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The *Escherichia coli* Regulator of Sigma 70 Protein, Rsd, Can Up-Regulate Some Stress-Dependent Promoters by Sequestering Sigma 70[▽]

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The *Escherichia coli* Rsd protein forms complexes with the RNA polymerase σ^{70} factor, but its biological role is not understood. Transcriptome analysis shows that overexpression of Rsd causes increased expression from some promoters whose expression depends on the alternative σ^{38} factor, and this was confirmed by experiments with *lac* fusions at selected promoters. The LP18 substitution in Rsd increases the Rsd-dependent stimulation of these promoter-*lac* fusions. Analysis with a bacterial two-hybrid system shows that the LP18 substitution in Rsd increases its interaction with σ^{70}. Our experiments support a model in which the role of Rsd is primarily to sequester σ^{70}, thereby increasing the levels of RNA polymerase containing the alternative σ^{38} factor.

The requirement of σ factors for the recognition of bacterial promoters by RNA polymerase (RNAP) is well known (9). Most bacteria contain a principal σ factor which assures the expression of most genes and minor σ factors which are needed for the expression of subsets of genes, often in response to specific stresses (10). Thus, in *Escherichia coli* K-12, σ^{70}, encoded by *rpoD*, is the principal σ factor, while six alternative σ factors are present at lower levels. One of these alternatives, σ^{38}, encoded by *rpoS*, accumulates when cell growth ceases and in response to certain stresses, and is regarded as the stationary-phase σ factor (11). Although the role of σ^{38} is understood, it is not clear how it captures sufficient RNAP in order to ensure expression of the σ^{38} regulon. This is because σ^{38} has a weaker affinity for RNAP than σ^{70}, and its level is always less than that of σ^{70} (14, 22). Studies from several laboratories have identified different factors that might favor the formation of RNAP containing σ^{38}, and its activity, in stationary phase (7, 8, 17). One such factor, discovered by Jishage and Ishihama (15), is the Rsd protein (*r*egulator of *s*igma *D*) that was found to accumulate in stationary phase and to bind to free σ^{70}. Jishage and Ishihama (16) showed that Rsd could increase expression from the σ^{38}-dependent *bolA* promoter and reduce expression from certain σ^{70}-dependent promoters and argued that by sequestering σ^{70}, Rsd permits σ^{38}, and possibly other alternative σ factors, to access RNAP. Recent studies showed that, as well as forming a 1:1 complex with σ^{70}, Rsd can also interact with RNAP in the absence of σ, raising the possibility that Rsd might also affect gene expression in *E. coli* by direct interactions with RNAP (13, 27). Thus, in this work, we used transcriptomics to assess possible roles for Rsd and genetic analysis to investigate the mechanism of action of Rsd. We also describe the use of the bacterial adenylyl cyclase two-hybrid system (BACTH; reference 18) to investigate Rsd-σ^{70} and Rsd-Rsd interactions.

MATERIALS AND METHODS

Bacterial strains. The starting *E. coli* K-12 strains used in this work were MG1655 (2), the Δ*lac* strain MC4100 (24), and the *cya* strain BTH101 (19). The method of Datsenko and Wanner (5) was used to delete the *rsd* gene of MG1655 and insert a kanamycin resistance cassette. To do this, the *kan* cassette in plasmid pKD4 was amplified by PCR with primers RSD P1 and RSD P2 (see Table 2) and the product was electroporated into MG1655. The Δ*rsd*::*kan* insertion was confirmed by PCR with the flanking primers RSD P1 screen and RSD P2 screen. The Δ*rsd*::*kan* allele was transferred into MC4100 by P1 transduction. The Datsenko and Wanner (5) method was also used to remove the *kan* insertion, and the *rpoS-359* allele (*rpoS*::*kan* [4]) was introduced by P1 transduction to generate an MC4100 Δ*rsd rpoS*::*kan* derivative.

BACTH assays. The *E. coli cya* strain BTH101 was transformed with derivatives of plasmids pU-T18 and pK-T25 encoding the T18 and T25 fragments of *Bordetella pertussis* adenylyl cyclase (Table 1). Transformants were plated on MacConkey maltose plates containing 100 μg/ml ampicillin and 50 μg/ml kanamycin. For β-galactosidase assays, transformants were grown aerobically at 30°C in LB medium containing 100 μg/ml ampicillin and 50 μg/ml kanamycin. Overnight cells were harvested and lysed with toluene, and activities were measured as described by Miller (23).

