JOURNAL OF BACTERIOLOGY, Nov. 1991, p. 7275–7282
0021-9193/91/227275-08$02.00/0
Copyright © 1991, American Society for Microbiology
Vol. 173, No. 22

Growth Stage Signal Transduction and the Requirements for *srfA* Induction in Development of Competence

J. HAHN AND D. DUBNAU*

Public Health Research Institute, 455 First Avenue, New York, New York 10016

Received 17 June 1991/Accepted 16 September 1991

srfA is an operon needed for the development of genetic competence in *Bacillus subtilis*. This operon is normally expressed at a low level during growth, and its transcription increases sharply just before the transition to stationary phase. The genetic requirements for the full expression of *srfA* were previously examined in several laboratories and shown to include *spo0A*, *spo0H*, *spo0K*, *comQ*, and *comA*. In the present study these results were confirmed with an isogenic set of strains. We have also shown that *comP* is needed for *srfA* expression but that other regulatory genes required for competence (*degU*, *sin*, and *abrB*) are not needed for the expression of *srfA*. We have used the expression of *srfA* under control of the regulatable P_{spac} promoter to study the kinetics of competence development and to determine whether the genes ordinarily required for expression of *srfA* are needed for any additional roles during the development of competence. When expression of *srfA* was driven from P_{spac}, competence was expressed constitutively throughout growth. Furthermore, when *srfA* was expressed from P_{spac}, the *spo0K*, *comQ*, *comP*, and *comA* determinants were no longer required for the expression of competence. We conclude therefore that the multiple signals which trigger the initiation of competence development in relation to growth stage are ordinarily received prior to the increase in *srfA* expression. We propose that these signals are mediated by the products of *spo0K*, *comQ*, *comP*, and *comA*, resulting in the phosphorylation of ComA by ComP. This in turn would enable ComA to function as a positive transcription factor for *srfA*, leading to the elaboration of the *srfA* product(s) and the consequent initiation of competence. We also propose that this is the major, and possibly the only, role for the *spo0K*, *comQ*, *comP*, and *comA* products during competence development.

Genetic competence is defined as a physiological state which enables bacterial cells to bind and take up high-molecular-weight transforming DNA. In *Bacillus subtilis* competence develops postexponentially in glucose minimal salts-based media but not in complex media and in a distinct minor fraction of the cells of a competent culture (reviewed in references 4 and 5). The existence of growth stage-specific, nutritional, and cell type-specific regulation implies that the control of competence is complex and responds to diverse signals. The development of competence is known to require the control of transcription of several so-called late competence genes, which are presumably required either directly for the binding and transport of DNA or for the assembly of a cell surface device which can accomplish this transport. Several loci that are needed for the transcription of the late competence genes but do not appear to act directly at the late competence gene promoters have been identified. This (and other data) demonstrates the existence of a signalling cascade that transduces nutritional and other types of information to the competence-specific transcriptional machinery. Several studies have begun to explore the relationships among the competence regulatory genes, and determination of the DNA sequences of these genes and biochemical analyses of their products have permitted specific inferences to be drawn concerning the mechanisms involved.

Several of the regulatory genes have been identified as histidine kinase and response regulator (RR) members of the bacterial two-component regulatory systems (reviewed in reference 43). These respond to appropriate signals by phosphorylation of the RR proteins, which in most cases behave as transcriptional activators. The *comP* and *comA* loci appear to encode histidine kinase and RR proteins, respectively, and are essential for the development of competence as well as for the postexponential expression of surfactin (30, 31, 48, 50). DegS and DegU are histidine kinase and RR proteins, respectively, that function in the regulation of a family of postexponentially expressed degradative enzymes (3, 16, 19, 24, 26, 44). DegU but not DegS is required for the development of competence (24, 38, 44). Spo0A, an RR protein that plays a central role in the regulation of several forms of postexponential expression, is also needed for the development of competence. In addition to these signal transduction proteins, a number of known DNA-binding and transcriptionally active molecules are required. These include Sin (11) and AbrB (1), which function as negative regulators of sporulation but act positively in the development of competence (11, 21, 22, 34, 53). Another sporulation locus that plays a role in competence is *spo0K* (40, 41). This operon encodes an oligopeptide permease system (33, 40), and at least the first, fourth, and fifth open reading frames of this five-cistron operon are required for competence (39, 40).

The *srfA* locus is also needed for competence (17, 27, 28, 46). This operon has been identified by mutations that have been called *csh-293* (17) and *comL* (46). *srfA* encodes proteins that are similar to others known to be responsible for the nonribosomal assembly of peptides and is required for the expression of surfactin, an extracellular peptide antibiotic (27, 45). Unlike most of the other regulatory genes listed above, which are expressed throughout growth, *srfA* expression is relatively low during exponential growth and increases as the stationary phase begins (17, 46). Since this increase is dependent on *comA* (46), *spo0K* (12, 20), and *comQ* (49) and since the expression of *srfA* is essential for

* Corresponding author.

that of the late competence genes (38, 46), it appears that this operon functions as an intermediate component of the regulatory pathway.

It had been previously shown that *comA* (30, 31, 46), *spo0H* and *spo0A* (17), *comQ* (49), and *spo0K* (12, 20) are required for the full expression of *srfA*. The present study extends the previous observations on the epistatic relationships among these regulatory genes, in order to permit a further delineation of the pathways involved in competence regulation. It is shown that *comP* is also required for the expression of *srfA* whereas *degU*, *abrB*, and *sin* are not. Furthermore, it is shown that competence can be expressed in the absence of the *spo0K*, *comA*, *comP*, or *comQ* product when *srfA* expression is driven by the P_{spac} promoter. The implications of these observations for the regulation of competence are discussed.

MATERIALS AND METHODS

Strains. The strains used were all derivatives of *B. subtilis* 168 and are listed in Table 1. The strains were constructed either by transformation or by transduction using phage PBS1 as described elsewhere (6). In some cases the P_{spac}-*srfA* strain (BD1916) was grown to competence in the presence of 2 mM isopropyl-β-D-thiogalactopyranoside (IPTG), and a second competence mutation was introduced by transformation. This was possible only if this mutation was selectable with a marker other than chloramphenicol or phleomycin resistance, since both of these markers are linked to the P_{spac}-*srfA* construct. In other cases, plasmid pMMN78, kindly provided by M. Nakano and P. Zuber, was used to integrate the P_{spac}-*srfA* construct by Campbell-like integration into a strain carrying a competence mutation, with selection for phleomycin resistance (1 μg/ml). This could be accomplished by transformation, since each regulatory mutant exhibits a characteristic residual level of transformability. Comparisons of competence levels were made only between strains that were isogenic except for the competence mutations being studied. The background used was that of the parent strain IS75 (*hisB2 leu-8 metB5*).

