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Biochemistry

An additional function for bacteriophage λ *rex*: The rexB product prevents degradation of the λ O protein

(phage λ development/protein stability/proteolytic regulation)

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ABSTRACT **The *rex* operon of bacteriophage λ excludes the development of several unrelated bacteriophages. Here we present an additional λ *rexB* function: it prevents degradation of the short-lived protein λ O known to be involved in λ DNA replication. We have shown that it is the product of *rexB* that is responsible for the stabilization of λ O: when a nonsense mutation is present in *rexB*, λ O protein is labile; suppression of the mutation by the corresponding nonsense suppressor causes partial restabilization of λ O. λ *rexB* also stabilizes λ O in trans. We discuss our results in relation to the function of rexB in λ DNA replication and its role in the protein degradation pathways of bacteriophage λ.**

When phage λ is in the lysogenic state in its host *Escherichia coli*, the only phage genes expressed are the adjacent genes *c*I and *rex* (1). The product of *c*I, the λ repressor, prevents vegetative development of the prophage and also further infection by homologous phages (2). λ *rex* expresses the Rex function, shown to exclude the development of several unrelated phages (3–11).

The first-described Rex function was the exclusion by λ prophage of the development of phage T4 *r*II mutants. Rex does not exclude wild-type T4 (3). The system for T4 *r*II exclusion (*rex*) is a landmark in the history of molecular biology: it was used for the first fine-structure analysis of a genetic region (T4*r*II) (3), for defining the cistron (12), and also for elucidation of the triplet nature of the genetic code (13). Later, the λ *rex* exclusion function was found to include the restriction of mutants of other phages as well as of T4 (6–11). Overexpression of the *rex* function causes exclusion of the development of wild-type phages (14). Furthermore, λ *rex* overexpression will inhibit the function of the *E. coli* host even without superinfection (15).

The *rex* exclusion function is performed by the products of two adjacent genes, *rexA* and *rexB* (16, 17). The genes *rexA* and *rexB* can be expressed coordinately with the λ *c*I repressor gene from promoters p_{RM} and p_{RE} (16, 18). There is a third promoter, p_{LIT}, which overlaps the region encoding the carboxyl terminus of rexA (Fig. 1*A*). Transcription from p_{LIT} results in a 470-nucleotide-long lit mRNA that permits expression of *rexB* without that of *rexA* (16, 20). When λ DNA replication is initiated at the λ origin, >10-fold increase in lit mRNA transcription has been detected (20). This shift from coordinate to discoordinate expression of *rexB* over *rexA* implies that λ *rexB* has another function, perhaps connected to λ DNA replication, and independent of that of *rexA* (16).

Here we report an additional function for the product of λ *rexB*: it prevents degradation of the λ O protein. λ O is a short-lived protein involved in λ DNA replication (21–25). We shall discuss our results both in relation to the role of rexB in λ DNA replication and in relation to the mechanism of protein degradation–antidegradation as a regulatory device in λ development.

MATERIALS AND METHODS

Materials and Media. [^{35}S]Methionine (>800 Ci/mmol; 1 Ci = 37 GBq) was obtained from Amersham. Antibodies to λ O protein were kindly provided by R. McMacken (The Johns Hopkins University, Baltimore). Bacteria were grown in LB or M9 medium with or without Casamino acids (26). Plasmid-carrying strains were grown in media containing ampicillin at 50 μg/ml, tetracycline at 15 μg/ml, or chloramphenicol at 30 μg/ml.

Bacterial and Phage Strains and Plasmid Derivatives. The following bacterial and phage λ strains were used: *E. coli* CSR603 (*recA1, uvrA6, phr-1, supE44, thr-1, leuB6, proA2, argE3, thi-1, ara-14, lacY1, galK2, xyl-5, mtl-1, rpsL31, tsx-33*) (27); *E. coli* MY320 [kindly provided by M. Yarus (Boulder, CO)]; *E. coli* N99 (*su°galK*$^-$). *E. coli* N99 and also phage λ strains λcI857S_{am}7 and λcI857 were kindly supplied to us by M. Belfort (Albany, NY). The temperature-inducible lysogens CSR603 (λcI857S_{am}7) and N99 (λcI857) were constructed by us. We used these lysogens as hosts for the λ p_L-containing pKC30 plasmid derivatives. We constructed the F'*lacI*^{q} derivatives by conjugation with *E. coli* MY320, which carries an F'*lacI*^{q} episome. The F'*lacI*^{q}-containing bacterial strains were used as hosts for the expression of λ *O* regulated by the *lac* promoter.

