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The CpxRA Signal Transduction System of *Escherichia coli*: Growth-Related Autoactivation and Control of Unanticipated Target Operons

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In *Escherichia coli*, the CpxRA two-component signal transduction system senses and responds to aggregated and misfolded proteins in the bacterial envelope. We show that CpxR-P (the phosphorylated form of the cognate response regulator) activates *cpxRA* expression in conjunction with RpoS, suggesting an involvement of the Cpx system in stationary-phase survival. Engagement of the CpxRA system in functions beyond protein management is indicated by several putative targets identified after a genomic screening for the CpxR-P recognition consensus sequence. Direct negative control of the newly identified targets *motABcheAW* (specifying motility and chemotaxis) and *tsr* (encoding the serine chemoreceptor) by CpxR-P was shown by electrophoretic mobility shift analysis and Northern hybridization. The results suggest that the CpxRA system plays a core role in an extensive stress response network in which the coordination of protein turnover and energy conservation may be the unifying element.

The CpxRA two-component system, together with σ^E and σ^{32}, regulates the synthesis of a number of enzymes that are involved in the folding and degradation of periplasmic proteins in *Escherichia coli* (4, 6, 7, 30). For example, the expression of *degP* (encoding a periplasmic protease) is activated by CpxRA and $E\sigma^E$, whereas the expression of *ppiD* (encoding a periplasmic peptidyl-prolyl *cis-trans* isomerase) is activated by CpxRA and $E\sigma^{32}$ (4, 6, 7, 30). Moreover, CpxRA appears to play a role in sensing and responding to envelope protein distress, since it also activates the expression of *ppiA* (encoding another periplasmic peptidyl-prolyl *cis-trans* isomerase), *dsbA* (encoding a periplasmic disulfide oxidoreductase), and *cpxP* (encoding a periplasmic protein of unknown function) (4, 5, 30; for a review, see reference 33).

Certain mutant versions of the CpxA sensor kinase (CpxA*) cause a variety of seemingly unrelated phenotypes. These include a decreased ability to perform conjugation (16, 35, 36); diminished assembly of lipoprotein and OmpF in the cell envelope (19, 20); random septum positioning during cell division (29); lost ability to grow on succinate (31), L-lactose (3, 28), and L-proline (27); acquired ability to grow on L-serine (24, 25, 38); partial isoleucine and valine auxotrophy (18, 39); increased sensitivity to high temperature (16), sodium dodecyl sulfate (3), and hydrogen peroxide (8a); enhanced tolerance to high pH (5) colicins A and K (26); increased resistance to the aminoglycoside antibiotics amikacin and kanamycin (31, 40); and decreased sensitivity to $CuCl_2$ (8a, 43). CpxA* proteins were shown to retain their kinase activity but to lack the ability to dephosphorylate CpxR-P, the phosphorylated form of the cognate response regulator (32). The resulting rise in CpxR-P levels apparently underlies the numerous aberrant CpxA* phenotypes, hinting that the Cpx regulatory system may play a physiological role that is more extensive than hitherto recognized.

Since a putative CpxR-P recognition consensus box has been reported (30), we screened *E. coli* promoters for the presence of this box to identify additional target operons. In this study, we present evidence that a number of genes that are not related to protein management are under the direct control of CpxR-P.

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MATERIALS AND METHODS

Strains and plasmids. The *E. coli* strains and plasmids used in this study are listed in Table 1. Standard molecular biological techniques were applied for their manipulation and construction (34).

Growth media. Cells were grown on Luria-Bertani (LB) or glucose (0.2%) minimal medium (pH 7.0) comprising 34 mM NaH_2PO_4, 66 mM K_2HPO_4, 20 mM $(NH_4)_2SO_4$, 1 μM $FeSO_4$, 30 mM $MgSO_4$, 1 mM $ZnCl_2$, 10 μM $CaCl_2$, 0.3 mM isoleucine, 0.3 mM valine, and 2 mM thiamine.