pU-T18 derivatives encoding Rsd-T18 fusions were made by cloning PCR-amplified DNA fragments encoding *rsd* into vector pU-T18 that had been digested with XbaI and KpnI. Wild-type and mutant *rsd* inserts were amplified with primers RSD NT Xba and RSD CT Kpn (Table 2), and the products were cleaved with XbaI and KpnI and purified prior to cloning. pK-T25 derivatives encoding T25-Rsd fusion derivatives were made by a similar procedure with the RSD NT Xba and RSD CT Kpn-Stop primers. The DNA sequences of recombinants were checked with two *rsd* internal primers with opposite orientations, RIPF and RIPB. Primers RpoD NT Xba and RpoD CT Kpn-Stop were used to amplify the full-length MG1655 *rpoD* gene by PCR. The product was cleaved with XbaI and KpnI and cloning into pK-T25 to create a derivative encoding a T25-σ^{70} fusion protein.

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TABLE 1. Plasmids used in this work

Bacterial plasmid	Description	Reference
pRW50	Broad-host-range *lacZ* fusion vector for cloning promoters on EcoRI-HindIII fragments, contains RK2 origin of replication, encodes Tet^r	21
pACYC184	Cloning vector; Cm^r Tet^r	3
pACYC184ΔHN	Derivative of pACYC184 with HindIII-NruI fragment removed; Cm^r Tet^s	This work
pACYC-Rsd	*E. coli rsd* gene cloned between BamHI and SphI sites of pACYC184	16
pACYC-RsdB	Derivative of pACYC-Rsd with shorter *rsd* insert cloned between HindIII and SphI sites of pACYC184, unique BglII site immediately upstream of *rsd* start codon	This work
pU-T18	Contains *B. pertussis cya* gene T18 fragment, encodes Amp^r	18
pK-T25	Contains *B. pertussis cya* gene T25 fragment, encodes Kan^r	19

Transcriptomics. To prepare RNA from bacteria in stationary phase, a 0.2-ml aliquot of an overnight culture grown aerobically at 37°C in LB medium (pH 7.4) supplemented with antibiotics as appropriate was inoculated into 50 ml of fresh medium in a 300-ml flask and grown to stationary phase while shaking at 180 rpm at 37°C for 8 h. The culture was mixed with 2 volumes of RNAprotect (QIAGEN) and incubated for 5 min, and cells were collected by centrifugation and stored at −80°C. Total RNA was purified with a QIAGEN RNeasy mini-kit according to the manufacturer's instructions. For each experiment, 20 μg total RNA was used to produce Cy3- and Cy5-labeled cDNA with the Amersham Cyscribe Postlabeling kit. To analyze the RNA, an *E. coli* oligonucleotide library (Sigma-Genosys) was printed by a Lucidea microarray spotter on Corning UltraGAPS slides. Full details of the preparation of the arrays, prehybridization, hybridization, and washing procedures were described by Kershaw et al. (20). In our experiments, plus and minus Rsd samples were labeled with Cy5 and Cy3 dyes, respectively. After hybridization and washing, microarray slides were scanned with a Fuji FLA8000 scanner and data for 4,289 genes were collated in scatter plots. Probes for each gene were spotted in duplicate, and all experiments were performed twice; hence, four datum points were measured for each gene. Data were analyzed and are presented as MA plots (described in reference 12) that display the effects of Rsd as a function of signal strength for each transcript, in order to identify genes where the expression changes are most significant. Our cutoff criterion for Rsd-dependent up-regulation was an intensity value of >1 and a relative log ratio (with and without Rsd) of >0.4 for at least three datum points.

Promoter cloning and activity measurements. The *E. coli gadA*, *gadB*, and *ybaS* promoters were amplified by PCR from MG1655 genomic DNA with the primer pairs pgadAF and pgadAR, pgadBF and pgadBR, and pybaSF and pybasR. The PCR products were cleaved with EcoRI and HindIII, and the resulting fragments were purified and cloned between the EcoRI and HindIII sites of the broad-host-range *lac* expression vector pRW50 (Table 1) to create promoter-*lac* fusions. The control p*melR* EcoRI-HindIII fragment was as described by Webster et al. (26). To assay expression from the different promoters, host strains were transformed with pRW50 derivatives and β-galactosidase activities were measured with the Miller protocol (23). Cells were grown aerobically in LB medium containing 35 μg/ml tetracycline (to maintain pRW50 derivatives) and 35 μg/ml chloramphenicol (to maintain pACYC-Rsd and derivatives). β-Galactosidase activities are reported as nanomoles of *o*-nitrophenyl-β-D-galactopyranoside hydrolyzed per minute per milligram of dry cell mass. Each experiment was performed independently three times.

