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Genetics

Induction of the *Bacillus subtilis* SOS-like response by *Escherichia coli* RecA protein

(Recbs protein/*din* operon/SOB response/competence)

PAUL E. LOVE AND RONALD E. YASBIN

Department of Microbiology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642

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ABSTRACT **A plasmid that expresses the *Escherichia coli* RecA protein partially restored DNA repair and recombination capability and induction of the SOS-like (SOB) response in a *recE4* mutant of *Bacillus subtilis*. In the presence of DNA-damaging agents, the *E. coli* RecA protein induced *din* operon expression, Weigle-reactivation activity, and synthesis of a *B. subtilis* recombination protein (Recbs) analogous to RecA but was unable to stimulate prophage induction. In addition, the RecA protein was capable of inducing the SOB response in competent *recE4* strains of *B. subtilis*, independent of exposure to DNA-damaging agents. The results suggest that (*i*) the SOS response of *E. coli* and the SOB response of *B. subtilis* are strikingly similar from both a phenotypic and a regulatory standpoint and that RecA and LexA protein analogs exist in *B. subtilis*, (*ii*) the Recbs protein is capable of regulating its own production, and (*iii*) SOS-inducing (RecA-activating) signals are generated in *B. subtilis* following either DNA damage or the development of physiological competence.**

DNA-repair processes play a fundamental role in the maintenance of genetic integrity and fidelity in living systems. Given the generality of the principles of DNA organization throughout nature and the virtually universal exposure of organisms to similar environmental mutagens such as UV radiation and oxidizing agents, it seems reasonable to assume that there may be functional similarities between essential components of the DNA-repair systems of widely divergent organisms.

Recent evidence indicates that the recombination or postreplication repair system in prokaryotes is highly conserved and depends primarily upon the activities of a single multifunctional enzyme (1–4). The *Escherichia coli* RecA protein, the most extensively characterized enzyme of this type, functions in DNA repair, genetic exchange, and the regulation of a complex cellular response to DNA damage (5–7). When expressed constitutively, RecA catalyzes DNA strand transfer, a requirement for both general genetic recombination and postreplication repair of damaged DNA (6, 7). However, in response to specific types of cellular insult, RecA protein undergoes a reversible alteration that enables it to facilitate the cleavage of a cellular repressor, the LexA protein (8), and of the repressors of certain temperate bacteriophages (9, 10). The increased transcription of DNA damage-inducible (*din*) genes that ensues results in the coordinate expression of a diverse set of cellular phenomena that are collectively termed the SOS response (5, 6, 11).

Several other genera of Enterobacteriaceae synthesize multifunctional proteins that play a singular role in genetic exchange, DNA repair, and the regulation of SOS-like responses and are capable of performing these activities in *E. coli* (1–3, 12). That the cloned *recA*$^+$ gene from *Pseudomonas aeruginosa* complements RecA functions in *E. coli* (4) indicates further that these proteins are functionally conserved in divergent species of Gram-negative eubacteria.

A RecA analog has also been detected in *Bacillus subtilis*, a Gram-positive soil organism that is phylogenetically distinct from *E. coli*. Lovett and Roberts (13) have purified a *B. subtilis* protein, herein designated Recbs, that crossreacts with antisera raised to *E. coli* RecA protein. Like RecA, the Recbs protein catalyzes DNA-dependent hydrolysis of dATP and DNA strand transfer *in vitro* (13). Furthermore, *B. subtilis* exhibits an inducible response, following DNA damage or the inhibition of DNA replication, that resembles phenotypically the SOS response of *E. coli* (14, 15). The characterization of the *B. subtilis* SOS-like (SOB) response has revealed that specific chromosomal loci (*din* genes) are activated transcriptionally in response to cellular insult or the development of physiological competence (16) and that Recbs induction accompanies these cellular events (13, 17). The expression of the SOB response is abolished in *B. subtilis* strains carrying a single mutation (*recE4*) in a gene that is essential for both efficient DNA repair and genetic recombination (15, 18).