Expression of competence. The medium used for the expression of competence was a glucose-minimal salts medium, supplemented with casein hydrolysate and yeast extract (1). For some experiments competence was tested during growth in L broth. Overnight cultures were grown in each medium with shaking at 30°C and then diluted 100-fold into fresh competence medium or L broth at 37°C, plus or minus IPTG (2 mM). Incubation was continued with vigorous shaking, and samples were withdrawn at intervals for measurements of transformability. Aliquots (0.5 ml) were added to 0.5 μg of prototrophic DNA, and after 30 min of incubation at 37°C the cells were plated to determine the numbers of Leu^+ transformants and of viable CFU. The minimal medium plates used for the selection of transformants contained antibiotics to ensure against the loss of the mutational constructions present in the recipient strains. This was considered to be particularly important in the case of the P_{spac}-*srfA* construct inserted by Campbell-like integration, which could be lost in the absence of continual selection with either chloramphenicol or phleomycin.

Determination of β-galactosidase activity. Samples were taken during growth in competence medium, and β-galactosidase activity was determined as described previously (14). β-Galactosidase activities are expressed as units per milligram of protein.

TABLE 1. Strains

Strain	Genotype[a]	Source[b]
BD1890	*csh-293*::Tn*917 lacZ* (Em^r)	17
BD1891	*csh-293*::Tn*917 lacZ* (Cm^r)	17
BD1902	*comA124 csh-293*::Tn*917 lacZ* (Em^r)	48
BD1904	*comP*::Cm *csh-293*::Tn*917 lacZ* (Em^r)	50
BD1905	*comPΔK1 csh-293*::Tn*917 lacZ* (Km^r)[c]	Y. Weinrauch
BD1906	*abrB*::Cm *csh-293*::Tn*917 lacZ* (Em^r)	35
BD1907	*sin*::Cm *csh-293*::Tn*917 lacZ* (Em^r)	11
BD1908	*spo0A*::Cm *csh-293*::Tn*917 lacZ* (Em^r)	A. D. Grossman
BD1909	*spo0H*::Cm *csh-293*::Tn*917 lacZ* (Em^r)	J. Healy
BD1911	*spo0K141 csh-293*::Tn*917 lacZ* (Cm^r)	2
BD1912	*degU* {pDH55 (CM^r)} *csh-293*::Tn*917 lacZ* (Em^r)	16
BD1913	*degS* {pDH64 (Cm^r)} *csh-293*::Tn*917 lacZ* (Em^r)	16
BD1914	*degU32*(Hy) *csh-293*::Tn*917 lacZ* (Em^r)	3
BD1915	*degS200*(Hy) *csh-293*::Tn*917 lacZ* (Em^r)	16
BD1916	P_{spac}-*srfA*	32
BD1917	*comA124* P_{spac}-*srfA*	48
BD1918	*comQ*::Km P_{spac}-*srfA*	49
BD1919	*spo0KE*:Cm P_{spac}-*srfA*	40
BD1920	*spo0KA*::Tn*917* P_{spac}-*srfA*	D. Rudner, A. D. Grossman
BD1921	*abrB*::Cm P_{spac}-*srfA*	35
BD1922	*degU* {pDH55(Cm^r)} P_{spac}-*srfA*	16
BD1923	*spo0H*::Cm P_{spac}-*srfA*	J. Healy, R. Losick
BD1924	*spo0A*::Cm P_{spac}-*srfA*	A. D. Grossman
BD1925	*degU32*(Hy) P_{spac}-*srfA*	3
BD1929	*comA comP* P_{spac}-*srfA*[d]	50
BD1931	*comP*::Cm P_{spac}-*srfA*	50
BD1932	*comPΔK1* P_{spac}-*srfA*	Y. Weinrauch
BD1933	*degU118*(Hy) *csh-293*::Tn*917 lacZ* (Cm^r)	16
IS75	*hisB2 leu-8 metB5*	

[a] All of the strains are isogenic with IS75 (*hisB2 leu-8 metB5*), although the auxotrophic markers are not shown in the table. The colons represent disruptions by insertion of the elements described after the colons. Campbell-like disruptions are represented by braces with insertion of the elements described within the braces.

[b] The source given in each case (except BD1890, BD1891, and BD1916) is for the mutation other than *csh-293* or P_{spac}-*srfA*.

[c] The *comPΔK1* mutation is an in-frame deletion constructed by Y. Weinrauch and linked to a kanamycin resistance element.

[d] The *comP comA* mutation is a replacement of major portions of both genes by a Cm^r cassette (50).

RESULTS

Genetic requirements for *srfA* expression. The *csh-293* construct of Jaacks et al. (17) was used to determine the dependence of *srfA* transcription on other regulatory genes. *csh-293* consists of an insertion of the transposon Tn*917-lacZ* into the coding sequence of *srfA* (27), so that expression of the promoterless *lacZ* element of the transposon (52) is driven by the *srfA* promoter. Double-mutant strains carrying both *csh-293* and other regulatory mutations were tested for β-galactosidase activity as a function of growth in competence medium (Fig. 1). In the otherwise wild-type background, enzyme expression increased markedly just before T_0, as reported previously (17, 27) (Fig. 1A, B, D, E, and F). (T_0 is defined as the time of transition from exponential growth to the stationary phase). This was also true of the *degU*, *degS*, *abrB*, and *sin* strains, indicating that these genes are not absolutely needed for the transcription of *srfA*, although in the *sin* and *abrB* strains a slight decrease in

