JOURNAL OF BACTERIOLOGY, Mar. 1997, p. 1787–1795 Vol. 179, No. 5
0021-9193/97/$04.00+0
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Mutations in the Alpha and Sigma-70 Subunits of RNA Polymerase Affect Expression of the *mer* Operon

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Received 10 October 1996/Accepted 17 December 1996

The mercury resistance (*mer*) operon is transcribed from overlapping, divergent promoters: P_R for the regulatory gene *merR* and P_{TPCAD} for the structural genes *merTPCAD*. The dyadic binding site for MerR lies within the 19-bp spacer of the σ^{70}-dependent P_{TPCAD}. Unlike typical repressors, MerR does not exclude RNA polymerase from P_{TPCAD} but rather forms an inactive complex with RNA polymerase at P_{TPCAD} prior to addition of the inducer, the mercuric ion Hg(II). In this "active repression" complex, MerR prevents transcriptional initiation at *merTPCAD* until Hg(II) is added. When Hg(II) is added, MerR remains bound to the same position and activates transcription of *merTPCAD* by distorting the DNA of the spacer region. MerR also represses its own transcription from P_R regardless of the presence or absence of Hg(II). To explore the role of MerR-RNA polymerase in these processes, we examined mutations in the σ^{70} and α subunits of RNA polymerase, mutations known to influence other activators but not to impair transcription generally. We assessed the effects of these σ^{70} and α mutants on unregulated P_{TPCAD} and P_R transcription (i.e., MerR-independent transcription) and on the two MerR-dependent processes: repression of P_{TPCAD} and of P_R and Hg(II)-induced activation of P_{TPCAD}. Among the MerR-independent effects, we found that mutations in regions 2.1 and 4.2 of *rpoD* suppress the deleterious effects of nonoptimal promoter spacing. Some C-terminal *rpoA* mutants also have this property to a considerably lesser degree. Certain "spacer suppressor" variants of *rpoA* and of *rpoD* also interfere with the MerR-dependent repression of P_{TPCAD} and P_R. MerR-Hg(II)-mediated transcriptional activation of P_{TPCAD} was also affected in an allele-specific manner by substitutions at position 596 of σ^{70} and at positions 311 and 323 of α. Thus, certain changes in σ^{70} or α render them either more or less effective in participating in the topologically novel transcriptional control effected by MerR at the divergent *mer* operons.

MerR, the regulatory protein of the bacterial mercury resistance operon, is unusual among prokaryotic activator proteins in that its dyadic binding site lies between the recognition elements of the σ^{70}-dependent *mer* structural gene promoter, P_{TPCAD} (34) (Fig. 1A). This promoter has −35 and −10 hexamers which closely approximate consensus, but it deviates from consensus in that there are 19 bp separating the σ^{70} recognition hexamers (26). Single and double base pair deletions in the interhexamer spacer result in stronger MerR-independent expression from P_{TPCAD} (22, 27).

MerR, when bound to the *mer* operator, bends the DNA (ca. 25° [2]) and represses transcription of the structural genes to a level 10-fold below that observed for a *merR* deletion strain. Nonetheless, in this repressed condition, MerR fosters the stable binding of RNA polymerase, which can be clearly seen to occupy the −35 region (but not the −10 region [1]) of the *merTPCAD* promoter, even before the inducer, Hg(II), is added (14). A similar phenomenon in which an inactive preinitiation complex is established has been observed with certain eukaryotic promoters (9, 11) and is referred to as "active repression" to distinguish it from "passive repression," in which the repressor simply occludes the binding site for the polymerase at the promoter. Paradoxically, MerR remains bound to the dyadic operator site (14) when it subsequently activates transcription of the structural genes (to a level as much as 100-fold above the repressed level [29]) in response to Hg(II). When added, Hg(II) provokes MerR to unwind the spacer DNA (ca. 55°) thereby allowing RNA polymerase to contact the −10 sequence and initiate transcription (1, 2, 25). The tightly overlapped divergent promoter P_R directs the synthesis of the *merR* transcript in a Hg(II)-independent fashion. The P_R promoter also has near-consensus −10 and −35 hexamers but differs from consensus in having a 15-bp spacer (26). When bound to the dyadic operator, MerR occludes the +1 position but not the −10 or −35 hexamers of P_R.

The close juxtaposition of the MerR binding site and the recognition elements for σ^{70} RNA polymerase suggested that there might be some physical contact(s) between these proteins which could be important both in repression and in Hg(II)-dependent activation of transcription. Indeed, three pairs of acidic residues clustered within 17 amino acids in the central region of MerR (D68 and E69, E77 and D78, and E83 and E84) resemble an acidic patch found on other activator proteins (6, 7, 15). Replacement of one pair of these residues with neutral residues (E83Q and E84Q) resulted in a MerR protein deficient in repression; however, neither the DNA binding affinity (K_d, $\sim 10^{-10}$M) nor the ability to activate transcription was altered (8). This suggests that these acidic residues might be important during repression rather than activation.

The *mer* operon's topologically novel regulatory mechanism makes unusual demands on RNA polymerase. During repression, the holoenzyme ($E\sigma^{70}$) engages in interactions with either