Utilizing a plasmid that expresses the RecA protein of *E. coli*, deVos *et al.* (19) demonstrated that recombination proficiency and mitomycin C resistance could be restored in a *recE4* mutant strain of *B. subtilis*. In this report, we relate the results of investigations on the ability of the *E. coli* RecA protein to function in the induction of the SOB response in *recE4* mutants of *B. subtilis*. A particular interest was to determine whether the RecA protein could respond to the SOB-inducing signal(s) known to be present during the development of physiological competence (16, 17, 20), since it is conceivable that these signals might interact specifically with components only of the *B. subtilis* regulatory system.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The bacterial strains used are listed in Table 1. Isolation and characterization of *din* operon fusions in *B. subtilis* have been described (16). Plasmid pPL608-recA (19), which contains the *E. coli recA*$^+$ gene placed under the transcriptional control of the *B. subtilis* bacteriophage SPO2 promoter, was obtained from G. Venema. Plasmid pPL608-recA and its parental plasmid, pPL608 (24), confer resistance to chloramphenicol and kanamycin in *B. subtilis*.

Media. *B. subtilis din* operon fusion strains were maintained on NBA medium (16). Strains carrying plasmids pPL608 or pPL608-recA were maintained and propagated in media containing chloramphenicol (5 μg/ml) and kanamycin (5 μg/ml). *E. coli* strains were maintained on Luria–Bertani medium (25). Competence media (GM1 and GM2) and minimal media were prepared and used as described (26).

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Abbreviation: EtMes, ethyl methanesulfonate.

Table 1. Bacterial strains

Strain	Relevant genotype	Ref.
B. subtilis		
YB886	*metB5*, *trpC2*, *xin-1*, SPβ⁻	21
YB1015	*metB5*, *trpC2*, *recE4*, *xin-1*, SPβ⁻	21
YB886/*din22*	*din22*::Tn*917-lacZ*, *recE*⁺	16
YB1015/*din22*	*din22*::Tn*917-lacZ*, *recE4*	16
YB886/*din76*	*din76*::Tn*917-lacZ*, *recE*⁺	16
YB1015/*din76*	*din76*::Tn*917-lacZ*, *recE4*	16
E. coli		
JM83	*recA*⁺	22*
DH1	*recA1*	23*
HH49	[del (*srlR-recA1*)$_{306}$::Tn*10*]	†

*Provided by M. Wojciechowski.
†Provided by C. Lovett.

Procedures. β-Galactosidase assays (27) were performed on cultures of fusion strains grown in supplemented GM1. Procedures for Renografin density gradients (28) and the growth and transformation of competent cells (26) have been detailed. Methods for the determination of UV and ethyl methanesulfonate (EtMes) survival (21) and for Weigle (W)-reactivation and prophage induction (15) have also been described. NaDodSO$_4$/polyacrylamide gel electrophoresis and immunoblot transfers were performed using the methods of Lovett and Roberts (13). Affinity-purified goat anti-rabbit horseradish peroxidase conjugate, anti-β-galactosidase, and horseradish peroxidase reagent (4-chloro-1-naphthol) were purchased from Bio-Rad. RecA antiserum was the generous gift of C. Lovett and J. Roberts.

RESULTS

Synthesis of the 38-kDa RecA Protein in *B. subtilis*. Immunoblot analysis of cell extracts from strains YB1015-(*recE4*)(pPL608-recA) and YB886(*recE*⁺)(pPL608-recA) revealed the presence of an ≈38-kDa protein that crossreacted strongly with antiserum raised against the *E. coli* RecA protein (Fig. 1, lanes 1 and 3). The 38-kDa protein comigrated electrophoretically with chromosomally encoded RecA protein (lane 6) and was not produced in *B. subtilis* strains harboring the parental plasmid, pPL608 (lanes 2 and 4). To determine whether the plasmid-encoded protein exhibited RecA activities in its natural host, pPL608-recA was inserted into the *E. coli* plasmid pBR322 (using the unique *Hin*dIII site in each plasmid) and then introduced into a *recA1* mutant of *E. coli* (strain DH1; Table 1). DH1 strains carrying the chimeric plasmid pPL608-recA/pBR322 were found to be as resistant to UV radiation as strain JM83(*recA*⁺), and UV induction of λ prophage was restored to wild-type levels (data not shown).

Since RecA antiserum crossreacts with the 42-kDa Recbs protein (13), this protein was also detectable in *B. subtilis* cell extracts. The Recbs protein was produced in the *recE4* mutant strain (Fig. 1, lane 2), although in significantly lower amounts than in the *recE*⁺ strain (lane 4). These findings are consistent with those of Lovett and Roberts (13), suggesting, together with the phenotypic characterization of the *recE4* mutant (15–19), that this strain synthesizes a nonfunctional Recbs protein. In this context, it is notable that the Recbs protein was present in greater amounts in strain YB1015-(pPL608-recA) than in strain YB1015(pPL608) (lanes 1 and 2), indicating that the RecA protein may influence the synthesis of Recbs.