Multicopy plasmids encoding wild-type and mutant Rsd. Vector plasmids pACYC184 and pACYC-Rsd were a gift from Akira Ishihama (Table 1). For use as a control, a Cm^r Tet^s derivative of pACYC184, pACYC184ΔHN, was con-

TABLE 2. Primers used in this work

Primer use and name	Sequence
Creation and screening of *rsd*::*kan* allele	
RSD P1	5′-TGAGCAGTTTTTGAATACAAACTTGCGGAGTCAATCGTGTAGGCTGGAGCTGCTTC-3′
RSD P2	5′-GCATTGAATGTAAATTACGCGTTAACAGCGCAGAACCATATGAATATCCTCCTTAG-3′
RSD P1 screen	5′-GATCCATAGCTCTTGCACTACC-3′
RSD P2 screen	5′-GATTAACTCTTGTTCCCTTCGC-3′
Constructs for BACTH analysis	
RSD NT Xba	5′-GACTCTAGAGATGCTTAACCAGCTCG-3′
RSD CT Kpn	5′-CTCGGTACCCGAGCAGGATGTTTGACGC-3′
RSD CT Kpn-Stop	5′-CTCGGTACCCGTCAAGCAGGATGTTTGACGC-3′
RpoD NT Xba	5′-GACTCTAGAGATGGAGCAAAACCCGCAG-3′
RpoD CT Kpn-Stop	5′-TTAGGTACCCGTTAATCGTCCAGGAAGCT-3′
RIPF	5′-GCATCTGCTCGTGGCT-3′
RIPB	5′-AAAACCTGTTGAAACTC-3′
Cloning of promoters	
pgadAF	5′-GCAGAATTCAGCAATGTTTGGGCGATTTTTATTAC-3′
pgadAR	5′-GCAAGCTTTATTTGAAGGCAATAAAAAAGTAGG-3′
pgadBF	5′-GCAGAATTCGATAATTCAGGAGACACAGAATGC-3′
pgadBR	5′-GCAAGCTTATGATTGGATCGCATTAAAAAAGTAGG-3′
pybaSF	5′-CGCAGAATTCAGGGTCAGGTCGATAGTTTGTG-3′
pybaSR	5′-GCACAAGCTTCCACTGCCTGCTGTAATTTG-3′
Construction of pACYC RsdB and mutagenesis	
D49244	5′-CGATAAGCTTGCTGTTGTACTAACCAAACAGGTTC-3′
D49245	5′-CGATCGGATCCAGATCTGCTCAGTGAGAAATGTAAAAACCATG-3′
D49382	5′-GAGCAGATCTTGAATACAAACTTGCGGAGTCAATCATGC-3′
D49383	5′-TGGTGCATGCGTTAACAGCGCAGAACTCAAG-3′