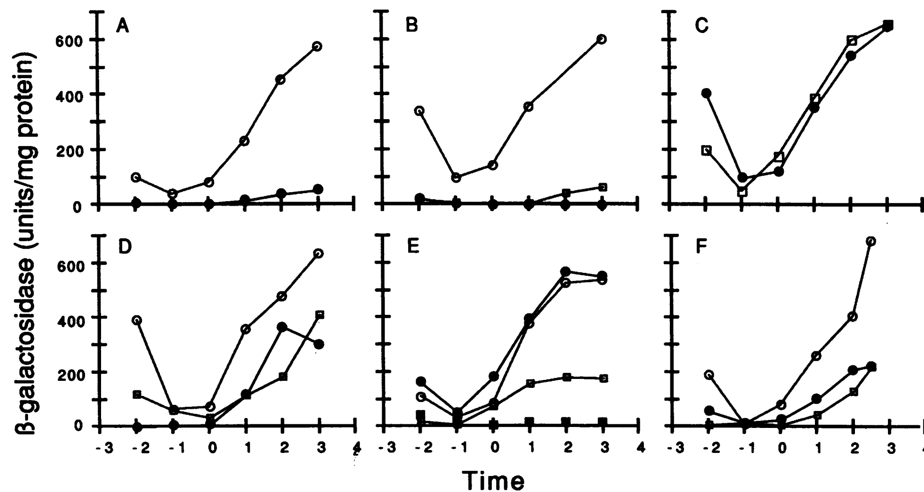

FIG. 1. Dependence of *srfA* expression on competence regulatory genes. (A, B, D, E, and F) Expression of β-galactosidase driven by the *srfA* promoter, measured in an otherwise wild-type strain as a function of growth in competence medium (○). The effects of point or disruption mutations on *srfA* expression are shown in the various panels: (A) *spo0K141* (BD1911) (●); (B) wild-type *comPΔK1* (BD1905) (□) and *comA* (BD1902) (●); (C) *degU* (BD1912) (●) and *degS* (BD1913) (□); (D) *abrB* (BD1906) (●) and *sin* (BD1907) (□); (E) *degS200*(Hy) (BD1915) (●), *degU32*(Hy) (BD1914) (□), *degU118*(Hy) (BD1933) (■); (F) *spo0A* (BD1908) (●) and *spo0H* (BD1909) (□). The numbers on the abscissa refer to hours before and after T_0.

expression to about two-thirds of the wild-type level was consistently observed (Fig. 1D). In contrast, the *comA*, *comP*, and *spo0K* mutants showed greatly reduced expression of β-galactosidase (Fig. 1A and B), although the residual levels of expression in the *comP* and *spo0K* backgrounds were slightly higher than in the *comA* mutant. We conclude that *srfA* expression is dependent on the products of these three genes. These results confirm those previously reported in the cases of *comA* (30, 46) and *spo0K* (12, 20). In another study it was shown that *comQ*, a newly identified competence regulatory gene, is also required for *srfA* expression (49). Finally, the dependence on *spo0A* and on *spo0H* was found to be partial, as previously reported (17) (Fig. 1F).

Mutations in *degS* [*degS*(Hy)] and in *degU* [*degU*(Hy)] which cause overproduction of degradative enzymes have been described previously (16, 24). These mutations also result in decreased competence (24, 42) and in decreased expression of late competence genes (38). The effects of a *degS*(Hy) and of two *degU*(Hy) mutations on *srfA* expression are shown in Fig. 1E. Only *degU32*(Hy) and *degU118* (Hy) exhibited decreased expression. The effect of *degU118* (Hy) on *srfA* transcription was greater than that of *degU32* (Hy), as is its effect on transformability (38).

Competence of the P_{spac}-*srfA* strain. Plasmid pMMN78 carrying a P_{spac}-*srfA* construct was provided by M. M. Nakano and P. Zuber (32). This plasmid carries part of the first reading frame of the *srfA* operon downstream from the IPTG-inducible P_{spac} promoter (51). Integration of pMMN78, which cannot replicate in *B. subtilis*, results in the insertion in the chromosome of a truncated copy of the first *srfA* open reading frame driven by the *srfA* promoter and also places an entire copy of the *srfA* coding sequence under P_{spac} control. Strains constructed in this fashion were shown by Nakano and Zuber (32) to express competence poorly in the absence of IPTG but at an elevated level when grown in the presence of this inducer of P_{spac} activity. This result was expected, since *srfA* is known to be an essential competence locus (17, 27, 28, 46).

The panels of Fig. 2 allow comparison of the development of competence during growth of the P_{spac}-*srfA* and wild-type strains in competence medium. Under these conditions the wild-type strain (IS75) develops transformability beginning

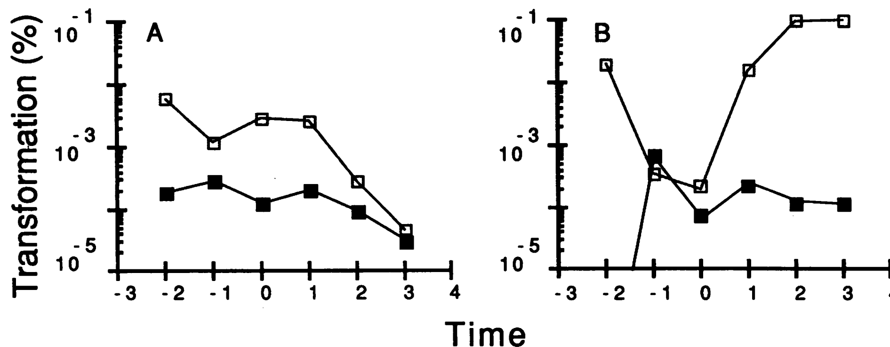

FIG. 2. Kinetics of competence development in competence medium. (A) Competence of the P_{spac}-*srfA* strain growing in competence medium in the presence (□) and absence (■) of IPTG; (B) development of competence of the wild-type strain (□) and the *csh-293* (*srfA*) strain (■) growing in competence medium. The numbers on the abscissa refer to hours before and after T_0.

at about T_0 and reaches a level about 20-fold higher than that of the P_{spac}-*srfA* strain grown with 2 mM IPTG. The transformability of the P_{spac}-*srfA* strain grown in competence medium and in the presence of IPTG in this experiment was about 20-fold higher than in its absence and is expressed throughout growth, although it declines after about T_1 (Fig. 2A). In repeated experiments the stimulatory effect of IPTG was found to be 20- to 100-fold, and expression of competence was always observed throughout growth. The cause of the decline noted during the stationary phase is unknown. It is not due to a loss of IPTG, since the addition of more inducer during growth does not prevent the decline (15). Also, the decline cannot be due simply to dilution of the cultures by growth, since the extent of the decrease is often 30-fold or more while the viable count of the culture rises only about 2- to 3-fold after T_0 (15). In the wild type, competence peaks at about T_2 and declines only after about T_3 to T_4, somewhat later than shown by the data presented here. Apparently the competent state is unstable, and the maintenance of competence after the cessation of growth may impose a special requirement that the low-level induction of *srfA* expression driven by P_{spac} cannot provide.

The lower level of competence exhibited by the induced cultures growing in competence medium compared with that of the wild-type strain at T_2 may be ascribed to two causes. First, all the selections for transformation involving the P_{spac}-*srfA* strains were carried out with chloramphenicol included in the selective plates to ensure the presence of the P_{spac}-*srfA* construction, which had been integrated by a single reciprocal recombination event and therefore might be prone to loss. We have noted consistently that the presence of antibiotic in the selective medium reduces the apparent transformation frequency severalfold. Second and most important, the lower level of expression of competence in the P_{spac}-*srfA* strains can also be explained by the relative inefficiency of the P_{spac} promoter, which supports less expression of *srfA* than does the *srfA* promoter itself (32). In agreement with this explanation, we have found that the use of 10 mM rather than 2 mM IPTG results in a slightly higher level of transformability (15).