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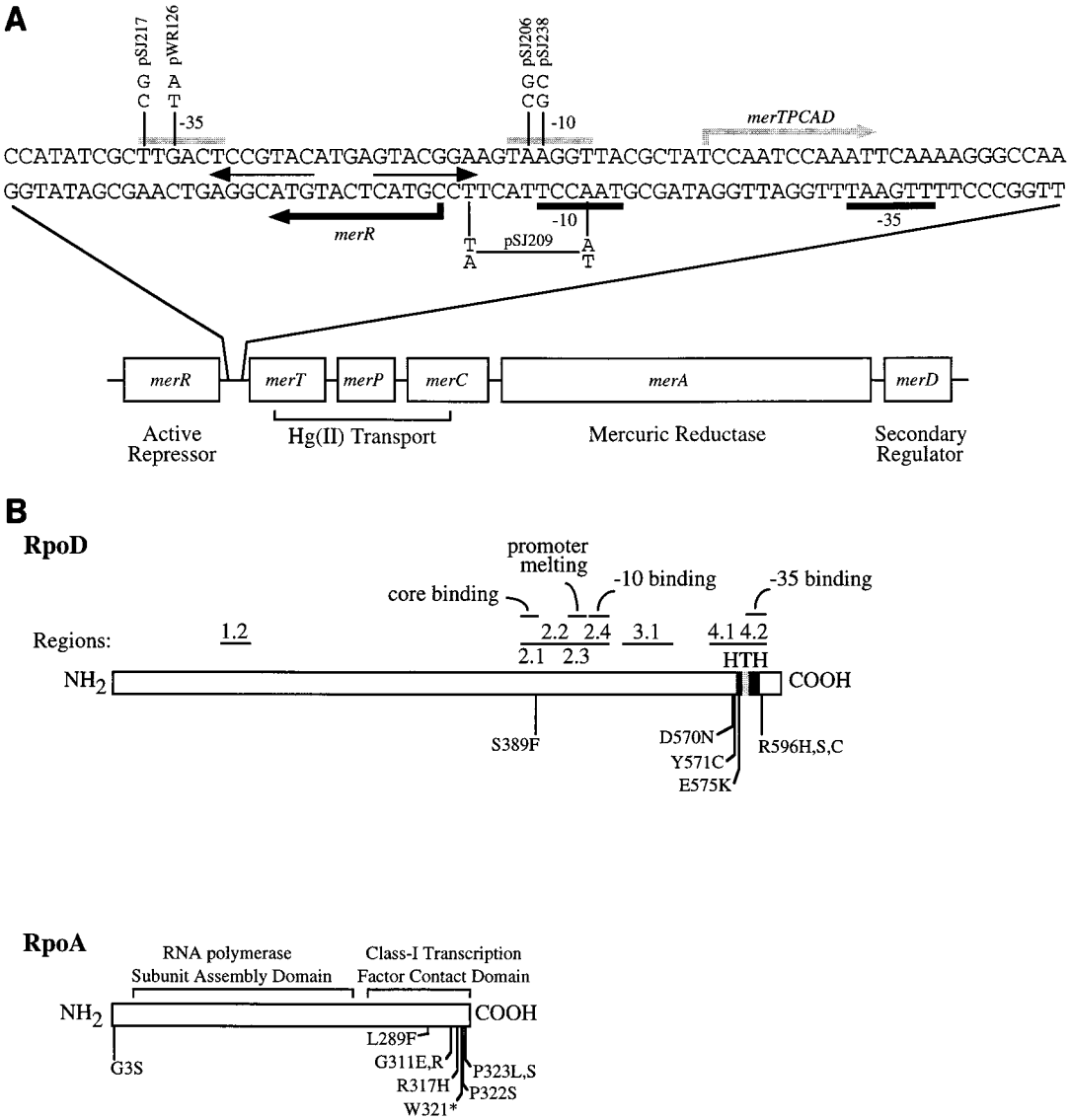

FIG. 1. (A) Sequence of wild-type Tn*21 merOP*. Previously isolated mutants (26, 29) used in this study are indicated. The solid arrows between the strands indicate the palindromic MerR binding region. The −10 and −35 recognition hexamers of the structural gene promoter (top strand) and the *merR* promoter (bottom strand) are indicated by bars whose shading corresponds to the relevant transcript (indicated by the large grey and black arrows). (B) Linear map of the RpoD and RpoA subunits of RNA polymerase. The locations of mutations used in these studies are indicated below each polypeptide; amino acids are shown in single-letter code. Mutations are designated by a letter representing the wild-type amino acid followed by the residue position in the protein and a letter representing the mutant amino acid. The four previously defined regions of RpoD (13) and the two previously defined regions of RpoA (18) are shown. HTH, helix-turn-helix.

MerR or DNA or both, which allow it to stably occupy an inactive promoter. Upon exposure to the inducer, Hg(II), MerR changes its own conformation and thereby effects a change in DNA conformation. RNA polymerase must be able to adjust its contacts accordingly (with either MerR or the DNA or both) in order to begin transcription. We expected that the regions of RNA polymerase engaged in such interactions at *mer* might in some cases, but not in others, be the same as those previously defined as important in other activation processes. The availability of mutations of the genes encoding the *Escherichia coli* σ^{70} (σ^{70}) (*rpoD*) (31, 32) and *E. coli* and *Salmonella typhimurium* α (*rpoA*) (21, 33) subunits of RNA polymerase (Fig. 1B) with well-defined specific effects on activator-dependent transcription in other systems provided us with an opportunity to assess the overlap between *mer*-related domains of RNA polymerase and domains figuring in other activation processes. Measurement of the MerR-independent (i.e., derepressed) expression and the MerR-dependent repression of P_{TPCAD} and P_R and of the MerR-dependent Hg(II)-induced activation of P_{TPCAD} provided an assessment of the ability of these RNA polymerase mutants to exploit the unusual aspects of transcriptional control in this system.

Because of the proximity of the MerR binding site to the σ^{70} binding positions at −10 and −35, we expected to see greater effects of mutations in σ^{70} than of mutations in α, which has a binding site more distant from MerR. Moreover, in vitro data obtained with truncated α proteins have indicated no role for the C terminus of α in MerR-mediated activation (data cited in reference 17). Surprisingly, we found allele-specific effects of both α and σ^{70} mutants on MerR-mediated processes. We also observed a new property (the ability to suppress the ill effects of nonideal spacers) in one group of σ^{70} mutants.

TABLE 1. Bacterial strains and plasmids

Strain or plasmid	Genotype or phenotype	Source	Reference
Strains			
E. coli			
CAG1574	*araD139* Δ(*ara leu*)*7697* Δ*lacX74 galU galK hsdR rpsL recA56 srl*	C. A. Gross	
CAG7365	*gal3* Δ*cya* Δ(*pro*, *lac*)$_{XIII}$ *zaj*::Tn*10 rpoD*$^+$ *zgh*::Tn*10* Kan, derived from MG1655	D. A. Siegele	32
CAG7366	*rpoD901*, derived from CAG7365 (S389F)	D. A. Siegele	32
CAG7367	*rpoD904*, derived from CAG7365 (Y571C)	D. A. Siegele	32
CAG7368	*rpoD911*, derived from CAG7365 (E575K)	D. A. Siegele	32
CAG7369	*rpoD912*, derived from CAG7365 (D570N)	D. A. Siegele	32
MS4274	Δ*lac*$_{chi}$*74 galOP308*::IS2 *rpsL rpoD*$^+$ *zgh*::Tn*10*, derived from RV308	M. M. Susskind	20
MS4276	*rpoD2*, derived from MS4274 (R596H)	M. M. Susskind	20
MS4278	*rpoD807*, derived from MS4274 (R596C)	M. M. Susskind	20
MS4280	*rpoD801*, derived from MS4274 (R596S)	M. M. Susskind	20
JMS4540	*araD*$^+$ Φ(*ompF′-lacZ*$^+$)*16-13 zhc-3*::Tn*10* (*rpoA*$^+$), derived from MC4100	T. J. Silhavy	33
JMS4542	*rpoA85*, derived from JMS4540 (P323L)	T. J. Silhavy	33
JMS4544	*rpoA52*, derived from JMS4540 (P323S)	T. J. Silhavy	33
JMS4545	*rpoA53*, derived from JMS4540 (G3S)	T. J. Silhavy	33
JMS4546	*rpoA54*, derived from JMS4540 (P322S)	T. J. Silhavy	33
S. typhimurium			
TN2262	*leuBCD485 pepT7*::MudJ (*rpoA*$^+$)	C. G. Miller	
TN2758	*leuBCD485 pepT7*::MudJ *zhb-1624*::Tn*10*Δ*rpoA8* (*oxrB8*) (G311R)	C. G. Miller	21
TN3567	*rpoA151*, derived from TN2262 (G311E)	C. G. Miller	21
TN3569	*rpoA155*, derived from TN2262 (L289F)	C. G. Miller	21
TN3570	*rpoA153*, derived from TN2262 (R317H)	C. G. Miller	21
TN3571	*rpoA154*, derived from TN2262 (W321*)	C. G. Miller	21
Plasmids			
pACYC177 AmpΔ	Kan^r p15A replicon	W. Ross	29
pACYC184	Tet^r Cml^r p15A replicon		
pNH9	*merR*$^+$ *merOP*$^+$; Kan^r p15A replicon	N. Hamlett	12
pSA1	*merR*$^+$ *merOP*$^+$; Cml^r p15A replicon		This study
pSJ43	*phoA-R*(Δ*10*)-*T′-lacZ*$^+$; Amp^r pMB1 replicon	S.-J. Park	26
pSJ206, pSJ209, pSJ238	Derivative of pSJ43 with single base pair substitutions in the −35 and −10 hexamers	S.-J. Park	26
pSJ51	*merR*$^+$ *merOP*$^+$ *merT′-lacZ*$^+$; Amp^r pMB1 replicon; *Hin*dIII linker replaces *Eco*RI at nucleotide 26 of pWR2	S.-J. Park	26
pSJ217	Derivative of pSJ51 with a single base pair substitution in the −35 hexamer	S.-J. Park	26
pWR2	*merR*$^+$ *merOP*$^+$ *merT′-lacZ*$^+$; Amp^r pMB1 replicon	W. Ross	29
pWR10	*merOP*$^+$ *merT′-lacZ*$^+$; Amp^r pMB1 replicon	W. Ross	29
pWR126	Derivative of pWR2 with a single base pair substitution in the −35 hexamer	W. Ross	29

