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Characterization of the σ^{38}-Dependent Expression of a Core *Escherichia coli* Starvation Gene, *pexB*

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A reverse genetics approach was used to clone a *pex* starvation gene that codes for an 18-kDa polypeptide, designated PexB. Single-copy *pexB-lacZ* operon fusions were constructed to study transcriptional regulation and the promoter region of this gene. The induction by carbon starvation or osmotic stress was transcriptional and controlled by σ^{38} but was independent of this sigma factor by the oxidative stress; presumably, it was σ^{70} mediated under the latter stress. During nitrogen starvation, the induction was controlled at the posttranscriptional level. The *pexB* upstream region contained 245 nucleotides within which sequences approximating the consensus for cyclic AMP receptor protein and integration host factor binding sites were discernible. Deletion of 164 bp of the upstream region, which included these consensus sequences, did not affect starvation- or osmotic stress-mediated induction of *pexB* but abolished its induction by oxidative stress. The same start site was used in transcription during carbon starvation, osmotic stress, or oxidative stress, suggesting that the *pexB* promoter can be recognized in vivo by both σ^{38} and σ^{70}, depending, presumably, on the presence of appropriate transcriptional factors. The −10 and −35 regions of *pexB* resembled those of some but not all genes known to be controlled by σ^{38}.

At the onset of carbon starvation, *Escherichia coli* induces some 50 polypeptides, which fall in several temporal classes. This induction is associated with the development of marked general resistance (7, 11–13, 21–23, 34). We are attempting to identify and characterize the individual polypeptides that contribute to this resistance. A core set of 15 polypeptides is induced regardless of whether the cells are starved for carbon, nitrogen, or phosphorus, and since this set includes many heat, oxidative, and osmotic shock proteins (7), we have focused on these polypeptides. This core set of proteins is regulated differently from most of the other carbon starvation proteins. First, their induction is independent of cyclic AMP (cAMP), while that of the rest requires this nucleotide. On this basis, we differentiated starvation proteins into Pex (cAMP independent) and Cst (cAMP dependent) (22, 39). Second, the induction of several Pex proteins requires secondary σ factors. Thus, σ^{32} is required for the induction of at least three Pex proteins, DnaK, GroEL, and HtpG, and mutants deficient in this sigma factor survive starvation poorly (11). Similarly, σ^{38} (also referred to as σ^{s} and KatF [29]) is required for the induction of at least six Pex proteins (25), and *E. coli* mutants deficient in σ^{38} (*rpoS*) are greatly compromised in the development of starvation-mediated resistance (18, 25).

This report deals with an 18-kDa membrane-associated (39) Pex protein that we identified in 1986 as spot 19 on our two-dimensional (2-D) gel electrophoresis maps (7, 22, 39) and subsequently named PexB (15). This protein is induced early in starvation and exhibits a sustained induction during carbon starvation: at 4 h of glucose or succinate starvation, it constitutes a major portion of the residual cellular bulk protein synthesis (7, 39). Its induction is σ^{38} dependent (25, 26).

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We reported earlier the cloning (15) of the gene encoding this protein and discuss here its regulation by different stresses and the nature of its promoter region. Different stresses induce it at the transcriptional level in either a σ^{38}-dependent or -independent manner or at a posttranscriptional level. All elements of the control of σ^{38}-dependent induction reside within 58 nucleotides upstream of the transcriptional start site. Recently, Almiron et al. (2) published their results on the role of this protein (referred to by them as Dps) in starvation.

MATERIALS AND METHODS

Bacterial strains, plasmid constructs, and growth media. The *E. coli* strains and plasmid constructs used in this study are listed in Table 1. M9 minimal, morpholinepropanesulfonic acid (MOPS) minimal, and LB media were prepared as described previously (27). When specified, the media were supplemented with ampicillin or tetracycline (50 or 15 μg/ml, respectively).

Starvation and other stress protocols. Carbon and nitrogen starvation experiments were conducted as previously described (3, 7, 39) except that MOPS minimal medium was used for carbon starvation. To determine the effect of acetate on *pexB* induction during glucose starvation, exponentially growing cells (in 0.05% glucose MOPS medium) were harvested by centrifugation at 37°C, washed twice with ice-cold MOPS medium devoid of a carbon source, and resuspended in MOPS medium minus glucose with or without 0.009% acetate. As a control, an aliquot of these cells was also suspended in MOPS medium supplemented with glucose. All cultures were incubated at 37°C in a shaking water bath at 150 rpm. For osmotic or oxidative stress, cells were grown in M9 minimal medium supplemented with 0.4% glucose. When the cultures had attained an A_{660} of 0.15 to 0.20, sterile NaCl crystals or H_2O_2 was added to a final concentration of 0.35 M or 120 μM, respectively, to induce the stress (12). β-Galactosidase assays were performed in duplicate as described previously (7) except that 20 μl of chlorophenolred-β-*o*-galactopyranoside (CPRG; 31 mg/ml) was used instead of *o*-nitrophenyl-β-D-galactopyranoside (ONPG). After sufficient red color developed (A_{574} of

