 27
BD1243	<i>comA124</i> ::Tn917 <i>lacZ</i> ⁺ <i>hisA1 leuA8 metB5</i>	12, 27
BD1248	<i>comG12</i> ::Tn917 <i>lacZ</i> ⁺ <i>hisA1 leuA8 metB5</i>	1, 2
BD1453	<i>comG12</i> ::Tn917 <i>lacZ</i> ⁺ <i>mecB31 aroD120 acf-2</i>	7
BD1454	<i>comG12</i> ::Tn917 <i>lacZ</i> ⁺ <i>mecB23 aroD120 acf-2</i>	7
BD1461	<i>comG12</i> ::Tn917 <i>lacZ</i> ⁺ <i>mecA42 aroD120 acf-2</i>	7
BD1512	<i>comG12</i> ::pTV21 Δ 2(Campbelled) <i>hisA1 leuA8 metB5</i>	1, 2
BD1626	<i>comA124</i> ::Tn917 <i>LacZ</i> ⁻ <i>hisA1 leuA8 metB5</i>	11
BD1630	<i>comB138</i> ::Tn917 <i>LacZ</i> ⁻ <i>hisA1 leuA8 metB5</i>	11
BD1658	<i>comP</i> ::Cm <i>hisA1 leuA8 metB5</i>	28
BD1820	<i>csh-293</i> ::Tn917 <i>LacZ</i> ⁻ <i>trpC2 pheA1</i>	This work
BH41	<i>spo0H</i> ::Cm(Δ HindIII-EcoRI) <i>trpC2 pheA1</i>	J. Healy and R. Losick
IS233	<i>spo0H</i> (Δ HindIII) <i>trpC2 pheA1</i>	I. Smith
IS432	<i>sin</i> ::Cm(Δ Ball-NruI) <i>hisA1 leuA8 metB5</i>	9
IS1388	<i>sin</i> ::Cm(Δ Ball-NruI) <i>hisA1 leuA8 metB5</i> (pIS21)	N. K. Gaur and I. Smith
JH12586	<i>abrB</i> ::Cm	20
KJ388	<i>csh-293</i> ::Tn917 <i>lacZ</i> ⁺ <i>trpC2 pheA1</i>	15

^a Double colons indicate an insertion of a plasmid, a Cm^r cassette, or a Tn917 element. In some cases the Cm^r cassette replaces a deleted restriction fragment, as indicated.

Tn917*lacZ* insertion into open reading frame 1 of the *comG* operon (in strain BD1248) (1). The mutation in strain BD1512 was derived by replacing the Tn917*lacZ* insertion in *comG12* by pTV21 Δ 2 (29), which confers chloramphenicol resistance (Cm^r), cloning this chromosomal construct in *Escherichia coli* as a plasmid, and reintroducing the cloned construct into the *B. subtilis* chromosome by a Campbell-like recombination event (2). The resulting mutation is referred to as *comG12*(Campbelled). It carries both a disrupted *lacZ* fusion to *comG* and an intact *comG* operon and is consequently competence proficient. The experiments described were, except where explicitly noted, conducted with a set of isogenic strains, constructed in the BD630 (*hisA1 leu-8 metB5*) background. The various markers were moved into BD630, or into derivatives of BD630, by transduction or by transformation, and these strains are described in the text where appropriate. For mutations marked by the insertion of antibiotic resistance cassettes or by Tn917 insertions, selection was for the resistance phenotype. Otherwise, markers were moved by congressional transformation. In these

cases, *spo* recombinants were detected by colonial morphology on TBAB plates, *degS*(Hy) and *degU*(Hy) markers were detected by the use of skim milk plates, and *mec* markers were detected by plating appropriate strains on L broth agar containing 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal). To move the *sin*::Cm marker by transduction, IS1388 was used. This strain (Table 1) carries the multicopy *sin* plasmid pIS21, in addition to the chromosomal *sin*::Cm mutation, and is therefore phenotypically Sin⁺. It was thus possible to prepare PBS1 transducing lysates on IS1388, which was impossible with nonmotile Sin⁻ strains. The Lac⁻ strain BD1820 was prepared by transducing KJ388 with PBS1 grown on a strain carrying pTV55 Δ 2 (11). This resulted in replacement of the Em^r Lac⁺ Tn917*lacZ* element by a Cm^r Lac⁻ derivative.