MATERIALS AND METHODS

Bacterial strains and plasmids. All plasmids used or constructed and the *E. coli* and *S. typhimurium* strains used in this work are listed in Table 1. The growth rates of isogenic strains with mutations in the σ^{70} and α subunits were similar to those of their respective parental strains. Therefore, the effects on *mer* expression are not due to any general changes in growth rate caused by these *rpo* mutants.

The PhoA-LacZ reporter construct pSJ43 has lower absolute expression of P_{TPCAD} than does pWR2 (26, 29). However, since the induction ratios of these two reporter plasmids are similar, we infer that *merT′-lacZ* expression in pWR2 and *merT′-lacZ* and *merR′-phoA* expression in pSJ43 and its mutant derivatives reflect the natural expression of the *mer* operon to the same degree.

The levels of activated expression of *mer* in all of the wild-type parental strains used here average fivefold higher than the derepressed levels, rather than the 10-fold increase seen with our benchmark strain CAG1574 (26, 29). Since each of these parental *rpoD*$^+$ strains differs from CAG1574 at several loci (Table 1), the basis for their differences in activated *mer* expression is not obvious. For this reason each series of mutants is always compared to its otherwise isogenic parental strain.

General methods and media. Plasmid DNA was prepared by the alkaline lysis method described by Birnboim and Doly (4). Restriction enzymes and T4 DNA ligase were from Promega Corporation (Madison, Wis.) and New England BioLabs (Beverly, Mass.), respectively, and were used according to the specifications of the manufacturer. Competent cells for electroporation were prepared according to the method described by the manufacturer (Bio-Rad, Inc.). Ampicillin (50 μg ml^{-1}), kanamycin (50 μg ml^{-1}), and chloramphenicol (25 μg ml^{-1}) were added, where appropriate, for the selection and maintenance of plasmids. Luria-Bertani (LB) medium and agar plates were prepared as described by Miller (24).

Plasmid construction and plasmid load. pSA1 was constructed by digesting pWR2 (29) with *Eco*RI and pACYC184 with *Eag*I and by filling in the 5′ overhanging ends with the Klenow fragment of DNA polymerase. The linearized plasmids (pWR2 and pACYC184) were then digested with *Bam*HI. After gel purification, the 670-bp *Eco*RI(blunt)-*Bam*HI fragment of pWR2 was ligated to the 3.68-kbp *Eag*I(blunt)-*Bam*HI fragment from pACYC184. pSA1 contains Tn*21 merR*, *merOP*, and 29 codons of *merT* (*mer* nucleotides 26 to 693 [3]).

β-Galactosidase and alkaline phosphatase assays with pSJ43 or one of its mutant derivatives as the reporter plasmid were done with MerR supplied in *trans*. In the derepressed condition (i.e., no MerR) a second plasmid was also present to equalize the number of plasmids present in each strain. The plasmid copy number of each strain was determined by alkaline lysis extraction of plasmid DNA from 1.5 ml of cells removed from the Klett flask just prior to Hg(II) induction. A photographic negative of the ethidium bromide-stained agarose gel of linearized plasmid DNA was scanned on a Molecular Dynamics computing densitometer, model 300A. The copy number of the reporter plasmid in each strain relative to that in the strain carrying wild-type σ^{70} and α was determined densitometrically. This number was used to correct the reporter activity in each of the mutant strains to that of the strain carrying wild-type σ^{70} and α.

β-Galactosidase assay. Overnight cultures in LB broth were diluted 1/20 with fresh LB broth with the appropriate antibiotic. The cells were grown an additional 50 Klett units and aliquoted into separate flasks for induction with Hg(II) (1 μM for 20 min). The cultures then were chilled rapidly on ice to prevent