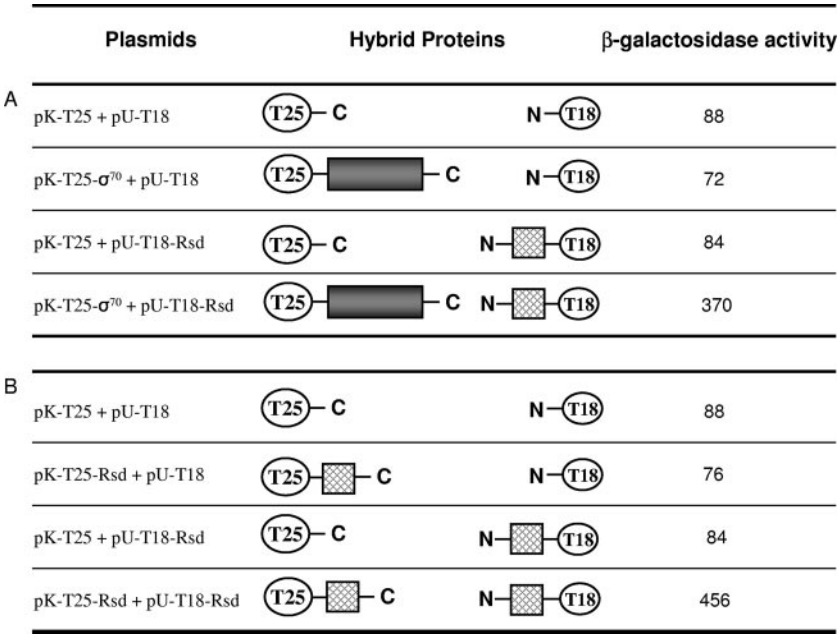

FIG. 1. BACTH experiments to investigate Rsd-σ^{70} and Rsd-Rsd interactions. Each line illustrates an experiment in which β-galactosidase expression was measured in BTH101 *cya* cells containing pK-T25 and pU-T18 and derivatives. The first column lists the plasmids present in each experiment, and the extreme right-hand column lists the measured β-galactosidase activity. The central part illustrates the different proteins encoded by the plasmids. The *B. pertussis* adenylyl cyclase T18 and T25 domains are illustrated as ovals. Rsd fused to T18 or T25 is illustrated by hatched squares with N and C indicating the N- and C-terminal ends of fusion proteins. Similarly, σ^{70} fused to T18 or T25 is illustrated by a dark-shaded rectangle. Panels A and B illustrate experiments designed to monitor Rsd-σ^{70} and Rsd-Rsd interactions, respectively. Data shown are the average of six independent experiments.

structed by removing DNA between the HindIII and NruI sites. pACYC-RsdB is a derivative of pACYC-Rsd carrying a shorter insert carrying *rsd* and its regulatory region, with a unique BglII site immediately upstream of the *rsd* translation initiation codon. To construct pACYC-RsdB, the *rsd* regulatory region was amplified by PCR with primers D49244 and D49245 (Table 2) and the product was digested with HindIII and BglII. Similarly, the *rsd* open reading frame (ORF) was amplified with primers D49382 and D49383 and the product was cleaved with BglII and SphI. The HindIII-BglII and BglII-SphI fragments covering the *rsd* regulatory region and the *rsd* ORF were purified and cloned between the HindIII and SphI sites of pACYC184 to give pACYC-RsdB. To make libraries of random mutations in *rsd*, error-prone PCR by the protocol described by Barne et al. (1) was used, with primers D49382 and D49383 and *Taq* DNA polymerase (Bioline) to amplify BglII-SphI fragments covering the *rsd* ORF. These fragments were then recloned into pACYC-RsdB, and the resulting plasmid libraries were screened for mutations that altered Rsd function. Western blotting, with rabbit anti-Rsd sera kindly provided by Akira Ishihama, was used to check levels of Rsd expressed from mutant pACYC-RsdB derivatives. The Western blot assay was calibrated with purified Rsd prepared according to Westblade et al. (27).

Nucleotide sequence accession number. Raw data from the transcriptome experiments described here (see Fig. 2) were deposited in the NCBI GEO database under accession number GSE5981.

RESULTS AND DISCUSSION

A two-hybrid system can be used to detect Rsd-σ^{70} and Rsd-Rsd interactions. Biochemical studies with purified proteins have shown that Rsd can form a 1:1 complex with σ^{70} or a 1:1 complex with itself to form a dimer (27, 28). To measure Rsd-σ^{70} and Rsd-Rsd interactions in vivo, we used the BACTH assay system, which relies on the fact that activity of *B. pertussis* adenylyl cyclase requires that two independently folding domains, T18 and T25, be brought together (18). Compatible plasmids pU-T18 and pK-T25 encode T18 and T25, respectively, and contain restriction sites to permit the cloning of DNA encoding Rsd or σ^{70} to give protein fusions to T18 and T25. Figure 1 illustrates the different fusions that were constructed. Combinations of pU-T18 and pK-T25 and their derivatives were introduced into *E. coli cya lac*$^+$ strain BTH101, and functional adenylyl cyclase activity, reconstituted from the T18 and T25 fragments, was measured by the induction of the *lac* operon (which is dependent upon cyclic AMP). Figure 1A lists the β-galactosidase activities measured in an experiment designed to monitor Rsd-σ^{70} interactions; activities above the background level were found only in cells carrying both an Rsd-T18 and a T25-σ^{70} fusion. Similarly, Fig. 1B lists the β-galactosidase activities measured in an experiment designed to monitor Rsd-Rsd interactions; activities above the background level were found only in cells carrying both an Rsd-T18 and a T25-Rsd fusion.

Attempts to study *rsd* function by transcriptomics. As a first step to studying the function of Rsd in vivo, a derivative of *E. coli* strain MG1655 in which the *rsd* gene was deleted and replaced with a kanamycin resistance cassette was made. Bacteriophage P1 transduction was then used to transfer the Δ*rsd*::*kan* allele into *E. coli* strain MC4100. The profiles of transcripts in MC4100 and the Δ*rsd*::*kan* mutant were then compared in cells growing exponentially and in stationary phase in LB medium. To do this, RNA was extracted from wild-type and mutant cells, differentially labeled, and analyzed with microarrays (see Materials and Methods). This analysis revealed no significant differences due to the *rsd* disruption (data not shown). The experiment was therefore repeated with