TABLE 1. *E. coli* strains, plasmids, and phages used in this study

Strain, plasmid, or phage	Genotype or description	Reference or source
E. coli strains		
AMS6	Δ*lacU169*	39
AMS150	AMS6 *rpoS*::Tn*10*	25
AMS161	AMS6(pAMC35)	This study
AMS169	AMS6 (λOLS1)	This study
AMS170	AMS150 (λOLS1)	This study
AMS171	AMS6 (λOLS2)	This study
AMS172	AMS150 (λOLS2)	This study
AMS173	AMS6 (pAMC39)	This study
AMS174	AMS150 (pAMC35)	This study
Plasmids		
pBluescript KS II		Stratagene
pAMC35	pBluescript *pexB*	This study
pRS415		40
pAMC37	pRS415 with *lacZ* operon fusion to −245 of *pexB*	This study
pAMC38	pRS415 with *lacZ* operon fusion to −82 of *pexB*	This study
pAMC39	pRS415 with *lacZ* operon fusion to −59 of *pexB*	This study
Phages		
P1 *vir*		Our laboratory strain
λRS45		40
λOLS1	λRS45 with *lacZ* operon fusion to −245 of *pexB*	This study
λOLS2	λRS45 with *lacZ* operon fusion to −82 of *pexB*	This study

ca. 0.4 to 0.6, which is within the linear part of the activity curve), samples were centrifuged and the optical density was recorded. β-Galactosidase activity is expressed in Miller units except that it is based on CPRG rather than ONPG conversion (27); the CPRG assay is about 10-fold more sensitive than the ONPG assay.

2-D gel electrophoresis. Proteins were pulse-labeled and separated on 2-D gels as previously described (7, 24). Labeled proteins were visualized by autoradiography on Hyperfilm-MP (Amersham Corp., Arlington Heights, Ill.).

Protein isolation and peptide sequencing. One liter of *E. coli* K-12 culture, grown in 0.025% glucose M9 medium, was starved for 2 h, pelleted (4°C), and resuspended in 30 ml of ice-cold 50 mM potassium phosphate buffer (pH 6.6). Cells were broken at 16,000 lb/in^2 in a French Press (American Instrument Co., Silver Spring, Md.). Following removal of unbroken cells (1,100 × *g*, 5 min), the supernatant was centrifuged at 17,300 × *g* (30 min) to pellet the membrane fraction. After suspension in 1 ml of 50 mM potassium phosphate buffer (pH 7.5) and addition of 300 μl of 50% trichloroacetic acid (TCA), the pellet was placed on ice for 2 h. The precipitated proteins were pelleted in a microcentrifuge (6 min, 4°C), washed sequentially in 10% TCA, 5% TCA, and acetone at −20°C, dried, and stored at −70°C. One hundred milligrams was later separated by 2-D gel electrophoresis. The gel was stained with 0.15% Coomassie brilliant blue R-250 in 10% acetic acid–45% methanol and destained (7% acetic acid and 45% methanol). The excised PexB spot was cleaved with cyanogen bromide (31), and the products, after separation by electrophoresis (28), were transferred to Westran polyvinylidene difluoride membrane (Schleicher & Schuell Inc., Keene, N.H.) as described previously (10). The membrane was stained with Coomassie brilliant blue, and approximately 1.5 mg of a 10-kDa peptide was excised and sequenced at the Stanford University Protein and Nucleic Acid Facility.

Construction of *lacZ* fusions. To study the regulation of the *pexB* gene, single-copy *pexB-lacZ* fusions were constructed by using the system of Simons et al. (40). Different-length fragments of *pexB* upstream sequence were generated by PCR; these were flanked by appropriate restriction sites to permit directional cloning. The following primers, complementary to the upstream *pexB* region (see Fig. 6), were used: −245GGGG AATTCCCTCTACACCGTCTTTAT−228; −82GGGGAAT TCAGCCAGAATAGCGGAACA−59; and −59GGGGAAT TCCCGGTGCTATACTTAATC−42. The fourth primer, complementary to the internal sequence of the *pexB* gene, was +44 0GG GGG AT CC ATA TCT GC GGT GT CGT CA+413. (The numbering is relative to the translational start site of the *pexB* gene.) Primers were synthesized at the Protein and Nucleic Acid Facility of Stanford University. Control sequencing ensured that the PCR procedure did not result in mutation.

The resulting fragments were cloned into the operon fusion vector pRS415. Each was recombined in phage RS45, which was used to lysogenize AMS6 or AMS150 to yield various fusion strains (Table 1).

Molecular biology protocols and materials. Restriction digests, ligations, and DNA purifications were performed as described previously (20). Plasmids were introduced into bacteria by electroporation (20). T4 DNA ligase and restriction endonucleases were purchased from New England Biolabs (Beverly, Mass.). Dideoxy sequencing was performed with the Sequenase version 2.0 DNA sequencing kit (United States Biochemical, Cleveland, Ohio). Geneclean II was purchased from Bio 101, La Jolla, Calif.), and plasmid pBluescript II was purchased from Stratagene, La Jolla, Calif.

Transcript mapping to locate the *pexB* transcriptional start site was performed as described previously (20). Total RNA was isolated, and a primer (5′TGCGGGTATAAAGCAGAT TGGTCGCTTTTG) complementary to 25 to 55 bases downstream of the translational start site of the *pexB* mRNA was used for primer extension and sequencing reactions. Transcript mapping of the *bla* gene (which encodes ampicillin resistance) was used as a control for the amount of mRNA in starved cultures (see Results), using the primer 5′TAAGGGCGA CACGGAAATGTTGAATAC, which is complementary to 5 to 31 bases downstream of the translational start site of the *bla* gene.