In the wild-type strain, competence is expressed poorly and constitutively in complex media such as L broth at a level of about $10^{-4}\%$ (8). The P_{spac}-*srfA* strain in L broth exhibited a lower level of competence (about $10^{-5}\%$), also expressed throughout growth. When IPTG was added to L broth, the level of constitutively expressed competence rose to 1×10^{-4} to $3 \times 10^{-4}\%$, about the same as that exhibited by the wild type in L broth (15). These results indicate that the competence expressed by the P_{spac}-*srfA* strain in L broth in the absence of induction was limited by the amount of *srfA* expression, since it was lower than that of the wild-type strain growing in the same medium. When *srfA* expression was induced by the addition of IPTG, competence was no longer limited by the availability of the *srfA* products and rose to the level characteristic of the wild-type strain in L broth or slightly higher but not to the level of the induced P_{spac}-*srfA* strain in competence medium. We conclude that the signals present in L broth that are responsible for the repression of competence development are primarily received after the point of the normal rise in *srfA* transcription. This will be discussed further below.

The data in Fig. 2 demonstrate that induction of the P_{spac}-*srfA* construct leads to the constitutive expression of competence. The ability of *srfA* expression under P_{spac} control to bypass the growth stage-specific regulation of competence implies that signals for this mode of competence control are received prior to the point at which *srfA* expression normally increases. Understanding the nature of these signals and the means by which they are interpreted, therefore, largely reduces to an understanding of the regulation of *srfA* expression.

Expression of *srfA* under P_{spac} control bypasses certain regulatory mutations. The P_{spac}-*srfA* construct was combined with mutations in the various competence regulatory genes. The expression of competence in the presence and absence of IPTG in these strains is shown in Fig. 3 as a function of growth. In the cases of *spo0K*, *comA*, *comP*, and *comQ*, the addition of inducer restored competence to about the induced level characteristic of the P_{spac}-*srfA* strain which was otherwise wild type (compare Fig. 2 and 3). This was true of two different *spo0K* mutations, one with a disruption of *spo0KE* (15) and the other with a Tn*917* insertion in the first open reading frame of *spo0K*, which was presumably polar on the entire *spo0K* operon (Fig. 3D). *spo0KA*, *spo0KD*, and *spo0KE*, at least, are known to be required for competence (39, 40). The bypass of *comP* was obtained with a disruption mutation which is slightly polar on *comA* (15, 50) in addition to the in-frame deletion mutation (Fig. 3E). A double *comA comP* mutation was also bypassed by *srfA* induction (Fig. 3A). In contrast, the *degU* (15) and *abrB* (Fig. 3I) null mutations were not bypassed by induction of P_{spac}-*srfA*. Bypass of a *sin* mutation was not tested, since for an unknown reason we experienced difficulty in constructing a *sin* P_{spac}-*srfA* strain.

In the cases of *spo0A* (Fig. 3H) and *spo0H* (Fig. 3G), a partial bypass by induction of P_{spac}-*srfA* was observed. Although the extent of increase in competence noted in the presence of IPTG was dramatic in both of these mutant strains, the final levels of transformability were lower than that of the induced P_{spac}-*srfA* strain. Also, the competence levels of the uninduced *spo0H* P_{spac}-*srfA* and *spo0A* P_{spac}-*srfA* strains were extremely low, much lower than the levels of the single P_{spac}-*srfA*, *spo0A*, and *spo0H* mutants. This can be seen directly in Fig. 3H for the case of *spo0A* and can be inferred from the fact that the decrease usually observed for *spo0H* mutants is only about 20-fold compared with the wild-type level (1). This behavior was in marked contrast to that of the *comA*, *spo0K*, *comQ*, *comP*, and *degU32*(Hy) mutants, which exhibited about the same levels of transformability when alone or when combined with P_{spac}-*srfA* (Fig. 3). Finally, Fig. 3F demonstrates that the effect of the *degU32*(Hy) mutation was bypassed by induction of P_{spac}-*srfA*.

Although these results measuring the bypass of competence regulatory mutations by P_{spac}-driven *srfA* expression will be discussed in more detail below, we can conclude at this point that they are at least consistent with the dependencies of *srfA* expression on the various regulatory genes. Thus, bypass of the *comA*, *comQ*, *comP*, and *spo0K* null mutations and of the *degU*(Hy) mutation but not of *abrB* and *degU* (null) mutations was observed. The first five mutations markedly depressed *srfA* expression, but the last two did not (Fig. 1). The partial bypass noted in the cases of *spo0H* and *spo0A* are consistent with the partial dependency of *srfA* expression on these two genes.

DISCUSSION

The competence hierarchy and the dependence of *srfA* expression on other regulatory genes. The promoter-proximal portion of the *srfA* operon is required for the expression of competence and of the known late competence genes (17, 27,