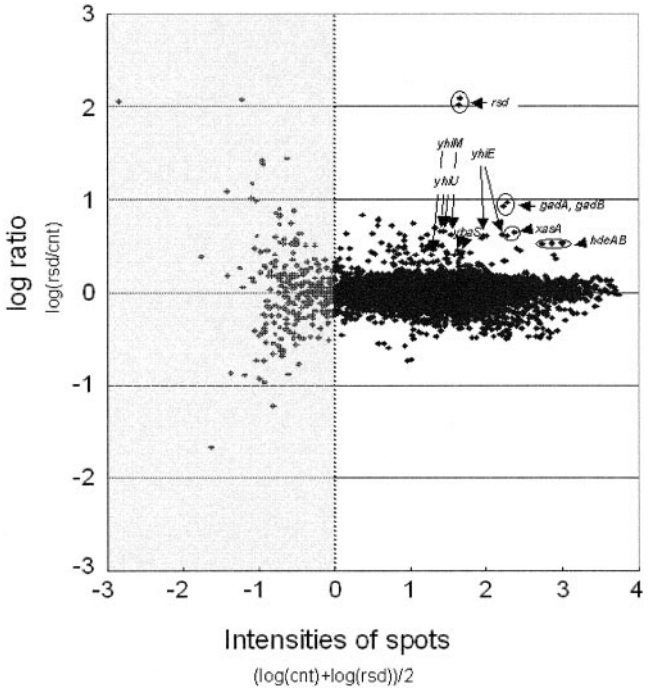

FIG. 2. Transcript analysis of the effects of Rsd. Shown is a graphic representation of an experiment done to compare the abundance of RNA corresponding to 4,289 probes in MC4100 Δ*rsd*::*kan* cells grown to stationary phase and carrying either pACYC-Rsd or the empty vector. Data are plotted vertically as the log of the relative ratio of the signal for each RNA in the presence (rsd) or absence (cnt) of overexpressed Rsd. The data are plotted horizontally as a function of the overall signal intensity (as in reference 12), thus permitting the identification of transcripts for which changes are significant. The subset of data where the signal intensity is insufficient for reliable analysis is shaded light gray. Fifteen genes were judged to be significantly up-regulated by Rsd (see text). Of these 15, 9 are known to be dependent on σ^{38}, and the corresponding datum points are identified and annotated.

the MC4100 Δ*rsd*::*kan* strain carrying pACYC-Rsd, a multicopy plasmid carrying the *rsd* gene and regulatory region, or a control empty plasmid. The results of this experiment, illustrated in Fig. 2, show that the expression of a small number of transcripts in stationary phase is increased 3- to 10-fold by the overexpression of Rsd. Fifteen genes satisfied our criteria for increased expression (see Materials and Methods), i.e., *ybaS*, *rmf*, *b0964*, *xasA*, *gadA*, *gadB*, *sufS*, *yhiM*, *hdeB*, *hdeA*, *yhiE*, *yhiU*, *yiiE*, *mopB*, and *pyrL*. These include nine genes whose expression is known to be controlled by σ^{38}, i.e., *ybaS*, *xasA*, *gadA*, *gadB*, *yhiM*, *hdeB*, *hdeA*, *yhiE*, and *yhiU*. Many of the products of these genes play key roles in ensuring the survival of *E. coli* in low-pH media (6, 25). These effects were observed in stationary phase but not in exponentially growing cells (data not shown).

Effects of Rsd measured by *lac* fusions. To confirm some of the effects of Rsd found by transcriptome analysis, PCR was used to amplify DNA fragments carrying the *gadA*, *gadB*, and *ybaS* promoters and the different fragments were cloned into pRW50, a broad-host-range promoter probe vector, to create promoter-*lac* fusions (Table 1). The resulting recombinant plasmids were transformed into the MC4100 Δ*rsd*::*kan* strain carrying pACYC-Rsd or pACYC184ΔHN, and β-galactosidase activities were measured throughout the growth of each strain in LB medium. The results, illustrated in Fig. 3A, show that Rsd has a clear stimulatory effect on the *gadA*, *gadB*, and *ybaS* promoters. As a control, the experiment was repeated with the *melR* promoter, and as expected, Rsd had little or no effect on expression of the p*melR*::*lacZ* fusion. Data from complementary experiments, illustrated in Fig. 3B, show that expression from the *gadA*, *gadB*, and *ybaS* promoters is substantially reduced by mutation of the *rpoS* gene, while expression from the *melR* promoter is unaffected.