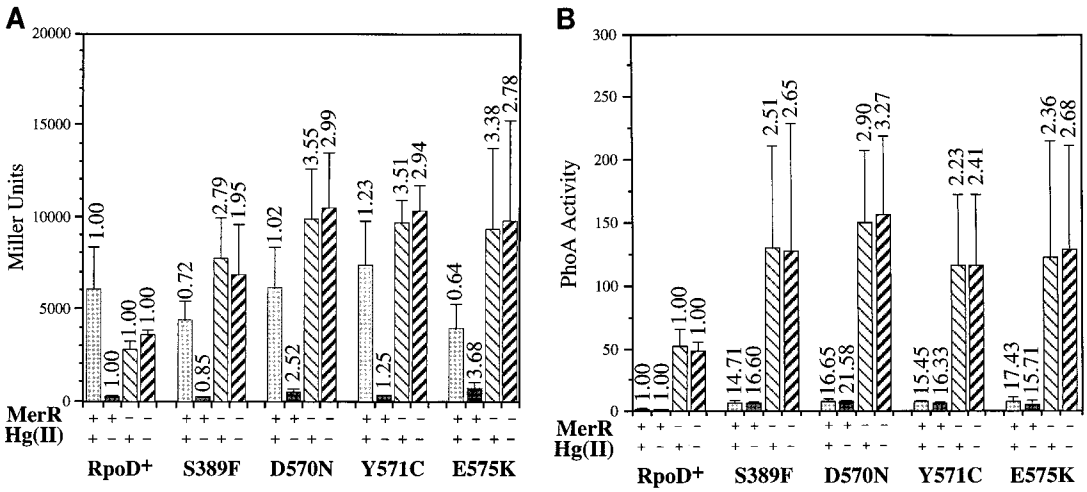

FIG. 2. (A) LacZ activities from the *merT′-lacZ* fusion (P_{TPCAD}) in isogenic strains containing mutant *rpoD* alleles. The reporter constructs were pWR2 (with MerR) and pWR10 (without MerR). Only the *rpoD* mutation is shown for clarity. Activity of β-galactosidase was determined as described in Materials and Methods. Each bar represents the average β-galactosidase activity in two independent assays. (B) PhoA activities from the *merR′-phoA* fusion (P_R) in isogenic strains containing mutant *rpoD* alleles. Each strain contained the reporter construct pSJ43 with either pSA1 (supplying MerR in *trans*) or pACYC184 (MerR$^-$ control for nonspecific plasmid burden on growth). Only the *rpoD* mutation is shown for clarity. Activity of alkaline phosphatase was determined as described in Materials and Methods. Each bar represents the average alkaline phosphatase activity in two independent assays. Numbers shown above each bar are the fold differences between the mutant strains and the parental strain under each condition tested. The standard deviations are shown.

further induction and/or growth, washed with minimal medium basal salts (24), and then assayed for β-galactosidase activity (24).

Since the *E. coli* strains designated JMS (33) have a chromosomal *lacZ* gene, the average background β-galactosidase activity for each strain was subtracted from MerR-Hg(II)-induced, MerR-repressed, and MerR-independent activities.

Alkaline phosphatase assay. Cells were prepared as for the β-galactosidase assay except that after the induction period, the cells were washed with 1 mM Tris-HCl, pH 8.0. The washed cells were resuspended in 0.7 ml of 1 mM Tris-HCl, pH 8.0. After lysis of the cells with a solution containing 40 μl of chloroform per ml and 0.002% sodium dodecyl sulfate, the reactions were started by addition of 50 μl of 0.4% Sigma 104 phosphatase substrate (Sigma Chemical Company), and the reaction mixtures were incubated at 37°C. To stop the reactions, 100 μl of KH_2PO_4 (1 M, pH 8.0) was added. Alkaline phosphatase activity was determined as described by Schneider and Beck (30). The alkaline phosphatase activities of plasmid-free strains were determined to be negligible (<0.001 U).

RESULTS AND DISCUSSION

The response of *mer* expression to mutations in *rpoD*. (i) Alterations in regions 2.1 and 4.2 of *E. coli* σ^{70} suppress nonoptimal spacers in P_{TPCAD} and P_R. The amino acid substitutions D570N, Y571C, and E575K are encoded in σ^{70} region 4.2, while the mutation yielding S389F lies in region 2.1. In the absence of MerR, all four of these Eσ^{70} mutants increased expression from P_{TPCAD} as much as 3.5-fold compared to wild-type Eσ^{70} (Fig. 2A) in a Hg(II)-independent manner. Moreover, D570N and E575K also overcame MerR repression (2.5- and 3.7-fold increases respectively, compared to Eσ^{70}). Surprisingly, in the activated condition [with both MerR and Hg(II)], these region 2.1 and 4.2 Eσ^{70} mutants did not significantly alter P_{TPCAD} expression compared to that of their parental strain, CAG7365.

With the divergent P_R promoter, holoenzymes containing these σ^{70} mutants similarly effected a two- to threefold increase in expression compared to Eσ^{70} when MerR was absent (Fig. 2B), and they also overcame MerR repression of its own promoter 15- to 20-fold, although the absolute activities were low (<8 U). As seen with the wild-type holoenzyme, neither repressed nor derepressed expression of P_R was affected by Hg(II).

(ii) The region 2.1 and 4.2 σ^{70} mutants still use canonical contacts in P_{TPCAD} and P_R. There are two primary possibilities for the enhanced expression by these mutants under derepressed conditions of both P_{TPCAD} and P_R: these σ^{70} proteins may (i) be utilizing alternate recognition elements or (ii) be less sensitive to the long interhexamer spacer. To distinguish between these two alternatives, we analyzed expression by Eσ^{70}-S389F and Eσ^{70}-E575K from promoters with nucleotide substitutions in the −35 (pSJ217 and pWR126) (Fig. 3) or the

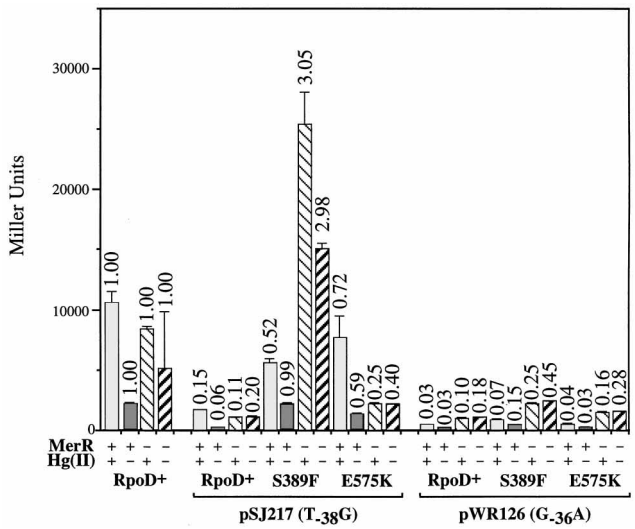

FIG. 3. LacZ activities from the *merT′-lacZ* fusion (P_{TPCAD}) in isogenic strains containing mutant *rpoD* alleles and nucleotide substitutions in the −35 recognition hexamer. The reporter constructs were pSJ217 (with MerR), pSJ217ΔR (without MerR), pWR126 (with MerR), and pWR126ΔR (without MerR). The wild-type promoter constructs are pWR2 (with MerR) and pWR10 (without MerR). Only the *rpoD* mutation is shown for clarity. Activity of β-galactosidase was determined as described in Materials and Methods. Each bar represents the average β-galactosidase activity. β-Galactosidase assays were performed on three days, and relative values were consistent. Numbers shown above each bar are the fold differences between the mutant strains and the parental strain under each condition tested. The standard deviations are shown.