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 tryptose blood agar base (TBAB; Difco), L broth, or minimal medium (4). When required, nutritional supplements were added to 50 μ g/ml. Erythromycin and chloramphenicol were added to 5 μ g/ml as required. X-Gal was added to L broth agar at 80 μ g/ml, and skim milk was added to 1% (wt/vol).

Transformation and transduction. Competent cells were prepared by the one-step protocol using CM and transformed as described previously (3). Transduction using bacteriophage PBS1 was carried out as described elsewhere (6).

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 (10) and for protein with the Bio-Rad reagent as specified by the manufacturer. Results were expressed as units of β -galactosidase per milligram of protein.

RESULTS

Effects of *degU*, *degS*, *spo0K141*, and *csh-293* mutations on competence. Regulatory mutations have been identified which prevent the normal development of competence and the expression of late competence genes (reviewed in reference 5, and see below). They include alterations in *comA*, *comB*, *comP*, *sin*, *spo0H*, *spo0A*, and *abrB*. In addition to these, other genes have been implicated in the regulation of competence. For instance, inactivation of *degU* results in greatly reduced competence (26). Also, point mutations known as *degU*(Hy) and *degS*(Hy), which elevate degradative enzyme synthesis, have also been reported to reduce competence (17). Jaacks et al. (15) have described a Tn917*lacZ* insertion (*csh-293*) that confers both Com⁻ and oligosporogenic phenotypes. Finally, Sadaie and Kada (22) and A. D. Grossman (personal communication) have observed that *spo0K141* strains are Com⁻. We have confirmed these observations concerning *degU*, *degS*, *csh-293*, and *spo0K* by using an isogenic set of strains (Table 2). The various mutations were moved by transformational congression into the BD630 background, except for the *degU* Δ 55 and *csh-293* mutations, which were moved by transformation and selection for Cm^r and Em^r, respectively, and for *spo0K141*, which was moved by selection for a linked Tn917 insertion that conferred Em^r. In addition to these mutations that have been reported to reduce competence, the *degS* Δ 64 null mutation was also introduced by selection for Cm^r. Each of the mutations tested had a marked effect on transforma-

TABLE 2. Transformability of regulatory mutants

Mutation ^a	Transformation frequency ^b
<i>com</i> ⁺	1.0
<i>degU</i> Δ55.....	3.4×10^{-3}
<i>degS</i> Δ64.....	1.6×10^{-1}
<i>degU</i> 32(Hy).....	2.2×10^{-2}
<i>degU</i> 118(Hy).....	3.1×10^{-5}
<i>degS</i> 200(Hy).....	2.1×10^{-4}
<i>csh</i> -293.....	1.95×10^{-3}
Tn917 <i>spo0K</i> 141.....	3.5×10^{-3}
Tn917.....	2.7×10^{-1}

^a The various mutations were introduced into a *hisA1 leuA8 metB5* background, and transformability was determined in these isogenic strains.

^b Transformability was determined by using the one-step protocol, at *T*₂, defined as 2 h after the transition from exponential growth (*T*₀). The transformation frequency for *Leu*⁺ was normalized to the value determined for the *com*⁺ strain. The results of several independent experiments have been combined. In these experiments, the *com*⁺ frequency averaged about 0.1%.

tion efficiency, except for *degS*Δ64, which decreased transformability only severalfold. Since the *spo0K*141 mutation was moved by selection for a linked Tn917 element, it was necessary to demonstrate that the Tn917 insertion by itself had little or no effect on transformability (Table 2). It should be noted that the level of competence attained in a given experiment is somewhat variable, so that effects of less than about 10-fold are difficult to document. This variability places special emphasis on the importance of comparing only isogenic strains.