Substitutions that affect the activity of Rsd at σ^{38}-dependent promoters. Two different mechanisms might account for the Rsd-dependent stimulation of expression from σ^{38}-dependent promoters. One possibility is that the sequestration of σ^{70} by Rsd permits σ^{38} to capture more RNAP. The second possibility is that the direct interaction of Rsd with RNAP favors the recognition of certain σ^{38}-dependent promoters. To distinguish between these possibilities, we searched for an Rsd mutant that was better able to stimulate expression from σ^{38}-dependent promoters. Such a mutant would show improved binding to σ^{70} if effects are due to σ^{70} sequestration. Alternatively, if Rsd functions via interaction with RNAP, it would be unlikely that the mutant has improved affinity for σ^{70}. To search for such mutations, we constructed a derivative of pACYC-Rsd carrying a BglII site immediately upstream of the *rsd* ORF and an SphI site immediately downstream. With this derivative, pACYC-RsdB, as a template, error-prone PCR was used to amplify the BglII-SphI fragment carrying the *rsd* gene. This fragment was then cloned back into pACYC-RsdB to give a library of plasmids carrying random base changes in the *rsd* gene. Three independent libraries were constructed, and these were transformed into the MC4100 Δ*rsd*::*kan* strain carrying pRW50 with the p*ybaS*::*lacZ* fusion and screened on MacConkey lactose indicator plates. Transformants carrying pACYC-RsdB and the control pACYC184ΔHN appear pink and white, respectively. After screening 1,000 colonies from each of the libraries, we identified 1 colony that exhibited a deeper pink Lac^+ phenotype. Further investigation showed that this phenotype was due to a mutation that created the LP18 substitution in Rsd encoded by pACYC-RsdB. After purification, the pACYC-RsdB derivative encoding LP18 was transformed into MC4100 Δ*rsd*::*kan* carrying the p*ybaS*::*lac*, p*gadA*::*lac*, or p*gadB*::*lac* fusion cloned in pRW50. Figure 4A illustrates the results of an experiment designed to quantify the effect of the LP18 substitution at the *ybaS*, *gadA*, and *gadB* promoters. These results show that the Rsd-dependent stimulation of expression from each promoter is enhanced by the LP18 substitution. Western blotting was used to check the levels of wild-type and LP18 mutant Rsd in this experiment. Data in Fig. 4B show that the level of Rsd is unaffected by the LP18 substitution.

To use the BACTH system to assess the effect of the LP18 substitution, a derivative of pU-T18 that encodes an LP18 Rsd-T18 fusion was constructed. Table 3 shows the results of a BACTH experiment designed to investigate the effects of the LP18 change on Rsd-σ^{70} and Rsd-Rsd interactions. The data show that the LP18 substitution increases the signal due to the Rsd-σ^{70} interaction while reducing the signal due to Rsd-Rsd contacts.