Plasmid pRLM74 carries the 1.5-kilobase (kb) *Alu* I fragment of phage λ (nucleotides 38,453–39,956 of λ DNA) in which is found the λ *O* gene; this fragment is flanked in the plasmid by *Bam*HI sites. We used pRLM74 (kindly provided by R. McMacken) to construct plasmid derivatives carrying λ *O*. We constructed pRS1 by subcloning the 1.5-kb *Bam*HI fragment of pRLM74 into the *Hin*dIII site of plasmid pKK104 (28) [kindly provided by A. Klein (Heidelberg, F.R.G.)], placing λ *O* under the control of the *lac* promoter (Fig. 1*B*). Plasmid pKC30 carries the 2.4-kb *Bam*HI–*Hin*dIII fragment of phage λ (nucleotides 34,498–36,895). This *Bam*HI–*Hin*dIII fragment includes the λ p_L promoter as well as λ *rexB* and some of λ *rexA*; *rexB* is under the control of the λ promoter p_{LIT}. We constructed pRS2 by subcloning the 1.5-kb *Bam*HI fragment from pRLM74 to pKC30 (29), placing the λ *O* gene under the λ p_L promoter (Fig. 1*B*). Deleting most of the *rexA* region contained in the 0.6-kb *Hin*dIII–*Bst*EII fragment from pRS2 resulted in pRS3 in which *rexB* remains under the control of the p_{LIT} located in the small remaining fragment of the *rexA* gene. Deleting the *rexB*–*rexA* region contained in the 1.1-kb *Hin*dIII–*Bal* I fragment of pRS2 resulted in pRS4 (Fig. 1*B*). Both pRS2$_{UAA}$ and pRS2$_{UAG}$ carry a nonsense mutation in λ *rexB*. They were constructed from pRS2 by oligonucleotide-directed site-specific mutagenesis (see below).

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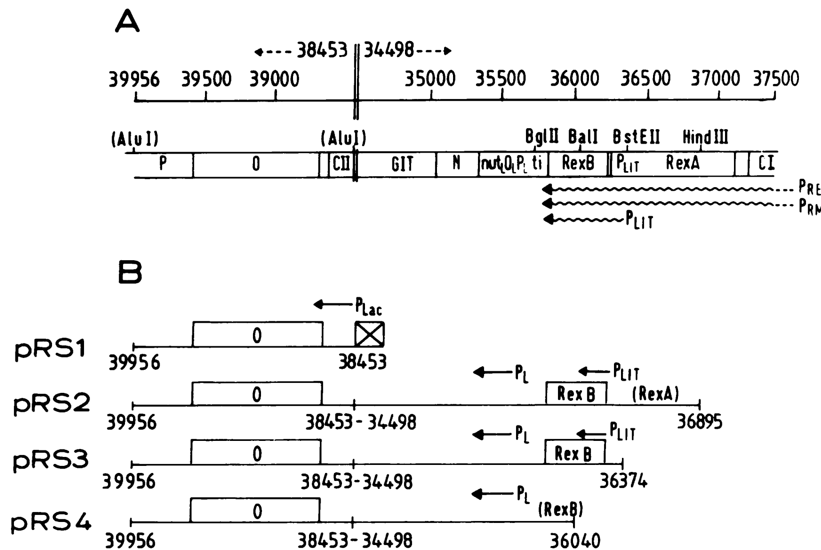