Nucleotide and protein sequence data were analyzed with the Genetics Computer Group sequence analysis software package. The TFASTA (19) and FASTA (33, 41) programs were used to compare the PexB amino acid sequence and deduced nucleotide sequence, respectively; the algorithm of Brendel and Trifonov (5) was used to locate transcriptional terminators.

Nucleotide sequence accession number. The sequence shown in Fig. 6 has been deposited in GenBank (accession number U04242).

RESULTS

Cloning of the *pexB* gene. As stated in Materials and Methods, a part of the PexB protein was sequenced. A FASTA data base search (33) of the deduced DNA sequence identified the location of the *pexB* gene upstream of the *glnHPQ* operon at 18 min on the *E. coli* chromosome (15, 32). Using the restriction map of this operon (32) and of the cloned DNA fragment, we determined that a 3.0-kb *Sac*II-*Pst*I fragment on the Kohara encyclopedia phage 10A8 contained the *pexB* gene (15, 16). This fragment was cloned into the high-copy-number

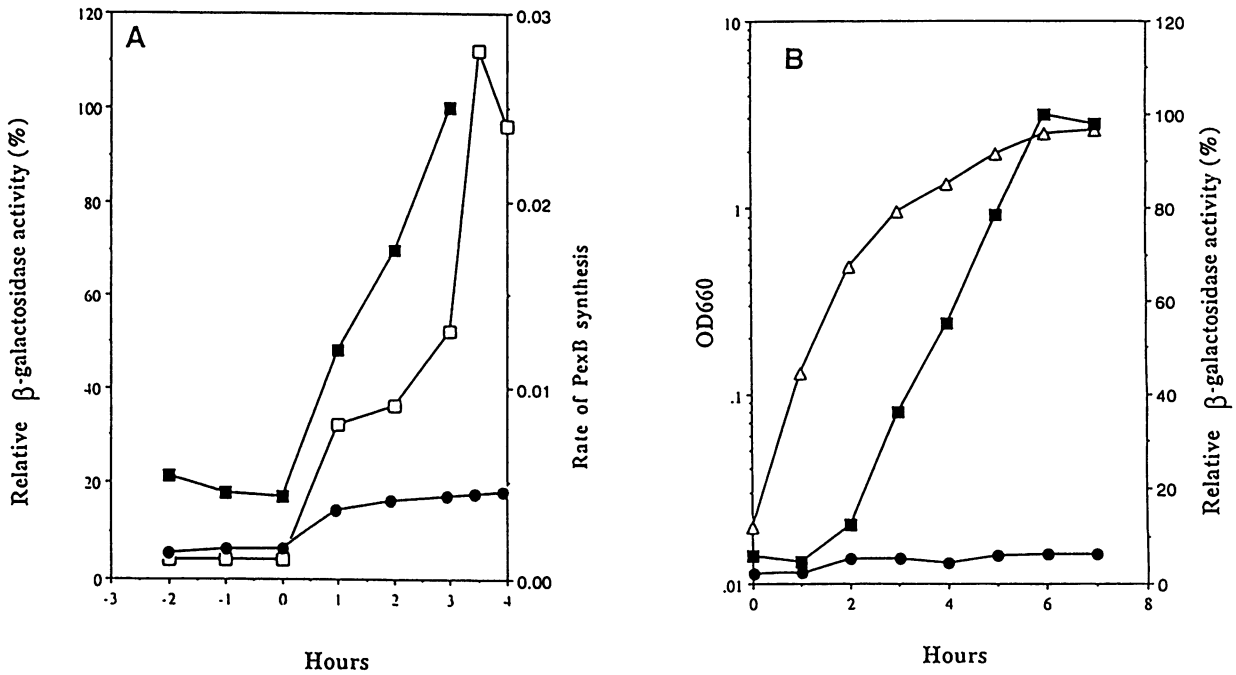

FIG. 1. Expression of the *pexB-lacZ* fusion during growth and starvation in *rpoS*-proficient (AMS169; ■) and -deficient (AMS170; ●) backgrounds. (A) Glucose starvation. Zero time denotes the onset of starvation. □, rate of PexB protein synthesis, replotted from reference 7 for comparison. One hundred percent β-galactosidase activity equals 24,000 U. (B) Starvation in LB medium. One hundred percent β-galactosidase activity equals 29,500 U. △, A_{660} of the culture. Growth rates of *rpoS*$^+$ and *rpoS* strains were essentially the same. The effect of *rpoS* mutation on *pexB-lacZ* expression is discussed in the text.

vector pBluescript KS II, generating pAMC35 (Table 1). Sequencing showed that this fragment contained two open reading frames, *pexB* and an upstream gene, designated open reading frame 1 (ORF1); a putative rho-independent transcription terminator (5) was present between ORF1 and *pexB*. ORF1, which exhibits no homology in the data base, is transcribed in the same direction as *pexB* and should encode a 35-kDa protein. An ORF1-*lacZ* fusion was expressed at low levels only during growth (data not shown).