The effects of these mutations were further investigated by testing their influences on expression of β-galactosidase from the *comG*12-*lacZ* fusion during growth in liquid CM (Fig. 1A to D). In agreement with their effects on compe-

tence, all of the mutations but *degS*Δ64 severely depressed expression of *comG*12. *degS*Δ64 decreased expression of *comG*12 only about twofold. None of the regulatory genes tested seemed to affect the expression of the early gene *comA*124-*lacZ* fusion (Fig. 1E to H). In addition to the experiments whose results are shown in Fig. 1, the same set of regulatory mutations was shown to have similar depressive effects on the expression of the late competence genes *comC*, *comD*, and *comE* (not shown). Also, none of the regulatory mutations affected the expression of β-galactosidase from the early gene *comB*138 fusion (results not shown).

Suppression of the early gene dependencies of *comG* expression. Although the expression of late competence genes as well as of competence itself is normally restricted to cells grown in glucose-minimal salts media, *mec* mutations permit this expression to occur in all media tested (7). To determine whether *mec* mutations can suppress the dependencies of late competence gene expression on various early regulatory gene products, a series of double and triple mutants were constructed. They all carried *comG*12, a mutation that consisted of Tn917-*lacZ* inserted in the first open reading frame of the *comG* operon (1, 2). This fusion construct permitted the use of β-galactosidase for monitoring *comG* transcription. A series of regulatory-gene mutations were combined with *comG*12. In these strains, β-galactosidase expression was expected to be markedly reduced, since expression of the fusion was known to be dependent on the various regulatory products. Finally, triple mutants were constructed that contained *mecA*42, *mecB*23, or *mecB*31 (7), in addition to the regulatory mutation and *comG*12. The expression of β-galactosidase in the isogenic *mec* and *mec*⁺ strains was then measured, to reveal the effects of the *mec*

FIG. 1. Epistatic effects of *degU*Δ55 (A and E) (Δ), *degS*Δ64 (A and E) (□), *degU*32(Hy) (B and F) (Δ), *degU*118(Hy) (B and F) (■), *degS*200(Hy) (B and F) (□), *csh*-293 (C and G) (□), and *spo0K*141 (D and H) (□) on expression of β-galactosidase from the *comG*12 (A, B, C, and D)- and *comA* (E, F, G, and H)-*lacZ* fusions in strains grown in CM. The expression of enzyme in single-mutant fusion strains not carrying early gene mutations is also shown (▲). Time is expressed in hours before and after *T*₀, which is defined as the time of transition from exponential growth.

FIG. 2. Suppression of the effects of the early gene mutations *comA124*, *comB138*, and *comP::Cm* on expression of β -galactosidase from the late gene fusion *comG12*. Strains carrying *mec*⁺ (□), *mecA42* (▲), *mecB31* (△), and *mecB23* (■) were grown in Cm and tested for expression of β -galactosidase. Time is expressed as described for Fig. 1.

mutation on regulatory-gene-dependent expression of *comG*.

The effects of *mecA42*, *mecB23*, and *mecB31* on the *comA*, *comP*, and *comB* dependencies of *comG* expression are shown in Fig. 2. Two conclusions are apparent. First, mutations in either *mecA* or *mecB* appeared to suppress the dependencies of *comG* expression on each of the three regulatory genes. Although the absolute levels of expression varied somewhat, they were roughly equivalent to the levels attained in the absence of regulatory-gene mutations. Second, the suppressed expression of *comG* exhibited apparently normal growth stage-specific regulation. Similar results were obtained with *sin* and *degU* (Fig. 3). Again, the *mec* mutations suppressed the dependency of *comG* expression on the early gene products, and growth stage-specific expression was not altered. The case of *csh-293* was somewhat more complex. *mecB23* appeared to suppress the dependence of *comG12* expression on *csh-293* (Fig. 3). The triple mutants carrying *comG12*, *mecA42* or *mecB31*, and *csh-293* were also constructed. These strains lysed when grown in CM and when grown on minimal medium agar, making it impossible to reliably measure β -galactosidase expression. However, the lytic colonies were blue when the minimal medium agar contained X-Gal. In L broth, both of