DNA Repair Capacity of *recE4* Mutants Harboring Plasmid pPL608-recA. deVos *et al.* (19) demonstrated that mitomycin C resistance was enhanced in several Rec⁻ mutants of *B. subtilis* by plasmid pPL608-recA. We decided to characterize further the DNA-repair functions of the RecA protein in *B. subtilis* by determining its effect on the repair of DNA lesions resulting from exposure to UV-radiation or EtMes. In these and subsequent experiments, the *B. subtilis* strains that were employed contained a *din*::Tn*917-lacZ* operon fusion that either does not affect DNA repair (*din22*) or that interrupts the excision repair system of *B. subtilis* (*din76*) (16). As expected, pPL608-recA was unable to complement the excision-repair deficiency in strain YB886/*din76*, but was capable of partially restoring both UV and EtMes resistance in the *recE4* derivatives (Fig. 2). Given the variety of DNA lesions that it is capable of repairing (Fig. 2 and ref. 19), it appears that the RecA protein can catalyze DNA strand exchange (recombinational repair) in *B. subtilis*. Additional evidence for RecA-promoted DNA-strand-exchange activity is the finding that pPL608-recA restores transformability in

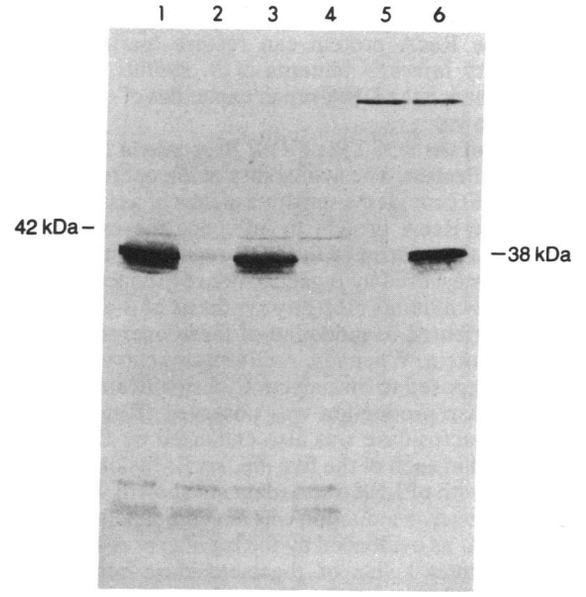

FIG. 1. Presence of the 38-kDa *E. coli* RecA protein and the 42-kDa Recbs protein in *recE*⁺ and *recE4* strains of *B. subtilis*. Crude extracts from 1 ml of cells at OD_{600} = 0.6 were electrophoresed in a NaDodSO$_4$/10% polyacrylamide gel, transferred to nitrocelluose, and incubated with *E. coli* RecA antiserum. Lanes: 1, YB1015-(pPL608-recA); 2, YB1015(pPL608); 3, YB886(pPL608-recA); 4, YB886(pPL608); 5, *E. coli* HH49[del(*recA*)]; 6, *E. coli* JM83(*recA*⁺). The high molecular mass band in *E. coli* cell extracts is presumably an unrelated crossreactive protein.

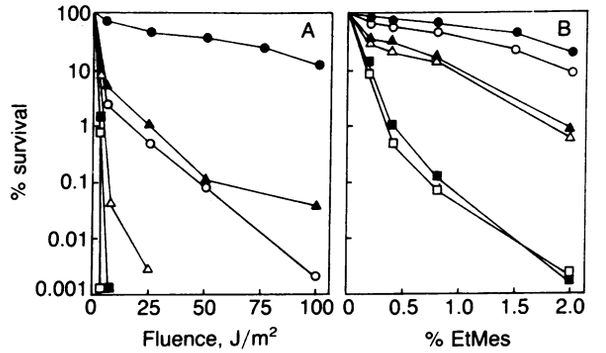

FIG. 2. DNA-repair capability of *B. subtilis* strains harboring plasmid pPL608-recA. (*A*) Survival of UV-irradiated cultures. (*B*) Survival of cultures exposed to EtMes. Solid symbols, *din22* fusion strains; open symbols, *din76*(*uvr*⁻) fusion strains; ● and ○, *recE*⁺(pPL608); ■ and □, *recE4*(pPL608); ▲ and △, *recE4*(pPL608-recA). UV and EtMes resistance of *recE*⁺ strains were unaffected by plasmid pPL608-recA (data not shown).

competent *recE4* strains of *B. subtilis* (Table 3 and ref. 19). Although the RecA protein can restore (partially) DNA-repair activity in *recE4* mutants of *B. subtilis*, it does not appear to enhance the DNA-repair capacities of *recE*$^+$ strains (data not shown).