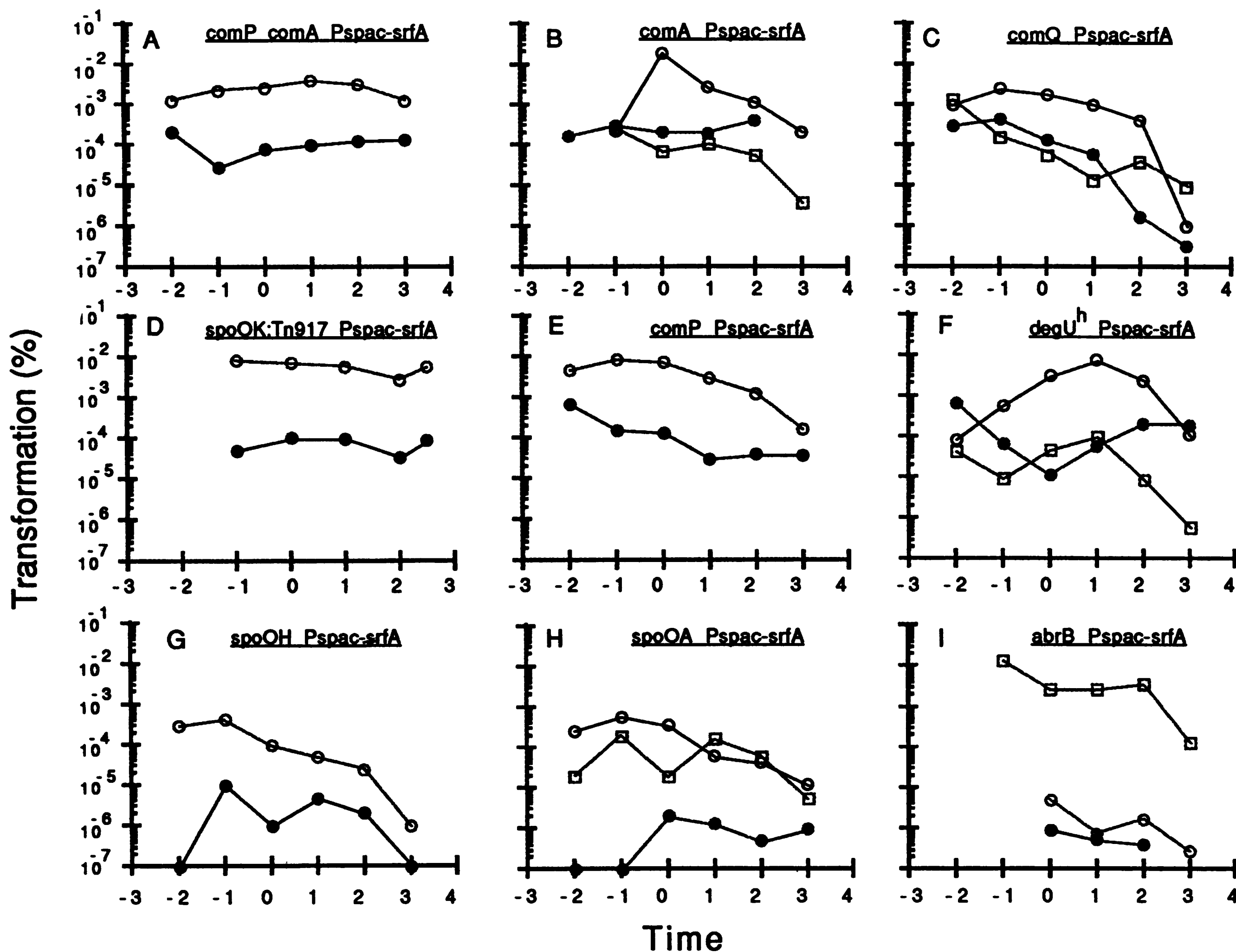

FIG. 3. Bypass of competence requirements by induction of P_{spac}-*srfA* during growth in competence medium. In each panel is shown the competence of the indicated double- or triple-mutant strain grown in competence medium in the presence (○) and absence (●) of IPTG. The following single mutant strains were also grown in competence medium, and their transformabilities are displayed (□): (B) *comA124*; (C) *comQ*; (F) *degU32*(Hy); (H) *spo0A*::Cm; (I) *abrB*. The *comP* mutant strain used in panel E carried the in-frame deletion. The *degU*(Hy) mutant used in panel F carried *degU32*(Hy). The numbers on the abscissa refer to hours before and after T_0.

38, 45, 46). The expression of competence throughout growth in competence medium when *srfA* expression is driven by the P_{spac} promoter suggests that in the wild-type strain the induction of *srfA* expression may be responsible for the appearance of competence at T_0 in competence medium. We have noticed consistently that the rise in *srfA* expression begins at about T_{-1}, shortly before the increase in competence (15). This is apparent in Fig. 1. β-Galactosidase fusion studies had previously shown that the transcription of *srfA* is dependent on expression of *comA* (30, 46), *spo0K* (12, 20), and *comQ* (49). In the present study, it is shown that this dependence extends to *comP* but not to *degU* and probably not to *sin* and *abrB* (Fig. 1). *srfA* expression also exhibits a partial dependency on *spo0A* and *spo0H* (Fig. 1) (17). These observations taken together suggest that the flow of information responsible for the onset of competence proceeds from an apparatus or pathway involving at least the Spo0K, ComP, ComQ, and ComA proteins to the sequences responsible for *srfA* transcription. The DegU, AbrB, and Sin products, on the other hand, must function principally either downstream from *srfA* or on a separate branch of the regulatory pathway (4, 5, 7). One of the possible schematic representations of these relationships is shown in Fig. 4. Since *mecA* and *mecB* mutations bypass mutations in most of the other regulatory genes shown in Fig. 4 (38), these regulatory loci probably act before *mecA* and *mecB*.

The P_{spac}-*srfA* mutant grown in the absence of IPTG; the *csh-293* mutant of *srfA*; the *spo0K*, *comA*, *comP*, and *comQ* mutants; and multiple-mutant strains deficient in various combinations of these markers are all about 100- to 500-fold deficient in transformability. These observations are consistent with the simple hypothesis that the last four loci are needed primarily to turn on expression of *srfA*. If so, the results would imply that the competence pathway is somewhat "leaky," allowing about 0.1 to 1% of the wild-type expression in the absence of the *srfA* signal. This residual leaky expression is not temporally controlled; expression of competence in the P_{spac}-*srfA* strain in the absence of IPTG is constitutive (Fig. 2 and 3), as is the residual level of

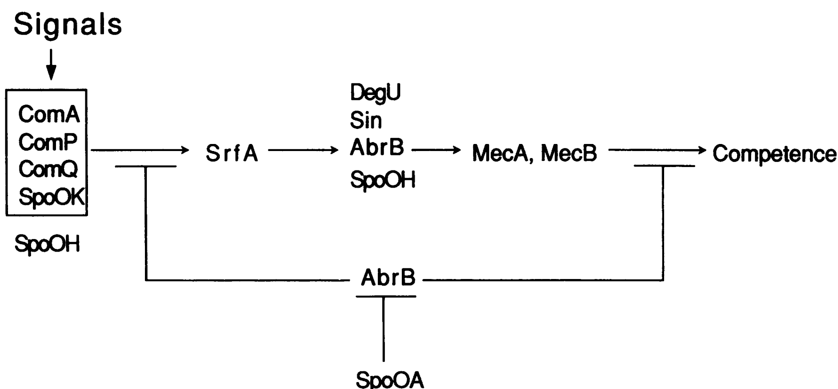

FIG. 4. Proposed pathway of information flow during the development of competence. As indicated, deficiencies in the Spo0H and Spo0A proteins exert effects on competence both before and after *srfA*. Overproduction of AbrB, which occurs in Spo0A-deficient strains (35), exerts a negative effect on competence (1, 37). This effect is exerted both before SrfA (see text) and after MecA and MecB (38) in the pathway. The lines terminated by arrowheads indicate positive effects and those terminated by perpendiculars indicate negative effects. It has not been shown that signal input occurs only at the point indicated.

competence observed in *comA*, *comP* (47), and *spo0K* (15) mutants. On the other hand, *degU*, *abrB*, *sin*, *spo0A*, and *spo0H* null mutants exhibit various levels of residual competence, ranging from about 5% of the wild-type level in the case of *spo0H* to 0.1% of the wild-type level in the cases of *spo0A* and *degU*. Double mutants of *srfA* with *spo0A*, *spo0H*, *abrB* (Fig. 3), and *degU* (15) exhibit residual levels much lower than that of the single mutants. These multiplicative effects are consistent with the inference that *degU*, *sin*, *abrB*, *spo0A*, and *spo0H* operate downstream of *srfA*, as shown in Fig. 4.