Construction of the Δ*cpxRA* strain. Strain BW21355, an isogenic derivative of MG1655 (12), was used for the mutant construction. First, an *argE*::Tn*10* mutation (closely linked to *cpxRA*) was P1 transduced from strain JP466 (30) into strain BW21355. The transductants were selected at 37°C for tetracycline resistance (20 μg/ml) and scored for arginine (0.6 mM) auxotrophy on glucose minimal medium, resulting in the isolation of strain ECL3500. $\Delta(cpxRA)_2$ was then P1 transduced from strain ECL1212 (29) into strain ECL3500. The transductants were selected for growth on glucose minimal medium free of arginine and scored for sensitivity to tetracycline and $CuCl_2$ (4 mM) (*cpx* deletion phenotype) (8a), resulting in the isolation of strain ECL3501. The presence of $\Delta(cpxRA)_2$ in ECL3501 was confirmed by PCR analysis and DNA sequencing (performed at the MicroCore Sequencing Facility of the Department of Microbiology and Molecular Genetics, Harvard Medical School).

Construction of the Φ(*cpx-lacZ*) reporter strains. Strains derived from ECL3501 and bearing the various Φ(*cpx-lacZ*) operon fusions were constructed by PCR amplification of $cpxRA^+$ and *cpxRA** (including 309 bp upstream of the *cpxR* start codon) from strains BW21355 and AE2293, respectively (*cpxA** allele: Leu38→Phe [TTT]) (30). For this procedure, *Taq* Plus Precision polymerase mix (Stratagene) was used with primers IFDRλ1 (5′-TCCCCCGGGTCGAACATATGGCTCTGCGTACTG-3′) and IFDRλ2 (5′-TACGGATCCGAAGTTTAACTCCGCTTATACAGC-3′); the PCR products (2,414 bp) were then restricted with *Sma*I and *Bam*HI (New England Biolabs), the recognition sites of which are present in the primers (underlined; *Sma*I site in IFDRλ1 and *Bam*HI site in IFDRλ2), and ligated into *Sma*I/*Bam*HI-restricted cloning vector pAlter-1 (Promega). This procedure yielded plasmids pAlter/R^+A^+ and pAlter/R^+A^*. The recombinant plasmids were restriction mapped, and the desired clones were sequenced to confirm the *cpxA** allele and the absence of PCR-introduced mutations.

To construct an in-frame deletion in *cpxR*, a sequence fragment (418 bp) was removed between the unique *Xho*I site in *cpxR* (bp 79) and bp 497, at which position a *Xho*I restriction sequence was introduced by PCR, allowing intragenic closure. First, a 1,212-bp fragment of *cpxRA* was PCR amplified from the BW21355 chromosome with primers CpxRXhoI (5′-GCCGCCGCTCGAGTTTACCCTGCTCTATTTG-3′) and Cpx14 (5′-GCCCATTTGCTCGGC-3′). Since

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

Strain or plasmid	Relevant characteristics or genotype	Reference or source
Strains		
BW21355	K-12 F$^-$ *rph-1* Δ(*lac*)*X74*	12
AE2293	K-12 F$^-$ *metB*$^+$ *glpK1 cpxA9*	31
JP466	MC4100 Δ*frd-101* F′ pOXgen *argE*::Tn*10*	30
ECL3500	BW21355 *argE*::Tn*10*	This study
ECL1212	MC4100 Δ*frd-101* F′ pOXgen Δ(*cpxRA*)$_2$	29
ECL3501	BW21355 Δ(*cpxRA*)$_2$	This study
ECL3502	ECL3501 Φ(*cpxR*$^+$*A*$^+$-*lacZ*)	This study
ECL3503	ECL3501 Φ(*cpxR*^{Δ}*A*$^+$-*lacZ*)	This study
ECL3504	ECL3501 Φ(*cpxR*$^+$*A**-*lacZ*)	This study
ECL3505	ECL3501 Φ(*cpxR*^{Δ}*A**-*lacZ*)	This study
GS015	K-12 *rpoS*::Tn*10*	1
ECL3506	ECL3502 *rpoS*::Tn*10*	This study
ECL3507	ECL3503 *rpoS*::Tn*10*	This study
Plasmids		
pRS415	*lac*-based promoter fusion vector	37
pRS415/*R*$^+$*A*$^+$	pRS415 containing the *cpxR*$^+$*A*$^+$ operon	This study
pRS415/*R*^{Δ}*A*$^+$	pRS415 containing the *cpxR*^{Δ}*A*$^+$ operon	This study
pRS415/*R*$^+$*A**	pRS415 containing the *cpxR*$^+$*A** operon	This study
pRS415/*R*^{Δ}*A**	pRS415 containing the *cpxR*^{Δ}*A** operon	This study
pAlter-1	Cloning vector	Promega
pAlter/*R*$^+$*A*$^+$	pAlter-1 containing the *cpxR*$^+$*A*$^+$ operon	This study
pAlter/*R*^{Δ}*A*$^+$	pAlter-1 containing the *cpxR*^{Δ}*A*$^+$ operon	This study
pAlter/*R*$^+$*A**	pAlter-1 containing the *cpxR*$^+$*A** operon	This study
pAlter/*R*^{Δ}*A**	pAlter-1 containing the *cpxR*^{Δ}*A** operon	This study