Induction of the SOS-Like (SOB) Response in *B. subtilis* by *E. coli* RecA Protein. The availability of *din* operon fusions in *B. subtilis* (16) provided a sensitive means of assaying for the ability of the RecA protein to influence the expression of genes under the control of the SOB regulon. Since *din* gene expression (measured by β-galactosidase production) is abolished in *recE4* mutants (16), any synthesis of β-galactosidase could be attributed to induction of these operons by the *E. coli* RecA protein. When *din, recE4* strains carrying pPL608-recA were exposed to mitomycin C, a significant increase in β-galactosidase production was observed (Fig. 3). Expression of β-galactosidase was also enhanced by UV radiation and occurred in each of the five *din, recE4* fusion strains that were tested with pPL608-recA (data not shown). However, in all cases *din* operon induction was not completely restored by pPL608-recA, as evidenced by the lag in expression time and lower (absolute) levels of β-galactosidase activity in the *recE4*, pPL608-recA derivatives relative to *recE*$^+$ strains (Fig. 3).

These initial observations prompted an investigation of the ability of the RecA protein to complement specific inducible functions in *B. subtilis*. We chose to examine two SOB phenomena, W-reactivation and prophage induction, because of their absolute dependence on Rec functions (14, 15, 18) and because they are presumably controlled by distinct transcriptional regulatory elements (repressors) (29).

The *E. coli* RecA protein partially restored W-reactivation activity in the *B. subtilis recE4* mutant strain (Fig. 4). As was the case with induction of *din* operon fusions (Fig. 3), pPL608-recA had no effect on the efficiency of W-reactivation in the *recE*$^+$ strain (data not shown). Conversely, the RecA protein was unable to effect the induction of prophage φ105 over a wide range of UV fluences, even though it was capable of inducing *din* operon expression in lysogenic strains (Table 2). Spontaneous levels of phage release were also unaffected by plasmid pPL608-recA (Table 2). Similar results were obtained using strains that were lysogenized with bacteriophage SPO2 [φ105 and SPO2 are heteroimmune bacteriophages (29)].

Induction of Recbs by *E. coli* RecA Protein. In *E. coli*, the SOS response is modulated by a single repressor (LexA) that

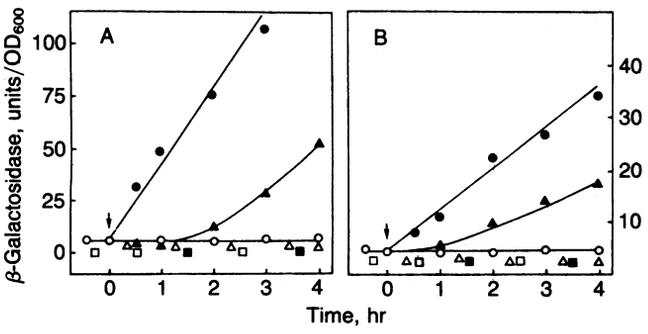

FIG. 3. Effect of plasmid pPL608-recA on induction of β-galactosidase in *din, recE4* fusion strains of *B. subtilis*. During exponential growth in liquid medium (GM1), cultures were divided and either challenged (at time indicated by arrow) with mitomycin C at 500 ng/ml (solid symbols) or left untreated (open symbols). At the designated intervals, 1-ml aliquots of each culture were assayed for β-galactosidase activity (27). (*A*) *din22* fusion strains. (*B*) *din76* fusion strains. ● and ○, *recE*$^+$(pPL608); ■ and □, *recE4*(pPL608); ▲ and △, *recE4*(pPL608-recA). Induction kinetics for *recE*$^+$(pPL608-recA) strains were identical to those shown for *recE*$^+$(pPL608) strains.