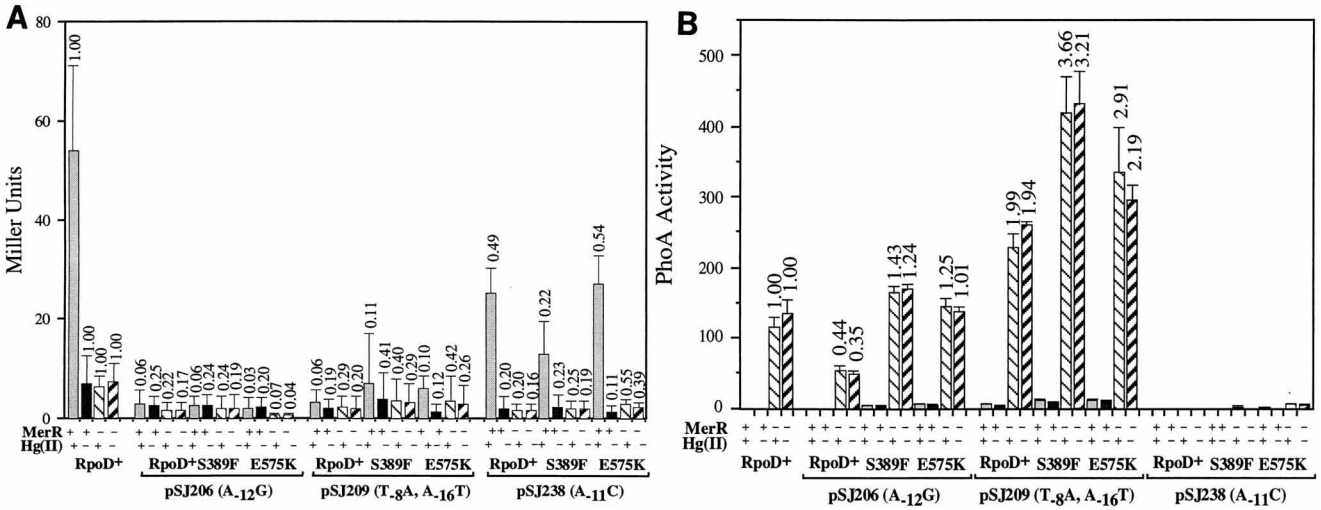

FIG. 4. (A) LacZ activities from the *merT′-lacZ* fusion (P_{TPCAD}) in isogenic strains containing mutant *rpoD* alleles and nucleotide substitutions in the −10 recognition hexamer. Each strain contained the reporter construct pSJ206, pSJ209, or pSJ238 with either pSA1 (supplying MerR in *trans*) or pACYC184 (MerR$^-$ control for nonspecific plasmid burden on growth). The wild-type promoter construct is pSJ43. Only the *rpoD* mutation is shown for clarity. Activity of β-galactosidase was determined as described in Materials and Methods. Each bar represents the average β-galactosidase activity in three independent assays. (B) PhoA activities from the *merR′-phoA* fusion (P_R) in isogenic strains containing mutant *rpoD* alleles and nucleotide substitutions in the −10 recognition hexamer. Each strain contained the reporter construct pSJ206, pSJ209, or pSJ238 with either pSA1 (supplying MerR in *trans*) or pACYC184 (MerR$^-$ control for nonspecific plasmid burden on growth). The wild-type promoter construct is pSJ43. Only the *rpoD* mutation is shown for clarity. Activity of alkaline phosphatase was determined as described in Materials and Methods. Each bar represents the average alkaline phosphatase activity. Alkaline phosphatase assays were performed on two days, and relative values were consistent. Numbers shown above each bar are the fold differences between the mutant strains and the parental strain under each condition tested. The standard deviations are shown.

−10 (pSJ206, pSJ209, and pSJ238) (Fig. 4A) recognition hexamers of P_{TPCAD}.

In the absence of MerR, Eσ^{70}-S389F expressed the −35 hexamer mutants at levels 27-fold (pSJ217) and 2-fold (pWR126) higher than those obtained with the wild-type Eσ^{70} (Fig. 3). Indeed, Eσ^{70}-S389F transcribes from pSJ217 (−35 hexamer GTGACT [mutant nucleotide underlined]) as well as it does from the wild-type promoter (TTGACT) (Fig. 2A). However, Eσ^{70}-E575K is severely inhibited in its MerR-independent transcription by changes at either of these positions in the −35 hexamer of P_{TPCAD}.

Nucleotide substitutions in two highly conserved positions of the −10 hexamer (pSJ206, TGAGGT, and pSJ209, TAAG GA) caused Eσ^{70} as well as Eσ^{70}-S389F and Eσ^{70}-E575K to lose the ability to transcribe from the derepressed mutant promoter (Fig. 4A). Moreover, the substitution in pSJ238, which changes the less highly conserved nucleotide at position −11 (to TACGGT), also abolishes MerR-independent expression from P_{TPCAD} for wild-type Eσ^{70} and both mutant holoenzymes, but all holoenzymes retain some activated expression.

Since the −10 hexamers of P_{TPCAD} and P_R overlap (Fig. 1A), this same set of −10 hexamer mutations can be used to assess the requirements of these σ^{70} mutant holoenzymes for canonical contacts in the P_R promoter with its short (15-bp) spacer. Two of the promoter mutations, one in pSJ209 and one in pSJ238, are located in the −10 hexamer of P_R, while the third, in pSJ206, lies immediately downstream of the −10 hexamer of P_R. The alteration in pSJ206 had no effect on the elevated expression seen with Eσ^{70}-S389F and Eσ^{70}-E575K (Fig. 4B); however, the wild-type holoenzyme exhibited twofold-lower expression from this mutant promoter. Wild-type Eσ^{70} expression from pSJ209, which carries a −10 hexamer more similar to consensus (TATCCT), was twofold higher than that from the wild-type promoter, and Eσ^{70}-S389F and Eσ^{70}-E575K further increased expression from this mutant promoter as much as 1.8-fold. The substitution in pSJ238 (TAA CCG), which changes one of the most highly conserved nucleotides in the −10 hexamer, abolishes expression from P_R with wild-type Eσ^{70} as well as Eσ^{70}-S389F and Eσ^{70}-E575K.

These mutants with point mutations in regions 2.1 and 4.2 of σ^{70} were originally isolated as allowing high-level expression of *lac* in an activator-independent manner in a Δ*cya* strain of *E. coli* (31). They are also able to transcribe P_{lac} with many different mutations in the −10 or −35 hexamers but are not specific to a particular position of the promoter (31, 32), suggesting that these changes affect DNA sequence-independent interactions. Recently, in vivo examination of some of these mutants has established that σ^{70} is also important as a "ruler" in sensing the dimensions of the spacer between the −10 and −35 recognition hexamers (10).