As a control for this experiment, we needed an Rsd mutant

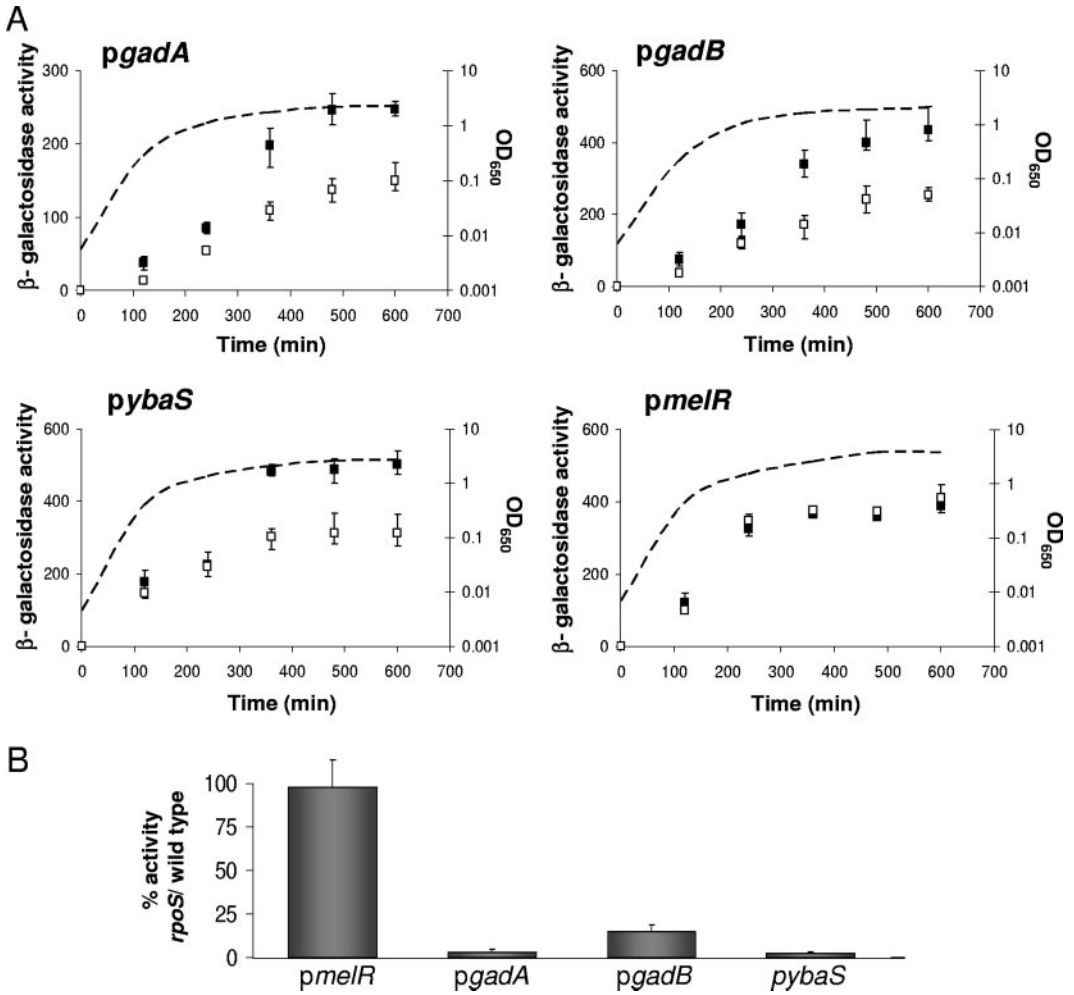

FIG. 3. Effects of Rsd on expression from different promoters. The four parts of panel A illustrate measurements of the expression of *gadA*::*lac*, *gadB*::*lac*, *ybaS*::*lac*, and *melR*::*lac* fusions during the growth of MC4100 Δ*rsd*::*kan* cells carrying either pACYC-Rsd (with Rsd; filled squares) or the empty vector pACYC184ΔHN (without Rsd; open squares). Activities were measured at different times (*x* axes), and the corresponding A_{650} values of cultures are indicated by the dashed lines. Panel B shows the effects of *rpoS* disruption on the activity of the *melR*::*lac*, *gadA*::*lac*, *gadB*::*lac*, and *ybaS*::*lac* fusions after overnight growth in MC4100 Δ*rsd*::*kan* cells carrying pACYC-Rsd. The histogram illustrates activities measured in the *rpoS*-359 mutant strain as a percentage of the activities in cells carrying wild-type *rpoS*. OD_{650}, optical density at 650 nm.

that was defective in the activation of expression from σ^{38}-dependent promoters. To find this, we screened for pACYC-RsdB derivatives that were unable to stimulate expression of the p*ybaS*::*lacZ* fusion carried by pRW50 in MC4100 Δ*rsd*::*kan* cells. This experiment yielded pACYC-RsdB derivatives carrying either the LP134 substitution or a nonsense triplet at codon 43. Western blotting showed that the LP134 substitution resulted in a 10-fold reduction in Rsd levels (data not shown), and hence neither mutant was studied further. Prompted by the high-resolution structure of the Rsd-σ^{70} domain 4 complex, which shows that D63 of Rsd makes a key contact with σ^{70} (G. A. Patikoglou, L. F. Westblade, E. A. Campbell, V. Lamour, W. J. Lane, and S. A. Darst, personal communication), a derivative of pACYC-RsdB encoding DA63 Rsd was constructed. Since Western blotting showed that the DA63 substitution does not alter Rsd levels (Fig. 4B), the effect of DA63 substitution on the *ybaS*, *gadA*, and *gadB* promoters was measured. Data in Fig. 4A show that Rsd carrying the DA63 substitution is defective in activation of expression from these promoters. Complementary BACTH assays (Table 3) confirmed that the DA63 substitution destroys the Rsd-σ^{70} interaction while hardly affecting Rsd-Rsd contacts.