these strains expressed β -galactosidase (not shown), although the level of expression from the *mecA42* strain was low (about 50 U/mg of protein). The expression in L broth exhibited the usual post-exponential-phase control. We therefore tentatively conclude that the *csh-293* requirement is bypassed by both *mecA* and *mecB* mutations. These results suggested that the *mecA* and *-B* products exerted their effects later than ComA, ComP, ComB, Sin, and the *csh-293* gene product in the competence-regulatory pathway. They also showed that the latter five gene products were not absolutely required for growth stage regulation of *comG*.

The effect of *mecA42* on the *abrB* dependency of *comG* expression is also shown in Fig. 3. Again, suppression of this dependency was observed, without noticeable alteration of growth stage-specific regulation. Attempts to test the effect of *mecB* mutations on *abrB* dependency encountered difficulties, since *mecB* and *abrB* are genetically linked (7, 21). Therefore, introduction of the *abrB::Cm* mutation by transduction would be expected to result in the loss of the recipient *mecB* mutation in some of the transductants. In fact, when such a cross was carried out with a *mecB23 comG12* recipient, only about 25% of the Cm^r transductants were blue on L broth agar containing X-Gal, suggesting that

FIG. 3. Suppression of the effects of mutations in the indicated early genes on expression of β -galactosidase from the *comG12-lacZ* fusion. Results are shown from *mec*⁺ (□), *mecA42* (▲), *mecB31* (△), and *mecB23* (■) strains grown in CM. Time is expressed as described for Fig. 1.

TABLE 3. Genetic test of suppression of *spo0K141* by *mecB* mutations

Mutation	No. of transformants ^a			
	Em ^r <i>spo0K141</i>		Em ^r <i>spo</i> ⁺	
	Blue	White	Blue	White
<i>mecB23</i>	0	10	8	0
<i>mecB31</i>	0	9	16	0

^a *mecB comG12*(Campbelled) recipients which also carried an intact copy of *comG* were transformed with DNA from strain AG881 (*spo0K141* Tn917). Selection was applied for the Em^r marker of Tn917. The latter is linked to *spo0K141*. The transformants were scored for β-galactosidase expression on X-Gal-containing plates and for sporulation on TBAB.

in the remaining 75%, the wild-type *mec*⁺ was cotransduced with *abrB*. The blue colonies implied that *mecB23* was able to suppress the *abrB* requirement. Although the blue color of these colonies was as intense as that of the equivalent *comG* *abrB*⁺ strain, it was not possible to grow the *abrB mecB23* strain in liquid CM for β-galactosidase assay. Growth proceeded to the mid- or late-exponential stage and was followed by lysis. However, on the basis of the behavior of these strains on X-Gal plates, we conclude that *mecB23* did suppress the *abrB* requirement for *comG* expression.

spo0H is also closely linked to *mecB*, and we were unable to readily construct a strain carrying mutations in both of these genes. *mecA42* clearly suppressed the *spo0H* dependency of *comG* expression (Fig. 3).

Finally, streaking on L broth agar containing X-Gal, as well as assays on L broth-grown cultures (not shown), revealed that the suppression of early gene dependencies by *mec* mutations (Fig. 2 and 3) was also manifested in complex medium.

The *spo0K141* dependency of *comG* expression was not suppressed by either *mecB23* or *mecB31*. This was demonstrated by the cross summarized in Table 3. Selection for the Tn917 Em^r marker linked to *spo0K141* yielded two types of transductants on X-Gal plates with *mecB comG12* recipients: blue Spo⁺ colonies and white Spo⁻ colonies. It thus appeared that the presence of the *spo0K141* mutation prevented the expression of *comG*, even in *mecB* strains.