In the absence of MerR, holoenzymes containing each of these σ^{70} mutants vigorously transcribed both *mer* promoters. At least some of the consensus promoter contacts are essential for this enhanced activity, since Eσ^{70}-S389F and Eσ^{70}-E575K are unable to suppress both a nonoptimal spacer and certain nonconsensus hexamers of P_{TPCAD} or of P_R (either −35 [Fig. 3] or −10 [Fig. 4]). The sole exception on this point was the ability of Eσ^{70}-S389F to transcribe P_{TPCAD} with the −35 hexamer mutation GTGACT. Amino acid 389 of σ^{70} lies in region 2.1, which is believed to contact the core RNA polymerase (19). Enhanced transcription by Eσ^{70}-S389F from a promoter with a nonconsensus −35 hexamer was also seen with P_{lac} (32). Without cyclic AMP receptor protein, Eσ^{70}-S389F expression from P_{lac} with a T-to-G transversion at −36 (analogous to the change in pSJ217) was similar to that from wild-type P_{lac}. Thus, σ^{70} region 2.1 alterations may be less sensitive to changes near the 5′ end of the −35 hexamer than to changes at other positions.

The vigorous transcription effected by these mutant holoenzymes was not entirely repressible by MerR at either P_{TPCAD}

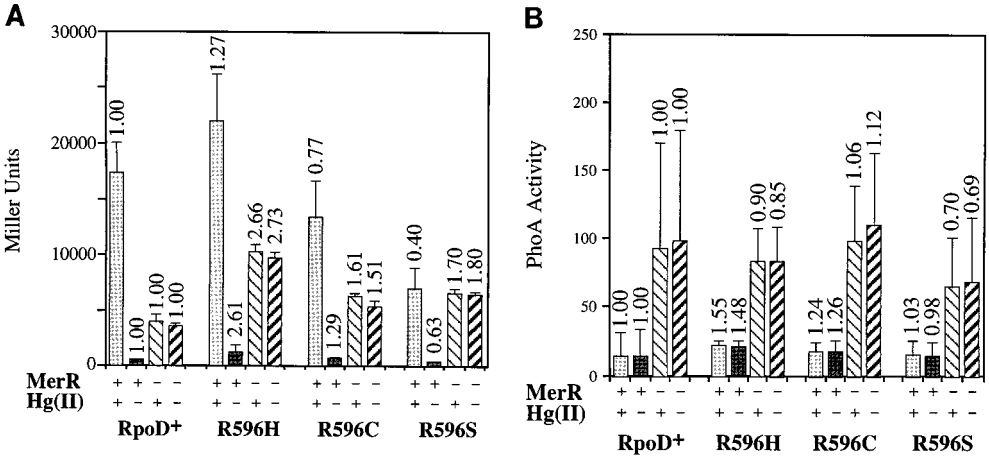

FIG. 5. (A) LacZ activities from the *merT'-lacZ* fusion (P_{TPCAD}) in isogenic strains containing mutant *rpoD* alleles. The reporter constructs were pWR2 (with MerR) and pWR10 (without MerR). Only the *rpoD* mutation is shown for clarity. Activity of β-galactosidase was determined as described in Materials and Methods. Each bar represents the average β-galactosidase activity. β-Galactosidase assays were performed on three days, and relative values were consistent. (B) PhoA activities from the *merR'-phoA* fusion (P_R) in isogenic strains containing mutant *rpoD* alleles. Each strain contained the reporter construct pSJ43 with either pNH9 (supplying MerR in *trans*) or pACYC177 AmpΔ (MerR$^-$ control for nonspecific plasmid burden on growth). Only the *rpoD* mutation is shown for clarity. Activity of alkaline phosphatase was determined as described in Materials and Methods. Each bar is the average alkaline phosphatase activity in three independent assays. Numbers shown above each bar are the fold differences between the mutant strains and the parental strain under each condition tested. The standard deviations are shown.

or P_R. This impairment of repression suggests that either directly (via overlapping binding sites or protein-protein contacts) or indirectly (via modulation of DNA flexibility or MerR-DNA contacts) these RNA polymerases are interfering with the action of MerR at P_{TPCAD} and P_R. In the presence of MerR-Hg(II), Eσ^{70}-S389F and Eσ^{70}-E575K can transcribe from all of these mutant promoters nearly as well as or better than wild-type Eσ^{70}. This suggests that when MerR-Hg(II) underwinds the spacer DNA, thereby compensating for the interhexamer spacing defect, Eσ^{70}-S389F and Eσ^{70}-E575K can tolerate nucleotide changes at these positions in the −35 and −10 recognition hexamers.

Data presented above show that these σ^{70} variants still require most of the canonical promoter contacts, and Dombroski et al. (10) have shown that they use the same start site with P_{tac} regardless of spacer length. However, none of these data rule out the possibility that these variants also require additional contacts in the promoter for their enhanced activity, some of which might be occluded when MerR is present. An alternative explanation for the behavior of these *rpoD* variants is that they can reconfigure either the DNA or MerR or both into an "activation-like" conformation in the absence of Hg(II) but are no more effective at forming an open complex and clearing the promoter than the wild-type polymerase in the presence of MerR-Hg(II).

(iii) Effects of alterations in the extreme carboxyl-terminal portion of *E. coli* σ^{70} on P_{TPCAD} and P_R. The amino acid of the σ^{70} product identified as contacting λcI and AraC is R596 in the carboxyl terminus (16, 20). In MerR-independent activity, holoenzymes containing any of three changes at this position (Eσ^{70}-R596H, Eσ^{70}-R596C, and Eσ^{70}-R596S) increased P_{TPCAD} expression (up to 2.7-fold, Eσ^{70}-R596H) (Fig. 5A) regardless of the presence of Hg(II). However, they show no increase above wild-type σ^{70} expression in their expression of the derepressed P_R (Fig. 5B).

For the two MerR-dependent functions, these C-terminal σ^{70} mutants displayed distinctly different behaviors. In active repression, Eσ^{70}-R596S did not compromise MerR function but Eσ^{70}-R596H overcame repression (2.6-fold). In Hg(II)-dependent activation, Eσ^{70}-R596S had only 40% of the MerR-Hg(II)-dependent P_{TPCAD} expression that wild-type Eσ^{70} had, whereas Eσ^{70}-R596H was slightly better at MerR-Hg(II)-dependent expression than wild-type Eσ^{70}. Finally, unlike the other two mutants, the R596C mutant was indistinguishable from wild-type Eσ^{70} in both MerR-dependent functions.

Thus, alterations in the extreme C-terminal position 596 of σ^{70} show allele-specific effects on both MerR-mediated activation and repression. While all three changes at σ^{70}-R596 yield mutants that are better than the wild type at derepressed expression of the 19-bp spacer of P_{TPCAD}, they show no improvement for the 15-bp spacer of P_R; thus, they are not general spacer suppressors. However, substitution with histidine results in slightly better MerR-Hg(II)-dependent activation and in overcoming of repression at both P_{TPCAD} and P_R; substitution with cysteine or serine impairs activation and has little or no effect on repression at either promoter. Thus, MerR-related processes may have requirements for contacts at R596 of σ^{70} which are distinct from the requirements of MerR-independent processes. Since the region in which residue 596 lies is only known to interact with other activator proteins and not with DNA, it is less likely that the effects of these mutants on MerR-related processes arise from simple competition for promoter binding. Alternatively, the R596H holoenzyme may interact very effectively with the MerR-DNA complex, perhaps even fostering an activation-like configuration before Hg(II) is added. In contrast, the R596C or R596S holoenzyme may interact less effectively with the activated configuration of the MerR-Hg(II)-DNA complex.

