Competence-Specific Induction of the *Bacillus subtilis* RecA Protein Analog: Evidence for Dual Regulation of a Recombination Protein

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The development of competence in *Bacillus subtilis* is accompanied by the transcriptional activation of DNA damage-inducible (*din*) operons and other SOS-like responses. We report here that *B. subtilis* Rec protein (the analog of *Escherichia coli* RecA), a DNA damage-inducible protein, is substantially induced when cells differentiate to a state of competence. We quantitated the induction of *B. subtilis* Rec protein and the *B. subtilis* *din-22* operon (representative of all known *B. subtilis* *din* operons) during competence development in Rec⁺ and DNA repair-deficient strains. We present two lines of evidence that Rec protein induction in competent cells is controlled by a competence-specific mechanism that is distinct from the SOS-like regulation that controls Rec induction following DNA damage: (i) Rec protein was significantly induced in *rec* mutants (*recA1* and *recE4*) that are highly deficient in Rec induction by DNA damage, and (ii) Rec protein induction during competence development was greater than maximum Rec induction by DNA damage. On the other hand, our results suggest that the *din-22* operon is induced by the same (SOS-like) mechanism both during competence development and after DNA damage.

The bacterium *Bacillus subtilis* differentiates to a discrete physiological state, termed competence, that is characterized by the ability to bind and take up exogenous DNA (2). Incoming DNA taken up by competent cells can be integrated into the host chromosome via general genetic recombination (i.e., genetic transformation) (9, 34), which presumably requires the DNA strand exchange activity (31) of the *B. subtilis* Rec protein, the analog of *Escherichia coli* RecA (26). Corresponding to the many physiological changes that occur during competence development (8), several competence-related proteins are induced or synthesized de novo (14) and competence-specific (*com*) genes are expressed (1). Competence development in *B. subtilis* is also accompanied by the induction of SOS-like responses (in the absence of DNA-damaging treatments), such as enhanced mutagenesis (33), prophage induction (37, 39), amplification of a putative recombination protein (6), and the transcriptional activation of DNA damage-inducible (*din*) operons (22).

The regulation of the SOS-like response to DNA damage in noncompetent *B. subtilis* cells appears to be similar to that of the well-characterized *E. coli* SOS system (20, 35). In *E. coli*, induction of the SOS system is the result of the RecA-mediated cleavage of LexA protein (19), the repressor of at least 17 *din* genes (including the *recA* gene) (35). We have presented evidence elsewhere that in *B. subtilis*, induction of both Rec protein and the *din-22* operon (representative of all known *B. subtilis* *din* operons) by DNA damage is controlled by a common, Rec-dependent mechanism (25). This mechanism is probably analogous to that of the *E. coli* SOS system because (i) purified Rec protein catalyzes the proteolytic cleavage of *E. coli* LexA in vitro (26) and (ii) *E. coli* RecA, expressed on a plasmid in *B. subtilis*, induces the *B. subtilis* SOS-like response (24, 25).

The induction of both Rec protein and the *din-22* operon

by either UV irradiation or mitomycin is completely abolished in *recE4* mutants and reduced more than 80% in *recA1* and *recM13* mutants (25). The *recA1* and *recE4* mutant induction deficiencies are probably due to mutations within the *rec* gene itself; both mutations are complemented by the cloned *rec* gene (27; unpublished results) and partially complemented by *E. coli* RecA protein expressed on a plasmid in *B. subtilis* (5, 24, 25). The *recM13* mutation apparently affects either the processing or utilization of the SOS-like inducing signals produced by mitomycin and UV because Rec induction by nalidixic acid (which probably produces a different inducing signal [28]) is nearly normal in *recM13* strains (25).

We demonstrate here that Rec protein is substantially induced in competent *B. subtilis* cells. We quantitated Rec induction and induction of the *din-22* operon during competence development in *rec*⁺, *recA1*, *recE4*, and *recM13* cells. Our results indicate that Rec induction in competent cells is controlled by a competence-specific mechanism that is Rec independent, whereas *din-22* induction during competence development is apparently regulated by a Rec-dependent, SOS-like mechanism.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *B. subtilis* strains used in all of the experiments are derivatives of YB886 (*metB5 trpC2 xin-1* SPβ[−]) (38). The isolation and characterization of *din* operon fusions in *B. subtilis* have been described before (22). The *rec* mutations were placed in the YB886 (*din22::Tn917-lacZ*) background by conjugation as described previously (22). Plasmid pPL608-*recA* (5), containing the *E. coli* *recA*⁺ gene under the transcriptional control of the *B. subtilis* bacteriophage SPO2 promoter, was obtained from G. Venema. Plasmid pPL608-*recA* and its parental plasmid pPL608 (36) confer resistance to chloramphenicol (5 μg/ml) and kanamycin (5 μg/ml) in *B. subtilis*.