primer CpxRXhoI contains an introduced *Xho*I restriction site at its 5′ end (underlined) and since a natural *Rsr*II site is present in the *cpxRA* sequence near the 3′ end of the PCR product, this product was treated with *Xho*I and *Rsr*II (New England Biolabs). The DNA fragment was then ligated to the backbone of *Xho*I/*Rsr*II-restricted pAlter/*R*$^+$*A*$^+$ and pAlter/*R*$^+$*A** (a 1,630-bp fragment was released from these vectors), yielding plasmids pAlter/*R*^{Δ}*A*$^+$ and pAlter/*R*^{Δ}*A**, which contained a 418-bp in-frame deletion in *cpxR*. The identity of the plasmid constructs was confirmed by restriction analysis and DNA sequencing.

All *cpx* operons were released from the pAlter-based plasmids with *Sma*I and *Bam*HI and ligated into *Sma*I/*Bam*HI-restricted operon fusion plasmid pRS415 (36). The resulting plasmids, pRS415/*R*$^+$*A*$^+$, pRS415/*R*^{Δ}*A*$^+$, pRS415/*R*$^+$*A**, and pRS415/*R*^{Δ}*A**, were transformed into strain ECL3501 (30°C, LB agar with 50 μg of ampicillin/ml) and transferred to λRZ5 (13) or λRS45 (37). Selection and transduction of recombinant phages to the *attB* site of strain ECL3501 were carried out as previously described (37). Single-copy lysogens ECL3502, ECL3503, ECL3504, and ECL3505 (Table 1) were confirmed by PCR analysis of the *attB* site (11) with primers P1-attλ (5′-TCAGAACGACGTTGATCGGGC GGGGTTG-3′), P2-attP (5′-AGTTTGTCTGCAAGACTCTATGAGAAGCA G-3′), P3-attP (5′-ATGTTGCATGGTGCACTGTTTATACCAACG-3′), and P4-attλ (5′-GCGCAATGCCATCTGGTATCACTTAAAGG-3′).

To address the role of stationary-phase transcription factor σ^S in *cpxRA* expression, an *rpoS*::Tn*10* mutation was P1 transduced from strain GS015 (1) into strains ECL3502 and ECL3503. Transductants were selected on LB agar containing tetracycline, leading to the respective isolation of strains ECL3506 and ECL3507.

Northern analysis of *motABcheAW* and *tsr* expression. Examination of *motABcheAW* and *tsr* expression was carried out by Northern analysis of total RNA from exponential-phase cultures (optical density at 600 nm [OD_{600}], 0.8 to 1.0) of strains ECL3502, ECL3503, ECL3504, and ECL3505 (LB medium, 37°C). Total RNA was isolated with the RNeasy Total RNA System (Qiagen) and

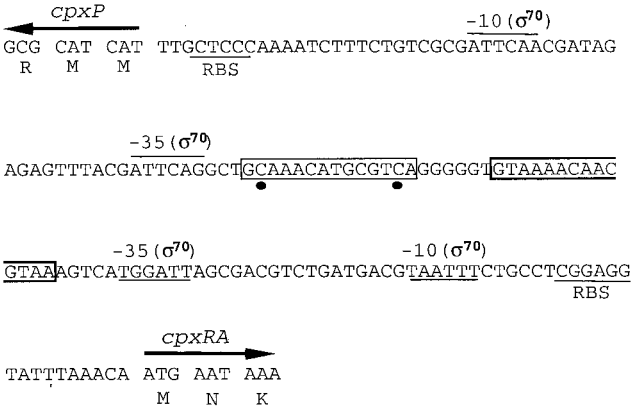

FIG. 1. Representation of the *cpxP-cpxRA* intergenic region. Putative recognition sequences for σ^{70} (single lines above or below the sequence) and ribosome binding (RBS) are shown. The perfect CpxR-P recognition consensus sequence is boxed in a bold line, whereas the consensus sequence with a 2-bp mismatch is boxed in a thin line. The consensus mismatches are indicated by black circles.