In summary, there are very dramatic effects of holoenzymes containing σ^{70} mutations in regions 2.1 and 4.2 on MerR-independent expression from P_{TPCAD} and P_R. While retaining the requirement for canonical contacts in the −10 and −35 recognition hexamers, holoenzymes containing these σ^{70} mutants behave as spacer suppressors capable of enhanced expression at interhexamer spacers of either 19 bp (P_{TPCAD}) or 15 bp (P_R). Some of these holoenzymes also interfere with MerR-mediated active repression at both promoters. In contrast, mutations in the extreme C terminus of σ^{70} enhance the derepressed expression from promoters with a long spacer but not from promoters with a short spacer. The latter mutants

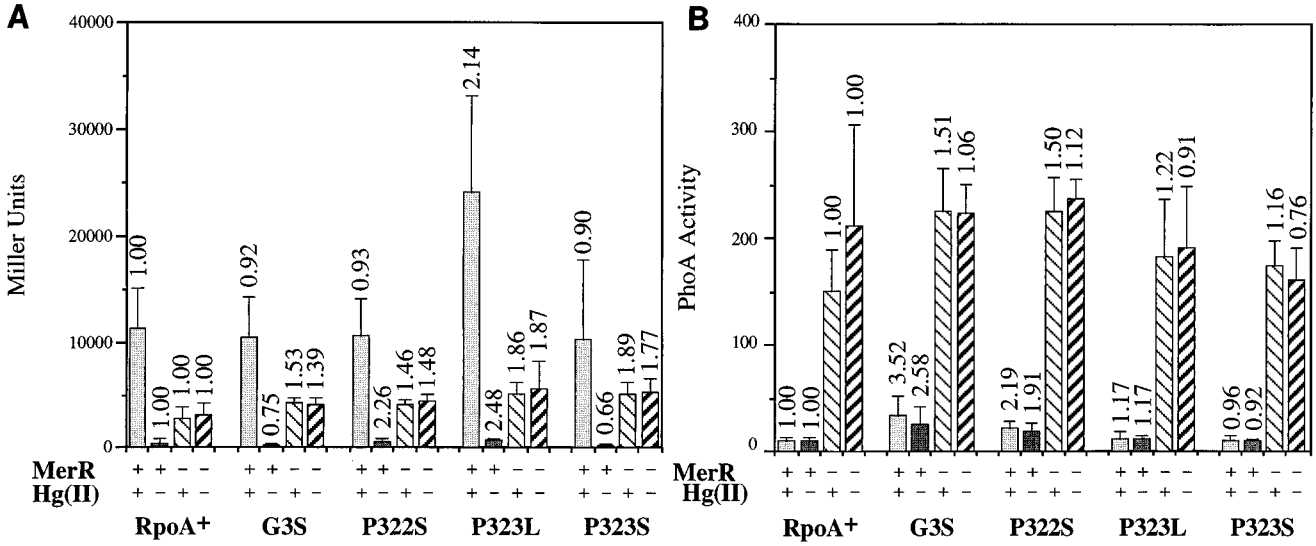

FIG. 6. (A) LacZ activities from the *merT′-lacZ* fusion (P_{TPCAD}) in isogenic strains containing mutant *rpoA* alleles. The reporter constructs were pWR2 (with MerR) and pWR10 (without MerR). Only the *rpoA* mutation is shown for clarity. Activity of β-galactosidase was determined as described in Materials and Methods. Each bar represents the average β-galactosidase activity in six independent assays. (B) PhoA activities from the *merR′-phoA* fusion (P_R) in isogenic strains containing mutant *rpoA* alleles. Each strain contained the reporter construct pSJ43 with either pNH9 (supplying MerR in *trans*) or pACYC177 AmpΔ (Mer^- control for nonspecific plasmid burden on growth). Only the *rpoA* mutation is shown for clarity. Activity of alkaline phosphatase was determined as described in Materials and Methods. Each bar represents the average alkaline phosphatase activity in two independent assays. Numbers shown above each bar are the fold differences between the mutant strains and the parental strain under each condition tested. The standard deviations are shown.

also have allele-specific effects on MerR-Hg(II)-mediated repression and activation at P_{TPCAD}.

Response of *mer* expression to mutations in *rpoA*. Effects of alterations in *E. coli* and *S. typhimurium* α on P_{TPCAD} and P_R. The C terminus of the α subunit of RNA polymerase interacts with DNA in the upstream regions of certain promoters (5) and with activators binding on the 5′ side of the −35 hexamer (reviewed in reference 17). Because MerR binds on the 3′ side of the −35 hexamer, we expected that alterations in α would not affect either of the MerR-dependent processes of active repression or transcriptional activation of *mer*. However, allele-specific effects on MerR-dependent functions were found at two amino acids in the C terminus of the α subunit.

Such effects were observed in a set of four α mutants originally isolated in *E. coli* as defective in OmpR-regulated transcription of *ompF′-lacZ* (33). All of these mutant holoenzymes have slightly increased activity in the derepressed condition at both P_{TPCAD} and P_R (Fig. 6). However, in MerR-dependent processes, the holoenzyme containing a leucine substitution (α-P323L) exhibits twofold higher activation and is also 2.5-fold more active in the repressed condition than is the wild-type holoenzyme (Fig. 6A). In contrast, a serine substitution at the same position (α-P323S) exhibits near wild-type levels of activity under both MerR-dependent conditions. A serine substitution at the adjacent position (α-P322S) interferes with MerR repression of P_{TPCAD} but has little effect on Hg(II)-induced activation. This latter mutant (α-P322S) also interferes with MerR repression at P_R as does α-G3S (Fig. 6B).

A second group of α mutants were originally isolated in *S. typhimurium* based on their diminished expression of OxrA-dependent genes during anaerobic growth (21). Holoenzymes containing these point mutations in the α subunit (α-L289F, α-G311E, α-G311R, α-R317H, and α-W321*; the asterisk refers to a nonsense codon) were significantly inhibited in expression from P_{TPCAD} under both MerR-repressed and -derepressed conditions (Fig. 7A). In the activated condition, only the holoenzyme containing α-G311R transcribes P_{TPCAD} at levels similar to those obtained with wild-type $E\sigma^{70}$, while a holoenzyme with a different substitution at the same position (α-G311E) is only half as active at P_{TPCAD}. At P_R this group of α mutants were all impaired in derepressed activity, variously allowing only 30 to 50% of wild-type activity, and none significantly overcame MerR-mediated repression of P_R (Fig. 7B).