The predicted linkage of *mecA42* and *spo0K141* complicated the simple construction of double-mutant strains (7, 21). To confirm this linkage and simultaneously test the bypass of *spo0K141* by *mecA42*, the transformation crosses summarized in Table 4 were carried out. The interpretation of these crosses in terms of map order is compromised by the relatively low linkage between the Tn917 element (Em^r) and

TABLE 5. Bypass of competence by *mecA42*

Early mutation	<i>mec</i> genotype	Transformation frequency ^a at:	
		T ₀	T ₂
None	<i>mec</i> ⁺	7 × 10 ⁻⁵	4 × 10 ⁻³
<i>comA124</i>	<i>mec</i> ⁺	3.4 × 10 ⁻⁶	1 × 10 ⁻⁵
	<i>mecA42</i>	2 × 10 ⁻⁴	2.7 × 10 ⁻³
<i>spo0HΔHindIII</i>	<i>mec</i> ⁺	1.3 × 10 ⁻⁶	1.1 × 10 ⁻⁵
	<i>mecA42</i>	1.1 × 10 ⁻⁵	5.4 × 10 ⁻⁵

^a Transformation frequency for Leu⁺, measured on samples withdrawn at T₀ and T₂ from cultures growing in CM.

the *mecA42* and *spo0K141* markers. In addition, since a fourth linked marker was not available, the map order in which the Em^r marker is in the middle could not be unambiguously ruled out. Nevertheless, the order given in Table 4 seems to be preferred. The order with the Em^r marker centrally located is less likely on the basis of the following argument. In both crosses, the major recombinant class resulted from transformation of the donor Em^r marker alone. This suggests that the *mecA42* and *spo0K141* markers are far from the Em^r marker, either on the same side of it or flanking it. However, the second most common recombinant class in both crosses resulted from the simultaneous transformation of all three donor alleles. This seems to imply strongly that *mecA42* and *spo0K141* are closely linked to one another and therefore on the same side of the Em^r marker. In addition, we conclude from Table 4 that unlike *mecB* mutations, *mecA42* bypasses *spo0K141*, since 3 and 10 recombinants were obtained from crosses A and B, respectively, that expressed β-galactosidase from the *comG12* fusion and exhibited a Spo⁻ phenotype (*mecA42 spo0K141*).

We also tested the suppression of *spo0A* dependency of *comG* expression. Bypass of *spo0A* dependency was not observed (Fig. 3). These experiments suggested that *spo0A* and *spo0K141* act later in the competence pathway than the *mec* products (only for *mecB* in the case of *spo0K141*) or on a separate branch of the pathway that joins later than the point of *mec* action.

Does *mec* suppression restore competence to early gene mutants? The experiments described so far have demonstrated the ability of *mec* mutations to suppress the dependencies of *comG* expression on certain early competence genes. It may be that this suppression extended to all the essential late competence genes. We tested this by directly measuring competence. To do this, strains were constructed that carried the early gene to be tested, a *mec* mutation, and the *comG12*(Campbelled) fusion. These constructs could be scored for β-galactosidase expression (to ensure the presence of the *mec* mutation) and for competence (since an intact copy of *comG* was present). Three such strains were constructed, carrying *comA124* and disruptions of *spo0H* and *sin*. *mecA42* bypassed the requirement for *comA* for the development of competence (Table 5). A partial bypass of the *spo0H* requirement was also obtained. The extremely rough colonial morphology typical of *sin* mutations was not suppressed by *mecA42*. This made it difficult to obtain reliable quantitative data for the transformability of the *mecA42 sin* strain, since successive dilutions of the transformed culture yielded aberrant colony counts due to cell clumping. This clumping was clearly visible microscopically. Nevertheless, although the *mec*⁺ *sin* strain gave rise to no more than 10 to 30 colonies when 0.1 ml of culture was plated without dilution, the double-mutant (*mecA42 sin*) strain yielded 200 to 400 colonies when plated in the same

TABLE 4. Mapping of *mecA42* and *spo0K141*

Donor genotype	Recipient genotype	Recombinant class ^a		No. of recombinants
		<i>mecA42</i>	<i>spo0K141</i>	
<i>spo0K141</i> Em ^r	<i>mecA42 comG12</i>	0	0	185
		0	1	3
		1	1	69
		1	0	28
<i>mecA42 comG12</i> Em ^r	<i>spo0K141 comG12</i>	0	0	115
		0	1	9
		1	1	59
		1	0	10