2-D gel analysis (not shown) revealed that at 1 h of starvation, strain AMS161 (which contains pAMC35) markedly overproduced an 18-kDa protein with the same isoelectric point as PexB; no unique protein spot in the region of 35 kDa was evident on these maps, which is consistent with the ORF1 fusion results mentioned above. Several starvation proteins in starved AMS161 were overinduced, but induction of others was diminished. Significantly, the latter class included all of the Pex proteins that were synthesized at this time point; according to the numbering scheme presented by Matin (22), these were Pex protein 17, 34, 35, 105, 113, and 114. It is not known if this is due to the overproduction of PexB and possible overtitration of a factor(s) needed for Pex protein induction in general.

Regulation of *pexB* transcription and translation during starvation. Our previous studies (7, 39) and those of Almiron et al. (2) have demonstrated that the PexB (Dps) protein is induced during carbon starvation. To determine if this induction occurs at the transcriptional level and to study the *pexB* gene promoter region, single-copy *pexB-lacZ* operon fusions were made as described in Materials and Methods. Strain AMS169 contained a fusion in which the region from −245 to +440 bp of the *pexB* gene (in relation to its translational start site) was fused to a promoterless *lacZ* gene (with its own Shine-Dalgarno sequence and ATG codon). The fusion in strain AMS171 contained only 82 bases upstream of the translational start site (i.e., it contained −82 to +440 bp of the *pexB* gene). Both strains AMS169 and AMS171 showed rapid induction of β-galactosidase upon carbon starvation in glucose minimal or LB medium, as illustrated for AMS169 in Fig. 1. An *E. coli* strain lysogenized with phage containing a DNA fragment with 59 bp upstream of the *pexB* translational start site (i.e., −59 to +440 bp of the *pexB* gene) showed no β-galactosidase activity during growth or starvation, indicating that this fragment did not contain the *pexB* promoter (data not shown).

We have previously determined the kinetics of the PexB protein synthesis during starvation by direct quantification of the PexB spot on 2-D gels (7); the kinetics under glucose starvation are reproduced here for comparison with induction of the transcriptional fusions (Fig. 1A). It is evident that PexB protein synthesis essentially parallels the induction of the transcriptional fusions. Thus, PexB synthesis under these conditions is transcriptionally regulated.

Nitrogen starvation, however, produced no induction of β-galactosidase in strain AMS169 or AMS171 (Fig. 2). Nevertheless, synthesis of the PexB protein itself increased 10-fold under these conditions. We have documented this finding in Fig. 2 by reproducing data from our previous work (39), in which PexB protein synthesis was directly quantified at various times after the onset of nitrogen starvation by computer-assisted densitometry. Thus, the induction of PexB by nitrogen starvation appears to be posttranscriptionally regulated.

Requirement for an extracellular compound. When an exponentially growing culture of strain AMS169 was washed and suspended in MOPS medium without glucose, it exhibited β-galactosidase induction (Fig. 3), but at a much lower level than during glucose exhaustion from the medium. This finding

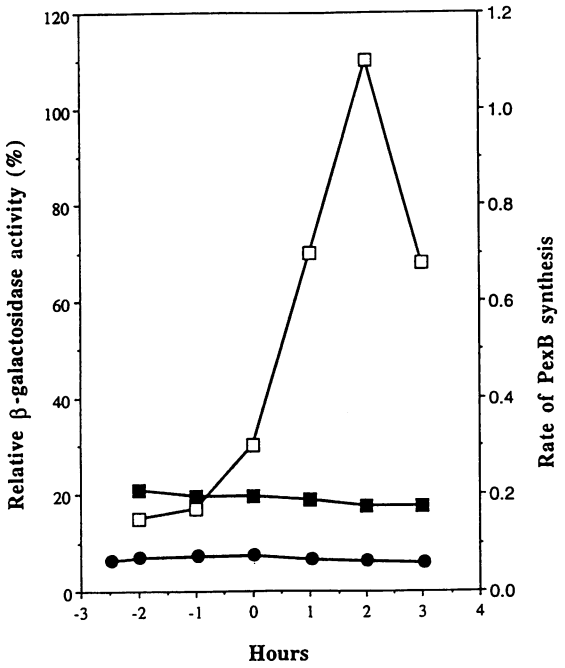

FIG. 2. Expression of the *pexB-lacZ* fusion in *rpoS*-proficient (AMS169; ■) and -deficient (AMS170; ●) backgrounds and rate of PexB protein synthesis in an *rpoS*-proficient background (□; reproduced from reference 39 for comparison) during nitrogen starvation. One hundred percent β-galactosidase activity equals 24,000 U. The effect of *rpoS* mutation on *pexB-lacZ* expression is discussed in the text.