The expression of *srfA* is partially decreased in a strain with a null mutation in *spo0H* (Fig. 1) (17), suggesting that the effect of *spo0H* deficiency on competence (1) is exerted both before and after *srfA* on the competence pathway. This is supported by the failure of P_{spac}-driven *srfA* expression to completely bypass a *spo0H* deficiency (Fig. 3G). Although *spo0H* encodes a minor sigma factor (9), primer extension mapping suggests that the *srfA* operon is driven by an $E\sigma^A$ polymerase (29), and the effect of the *spo0H* mutation on *srfA* expression is therefore most likely indirect.

It is known that Spo0A acts to downregulate *abrB* expression (10, 35), and we have obtained evidence that this is the only role for Spo0A in the competence system, implying that an excess of AbrB inhibits competence development (1, 37). The partial dependence of *srfA* expression on *spo0A* (Fig. 1) (17) therefore suggests that AbrB acts negatively on competence at points in the competence regulatory pathway both before and after the increased expression of *srfA*. The observation that the *spo0A* deficiency is only partially bypassed by P_{spac}-driven expression of *srfA* (Fig. 3H) further supports this conclusion. The notion that AbrB acts negatively on competence at multiple points was advanced previously (38).

degU(Hy) and *degS*(Hy) mutations stimulate degradative enzyme synthesis and inhibit competence (3, 24). The present data seem to indicate that the inhibitory effects of *degU118*(Hy) and *degU32*(Hy) are exerted before *srfA* (Fig. 1), and the bypass data (Fig. 3F) suggest that the inhibitory effect of *degU32*(Hy) on competence is entirely exerted before *srfA* in the competence regulatory pathway. The failure of the *degS*(Hy) mutation to also decrease *srfA* expression (Fig. 1) is unexpected and unexplained.

The major role of the ComA-ComQ-ComP-Spo0K signal transduction system is to turn on *srfA*. The dependence of *srfA* expression on *comA*, *comP*, *comQ*, and *spo0K* and the apparently complete bypass of mutations in each of the last four genes by P_{spac}-driven expression of *srfA* (Fig. 2 and 3) suggest that the major roles for the four regulatory loci in the development of competent cells is to turn on the transcription of *srfA*. As noted above, the constitutive expression of competence in the induced P_{spac}-*srfA* culture further suggests that the normal induction of *srfA* expression responds to growth stage-related signals. These conclusions imply that before reaching T_0 in competence medium, signals are received by the ComP-ComQ-ComA-Spo0K sensing device, which then increases the transcription of *srfA*, thereby inducing the development of competence via regulatory genes that act downstream of *srfA*. It has been shown previously that the response to at least some nutritional signals (involving the presence of glucose and glutamine) requires the action of ComP (50). Since the P_{spac}-driven induction of *srfA* bypasses growth stage-related control and since certain nutritional responses require ComP, we infer that growth stage and nutritional signals normally act prior to the end-of-growth increase in expression of *srfA*. It is reasonable to postulate that these signals are received by the ComP-ComQ-ComA-Spo0K sensing device, especially given what is known about the nature of these gene products (see below). Since the inhibition of competence development by L broth is not bypassed by *srfA* induction, it is likely that additional nutritional signals may be received downstream from *srfA*.

The proteins encoded by *spo0K* closely resemble components of the oligopeptide permease (*opp*) of gram-negative organisms (33, 40). Several of the *spo0K* proteins as well as ComP are likely to be membrane associated, and it is attractive to speculate that the ComP, ComQ, and Spo0K proteins interact at the *B. subtilis* cell surface, gathering environmental information and consequently regulating the phosphorylation of ComA. The organization of the *spo0K* oligopeptide permease resembles that of a large class of ATP-driven transport systems, each of which consists of several components. The role of *spo0K* in controlling the *com* regulon of *B. subtilis* is strikingly parallel to that of the *pst* system in controlling the *pho* regulon of *Escherichia coli* (36). The *pst* operon of *E. coli* encodes a high-affinity phosphate uptake system that is a member of the *spo0K*

class of transporters. The *pst* proteins apparently sense the level of phosphate in the periplasm and transduce this information across the membrane to the histidine kinase and RR proteins PhoB and PhoR. This resemblance has been pointed out previously by A. D. Grossman and his colleagues (12, 40). In particular, the *spo0K* system may respond to an extracellular peptide and may communicate across the cell membrane with the *com* system. A soluble competence factor has been reported in the case of *B. subtilis* (18), and a soluble sporulation factor in this organism has been described (13). Most relevant and suggestive is the report by Grossman et al. (12) that a soluble extracellular peptide factor may trigger the expression of *srfA*.

What then is the meaning of the reported resemblance of the predicted *srfA* proteins to those known to catalyze the nonribosomal assembly of extracellular peptide antibiotics (27, 45)? Perhaps *srfA* encodes enzymes required to produce a competence signalling molecule, and *spo0K* responds to this signal. This interaction may result in the further activation of *srfA* transcription, thus causing a sharp increase in the concentration of *srfA* products, particularly as growth proceeds. Alternatively, the *spo0K* receptor system may respond to an environmental molecule other than the *srfA* product, perhaps to a different peptide factor, again leading to an increase in *srfA* transcription. By either mechanism, the enhanced expression of *srfA* may result in increased synthesis of a peptide competence factor, in the first case identical to and in the second model different from the one postulated to activate the *spo0K*-dependent mechanism. A receptor for this factor must exist downstream of *srfA* in the competence pathway, leading to the switching on of competence correlated with the increase in cell density. The present evidence suggests that this receptor is not encoded by *spo0K*, since the major role of that locus is exerted upstream of *srfA*.