Materials. The *B. subtilis* Rec protein (26) and *E. coli* RecA protein (4) used as standards were purified as described before. Polyclonal *E. coli* RecA antiserum was

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prepared as described before (26). Affinity-purified goat anti-rabbit immunoglobulin-horseradish peroxidase conjugate and horseradish peroxidase reagent (4-chloro-1-naphthol) were purchased from Bio-Rad Laboratories.

Induction of *B. subtilis* Rec protein. *B. subtilis* cultures were grown to a stage of maximum competence (determined by plasmid and chromosomal transformation frequencies) as described previously (23) and then fractionated on Renografin density gradients as described before (15, 22). Samples containing equivalent numbers of cells as indicated (based on the approximate values of 3×10^8 cells per ml at an OD_{600} of 1.0, determined by counting cells under a microscope) were lysed in buffer containing 20 mM Tris (pH 7.5), 10% (wt/vol) sucrose, and 0.5 mg of lysozyme per ml. Samples were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, followed by immunoblot transfer with *E. coli* RecA antibody as described before (26). Rec protein was quantitated by densitometric scanning and interpolation from standard curves derived from similarly analyzed purified Rec standards.

Induction of β -galactosidase activity in *din* fusion strains. Cultures of *B. subtilis* strains containing the *din-22::Tn917-lacZ* operon fusion were grown to the stage of maximum competence and fractionated on Renografin density gradients as described above. β -Galactosidase assays were performed on samples of each fraction and on samples of unfractionated cultures as described before (22) by the procedure of Miller (29).

RESULTS AND DISCUSSION

When *B. subtilis* cells are grown under conditions conducive to competence development, only about 10 to 20% of the population typically become competent (40). Because the competent subpopulation has a lighter buoyant density than the noncompetent cells, the two fractions can be separated by sedimentation. In order to quantitate the levels of Rec protein and *din-22* promoter activity in competent cells, we fractionated competent cultures by centrifugation through Renografin gradients (15).

We quantitated the level of *B. subtilis* Rec protein by densitometric scanning of immunoblot transfers for fractionated competent and noncompetent cells (Fig. 1). Purified Rec protein was analyzed similarly to construct a standard curve. The *E. coli* RecA antiserum used to detect Rec protein cross-reacts with other *B. subtilis* proteins whose concentration is unaffected by induction and which therefore served as internal controls. The amount of Rec protein in wild-type competent cells was about 14-fold higher than in noncompetent cells. By contrast, the maximum induction of Rec protein by DNA damage, determined by the same assay, results in a fivefold increase in the level of the protein (25). We estimate that the induced level of Rec protein in competent *rec⁺* cells was about 60,000 molecules per cell, using the approximate value of 3×10^8 cells per ml at an OD_{600} of 1.0 (determined by counting cells under a microscope). This estimation is also subject to the uncertainty in the concentration of purified Rec protein (which could be off by a factor of 2) used as standards (26).

Rec protein was induced about 10-fold above its low basal level in competent *recE4* cells, and Rec induction in competent *recA1* and *recM13* cells was apparently the same as in *rec⁺* cells. By contrast, there was no induction of Rec protein by DNA damage in *recE4* strains, and Rec induction by DNA damage was reduced about 80% in *recA1* and *recM13* strains (25). *E. coli* RecA protein, expressed on

FIG. 1. Induction of Recbs protein in competent *rec⁺* and *rec* strains. *B. subtilis* cultures were grown to a stage of maximum competence, fractionated on Renografin density gradients as described in the text, and lysed. Samples corresponding to 7.5×10^7 cells were subjected to SDS-polyacrylamide gel electrophoresis, followed by immunoblot transfer with *E. coli* RecA antibody. (A) Strains without plasmid pPL608-*recA*. (B) Strains containing plasmid pPL608-*recA*. (Strains containing pPL608-*recA* produce three distinct polypeptides, with M_r s ranging from about 34,000 to 38,000, that all cross-react with *E. coli* RecA antibody to about the same degree.) Lanes: T, top band from Renografin gradient, containing predominantly competent cells; B, bottom band from Renografin gradient, containing predominantly noncompetent cells; 1 and 2, YB886 (*rec⁺*); 3 and 4, YB1260 (*recA1*); 5 and 6, YB1015 (*recE4*); 7 and 8, YB1017 (*recM13*).

plasmid pPL608-*recA* (5), had no detectable effect on the level of Rec protein in any of the strains tested (Fig. 1B).