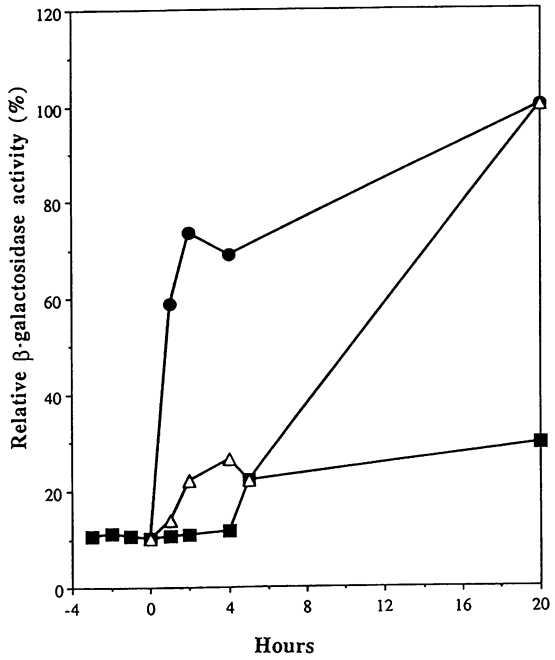

FIG. 3. Expression of the *pexB-lacZ* fusion in an *rpoS*$^+$ background (strain AMS169) after resuspension of the exponential-phase culture in MOPS medium with no addition (■), in MOPS medium supplemented with 0.009% acetate (●), or in MOPS medium containing 0.05% glucose (△). Induction of β-galactosidase activity at 4 h under these conditions is due to the exhaustion of glucose. One hundred percent β-galactosidase activity equals 18,000 U.

suggested a role for an extracellular factor, accumulating under the latter conditions, that facilitated *pexB* induction. When low levels of acetate (0.009%) were supplied to such resuspended starving cultures, induction of the *pexB-lacZ* fusion became much more pronounced (Fig. 3). No change in culture A_{660} occurred as a result of acetate addition. Control aliquots of the suspended culture to which glucose was added showed only low levels of β-galactosidase until glucose was exhausted from the medium. Provision of low levels of acetate during carbon starvation facilitates induction also of other carbon starvation genes (37, 38, 43), but it is not known whether this effect is specific for acetate or whether acetate acts as a source of energy for gene expression in starving cultures.

Regulation of the *pexB-lacZ* fusions by osmotic and oxidative stresses. The two fusion strains, AMS169 and AMS171, were subjected to osmotic or oxidative stress as described in Materials and Methods. Both strains were induced by the osmotic stress (Fig. 4A). In contrast, the oxidative stress induced only the strain containing the longer upstream region of *pexB* (AMS169); strain AMS171 was not induced by this stress (Fig. 4B), suggesting that a *pexB* sequence involved in responding to the oxidative stress is missing in this strain.

Effect of *rpoS* mutation. The σ^{38}-deficient strains, AMS170 and AMS172, containing fusions of the same length as in AMS169 and AMS171, exhibited markedly decreased synthesis of β-galactosidase during starvation and, to a lesser extent, also during growth (Fig. 1). For example, during glucose starvation, induction of the fusion strain was only 20% in AMS170 (*rpoS*) compared with AMS169 (*rpoS*$^+$). A small but reproducible induction during glucose starvation remained, however, in the *rpoS* background under these conditions (Fig. 1A).

The induction of β-galactosidase activity by osmotic stress in both the *pexB-lacZ* fusion strains (AMS169 and AMS171) was abolished by the introduction of an *rpoS* mutation (i.e., in strains AMS170 and AMS172; Fig. 4A). However, the induction of β-galactosidase activity produced by oxidative stress was similar in strains AMS169 (*rpoS*$^+$) and AMS170 (*rpoS*) (Fig. 4B), indicating that the *rpoS* mutation has no effect on *pexB* induction by oxidative stress.

***pexB* transcriptional start site.** The transcriptional start site of the *pexB* gene under different stresses was mapped by primer extension analysis (Fig. 5). RNA isolated from cells starved in minimal glucose or LB medium or from cells subjected to osmotic or oxidative stress was used. *rpoS*$^+$ and *rpoS* strains possessing the *pexB*-containing plasmid AMS35 (i.e., strains AMS161 and AMS174, respectively; Table 1) were used in these experiments. Two DNA bands were observed as reverse transcription products of the RNA isolated from cells starved in either medium (Fig. 5A). The same extension products were observed when mRNA from strain AMS6 was analyzed; this strain is devoid of pAMC35 and contains only the chromosomal copy of the *pexB* gene. As expected, the amount of the extension products was less in experiments with AMS6 (data not shown).

When the strain AMS174 (*rpoS*) was used as the source of RNA in extension experiments, both bands decreased dramatically, with the lower band disappearing altogether (Fig. 5A). This could be a reflection of decreased amount of total mRNA in the starved AMS174 cells. However, the amount of the *bla* gene mRNA extension product was similar in starved *rpoS*$^+$ (AMS161) and *rpoS* (AMS174) strains (Fig. 5B); this gene is present on pAMC35. Thus, the *rpoS* mutation-mediated reduc-