FIG. 1. Phage λ and plasmid maps. (*A*) DNA regions of λ included in our plasmids showing the λ regions and their regulatory elements. The nucleotides are numbered according to the λ genetic map (19). Note that the λ DNA in our plasmids includes nucleotides 34,498–36,895 and nucleotides 38,453–39,956 joined together in opposite directions as indicated by the dashed arrows. The junction is represented by a double vertical bar. Relevant restriction sites are indicated. The wavy arrows represent the direction and extent of the three transcripts initiated at p_{RE}, p_{RM}, and p_{LIT}. (*B*) pRS1 carries the 1.5-kb *Bam*HI fragment of pRLM74 [including the *Alu* I fragment of λ DNA (nucleotides 38,453–39,956)]. This fragment includes the λ *O* gene (nucleotides 38,686–39,582). Here the λ *O* gene is regulated by the *lac* promoter, represented by ⊠. pRS2 carries two separate regions of λ DNA joined by us: (*i*) the 2.4-kb *Bam*HI–*Hin*dIII fragment (nucleotides 34,498–36,895 of λ DNA) including p_L, *rexB*, and most of *rexA* and (*ii*) the 1.5-kb *Bam*HI fragment of pRLM74. Here the λ *O* gene is regulated by the λ p_L promoter. pRS3 and pRS4 are derivatives of pRS2, deleted in the *rex* regions (nucleotides 36,895–36,374 and 36,895–36,040, respectively).

We subcloned several genes into plasmid pSU27-18, which is compatible with pBR322 derivatives and in addition carries a chloramphenicol-resistence gene (30). This Cm^R gene-containing series of plasmids pLDG1 and pLDG2 includes (*i*) pLDG1, which carries the *rexB* gene in the 1.0-kb *Eco*RI–*Bst*EII fragment of pRLM74 cloned into the *Sma* I site of pSU27-18 (this *Eco*RI–*Bst*EII fragment also carries the last 60 nucleotides of λ *rexA* and the first 375 nucleotides of pBR322), and (*ii*) pLDG2, which carries the su^+7-UAG gene regulated by the *lac* promoter subcloned into the *Hin*dIII–*Eco*RI large fragment of pSU27-18 from the *Hin*dIII–*Eco*RI partial fragment from plasmid pMY228tet^R (31). (Plasmid pMY228 was kindly provided to us by M. Yarus.)

Molecular Cloning. All recombinant DNA manipulations were carried out by standard procedures (32). Restriction enzymes and other enzymes used in recombinant DNA experiments were obtained from New England Biolabs. Nonsense mutations were obtained using synthetic oligonucleotides in a site-specific mutagenesis reaction using an Amersham kit for phage M13 site-directed mutagenesis. DNA sequencing was carried out using a United States Biochemical sequencing kit.

Labeling and Identification of the *in Vivo* Synthesis of λ O Protein. We examined the synthesis of λ O protein directed by various plasmids. (*i*) Experiments with plasmid pRS1 were carried out in a *lacI*^{q} derivative of *E. coli* CSR603. (*ii*) Experiments with pRS2 to pRS4 were carried out in strains CSR603 (λ*c*I857S_{am}7) and N99 (λ*c*I857). CSR603 (F′*lacI*^{q}) cells were grown at 37°C, and the synthesis of λ O protein directed by pRS1 was induced by the addition of 1 mM isopropyl β-D-thiogalactopyranoside for 2 hr. Cells carrying temperature-sensitive λ*c*I857 lysogens were grown at 30°C to midlogarithmic phase in M9 medium supplemented with Casamino acids. In these lysogenic strains, the synthesis of λ O protein was induced by shifting the culture to 41°C for 30 min. Before labeling with [^{35}S]methionine, all cultures were washed as described previously (33). Cells were labeled for 2 min by the addition of L-[^{35}S]methionine at 15 μCi/ml. Labeling was terminated by freezing the samples in liquid nitrogen. In the pulse–chase experiments, cells were labeled for 2 min with L-[^{35}S]methionine, and then unlabeled methionine was added to a final concentration of 500 μg/ml, after which samples were removed at various times. Cells were lysed as described previously (33) and immunoprecipitated with antibodies to λ O protein as described by Oliver and Beckwith (34). Samples were applied to 0.1% SDS/15% polyacrylamide gels for electrophoresis. Labeled proteins on the gels were detected by autoradiography. Quantitation of the amount of λ O protein in the pulse–chase experiments was based on densitometric measurement of autoradiograms of the gels.