Prior to the work reported here there have been no published studies addressing the effect of α on regulators which bind downstream of the −35 region. As noted above, unpublished studies (cited in reference 17) had shown that deletion of contact site I of the α subunit did not interfere with MerR-Hg(II)-induced transcriptional activation in vitro. However, in contrast to the in vitro behavior of MerR with truncated α subunits, MerR is sensitive in vivo to alterations at G311 and P323. Changing either residue results in distinct MerR-dependent responses of the *mer* promoter (Fig. 6).

The *rpoA* mutants originally identified as impaired in OmpR activation (G3S, P322S, P323L, and P323S) are all more effective (50 to 90% more effective than the wild type) in expressing P_{TPCAD} but are no better than the wild type at P_R. Thus, they are not general spacer suppressors like the *rpoD* mutants described above but are simply able to do well on this promoter with a 19-bp spacer. Moreover, none of them is defective in MerR-mediated activation (Fig. 6A), so MerR does not require the contacts altered by these mutations in the same way as OmpR does. However, substitution of leucine for proline at position 323 stimulates twofold-higher expression of P_{TPCAD} under both activated and repressed conditions, although MerR-independent expression is unaffected (Fig. 6B). In contrast, a mutant with a serine substitution at this same position is little different from the wild type in these two MerR-dependent processes. Substitution of serine for the adjacent proline (position 322) results only in impairment of repression; it does not stimulate activation.

The group of α mutants originally selected for their effects on OxrA show lower MerR-independent expression of both the 19-bp (Fig. 7A) and 15-bp (Fig. 7B) spacers and do not

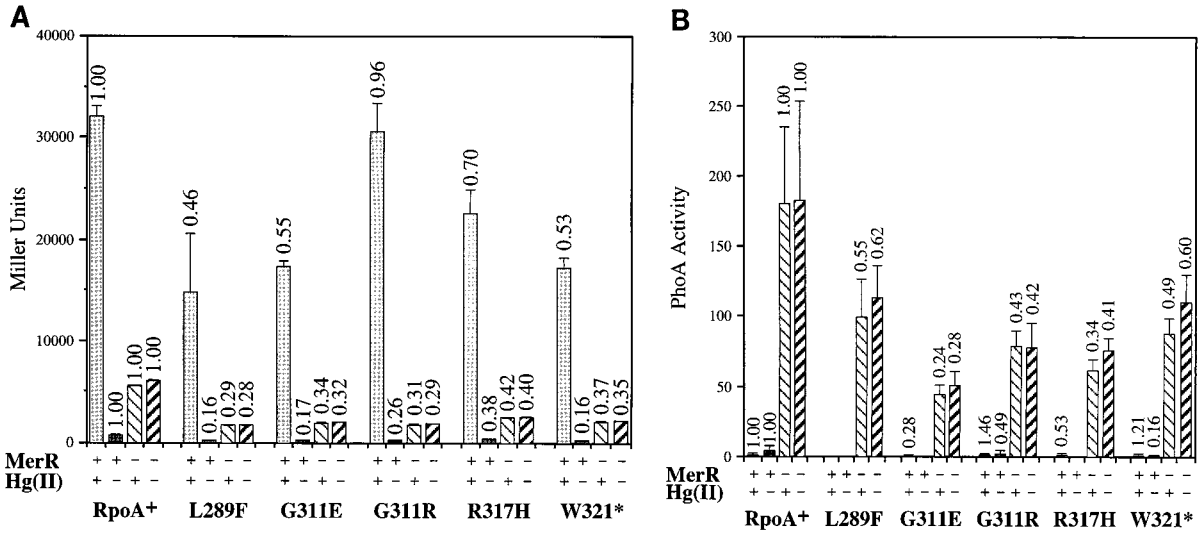

FIG. 7. (A) LacZ activities from the *merT'-lacZ* fusion (P_{TPCAD}) in isogenic strains containing mutant *rpoA* alleles. The reporter constructs were pWR2 (with MerR) and pWR10 (without MerR). Only the *rpoA* mutation is shown for clarity. Activity of β-galactosidase was determined as described in Materials and Methods. Each bar represents the average β-galactosidase activity. β-Galactosidase assays were performed on two days, and relative values were consistent. (B) PhoA activities from the *merR'-phoA* fusion (P_R) in isogenic strains containing mutant *rpoA* alleles. Each strain contained the reporter construct pSJ43 with either pSA1 (supplying MerR in *trans*) or pACYC184 (MerR$^-$ control for nonspecific plasmid burden on growth). Only the *rpoA* mutation is shown for clarity. Activity of alkaline phosphatase was determined as described in Materials and Methods. Each bar represents the average alkaline phosphatase activity in two independent assays. Numbers shown above each bar are the fold differences between the mutant strains and the parental strain under each condition tested. The standard deviations are shown.

overcome MerR repression of either promoter. With one exception (G311R), they are also defective in MerR-dependent activated expression of P_{TPCAD} (Fig. 7A). The transcription proficiency of the arginine-substituted holoenzyme at 311 compared to that of the glutamate-substituted variant suggests that a positive charge (but not a negative one) at 311 allows interaction with some MerR-related structure to restore transcriptional competence. However, a charge difference at position 311 is irrelevant in MerR-independent transcription of the promoter. Thus, the MerR-related processes of repression and activation of P_{TPCAD} may have residue-specific requirements in the regions around G311 and P323 of α. Such requirements suggest interactions, perhaps through the flexible linker arm of the α subunit (5, 28, 35), through another subunit of RNA polymerase (σ^{70} has been cross-linked to the C terminus of α between residues 209 and 329 [23]), or with some MerR-mediated DNA conformation.

In summary, we have examined the abilities of well-characterized *rpoD* and *rpoA* mutants to exploit the unusual aspects of transcriptional control in the *mer* operon. In addition to observing a novel general effect of certain *rpoD* mutants (the ability to function with nonoptimal spacers), we have also noted allele-specific effects of other mutants in σ^{70} and in α. The behaviors of strains with mutations at R596 of *rpoD* and at G311 and P323 of *rpoA* are consistent with there being required structures at these residues to support interactions either with MerR or with some MerR-related conformation of the promoter DNA. Mutations at these three residues can affect the ability of holoenzymes containing these altered subunits to engage in the MerR-dependent processes of *mer* transcriptional control. These observations suggest a certain intimacy in the relationship between MerR and RNA polymerase in the *merOP* region, perhaps even physical contact. Current studies with purified MerR and RNA polymerase are testing this hypothesis.

ACKNOWLEDGMENTS

We are grateful to Carol Gross, Mimi Susskind, Tom Silhavy, and Charles Miller for bacterial strains and to Alicia Dombroski for communication of results prior to publication. We thank Tim Hoover, Alicia Dombroski, John Helmann, Charlie Moran, Linc Sonenshein, and an anonymous reviewer for helpful discussions and/or critical reading of the manuscript. We thank Joy Wireman and Scott Jacobs for technical assistance with the activity assays.

This work was supported by NSF grant 9218636 to A.O.S.

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