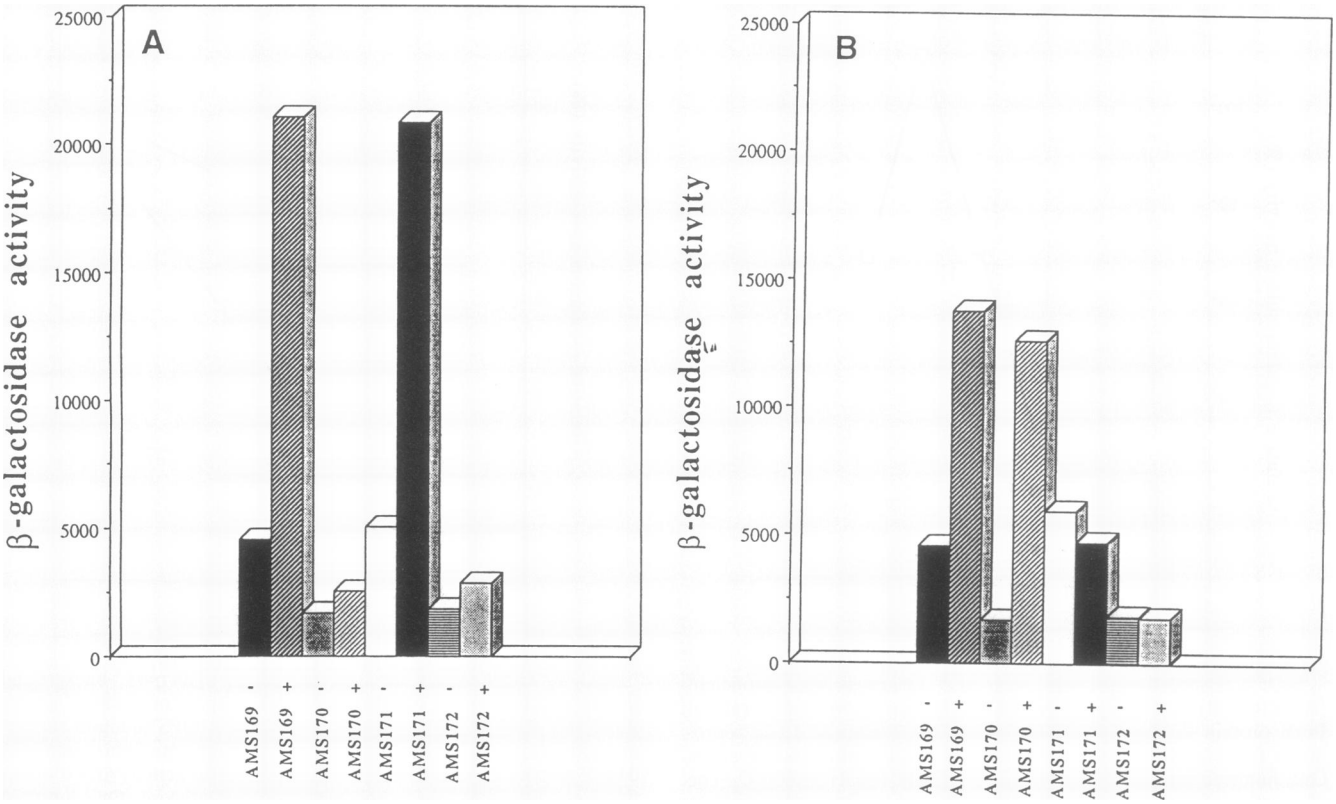

FIG. 4. Expression of two different-length *pexB-lacZ* fusions in response to 30-min osmotic (A) or oxidative (B) stress in *rpoS*$^+$ (AMS169 and AMS171) and rpoS (AMS170 and AMS172) backgrounds. +, addition of sodium chloride (0.350 M) or peroxide (120 μM) to exponentially growing cells; −, no addition. AMS169 and AMS170 contain a *lacZ* operon fusion to bp −245 of the *pexB* gene; AMS171 and AMS172 contain a *lacZ* operon fusion to bp −82 of the *pexB* gene.

tion in *pexB* mRNA is specific and appears not be a general phenomenon.

Primer extension analysis of RNA isolated from osmotically stressed cells showed only the upper band and only in an *rpoS*$^+$ background (Fig. 5C). Both the bands in *rpoS*-proficient background, and the upper band in *rpoS*-deficient background could, however, be detected if the gels were loaded with 10-fold more material. The extension product from oxidatively stressed cells showed the two bands in both *rpoS*$^+$ and *rpoS* backgrounds (Fig. 5D).

DISCUSSION

We postulated (7) and subsequently provided direct evidence (11, 26) that the induction of Pex class of proteins was involved in the general resistance that *E. coli* develops upon starvation (21, 22). Since PexB protein exhibits a strong induction in response to starvation to different individual nutrients and several other stresses, we postulated (15) that this protein is likely to have an important role in stress resistance. Consistent with this expectation, Almiron et al. (2) found that PexB (which they refer to as Dps) is a DNA-binding protein and is involved in starvation-mediated resistance to peroxide.

The *E. coli pexB-lacZ* transcriptional fusion strains studied here were rapidly induced upon glucose starvation, and their induction kinetics closely resembled those of PexB protein induction itself, determined previously by protein quantification. This finding extends previous work (7, 25) in showing that PexB protein induction under these conditions is controlled at the transcriptional level. Similarly, the synthesis and induction of PexB by osmotic and oxidative stresses, demonstrated previously at the protein level, are shown here to be also regulated at the transcriptional level.

However, PexB induction under nitrogen starvation is not transcriptionally regulated. The data show that the 10-fold induction of this protein that occurs under these conditions (39) is not accompanied by induction of gene transcription, as judged by the failure of *pexB-lacZ* operon fusions to be induced under these conditions. Whether this posttranscriptional regulation entails stabilization of the PexB protein or message or enhanced efficiency of translation is as yet not known.