RESULTS

Plasmid pRS2 Directs the Synthesis of a Stable λ O Protein. The λ O protein is rapidly degraded in *E. coli* host cells (28, 35). We have confirmed this by using plasmid pRS1 (Fig. 1*B*), which carries the λ *O* gene under the control of the *lac* promoter. As shown by a pulse–chase experiment, when pRS1 is in *E. coli* CSR603 (λ*c*I857S_{am}7) (data not shown) or in *E. coli* CSR603 (F′*lacI*^{q}) (Fig. 2, lanes C), the λ O protein is rapidly degraded. Quantitation of the labeled band of λ O protein in these experiments showed that the half-life of this protein is normally about 3 min; after 30 min of chase only 1% of the labeled λ O protein was detectable (data not shown).

When we studied the life-time of the λ O protein directed by the *O* gene on a different plasmid, pRS2 (Fig. 1, lanes B), we had unexpected results (Fig. 2, lanes A). Although λ O directed by pRS1 is rapidly degraded (Fig. 2, lanes C), λ O directed by plasmid pRS2 remains stable for 30 min (Fig. 2, lanes A). That plasmid pRS2 directs the synthesis of a stable λ O protein seemed important to us because of the wide interest in the pathways of degradation–antidegradation of proteins in bacterial cells. Why does plasmid pRS1, as do other previously described plasmids carrying λ *O* (28), direct the synthesis of a labile λ O protein, while pRS2 directs a stable λ O protein?

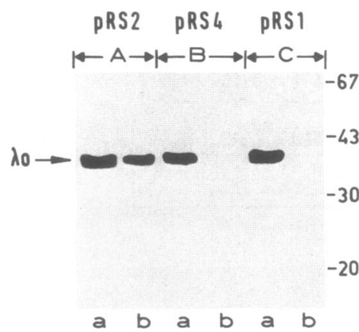

FIG. 2. Comparison of stability of the λ O protein directed by various plasmids. *E. coli* CSR603 (F′*lacI*^{q}) carrying plasmid pRS1 (lanes C) or CSR603 (λ*c*I857S_{am}7) carrying pRS2 (lanes A) or pRS4 (lanes B) was labeled with [^{35}S]methionine for 2 min (lanes a). An excess of unlabeled methionine was added and samples were removed after 30 min (lanes b). The cells were lysed, immunoprecipitated with antibodies against the λ O protein and applied to a 15% SDS/polyacrylamide gel for electrophoresis. Gels were analyzed by autoradiography. Molecular masses of protein standards are given (in kilodaltons) on the right. The arrow indicates the position of the O protein.