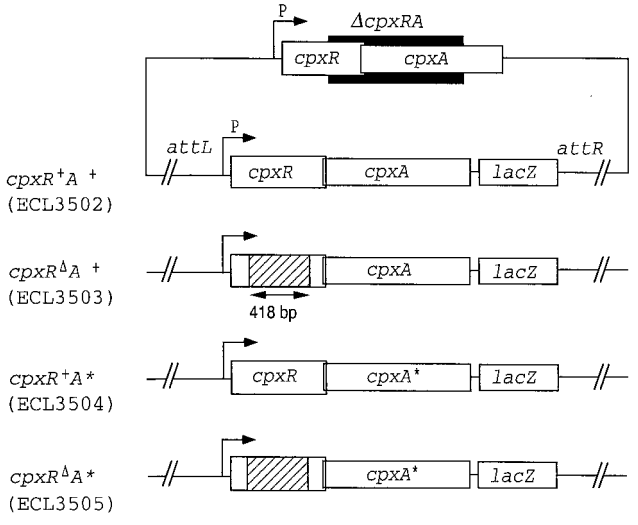

FIG. 2. Representation of the Φ(*cpx-lacZ*) operon fusion constructs integrated at the λ attachment site (*attB*) of strain ECL3501. The black box depicts the deleted region within *cpxRA*. The hatched boxes depict the in-frame deletion (418 bp) within *cpxR*. The Φ(*cpx-lacZ*) constructs contain 309 bp upstream from the *cpxR* translational start point and include the *cpxRA* promoter region (P) (Fig. 1).