Figure 2 shows a time course for *B. subtilis* Rec protein induction in unfractionated *rec⁺* and *recE4* cells grown from the late exponential phase to 90 min after the time of maximum competence (assessed by transformation frequencies). Because only a small percentage of the total cell population becomes competent, both the rate and extent of Rec induction are diluted by the noncompetent cells in the culture. Thus, the amount of Rec protein in *rec⁺* cells appeared to be only about fourfold higher. Comparison of the level of Rec protein in fractionated cells (Fig. 1A) with that from the corresponding time period in Fig. 2 (designated by arrow) indicated that about 19% of the cell population became competent. This value is consistent with previous estimates of 10 to 20% (40). Assuming that this value is constant throughout the time course, we estimate (from the maximum slope in Fig. 2) that the increase in the rate of Rec accumulation was more than 1,100 molecules per min per cell. Using the same assay, we determined that the maximum rate of Rec accumulation in *rec⁺* cells following DNA damage was about 700 molecules per min per cell (25).

The time course for *B. subtilis* Rec protein induction in unfractionated *recE4* cells during competence development was similar to that of the wild-type strain. This was surpris-

FIG. 2. Induction of RecBs protein during competence development in *B. subtilis* *rec*⁺ and *recE4* strains. *B. subtilis* cells were grown according to the standard competence regimen, and samples were removed at 30-min intervals from the late exponential phase of growth to 90 min after the time of maximum competence (designated by arrow). Samples containing 1.5×10^8 cells were lysed and subjected to immunoblot transfer, and Rec protein was quantitated. Symbols: $\square$, YB886 (*rec*⁺); $\circ$, YB1015 (*recE4*); $\bullet$, YB1015 *recE4*(pPL608-*recA*). Inset: Corresponding immunoblot transfers for YB886 (A) and YB1015 (B).

ing, because the level of Rec protein in fractionated competent *recE4* cells was reduced more than twofold (Fig. 1). The discrepancy between the nearly wild-type level of Rec protein in unfractionated competent cells and the reduced level in fractionated competent cells suggests that a larger percentage of *recE4* cells differentiate to competence. Based on the level of Rec protein in fractionated competent *recE4* cells, we estimate that nearly 50% of the cells in this mutant have become competent, but this was not tested further.

We also quantitated the induction of *B. subtilis* *din* operons in competent cells by measuring β -galactosidase activity in a set of *rec*⁺ and *rec* *din*::Tn917-*lacZ* fusion strains (22, 25). The results for the *din-22* fusion strains are reported here because they showed the greatest induction of β -galactosidase activity (22), although we observed similar effects of the *rec* mutations on the induction of other *din* operons in competent cells (data not shown). Table 1 shows the level of β -galactosidase activity, corresponding to *din-22* promoter activity, in fractionated competent cells containing the *din-22*::Tn917-*lacZ* operon fusion (22). In *rec*⁺ compe-

TABLE 1. β -Galactosidase induction in competent *rec*⁺ and *rec* *din-22*::Tn917-*lacZ* fusion strains

Strain	β -Galactosidase activity ^a (U/OD ₆₀₀ unit)					
	No plasmid			pPL608- <i>recA</i>		
	U	T	B	U	T	B
YB886 (<i>rec</i> ⁺)	50	197	7	35	215	5
YB1260 (<i>recA1</i>)	16	57	2	24	112	4
YB1015 (<i>recE4</i>)	0.5	0.1	0.9	11	62	7
YB1017 (<i>recM13</i>)	38	166	12	44	178	10

^a Activity was measured in unfractionated cells (U), the top band from the Renografin gradient, containing predominantly competent cells (T), and the bottom band from the Renografin gradient, containing predominantly non-competent cells (B).

tent cells, the level of β -galactosidase activity was essentially the same as that following DNA damage (25). The effects of the *recA1* and *recE4* mutations on β -galactosidase induction in competent cells were also similar to their corresponding effects on induction by DNA damage (25); β -galactosidase induction was abolished in the *recE4* mutant and reduced, relative to the wild-type level, by about 70% in the *recA1* strain. Moreover, *E. coli* RecA protein, expressed on plasmid pPL608-*recA*, partially complemented the deficiencies in both mutants; this complementation by *E. coli* RecA was similar to that following DNA damage. These results suggest that induction of the *din-22* operon during competence development is Rec-dependent and probably involves an SOS-like mechanism (i.e., the Rec-mediated inactivation of a LexA-like repressor). If an SOS-like mechanism is involved, the signal that triggers *din-22* induction in competent cells should be similar to that produced by DNA damage. Biochemical studies indicate that at least part of the SOS-inducing signal in *E. coli* is single-stranded DNA, which activates RecA protein to cause repressor cleavage in vitro (4, 30). *B. subtilis* Rec is also activated to cause *E. coli* LexA cleavage in vitro by binding single-stranded DNA (26). It is consistent with an SOS-like mechanism controlling *din-22* induction during competence development that chromosomal DNA in competent cells contains a high level of single-stranded gaps (17). Furthermore, incoming donor DNA molecules are single stranded, with an average size of about 8.5 kilobases (10).