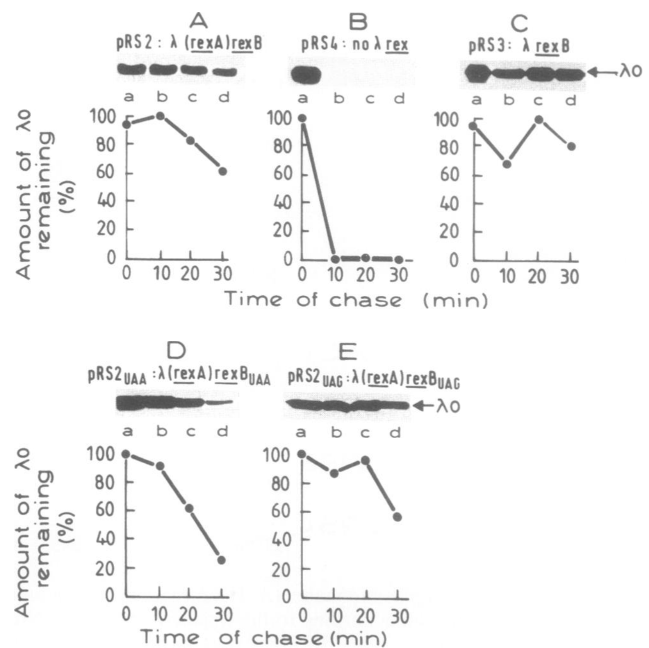

FIG. 3. Effect of λ *rex* on stability of the λ O protein in *E. coli* CSR603 (λ*c*I857S_{am}7). *E. coli* CSR603 (λ*c*I857S_{am}7) carrying plasmids pRS2 (*A*), pRS4 (*B*), pRS3 (*C*), pRS2$_{UAA}$ (*D*), and pRS2$_{UAG}$ (*E*) was labeled with [^{35}S]methionine for 2 min (points a). An excess of unlabeled methionine was added and samples were removed after 10 (points b), 20 (points c), and 30 min (points d). Samples were immunoprecipitated with antibodies against the λ O protein. Fractionation and autoradiography were carried out as described in Fig. 2 and in *Materials and Methods*. The concentration of labeled λ O protein was scanned densitometrically. The relative intensity of each band was determined and expressed as its ratio to the band with the highest intensity (100%) for each particular plasmid.

In addition to the differences in promoters between plasmids pRS1 and pRS2 [in pRS1 λ *O* is regulated by the *lac* promoter while in pRS2 it is regulated by the λ p_L promoter (Fig. 1)], pRS2 carries another λ DNA fragment not carried by pRS1. This fragment contains λ *rexB* and most of λ *rexA* (Fig. 1). We report below that the Rex function is indeed responsible for stabilization of the λ O protein.

λ *rex* Prevents λ O Protein Degradation. To examine whether the λ *rex* fragment present on pRS2 contributes to λ O protein stabilization we deleted most of its *rex* region to construct pRS4 (Fig. 1*B*). The results of a pulse–chase experiment show that λ O directed by plasmid pRS4 is labile (Fig. 2, lanes B). Its degradation pattern was similar to that for the O protein directed by plasmid pRS1 (Fig. 2, lanes C), which also lacks the λ *rex* gene (Fig. 1*B*).

Plasmid pRS2 carries the whole *rexB* gene but is missing part of *rexA* (Fig. 1*B*). This suggests that *rexB* causes the observed stabilization of the λ O protein. To test this hypothesis, we constructed pRS3 by deleting most of the *rexA* gene from pRS2. Plasmid pRS3 retains the *rexB* gene with its own promoter, p_{LIT} located at the end of the *rexA* gene (Fig. 1*B*). The results of a pulse–chase experiment show that pRS3-directed λ O is quite stable for 30 min after chase (Fig. 3*C*), behaving like pRS2-directed λ O (Fig. 3*A*). We conclude that *rexB* causes λ O protein stabilization.

The labeled λ O protein was quantitated in the pulse–chase experiments by densitometric scanning of the autoradiograms. The experiments suggest that pRS2-directed λ O is not completely stable during the 30-min period of chase. However, compared to the degradation of pRS1-directed λ O the decrease is minimal and ≈60% of the label remained 30 min after the chase (Fig. 3*A*). Similar results were obtained with plasmid pRS3, which lacks most of *rexA* but carries *rexB* (Fig. 3*C*). In contrast, pRS4, which lacks both *rexA* and most of *rexB*, directs the synthesis of a labile λ O protein. Fig. 3*B* shows an experiment representative of several others: only 1% of pRS4-directed λ O remained after 10 min of chase. Occasionally the degradation of pRS4-directed λ O was slower, but at most ≈15% of λ O protein remained after 30 min of chase.

The λ *rexB* Product Causes Stabilization of the λ O Protein. In several ways we further confirmed that *rexB* prevents degradation of the O protein and that it is the product of *rexB* that is involved in this process. First, we introduced either a UAA (ochre) or a UAG (amber) mutation into *rexB*. These nonsense mutations were derived by oligonucleotide-directed site-specific mutagenesis in which we changed a UAC to UAA or UAG at position 36231–36233 of the λ DNA in the N-terminal portion of *rexB* (Fig. 1). We call the plasmids that carry *rexB* with a nonsense mutation pRS2$_{UAA}$ or pRS2$_{UAG}$. As shown in Fig. 3*D*, when there is a UAA nonsense mutation in *rexB*, λ O is quite labile: only 25% of λ O protein remained after 30 min of chase. On the other hand, in this *E. coli* strain (CSR603), which carries the chromosomal UAG suppressor gene *supE44*, pRS2$_{UAG}$-directed λ O protein is as stable as that directed by pRS2 (Fig. 3*E*). These results further establish that *rexB* is responsible for O protein stabilization and also indicate that it is the protein product of the *rexB* gene that is involved in the process.