An equally novel finding of this work is that *pexB* requires σ^{38} for transcriptional induction under some but not all stresses. Thus, under starvation and osmotic stresses, the *pexB-lacZ* fusions required this sigma factor for induction, and the amount of *pexB* mRNA, as judged by transcript mapping, was markedly reduced when σ^{38}-deficient cells were exposed to these stresses. In contrast, neither the induction of the appropriate-length fusion nor the *pexB* mRNA levels were affected when cells deficient in this sigma factor were subjected to oxidative stress.

Primer extension analysis revealed two potential transcriptional start sites for *pexB* during growth and various stresses. We suspect, however, that the start site indicated by the lower band in Fig. 5 is an artifact arising from a reverse transcriptase pause site: the T nucleotide start site indicated by this band is

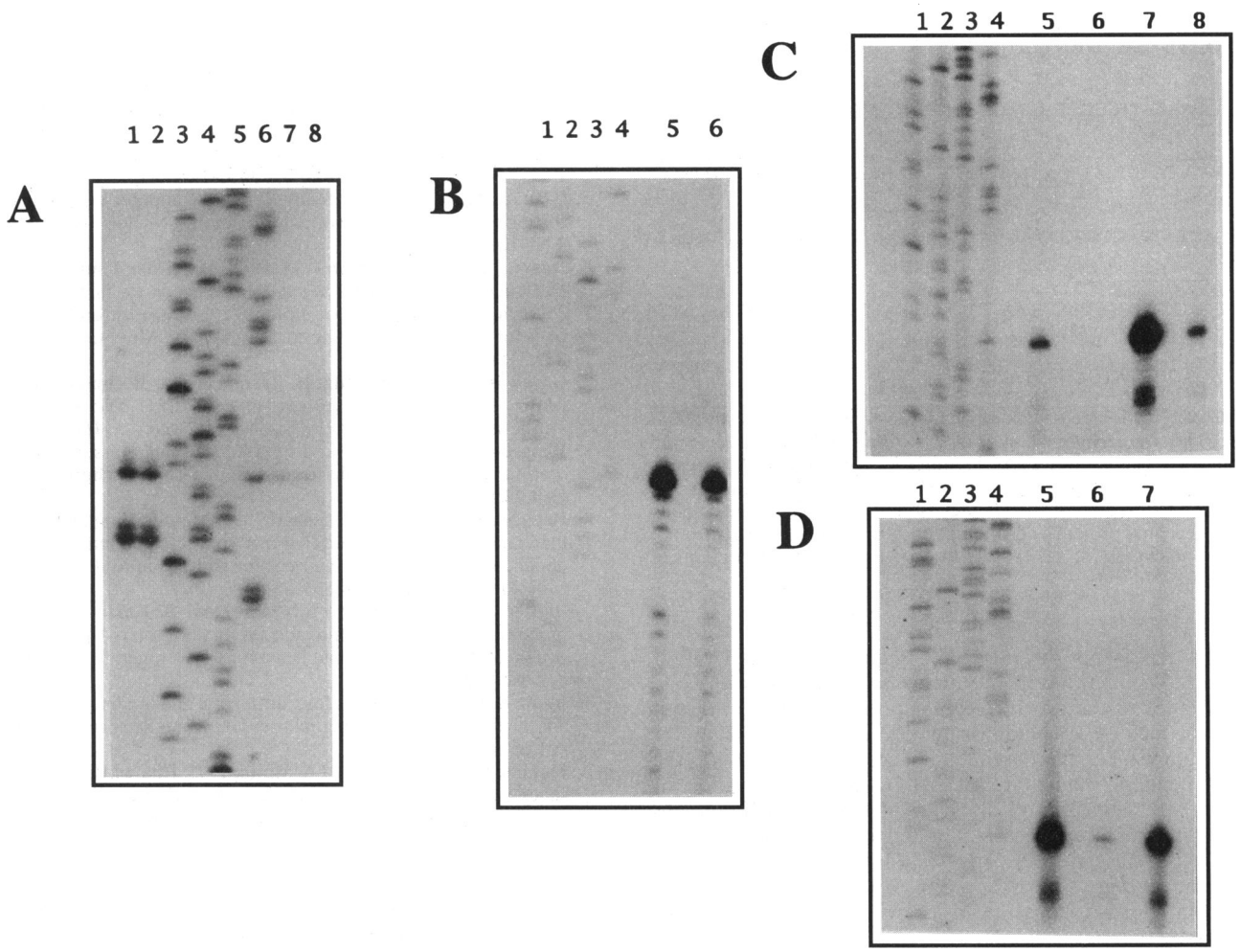

FIG. 5. Location of the *pexB* transcriptional start site by primer extention. A primer complementary to *pexB* mRNA from 25 to 55 bp downstream of the translational start site was used for both primer extention and sequencing reactions in this and other experiments except that of panel B. Lanes 3 to 6 in panel A and 1 to 4 in the other panels provide the sequencing ladder for G, A, T, and C reactions, respectively. (A) RNA was isolated from overnight cultures of *rpoS*$^+$ and *rpoS* strains possessing the *pexB*-containing plasmid pAMC35 (AMS161 and AMS174, respectively) grown in LB or minimal medium. Lanes 1 and 2, *rpoS*$^+$ stationary-phase cells in minimal and LB medium, respectively; lanes 7 and 8, *rpoS* stationary-phase cells in minimal and LB medium, respectively. (B) RNA isolated from the same strains analyzed for *bla* (ampicillin resistance) primer extension product after overnight culture in LB medium. A primer complementary to the *bla* mRNA from 5 to 31 bp downstream of the translational start site was used. Lanes 5 and 6, *rpoS*$^+$ and *rpoS* strains, respectively. The amounts of *bla* mRNA extension product are similar in the two strains. (C) RNA isolated from exponentially growing cultures of the same strains 30 min after the onset of osmotic stress. Lanes 5 and 6, *rpoS*$^+$ and *rpoS* strains, respectively; lanes 7 and 8, same as lanes 5 and 6 but with 10-fold more material loaded on the gel. (D) RNA isolated from exponentially growing cultures of the same strains 30 min after exposure to oxidative stress. Lanes 5 and 7, *rpoS*$^+$ and *rpoS* strains, respectively; lane 6, same as in lane 5 but with 10-fold less material loaded on the gel.

unusual. Furthermore, the intensity of the lower band varied more than that of the upper band in a given experiment. Thus, although we cannot exclude the possibility that two closely spaced start sites are involved, it is probable that the G nucleotide site, indicated by the upper band, is the only transcriptional start site for this gene. The −10 region of the relevant promoter (TATACT) resembles the −10 consensus sequence for σ^{70} promoters (Fig. 6). Although, as shown by deletion analysis reported here, the −35 region is an integral part of this promoter, it does not resemble any known consensus sequences. It does resemble the corresponding region in the *fic* gene and, to a lesser extent, that of the *osmY* gene (45), both of which are σ^{38} regulated.