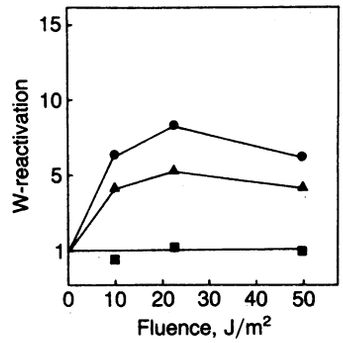

FIG. 4. Effect of plasmid pPL608-recA on W-reactivation of bacteriophage φ105. Filtered lysates of bacteriophage φ105 were irradiated at a fluence of 700 J/m^2 and mixed with aliquots of mid-exponential cultures of *B. subtilis*. UV fluences shown in the figure refer to the dose delivered to bacteria prior to infection by bacteriophage φ105. W-reactivation values were calculated as described (15). ●, *recE*$^+$(pPL608); ■, *recE4*(pPL608); ▲, *recE4*-(pPL608-recA).

controls the synthesis of a number of damage-inducible proteins, including RecA (11). If, as our data suggest, a LexA analog exists in *B. subtilis*, then Recbs induction and *din* operon induction should be coregulated by this protein and thus triggered by activated RecA. Working under the assumption that the *recE4* mutation does not disrupt the regulatory sequences that control the synthesis of Recbs, we looked for induction of the Recbs protein in strain YB1015/*din22*(pPL608-recA) following exposure to mitomycin C [previous reports (13, 17) have indicated that the Recbs protein is not induced by DNA-damaging agents in *recE4* mutants]. The results indicate that the RecA protein is capable of inducing the synthesis of Recbs in a *recE4* mutant of *B. subtilis* (Fig. 5*B*). These data, which are consistent with our previous observation that plasmid pPL608-recA increased the constitutive level of Recbs in strain YB1015-(*recE4*) (Fig. 1), imply that functional Recbs protein regulates its own production (Fig. 5*A*) and that RecA can substitute for

Table 2. Effect of plasmid pPL608-recA on UV induction of prophage φ105

φ105 lysogen	Fluence, J/m^2	pfu/ml	β-Galactosidase, units/OD$_{600}$
YB886/*din22*			
(pPL608)	0	3.6×10^3	7.2
	5	1.9×10^5	15.2
	10	4.7×10^5	28.6
	25	2.8×10^6	52.1
	50	3.9×10^6	74.2
YB1015/*din22*			
(pPL608)	0	3.0×10^1	0.1
	0.5	2.9×10^1	0.3
	1	3.7×10^1	0.4
	10	3.1×10^1	0.3
	50	1.1×10^1	0.2
YB1015/*din22*			
(pPL608-recA)	0	1.1×10^2	2.7
	0.5	4.4×10^1	3.1
	1	8.5×10^1	3.3
	10	2.3×10^2	14.0
	50	6.0×10^1	29.0

Prophage-induction experiments were performed as described (15). pfu, Plaque-forming units. β-Galactosidase assays were performed as described (27) on 1-ml aliquots of cultures taken at the time of plating for pfu. Results obtained with strain YB886/*din22*(pPL608-recA) were similar to those shown for strain YB886/*din22*(pPL608).