^a Donor and recipient alleles are designated 1 and 0, respectively. For all crosses, Tn917 class alleles were 1.

way. It appeared, therefore, that *mecA42* at least partially bypassed *sin* for competence. It was also clear that competence in the *mecA42 comA* and *mecA42 spo0H* strains developed with the usual growth stage-dependent kinetics (3). Transformability was relatively high early during exponential growth, dropped to a minimum at about T_0 , and then rose sharply (Table 5 and data not shown). Thus the behavior of *comG* in the experiments described above was typical of that of all the essential late competence genes, at least with respect to the early genes *comA*, *spo0H*, and probably *sin*.

DISCUSSION

The roles of *degU* and *degS* in the development of competence. In agreement with results in the literature (17, 26), we have confirmed that a *degU* disruption and several *degU*(Hy) and *degS*(Hy) mutations result in decreased competence. A disruption of *degS* has only a minor effect on competence. These effects are mirrored in the levels of late gene expression measured in the different mutant backgrounds, showing that the effects of the *deg* mutations on competence are manifested via effects on competence gene expression. These effects are exerted at least partly at the transcriptional level, since the *lacZ* fusion constructs used are transcriptional fusions.

Msadek et al. (19) have reported that *degS* and *degU* are transcribed from the same promoter but that a minor promoter embedded in the *degS* sequence also probably initiates transcription of *degU*. Transcription from the minor promoter occurs at about 20% of the rate of transcription from the major promoter. The slightly decreased competence of the *degS* insertion is therefore probably due to a polar effect on *degU* transcription. We conclude, therefore, that the *degS* gene product is not required for the development of competence. DegU, however, is required, since *degU* Δ 55 strains are poorly competent and greatly reduced in the expression of late competence genes. The similarities of DegS and DegU to known signal transduction proteins that are known to transmit information via phosphorylation suggest that DegS and DegU may operate in a similar manner. The *degU*(Hy) and *degS*(Hy) mutations may alter their gene products so that DegU is more often in the phosphorylated state or so that it behaves as an activator even when unphosphorylated (13). We propose therefore that the unphosphorylated form of DegU is required for competence and that excess phosphorylation inhibits competence. This may be due to a dearth of the unphosphorylated protein or because the phosphorylated form of DegU is directly inhibitory. Similar conclusions have been reached by Msadek et al. (19).

The role of *csH-293* in the development of competence. Jaacks et al. (15) isolated the *csH-293* fusion to Tn917*lacZ* and showed that this fusion resulted in a competence-deficient and conditionally oligosporogenic phenotype. The β -galactosidase activity of this fusion strain increased after addition of decoynine to cells growing in minimal medium and also at about T_0 when cells were grown in complex sporulation medium. Expression was largely dependent on the *spo0H* gene product. The apparent growth stage-specific regulation of *csH-293* transcription and the dependence of late *com* gene expression on the *csH-293* gene product suggest that this product may act after the other competence-regulatory genes, since the latter are expressed at relatively high levels during exponential growth and are apparently not dependent on one another for their expres-

sion. In support of this idea, we have recently observed that the expression of β -galactosidase from *csH-293* growing in CM is dependent on the products of *comA*, *comP*, and *comB* but not on DegU (J. Hahn and D. Dubnau, unpublished results). In these experiments, partial dependence on the *spo0H* and *spo0A* products was also noted, and in all cases *csH-293* expression increased sharply at about T_0 , although some expression was observed during exponential growth. We conclude that the product of the gene marked by the *csH-293* fusion is required for the expression of late competence genes and is itself expressed to high levels only when the ComA, -B, and -P proteins are present. The *csH-293* gene may therefore function as an intermediate in the competence-regulatory pathway.