Competence regulation involves the processing of multiple signals. We have observed that during exponential growth, competence is repressed by the addition of amino acids and that this repression is overridden after T_0 in competence medium (7). It is shown here that competence is expressed during exponential growth in the IPTG-induced P_{spac}-*srfA* strain, growing in the presence of amino acids included in the competence medium. The repression of competence by amino acids therefore probably acts before *srfA* in the competence cascade, and by implication, the ComQ-ComP-ComA-Spo0K machinery which turns on *srfA* may be responsive to the presence or absence of exogenous amino acids. Evidence for the involvement of ComA and ComP in the response of *degQ* expression to amino acids has been presented by Msadek et al. (25). This machinery responds to the presence of exogenous glucose as well (50). Finally, as discussed above, the involvement of *spo0K* in the regulation of *srfA* hints that a peptide may act as a regulator in this system. It therefore appears that multiple signals are interpreted by the regulatory apparatus for competence and that these multiple signals may be received by the Spo0K-ComQ-ComP-ComA machinery. We hypothesize that when the proper combination of signals is received, phosphorylation of ComA by ComP occurs, activating the RR molecule as a transcriptional factor and causing the increased expression of *srfA*. This in turn may result in the release of an extracellular peptide competence factor, which will cause the initiation of competence development in a minority of the cells in the culture. The competence regulatory genes (*degU*, *abrB*, *sin*, *comK* [46], and *ctf* [23]) which are not required for the expression of *srfA* may be involved in the downstream, factor-responsive segment of the competence regulation pathway (Fig. 4) and in the response to other signals, such as the inhibitory component(s) of L broth.

ACKNOWLEDGMENTS

We particularly thank M. Nakano and P. Zuber for their kind gift of pMMN78 and for communicating results before publication. We thank A. D. Grossman as well for providing information prior to publication. We also thank E. Dubnau, A. D. Grossman, A. Londoño, M. Nakano, M. Roggiani, I. Smith, Y. Weinrauch, and P. Zuber for valuable discussions.

This work was supported by NIH grant AI 10311.

REFERENCES

1. **Albano, M., J. Hahn, and D. Dubnau.** 1987. Expression of competence genes in *Bacillus subtilis*. J. Bacteriol. **169:**3110–3117.
2. **Coote, J. G.** 1972. Sporulation in *Bacillus subtilis*. Genetic analysis of oligosporogenous mutants. J. Gen. Microbiol. **71:**17–27.
3. **Dahl, M. K., T. Msadek, F. Kunst, and G. Rapoport.** 1991. Mutational analysis of the *Bacillus subtilis* DegU regulator and its phosphorylation by the DegS protein kinase. J. Bacteriol. **173:**2539–2547.
4. **Dubnau, D.** 1991. Genetic competence in *Bacillus subtilis*. Microbiol. Rev. **55:**395–424.
5. **Dubnau, D.** 1991. The regulation of genetic competence in *Bacillus subtilis*. Mol. Microbiol. **5:**11–18.
6. **Dubnau, D., R. Davidoff-Abelson, and I. Smith.** 1969. Transformation and transduction in *Bacillus subtilis*: evidence for separate modes of recombinant formation. J. Mol. Biol. **45:**155–179.
7. **Dubnau, D., J. Hahn, L. Kong, M. Roggiani, and Y. Weinrauch.** 1991. Genetic competence as a post-exponential global response. Semin. Dev. Biol. **2:**3–12.
8. **Dubnau, D., and M. Roggiani.** 1990. Growth medium-independent genetic competence mutants of *Bacillus subtilis*. J. Bacteriol. **172:**4048–4055.
9. **Dubnau, E., J. Weir, G. Nair, L. Carter III, C. Moran, Jr., and I. Smith.** 1988. *Bacillus* sporulation gene *spo0H* codes for σ^{30} (σ^H). J. Bacteriol. **170:**1054–1062.
10. **Fürbass, R., M. Gocht, P. Zuber, and M. A. Marahiel.** 1991. Interaction of AbrB, a transcriptional regulator from *Bacillus subtilis* with the promoters of the transition state-activated genes *tycA* and *spoVG*. Mol. Gen. Genet. **225:**347–354.
11. **Gaur, N. K., E. Dubnau, and I. Smith.** 1986. Characterization of a cloned *Bacillus subtilis* gene that inhibits sporulation in multiple copies. J. Bacteriol. **168:**860–869.
12. **Grossman, A. D., K. Ireton, E. F. Hoff, J. R. LeDeaux, D. Z. Rudner, R. Magnuson, and K. A. Hicks.** 1991. Signal transduction and the initiation of sporulation in *Bacillus subtilis*. Semin. Dev. Biol. **2:**31–36.
13. **Grossman, A. D., and R. Losick.** 1988. Extracellular control of spore formation in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **85:**4369–4373.
14. **Gryczan, T. J., M. Israeli-Reches, and D. Dubnau.** 1984. Induction of macrolide-lincosamide-streptogramin B resistance requires ribosomes able to bind inducer. Mol. Gen. Genet. **194:**357–361.
15. **Hahn, J., and D. Dubnau.** Unpublished data.
16. **Henner, D. J., M. Yang, and E. Ferrari.** 1988. Localization of *Bacillus subtilis sacU*(Hy) mutations to two linked genes with similarities to the conserved procaryotic family of two-component signalling systems. J. Bacteriol. **170:**5102–5109.
17. **Jaacks, K. J., J. Healy, R. Losick, and A. D. Grossman.** 1989. Identification and characterization of genes controlled by the sporulation-regulatory gene *spo0H* in *Bacillus subtilis*. J. Bacteriol. **171:**4121–4129.
18. **Joenje, H., M. Gruber, and G. Venema.** 1972. Stimulation of the development of competence by culture fluids in *Bacillus subtilis* transformation. Biochim. Biophys. Acta **262:**189–199.
19. **Kunst, F., M. Debarbouille, T. Msadek, M. Young, C. Mauel, D. Karamata, A. Klier, G. Rapoport, and R. Dedonder.** 1988.