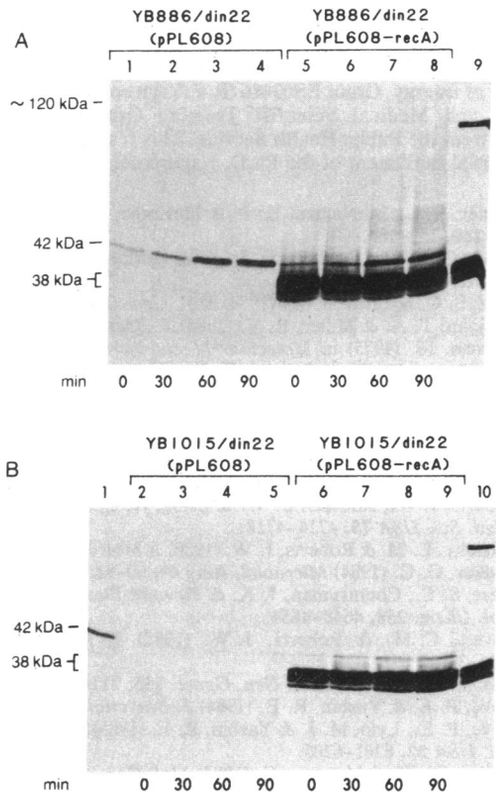

FIG. 5. Induction of Recbs by *E. coli* RecA protein. Mid-exponential phase cultures (OD_{600} = 0.3) of *B. subtilis* strains were treated with mitomycin C (500 ng/ml) and aliquots were taken at the times indicated. Crude extracts were prepared and electrophoresed as described for Fig. 1. Nitrocellulose filters were incubated with RecA antiserum (*B*) or sequentially incubated with affinity-purified β-galactosidase antiserum and RecA antiserum (*A*). The 120-kDa bands (and lower molecular mass proteolytic forms) reflect presence of β-galactosidase in crude cell preparations. Control lanes: (*A*) Lane 9, *E. coli* strain JM83, not treated with mitomycin C. (*B*) Lane 1, YB886/*din22*(pPL608) after 60 min of exposure to mitomycin C; lane 10, *E. coli* strain JM83, not treated with mitomycin C.

Recbs in this capacity. Finally, a comparison of the data in Figs. 3 and 5*A* demonstrates that induction of *din* operon expression occurs concomitantly with the amplification of Recbs following exposure to mitomycin C, suggesting further that these cellular events are coordinately regulated.

Induction of the SOB Response in Competent *B. subtilis* by RecA Protein. In *B. subtilis* the SOB response is elicited during the development of physiological competence independent of exposure to agents that damage DNA or inhibit DNA replication (16, 20). To gain a further understanding of the process by which this occurs, we examined the ability of the *E. coli* RecA protein to induce the expression of a *din* operon fusion in competent *recE4* mutant strains of *B. subtilis*. Remarkably, the RecA protein was activated by the development of competence in strain YB1015/*din22*(pPL608-recA), as evidenced by induction of β-galactosidase (Table 3). Further, the pattern of β-galactosidase expression in this strain was similar to that in the *recE*$^+$ strains and was therefore specific to the competent subpopulation of cells (top band isolated from Renografin gradients). The RecA protein was also capable of completely restoring transformation ability in the *recE4* mutant (Table 3), as previously demonstrated by deVos *et al.* (19).

Table 3. Effect of plasmid pPL608-recA on transformation frequency and β-galactosidase expression in competent *din22*::Tn*917-lacZ* fusion strains

Fusion strain	Met$^+$ cfu/ml	Transformation frequency	β-Galactosidase, units/OD_{600} T	B
YB886/*din22* (pPL608)	3.3×10^5	2.9×10^{-3}	105.2	5.5
YB886/*din22* (pPL608-*recA*)	3.1×10^5	3.4×10^{-3}	109.2	7.8
YB1015/*din22* (pPL608)	1.2×10^1	$<10^{-7}$	0.3	0.3
YB1015/*din22* (pPL608-*recA*)	1.5×10^5	1.7×10^{-3}	42.5	6.8

din fusion strains were grown to competence, transformed with *met*$^+$ DNA, and fractionated in Renografin density gradients as described (28). β-Galactosidase assays were performed on cells isolated from gradient bands after resuspension in GM1 medium. T, top band of Renografin gradient, containing predominantly competent cells; B, bottom band of Renografin gradient, containing predominantly noncompetent cells. Transformation frequencies (determined with unfractionated cells) were calculated by dividing the number of colony-forming units (cfu) of Met$^+$ transformants by the total number of viable cells.

DISCUSSION

The ability of the RecA protein to substitute for Recbs in the induction of the *B. subtilis* SOS-like (SOB) response demonstrates that the recombinational and regulatory properties of these proteins are highly conserved even though they function in distantly related prokaryotic organisms (30). The fundamental components of the SOS response of *E. coli* consist of a positive regulatory element (the RecA protein) and a negative regulatory element, or repressor (the LexA protein) (11). The data presented here suggest that analogs of both these elements exist in *B. subtilis* and serve identical roles in controlling the coordinate expression of the defined gene sets that compose the SOB regulon.