Implications of *mec*-induced suppression of early gene mutations. In most cases, the early gene requirements for late gene expression and even for the development of competence can be suppressed by *mec* mutations (Fig. 2 and 3; Table 4). Exceptions were the requirements for *spo0K* (in the case of *mecB*) and for *spo0A* (in the cases of both *mecA42* and *mecB*), which could not be suppressed. Otherwise, no difference was noted between the suppression capabilities of *mecA* and *mecB* mutations, although they differ in other phenotypic properties (7). The simplest interpretation of these results, which we favor, is that the *mec* mutations bypass the early gene requirements. An alternative explanation, in which suppression involves specific interactions of the two altered *mec* gene products with each of the suppressed mutant proteins, seems highly unlikely, and for the remainder of this discussion we will assume that the bypass interpretation is correct. The suppression noted when competence was measured implies that at least for Sin, ComA, and probably σ^H as well, all of the essential late competence genes are affected similarly. This result provides a preliminary justification for the simplifying working hypothesis that all late competence genes are regulated by a common mechanism, perhaps turned on by a single master activator of the competence regulon. The bypass results, together with the ability of *mec* mutations to permit competence development in all media tested, implies that the set of early regulatory genes that can be bypassed constitutes a signal-processing system responsible for activating the transcription of late genes and that the output from this system is processed via *mec*. An assumption in this discussion, and in what follows, is that the *Mec* products function normally as components of the competence-regulatory machinery. The alternative possibility, that the *mec* mutations activate a bypass machinery that is not normally functional, seems less likely.

In each of the bypass experiments, the expression of the late competence gene or of competence was seen to remain under growth stage-specific control. This compels the inference that *comA*, *comB*, *comP*, *degU*, *sin*, *csH-293*, *spo0H*, and *abrB* are not absolutely required for this mode of control. It also suggests that post-exponential-phase regulation is exerted at the point of *mec* action or later. The two regulatory loci *spo0A* and *spo0K* remain as candidates for involvement in growth stage regulation. *spo0A* is not bypassed by *mec* mutations. Although *spo0K141* is bypassed by *mecA42*, we do not know whether the expression of late genes under these conditions is subject to post-exponential-phase control.

The competence pathway. The bypass hypothesis permits inferences concerning the pathway of competence-specific regulation. Figure 4 outlines such a pathway, based on the data described in Results and on the unpublished data

FIG. 4. Scheme of information flow during the development of competence. The arrows and lines ending in perpendicular lines indicate stimulatory and inhibitory interactions, respectively. Asterisks indicate activated (presumably phosphorylated) forms of DegU and ComA. Some aspects of the scheme are arbitrary. For instance, SpoOK is shown acting via *mec* or later. In fact, it may act after *mecB* and before *mecA* (see text). It is possible that *mecA42* is an allele of *spoOK*. Also, Sin and AbrB (positive role) are shown as acting directly on the *mec* system. It is perhaps more likely that they exert their influences earlier, along the ComA or DegU pathways. The question mark indicates the possibility that SpoOA does not function as a signal-transducing protein for competence (see text). *spoOH* (σ^H) is not included. It must be required to transcribe at least one essential competence gene, which acts before or at the point of *mecA* action.

concerning *csh-293* described above in the Discussion. In this scheme, the lines represent the flow of information and not necessarily material transfer. In some cases both may be involved. For instance, phosphorylation of DegU and ComA may be the means by which information is transduced to an effector, and an increase in *csh-293* expression may mediate a later stage of information flow. The pathway involves at least three potential signal transduction branches: the *degU*, *comA*, and *spoOA* systems. We propose that the first two of these transmit information concerning the nutritional environment or the internal state of the cell to an information-processing system that can activate transcription of late competence genes or of genes for sporulation, degradative enzymes, motility, or surfactin production, all possible post-exponential-phase responses. Which combination of responses is selected depends on the nature of the output of the information-processing system, which in turn depends on the signals received.