The level of β -galactosidase activity in competent *recM13* cells was about 15% lower than the wild-type level (Table 1). By contrast, induction of both Rec protein and the *din-22* operon by mitomycin or UV was reduced more than 80% in *recM13* cells; however, there was only a slight reduction in Rec amplification in these cells following treatment with nalidixic acid (25). Thus, the *recM13* mutation appears to affect the processing or utilization of the signal(s) produced by mitomycin and UV, but not the signal(s) generated by nalidixic acid treatment. If *din-22* induction during competence development is regulated by an SOS-like mechanism, the *recM13* mutation does not significantly affect the activation of Rec protein.

The results presented here also argue strongly that both the *recA1* and *recE4* mutations lie within the *rec* gene. *recA1* and *recE4* mutants exhibit deficiencies in *din-22* operon induction during competence development that are essentially the same as their induction deficiencies following DNA damage, despite the presence of high levels of Rec protein. These induction deficiencies are complemented by *E. coli* RecA protein at a concentration that is more than fivefold lower than that of Rec protein. (RecA protein concentration was determined by the same assay used to quantitate Rec, with purified RecA used to construct a standard curve.) Moreover, competent *recA1* and *recE4* cells are deficient in recombination-dependent transformation, apparently due to inefficient formation of heteroduplex joint molecules (11). The low transformation frequencies of these mutants are increased to the wild-type level in the presence of pPL608-*recA* (5). Thus, the apparent inability of Rec protein in *recA1* and *recE4* strains to promote *din-22* operon induction and general genetic recombination in the same environment where *E. coli* RecA, at a much lower concentration, can function effectively indicates that the Rec protein in these mutants is defective.

The most significant result of this study derives from the distinct induction of *B. subtilis* Rec protein during competence development. We have presented evidence that Rec

induction in competent cells is independent of active Rec protein and therefore distinct from the SOS-like mechanism that controls Rec induction following DNA damage. Rec protein is significantly induced in competent *recA1* and *recE4* cells, in contrast to the pronounced induction deficiencies in these mutants following DNA damage (25). In particular, Rec is induced about 10-fold in competent *recE4* cells, which are completely deficient in all functions that presumably require active Rec protein: induction of Rec protein and *din* operons by DNA damage (25), induction of *din* operons in competent cells (22), prophage induction (23, 25), and recombination-dependent transformation (11). We have also shown that the extent of Rec induction during competence development is substantially higher than maximum Rec induction by DNA damage (25). A possible explanation for the increased Rec induction in competent cells is that competence is a better activator of Rec protein than DNA damage and therefore a better inducer of the *B. subtilis* SOS-like system. However, this does not seem to be the case, because the level of induction for all *din* operons in competent cells was essentially the same as that following maximum induction by DNA damage (22, 25; unpublished data). Thus, the high level of Rec induction in competent cells probably reflects the competence-specific induction mechanism.

Assuming that an SOS-like mechanism is activated during competence development (as the induction results for *din-22* indicate), SOS-like induction could be partly responsible for the induced level of Rec protein that we observed in competent cells. This would explain the relatively low amount of Rec protein in competent *recE4* cells. Our inability to detect additional Rec induction in these cells in the presence of the pPL608-*recA* plasmid does not preclude this possibility, because the low level of induction caused by *E. coli* RecA could be masked by the more pronounced competence-specific induction. On the other hand, SOS-like induction of Rec protein during competence development could be superseded by the competence-specific induction. In this case, the reduced level of Rec in competent *recE4* cells could reflect a less stable Rec protein in these cells.

Because the primary goal of competent cells is presumably to integrate donor DNA via general genetic recombination, it is not surprising that differentiation to competence would involve a separate induction mechanism to produce a high level of Rec recombination activity. It is noteworthy that a recombination protein is induced to a similar level in competent *Streptococcus sanguis* cells (32), although there is no evidence for SOS-like regulation in this bacterium.

Our results do not suggest any clues regarding the nature of competence-specific Rec regulation; however, it is possible that this regulation is common to a network of competence-specific proteins. The recent characterization of a set of *com* mutations indicates that several *com* gene promoters are activated during the development of competence (1). The regulation of many of these *com* promoters and the development of competence are dependent on the *spo0A* and *spo0H* gene products (1), which are also required for the expression of sporulation genes (3, 12, 13). Thus, the control of competence-specific gene expression, including Rec induction in competent cells, may overlap with the control of sporulation genes and therefore involve different RNA polymerase sigma factors as effectors of stage-specific regulation (7, 16, 18, 21).

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