Of major importance to the elucidation of the SOB regulatory system is an understanding of the specific alteration caused by the *recE4* mutation. Considerable insight has been obtained from previous studies in which numerous phenotypic similarities between *recE4* mutants of *B. subtilis* and *recA*$^-$ mutants of *E. coli* were delineated (15–18). The subsequent isolation of a RecA protein analog from *B. subtilis* that is inducible in *recE*$^+$ but not *recE4* strains suggested that this protein (Recbs) is synthesized but is nonfunctional in *recE4* mutants (13). Our findings are consistent with this hypothesis and imply that (*i*) the Recbs protein is autoregulated and serves as the effector molecule in the induction of the SOB response and (*ii*) *recE4* strains code for an altered form of Recbs which, although inducible by RecA protein, is incapable of regulating its own production (Fig. 5) or initiating prophage induction following amplification by RecA protein (Table 2). While the available data provide rather strong circumstantial evidence that the 42-kDa Recbs protein is the product of the *recE* gene, definitive proof awaits the cloning and characterization of this gene.

Since the RecA protein can partially restore *din* gene expression, W-reactivation, and induction of Recbs in *B. subtilis*, it is capable of (*i*) responding to (SOS) inducing signals generated in *B. subtilis* and (*ii*) catalyzing the derepression of specific genes under the control of the SOB regulon. Given the exclusive role played by the LexA protein in controlling the expression of SOS genes in *E. coli*, it seems reasonable to postulate that at least one cellular repressor exists in *Bacillus* that is functionally analogous to the LexA repressor. Additional support for this hypothesis is that the Recbs protein is capable of catalyzing the *in vitro* cleavage of LexA repressor in the presence of single-stranded DNA and deoxyribonucleoside triphosphate (13). Further, since the

LexA repressor influences the expression of SOS functions in several genera of Enterobacteriaceae (31), this protein is functionally conserved in a number of prokaryotic organisms.

Plasmid pPL608-recA was unable to effect the induction of prophages from *recE4* mutant strains (Table 2), indicating that the RecA protein does not efficiently bind to or cleave phage-specific repressors in *B. subtilis*. The differential activities of the RecA protein on cellular (LexA) and bacteriophage repressors in *E. coli* have been characterized (32–34). The unique binding and cleaving specificities that distinguish RecA/phage-repressor interactions may explain why these proteins are not inactivated as readily as is LexA in SOS-induced cells (33, 34). The functional dichotomy of the RecA protein is most evident in *recA430* mutants of *E. coli*, which synthesize an altered form of RecA that is capable of catalyzing the *in vitro* cleavage of the LexA protein but not the λ repressor cI (35). Interestingly, the Recbs protein exhibits an *in vitro* activity that is similar to that of the RecA430 protein (13). These findings, in conjunction with the observation that the *recA* gene from *Erwinia carotovora* complements all of the RecA functions in *E. coli* except prophage induction (1), suggest that bacteriophage repressors may have diverged considerably in prokaryotes.

An interesting result of this study is that the *E. coli* RecA protein stimulates *din* operon expression in competent *recE4* mutants of *B. subtilis* (Table 3). The most plausible explanation for these findings is that internal signals (presumably gapped or single-stranded DNA) are generated during the development of physiological competence in *B. subtilis* that resemble those that result from the metabolic processing of damaged DNA. These signals might originate either from DNA processing associated with genetic exchange (recombination) or the generation of gapped regions in the chromosome, both of which occur during the development of competence (36). In any event, it is apparent that the induction of the SOB response during competence is not due to some specific function of, or interaction with, the Recbs protein exclusively. Notwithstanding these findings, recent investigations indicate that a competence-specific mechanism operates in *B. subtilis* to amplify cellular levels of Recbs independent of the SOB regulatory pathway (data not shown), although SOB-mediated induction of Recbs occurs secondarily (16). In this regard, *B. subtilis* (and perhaps other naturally competent organisms) seems to have diverged from *E. coli* by evolving the capacity to invoke the expression of an essential recombination protein at a specific stage in cellular differentiation (competence).

That a multifunctional recombination protein such as RecA has been functionally conserved in *E. coli* and *B. subtilis* is certainly intriguing in light of the many differences between these organisms (e.g., the ability of *B. subtilis* but not *E. coli* to undergo discrete physiological changes leading to sporulation and competence). Given the importance of recombination and DNA repair in virtually all living systems, it is possible that an essential and versatile protein such as RecA may have been highly conserved throughout evolution. The detection of RecA-like activities in eukaryotic organisms such as *Ustilago* (37) and *Saccharomyces* (38–40) raises interesting questions as to the potential functional and structural similarities between these proteins and their counterparts in prokaryotic organisms.

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