For comparison, we carried out similar experiments in *E. coli* N99 (λ*c*I857). As shown in Fig. 4, in this strain also the presence of a functional *rexB* gene on a plasmid inhibits degradation of the λ O protein. A rather stable λ O is synthesized in cells carrying pRS2 (Fig. 4*A*). In contrast, a labile λ O protein is synthesized in N99 (λ*c*I857) cells carrying pRS4 (no *rex* function), pRS2$_{UAA}$ (with a UAA mutation in *rexB*), and pRS2$_{UAG}$ (with a UAG mutation in *rexB*) (Fig. 4 *B–D*, respectively).

We have also shown that the addition of the corresponding nonsense suppressor can partially reverse the defects of *rexB*$_{UAG}$ in the stabilization of λ O. To do this we cloned a *su7-UAG* gene into the pBR322-compatible plasmid pSU27-18 to construct plasmid pLDG2. When transformed into bacterial strains carrying nonsense mutations, pLDG2 suppresses amber mutations (data not shown). To test the effect of this nonsense suppressor on the function of λ *rexB*$_{UAG}$, we cotransformed *E. coli* N99 (λ*c*I857) with pRS2$_{UAG}$ and pLDG2 (Fig. 4*E*). The UAG suppressor gene partially restored the stabilization of pRS2$_{UAG}$-directed λ O

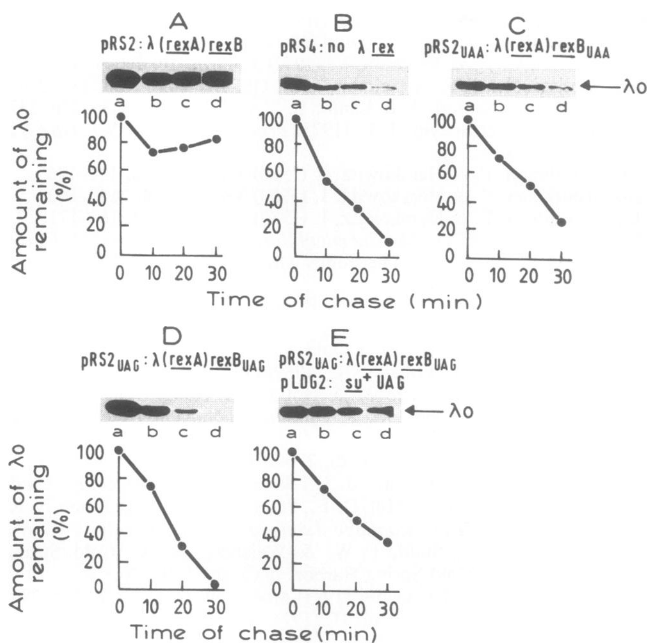

FIG. 4. Effect of λ *rex* on stability of the λ O protein in *E. coli* N99 (λcI857). *E. coli* N99 (λcI857) carrying plasmids pRS2 (*A*), pRS4 (*B*), $pRS2_{UAA}$ (*C*), $pRS2_{UAG}$ (*D*), and $pRS2_{UAG}$ with pLDG2 (*E*) was pulse labeled and chased. Experimental conditions were as described in Figs. 2 and 3 and in *Materials and Methods*.

protein in *E. coli* N99 (λcI857). In *E. coli* CSR603 (λcI857S_{am}7), which carries the chromosomal UAG suppressor gene *supE44*, $pRS2_{UAG}$-directed λ O protein is as stable as that directed by pRS2 (Fig. 3*E*). In the two *E. coli* strains, N99 (λcI857) and CSR603 (λcI857S_{am}7), the levels of the stabilization of λ O by a UAG suppressor tRNA acting on $rexB_{UAG}$ are different. This may be due to one or more of the following reasons: differences in these two bacterial strains, differences in the efficiency of the suppressors themselves (*supE44* is $su^{+}2$ whereas pLDG2 carries $su^{+}7$), or differences in amino acid replacement.