Deduced polypeptides encoded by the *Bacillus subtilis sacU* locus share homology with two-component sensor-regulator systems. J. Bacteriol. **170**:5093–5101.
20. **Magnuson, R., D. Z. Rudner, and A. D. Grossman (Massachusetts Institute of Technology).** 1991. Personal communication.
21. **Mandic-Mulec, I., N. K. Gaur, and I. Smith (Public Health Research Institute).** 1991. Personal communication.
22. **Marahiel, M. A., P. Zuber, G. Czekay, and R. Losick.** 1987. Identification of the promoter for a peptide antibiotic biosynthesis gene from *Bacillus brevis* and studies on its regulation in *Bacillus subtilis*. J. Bacteriol. **169**:2215–2222.
23. **Mohan, S., and D. Dubnau.** 1990. Transcriptional regulation of *comC*: evidence for a competence-specific factor in *Bacillus subtilis*. J. Bacteriol. **172**:4064–4071.
24. **Msadek, T., F. Kunst, D. Henner, A. Klier, G. Rapoport, and R. Dedonder.** 1990. Signal transduction pathway controlling synthesis of a class of degradative enzymes in *Bacillus subtilis*: expression of the regulatory genes and analysis of mutations in *degS* and *degU*. J. Bacteriol. **172**:824–834.
25. **Msadek, T., F. Kunst, A. Klier, and G. Rapoport.** 1991. DegS-DegU and ComP-ComA modulator-effector pairs control expression of the *Bacillus subtilis* pleiotropic regulatory gene *degQ*. J. Bacteriol. **173**:2366–2377.
26. **Mukai, K., M. Kawata, and T. Tanaka.** 1990. Isolation and phosphorylation of the *Bacillus subtilis degS* and *degU* gene products. J. Biol. Chem. **265**:20000–20006.
27. **Nakano, M. M., R. Magnusson, A. Myers, J. Curry, A. D. Grossman, and P. Zuber.** 1991. *srfA* is an operon required for surfactin production, competence development, and efficient sporulation in *Bacillus subtilis*. J. Bacteriol. **173**:1770–1778.
28. **Nakano, M. M., M. A. Marahiel, and P. Zuber.** 1988. Identification of a genetic locus required for the biosynthesis of the lipopeptide antibiotic surfactin in *Bacillus subtilis*. J. Bacteriol. **170**:5662–5668.
29. **Nakano, M. M., L. Xia, and P. Zuber.** 1991. Transcription initiation region of the *srfA* operon which is controlled by the *comP-comA* signal transduction system in *Bacillus subtilis*. J. Bacteriol. **173**:5487–5493.
30. **Nakano, M. M., and P. Zuber.** 1989. Cloning and characterization of *srfB*, a regulatory gene involved in surfactin production and competence in *Bacillus subtilis*. J. Bacteriol. **171**:5347–5353.
31. **Nakano, M. M., and P. Zuber.** 1990. Identification of genes required for the biosynthesis of the lipopeptide antibiotic surfactin in *Bacillus subtilis*, p. 397–405. *In* M. M. Zukowski, A. T. Ganesan, and J. A. Hoch (ed.), Genetics and biotechnology of bacilli, vol. 3. Academic Press, San Diego, Calif.
32. **Nakano, M. M., and P. Zuber.** 1991. The primary role of ComA in establishment of the competent state in *Bacillus subtilis* is to activate expression of *srfA*. J. Bacteriol. **173**:7269–7274.
33. **Perego, M., C. F. Higgins, S. R. Pearce, M. P. Gallagher, and J. A. Hoch.** 1991. The oligopeptide transport system of *Bacillus subtilis* plays a role in the initiation of sporulation. Mol. Microbiol. **5**:173–185.
34. **Perego, M., and J. A. Hoch.** 1988. Molecular cloning of the transcription inhibitor *abrB* of *Bacillus subtilis*, p. 129–134. *In* A. T. Ganesan and J. A. Hoch (ed.), Genetics and biotechnology of bacilli. Academic Press, Inc., New York.
35. **Perego, M., G. B. Spiegelman, and J. A. Hoch.** 1988. Structure of the gene for the transition state regulator *abrB*: regulator synthesis is controlled by the *spo0A* sporulation gene in *Bacillus subtilis*. Mol. Microbiol. **2**:689–699.
36. **Rao, N. N., and A. Torriani.** 1990. Molecular aspects of phosphate transport in *Escherichia coli*. Mol. Microbiol. **4**:1083–1090.
37. **Roggiani, M., and D. Dubnau.** Unpublished data.
38. **Roggiani, M., J. Hahn, and D. Dubnau.** 1990. Suppression of early competence mutations in *Bacillus subtilis* by *mec* mutations. J. Bacteriol. **172**:4056–4063.
39. **Rudner, D. Z., J. R. LeDeaux, K. Ireton, and A. D. Grossman (Massachusetts Institute of Technology).** 1991. Personal communication.
40. **Rudner, D. Z., J. R. LeDeaux, K. Ireton, and A. D. Grossman.** 1991. The *spo0K* locus of *Bacillus subtilis* is homologous to the oligopeptide permease locus and is required for sporulation and competence. J. Bacteriol. **173**:1388–1398.
41. **Sadaie, Y., and T. Kada.** 1983. Formation of competent *Bacillus subtilis* cells. J. Bacteriol. **153**:813–821.
42. **Steinmetz, M., F. Kunst, and R. Dedonder.** 1976. Mapping of mutations affecting synthesis of exocellular enzymes in *Bacillus subtilis*. Mol. Gen. Genet. **148**:281–285.
43. **Stock, J. B., A. J. Ninfa, and A. M. Stock.** 1989. Protein phosphorylation and the regulation of adaptive responses in bacteria. Microbiol. Rev. **53**:450–490.
44. **Tanaka, T., and M. Kawata.** 1988. Cloning and characterization of *Bacillus subtilis iep*, which has positive and negative effects on production of extracellular proteases. J. Bacteriol. **170**:3593–3600.
45. **van Sinderen, D., and G. Grandi (University of Groningen).** 1991. Personal communication.
46. **van Sinderen, D., S. Withoff, H. Boels, and G. Venema.** 1990. Isolation and characterization of *comL*, a transcription unit involved in competence development in *Bacillus subtilis*. Mol. Gen. Genet. **224**:396–404.
47. **Weinrauch, Y., and D. Dubnau.** Unpublished data.
48. **Weinrauch, Y., N. Guillen, and D. A. Dubnau.** 1989. Sequence and transcription mapping of *Bacillus subtilis* competence genes *comB* and *comA*, one of which is related to a family of bacterial regulatory determinants. J. Bacteriol. **171**:5362–5375.
49. **Weinrauch, Y., T. Msadek, F. Kunst, G. Rapoport, and D. Dubnau.** 1991. Sequence and properties of *comQ*, a new competence gene of *Bacillus subtilis*. J. Bacteriol. **173**:5685–5693.
50. **Weinrauch, Y., R. Penchev, E. Dubnau, I. Smith, and D. Dubnau.** 1990. A *Bacillus subtilis* regulatory gene product for genetic competence and sporulation resembles sensor protein members of the bacterial two-component signal-transduction systems. Genes Dev. **4**:860–872.
51. **Yansura, D. G., and D. J. Henner.** 1984. Use of the *Escherichia coli lac* repressor and operator to control gene expression in *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **81**:439–443.
52. **Youngman, P., P. Zuber, J. B. Perkins, K. Sandman, M. Igo, and R. Losick.** 1985. New ways to study developmental genes in spore-forming bacteria. Science **228**:285–291.
53. **Zuber, P., and R. Losick.** 1987. Role of *abrB* in *spo0A*- and *spo0B*-dependent utilization of a sporulation promoter in *Bacillus subtilis*. J. Bacteriol. **169**:2223–2230.