The ComP-ComA sensor-effector pair may be involved in detecting the availability of C and N sources (28). Since ComP is likely to be an integral membrane protein, based on its inferred amino acid sequence, it probable relays information from the external environment. Msadek et al. (19) have suggested that the DegS-DegU sensor-effector pair likewise detects information concerning N- and C-source availability. From its sequence, it appears that DegS is likely to be a cytoplasmic protein (13, 16). Since it seems that the phosphorylated form of ComA and the unphosphorylated form of DegU are required for expression of competence, these systems may respond to the same signals in a reciprocal fashion or respond to different signals. We prefer the latter possibility, since the expression of late competence genes in the *degS* Δ 64 mutant is still repressed by glutamine and by the substitution of glycerol for glucose in the CM (J. Hahn and D. Dubnau, unpublished observations). On the other hand, strains that express late competence genes in the absence of ComP are no longer repressed by these condi-

tions. That has been shown in the case of a *comP* mutant strain overexpressing ComA (28) and in *mec* strains (7). This difference in behavior in the absence of ComP or DegS suggests that although the two sensors may be responding to N- and C-source availability, the ligands that directly or indirectly affect the two systems are not the same.

An interesting aspect of the scheme is the dual role played by AbrB. This protein, known to bind to DNA (25), is both a positive and a negative regulator of competence (3) as well as of other post-exponential-phase forms of expression (25). It should be stressed that on the molecular level, AbrB may function in competence exclusively in a negative or positive fashion, and the apparent positive role may result from the repression of a repressor. The SpoOA protein functions in vivo and in vitro as a negative regulator of *abrB* (18, 20, 30). However, it is clear that *spoOA* plays a role in sporulation, in addition to down regulation of *abrB* expression, since *spoOA* *abrB* double mutants fail to sporulate. In contrast, the role of the *spoOA* product in competence seems to be restricted to its negative effect on the synthesis or on the function of AbrB as a negative repressor. This was inferred from the observation that the residual level of competence in an *abrB* mutant is the same whether the strain is wild type or deficient for *spoOA* (3). It has been postulated that the *spoOF*, *spoOB*, and *spoOE* gene products are required to convert the SpoOA protein to an active state for sporulation as well as for other post-exponential-phase forms of expression (14). By analogy with similar signal transduction proteins (24), it has been argued that this activation corresponds to a phosphorylation event. A point mutation in the *spoOA* coding sequence (*sof-1*) bypasses the requirements for SpoOF, SpoOB, and SpoOE (14) and thus behaves as though the mutant protein is more often in the phosphorylated state. The lack of dependence of competence on the products of *spoOF*, *spoOB*, and *spoOE* (3) suggests that activation of SpoOA is not absolutely needed for competence, or for repression of AbrB synthesis, at least in CM. We have observed that the *sof-1* mutant is not altered in competence or in the expression of late competence genes (J. Hahn and D. Dubnau, unpublished results), in contrast to the competence deficiency of the analogous *degU*(Hy) mutants (16, 19). This implies that competence may be indifferent to the phosphorylation state of SpoOA. These arguments raise the possibility that SpoOA may not function as a signal transducer for competence but may be required only to maintain AbrB concentrations below an inhibitory level. Therefore, in wild-type strains, AbrB may never exert a negative influence on competence, since its level of expression may always be repressed by SpoOA.

The ability of *mec* mutations to bypass the positive function of the *abrB* null mutant, but not to bypass the *spoOA* mutant, suggests that the positive and negative functions of AbrB are exerted before and after the point of *mec* action, respectively. More precisely, the negative AbrB function may be exerted either after the point of *mec* action or via *mecA* or *mecB*. Similarly, the *spoOK* gene product or products apparently act either at the point of *mecB* action or after, but either before or at the point of *mecA* action. The latter alternative is consistent with the intriguing possibility that *mecA* is an allele of *spoOK*, since these loci are closely linked.

The genetic approach employed in this study is capable of extension. For instance, bypass studies are under way with multicopy plasmids that overexpress various regulatory products such as ComA and Sin (Y. Weinrauch, M. Roggiani, R. Penchev, and D. Dubnau, unpublished results).

Although powerful, these genetic experiments must be supplemented by physiological and biochemical data before a complete understanding of signal transduction and information flow during competence regulation is achieved.

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