To confirm that it is the product of the *rexB* gene, and not the presence in cis of the gene itself, that increases the stability of the λ O protein, we asked whether the *rexB* gene can act in trans. For this purpose we cloned the *rexB* gene into plasmid pSU27-18 to construct plasmid pLDG1. *E. coli* CSR603 (λcI857S_{am}7) was cotransformed with pLDG1 and either pRS4 (no λ *rex*) or with $pRS2_{UAA}$. In both cases when *rexB* is supplied in trans by pLDG1 the stability of λ O is significantly increased and is similar to that of the pRS2-directed λ O protein (Fig. 5). To test whether the presence of a lysogen may affect the stability of λ O, we examined the action of λ *rexB* on λ O in trans in the nonlysogenic strain CSR603 (F′*lacI*^{q}). We again used pLDG1, this time with plasmid pRS1, which does not carry λ *rexB* but does carry λ *O* under control of the *lac* promoter. The expression of λ *O* was induced by the addition of isopropyl β-D-thiogalactopyranoside at 37°C. As a control, we used the plasmid pair pRS1 and pSU27-18, which does not carry *rexB*. We found that λ O was significantly stabilized under these experimental conditions (*rexB* on pLDG1) but not under the control conditions (pSU27-18, no *rexB*) (data not shown). However, here when λ O was directed by λ *O* on plasmid pRS1 under control of the *lac* promoter, stabilization by *rexB* was less efficient than when λ *O* was in a lysogenic strain on a plasmid and under the control of λ p_L.

DISCUSSION

Here we describe an additional function for λ *rex*: it prevents degradation of the λ O protein, known to be involved in λ DNA replication (22–25). We have demonstrated that *rexB* is responsible for this effect (Fig. 3). In addition, we have shown that the product of *rexB*, and not the presence in cis of the gene itself, is responsible for the described stabilization of the λ O protein. We have based our conclusion on several lines of evidence. First, nonsense mutations in *rexB* prevent stabilization of the λ O protein, and introduction of the corresponding nonsense suppressor gene partially restores the stabilization effect of *rexB* (Figs. 3 and 4). In addition, the stabilization effect of *rexB* on the λ O protein is manifested in trans (Fig. 5).

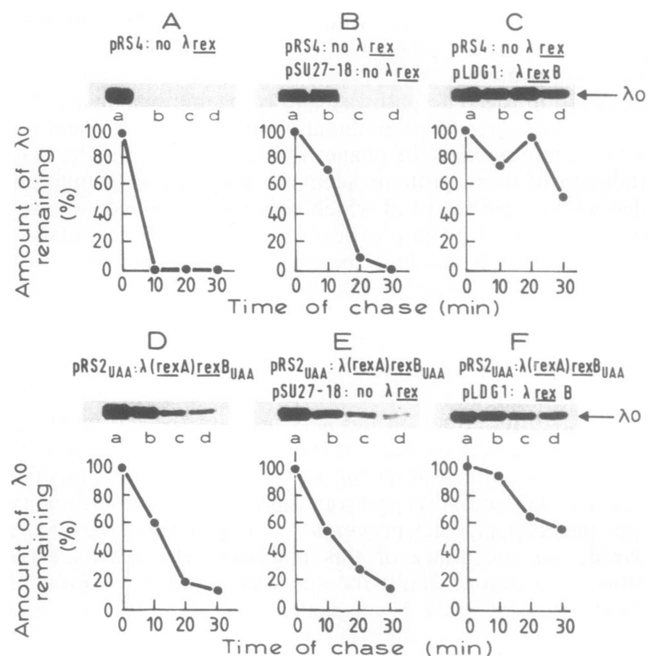

FIG. 5. Effect of *rexB* in trans on λ O protein stability. *E. coli* CSR603 (λcI857S_{am}7) carrying plasmids pRS4 (*A*), pRS4 and pSU27-18 (*B*), pRS4 and pLDG1 (*C*), $pRS2_{UAA}$ (*D*), $pRS2_{UAA}$ and pSU27-18 (*E*), and $pRS2_{UAA}$ and pLDG1 (*F*) was studied by pulse–chase experiment carried out as described in Figs. 2 and 3 and in *Materials and Methods*.