Conclusions. At first sight, the function of the *E. coli* Rsd protein as an anti-σ^{70} factor appears simple. However, its low level relative to σ^{70}, the absence of a phenotype in *rsd* mutants, and its interaction with RNAP lacking σ^{70} suggest that it might play a more subtle role (13, 16). In our study, we were able to measure biological effects of Rsd only when it was overproduced. Under these conditions, the expression of some σ^{38}-dependent promoters was increased. Our observation that these effects were enhanced by a substitution in Rsd that also improves its interaction with σ^{70} argues that, here, Rsd is functioning by biasing the competition between σ^{70} and σ^{38} for available RNAP by sequestering σ^{70}. The high-resolution structure of the Rsd-σ^{70} domain 4 complex shows that Rsd residue L18 plays no direct role in the Rsd-σ^{70} domain 4

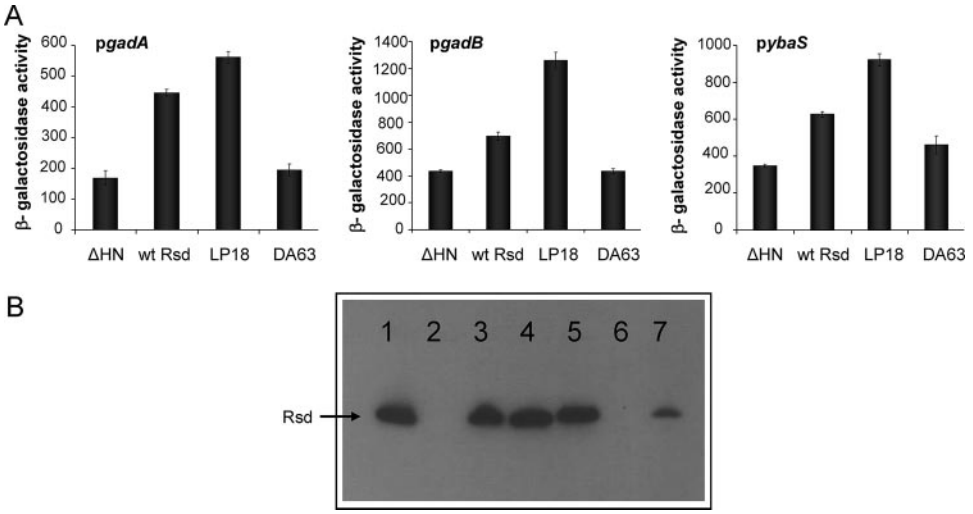

FIG. 4. Effects of Rsd substitutions on expression from different promoters. The bar charts in panel A illustrate measurements of expression of *gadA*::*lac*, *gadB*::*lac*, and *ybaS*::*lac* fusions during the growth of MC4100 Δ*rsd*::*kan* cells carrying either the empty vector pACYC184ΔHN; pACYC-RsdB, which encodes Rsd; or a derivative carrying the LP18 or DA63 substitution. β-Galactosidase activities were measured after overnight growth. Panel B illustrates a Western blot assay in which a sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel was probed with anti-Rsd antibodies. Purified Rsd (27) was loaded in lane 1. The other lanes contain extracts from stationary-phase cultures of MC4100 Δ*rsd*::*kan* pACYC184ΔHN (lane 2), MC4100 Δ*rsd*::*kan* pACYC-RsdB (lane 3), MC4100 Δ*rsd*::*kan* pACYC-RsdB LP18 (lane 4), MC4100 Δ*rsd*::*kan* pACYC-RsdB DA63 (lane 5), MC4100 Δ*rsd*::*kan* (lane 6), and wild-type MC4100 (lane 7).

interface (Patikoglou et al., personal communication). Thus, the effects of LP18 may be due to its destabilization of the Rsd dimer, thereby generating more monomer species to interact with σ^{70}, or due to an improvement with another domain of σ^{70}. Our results suggest that the role of Rsd may be to favor the expression of σ^{38}-dependent genes that are needed for *E. coli* to respond to lower-pH conditions (6, 25). However, it is unclear why Rsd-dependent activation is restricted to a subset of σ^{38}-dependent genes. We suggest that there may be some conditions under which the balance of levels of Rsd, σ^{70}, and σ^{38} is set such that the presence of Rsd makes an important difference.

TABLE 3. Effects of LP18 and DA63 substitutions in Rsd measured by BACTH[a]

Protein encoded by pU-T18	β-Galactosidase activity (Miller units)
In BTH101 cells carrying pK-T25 derivative encoding T25-σ^{70}	
T18	71
WT Rsd-T18	341
LP18 Rsd-T18	764
DA63 Rsd-T18	77
In BTH101 cells carrying pK-T25 derivative encoding T25-Rsd	
T18	75
WT Rsd-T18	370
LP18 Rsd-T18	78
DA63 Rsd-T18	319

[a] β-Galactosidase activities were measured in BTH101 *cya* cells carrying pK-T25 derivatives encoding a T25-σ^{70} or a T25-Rsd fusion protein and pU-T18 derivatives encoding T18 Rsd-T18 fusions. Cells were grown aerobically overnight at 30°C in LB medium supplemented with kanamycin and ampicillin. Data listed are averages of five to nine independent measurements that differed by no more than 20%. WT, wild type.

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