A unique consensus sequence for σ^{38} promoters was proposed (1, 30, 35, 36, 44), but its validity is now doubtful (4, 8, 17), since several σ^{38}-dependent genes, including *glgS* (9), *cyxA* (6), *osmB* (14), *fic* (42), and, as shown here, *pexB*, do not have such sequences. Tanaka et al. (42) and Nguyen et al. (30a) report that most promoters recognized by Eσ^{38} in vitro were also recognized by Eσ^{70} and that all contained a typical −10 σ^{70} region. It is noteworthy in this connection that in vivo, the *pexB* promoter appears to be recognized by both Eσ^{38} and Eσ^{70}. Thus, although diminished in amount, a message with the start site of this promoter is found in σ^{38}-deficient cells subjected to starvation or osmotic stress. More importantly, induction of this promoter by oxidative stress is completely independent of σ^{38} and depends, presumably, on σ^{70}.

The *pexB* upstream region contains 245 nucleotides (Fig. 6). Since the putative transcription terminator of ORF1 is immediately upstream of nucleotide −245, this entire region pre-

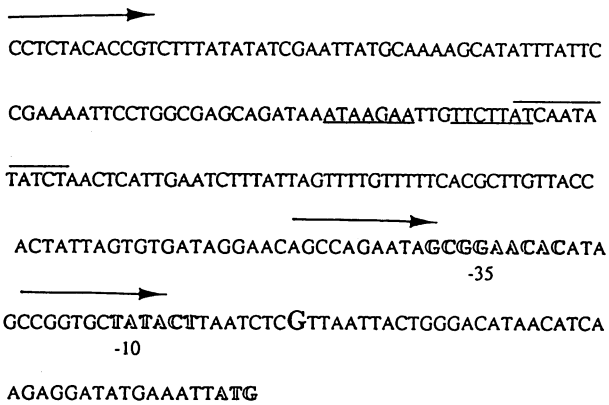

FIG. 6. Nucleotide sequence of the upstream region of the *pexB* gene. The transcriptional start site is indicated by the enlarged boldface G. The −10 (TATACT) and −35 (GCGTAACCC) regions are indicated by shadowed letters; the latter site resembles the corresponding region of the *fic* gene (42). The sequences resembling cAMP receptor protein and integration host factor binding sites are under- and overlined, respectively. Arrows indicate the start points of various *lacZ* fusions.

sumably represents the intergenic sequence between ORF1 and *pexB*. Within this region, palindromic sequences with some resemblance to the cAMP receptor protein (46) and integration host factor (9a) binding sites are discernible. Whatever their significance, these sequences are not needed for the *rpoS*-dependent induction of *pexB* by carbon starvation or osmotic stress: the fusion in strain AMS171, which contains neither of these sequences, exhibited normal induction patterns in response to these stresses. However, these or other upstream sequences have a role in oxidative stress-mediated induction of *pexB*, since the fusion in strain AMS171 is not induced by this stress.

These results highlight the complexities of the σ^{38} regulon. This sigma factor acts on promoters which are induced by several individual stresses, have as yet no unique feature, can evidently also be recognized by σ^{70}, presumably in the presence of appropriate transcriptional factors, and control genes whose main regulation can be at the posttranscriptional level (like that of σ^{38} itself [19a, 26]). Unravelling these complexities can be expected to provide new insights into biological regulation.

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