In general, we carried out these experiments in *E. coli* strains lysogenized with temperature-sensitive inducible lysogens, with both the λ *O* and λ *rexB* genes located on plasmids. Under the condition of induction by high temperature (41°C), λ O protein is directed only by λ *O* located on a plasmid (and not on the prophage); when we induced lysogenized cells carrying either pBR322 or no plasmid at all, no λ O protein was detected. Since *rex* exclusion has been described as a quantitative phenomenon (14), it is possible that the presence of the λ *rex* operon on the bacterial chromosome may play a role in the stabilization effect of *rexB* on the O protein. Furthermore, additional λ genes present on the lysogen and induced by high temperature may also contribute to the *rexB* function investigated here. To study this problem, we examined the effect of λ *rexB* in trans on λ O stabilization when λ *O* was under control of the *lac* promoter. These conditions permit the expression of λ *O* in a nonlysogenic strain. The results of our experiments suggest that the *rexB* product can stabilize the λ O protein in a nonlysogenized strain. However, stabilization of the λ O protein is less effective in this nonlysogenic host than when λ *O* is in a lysogenized strain on a plasmid and under the control of λ p_L. This suggests that some factor other than the *rexB* product alone may contribute to the process of the stabilization of λ O.

The development of phage λ is regulated through the turnover of several phage proteins. These include the λ cII

protein, involved in the switch between the lytic and lysogenic states of the phage (36, 37); the transcription antitermination protein λ N, required for expression of the genes involved in the lytic pathway and lysogenic responses (38–40); the site-specific recombination protein Xis (41); and the λ O protein needed for phage replication (21–25). The degradation of these proteins seems to occur through multiple degradation pathways of which only two have been characterized. The cII protein is degraded by the Hfl degradation pathway, which involves the *hflA* and *hflB* loci of *E. coli* (42–45). The product of the bacterial gene *hflA* has been identified as a protease that cleaves cII into small fragments (46). The λ cIII protein decreases cII degradation (44, 45, 47). The λ N protein is degraded by the Lon degradation pathway in which *E. coli lon*-mediated proteolysis is involved. In contrast to its effect on the λ N protein, the *lon* mutation causes only a 50% decrease in the half-life of the λ cII protein and does not affect either the λ O or the λ Xis proteins (48).

The λ O degradation pathway is not known. Our discovery that the *rexB* product prevents λ O degradation provides a handle for the study of this pathway. The results raise obvious questions about the specificity and mechanism of stabilization by rexB. We suggest that *rexB* protein acts in a degradation pathway different from those of the λ cII and λ N proteins. The λ *rexB* product may act as an inhibitor of a protease just as λ cIII acts to stabilize λ cII (46). If that is the case, λ *rexB* might act in one of two possible ways: (*i*) λ *rexB* protein might interact either directly or indirectly with a class of *E. coli* proteases other than HflA and Lon or (*ii*) *rexB* might interact with one or more of a class of proteolytic substrates such as λ O.

What is the role of the protective activity of Rex B on λ O protein degradation in phage λ development? Clearly, the antidegradative action can regulate cellular levels of λ O, a protein critical for λ DNA replication (see above). It has been reported that at the time of the initiation of λ DNA replication the transcription of lit mRNA, and thereby the noncoordinative expression of *rexB* (without *rexA*), is increased (20). This increase in λ *rexB* expression is dependent on host and phage replication genes, including the λ *O* gene. It has also been suggested that λ *rex* is involved in the switching from early to late phage DNA replication (11). The stabilization of the λ O protein by the *rexB* product could clearly be part of such a mechanism. Moreover, an increase in the level of λ O protein may influence synthesis of the product of *rexB*, which in turn may increase the level of λ O protein by preventing its degradation. Thus, λ O protein levels may be autogenously controlled by a positive regulatory loop.

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