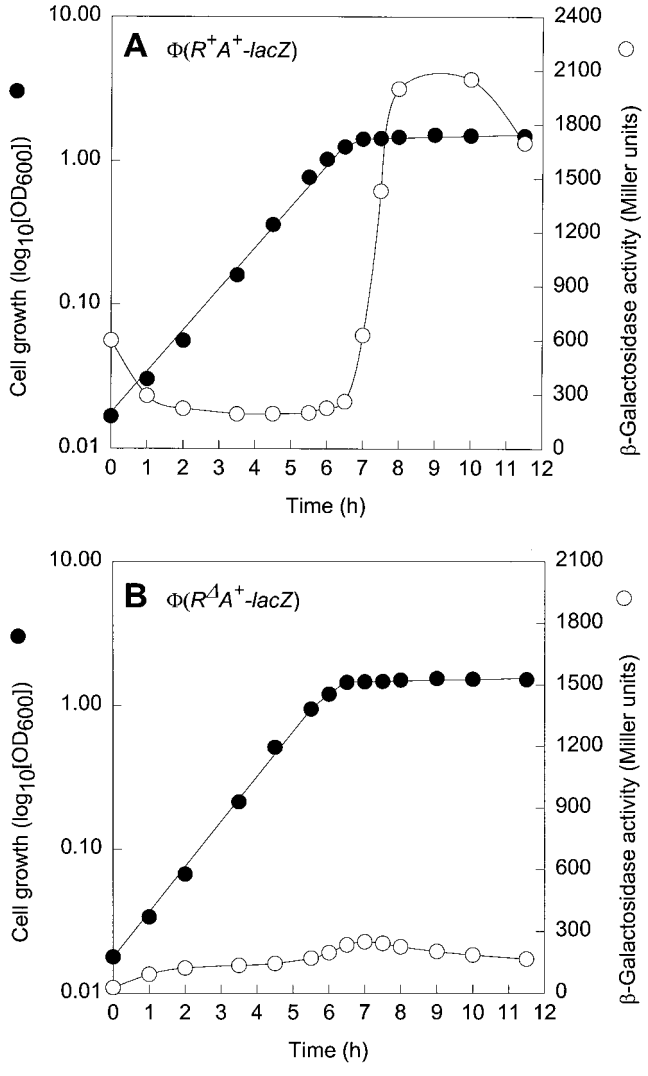

FIG. 3. Effect of CpxR-P on Φ(*cpxRA*$^+$-*lacZ*) expression. Growth curves and β-galactosidase activity profiles of strain ECL3502 [Φ(*cpxR*$^+$*A*$^+$-*lacZ*)] (A) and strain ECL3503 [Φ(*cpxR*$^\Delta$*A*$^+$-*lacZ*)] (B) are shown. The cells were grown in glucose minimal medium (pH 7.0) at 37°C.

separated by gel electrophoresis in Tris-acetate-EDTA–agarose (1%) containing guanidine thiocyanate (20 mM). Target mRNA was hybridized with randomly labeled ([α-^{32}P]ATP; NEN Life Science Products) DNA probes by use of the Klenow enzyme (New England Biolabs). For transcriptional analysis of *motABcheAW*, an 864-bp DNA fragment that covers the coding sequences of *motB* and *cheA* was PCR generated with primers MOTAB1 (5′-GAAGAGATTGAGAC GCACGAAAGC-3′) and MOTAB2 (5′-TCGATTTTGAGATGGGGACGTA ACG-3′). For analysis of *tsr* expression, an 895-bp *tsr* gene fragment was PCR generated with primers TSR1 (5′-CAGCGGCAGAGATCAAACGTAATTAC G-3′) and TSR2 (5′-GCTTTTAATTTCACGAGCCGCCTGGG-3′). The expression of *envZ* (not CpxR-P regulated) was used as the internal control as previously described (30).

Analysis of CpxR-P binding to the promoter regions of *motABcheAW* and *tsr*. The binding of CpxR or CpxR-P to the promoter regions of *motABcheAW* and *tsr* was examined by electrophoretic mobility shift analysis by a previously described method (32). A CpxR (or CpxR-P)/DNA ratio of 20 (200 pmol/10 pmol) was used in the presence of a 500-fold molar excess of competitor DNA (sheared herring sperm DNA; Promega) and a 100-fold molar excess of competitor protein (bovine serum albumin; New England Biolabs). CpxR was overproduced and purified as described before (30). CpxR was phosphorylated by incubation with acetyl phosphate (30). A 246-bp *motABcheAW* promoter fragment containing the putative CpxR-P recognition consensus box in the center was PCR amplified from the chromosome of strain BW21355 with primers MOT/EMS1 (5′-GGACATTGGTGCGGTTTGTTGAAAGTGG-3′) and MOT/EMS2 (5′-GCTGGAATGTTGCGCCTCACCGTATCAG-3′). A 212-bp *tsr* promoter fragment containing the CpxR-P recognition consensus box in the center was PCR amplified with primers TSR/EMS1 (5′-ATGTATTGATTAATAGTTGGCCGA AGCCG-3′) and TSR/EMS2 (5′-GATATGAATCACATATTTATCGTCACTT AAACG-3′). All primers were then 5′ labeled (30 min, 37°C) with [γ-^{32}P]ATP by use of T4 polynucleotide kinase (New England Biolabs). Radiolabeled promoter DNA was PCR generated by use of the labeled primers with the previously amplified DNA fragments as templates. The labeled promoter DNA was purified (Qiagen gel purification kit) after agarose gel electrophoresis (2% SeaKem Ultrapure Agarose; FMC Corp.) and used in binding assays.

β-Galactosidase assay. Specific β-galactosidase activities in the reporter strains grown in glucose minimal medium (20 ml, 37°C, 280 rpm) were assayed as previously described (22).

RESULTS AND DISCUSSION

CpxR-P autogenously activates *cpxRA* expression. The intergenic region of the divergently transcribed *cpxP* and *cpxRA* operons contains a perfect CpxR-P binding consensus sequence, 5′-GTAAA(N)$_5$GTAA-3′, which is located between bp 57 and 70 upstream from the CpxR translational start point (30) (Fig. 1). A second such sequence, with a 2-bp mismatch, is located between bp 77 and 90 (this study). The transcription of

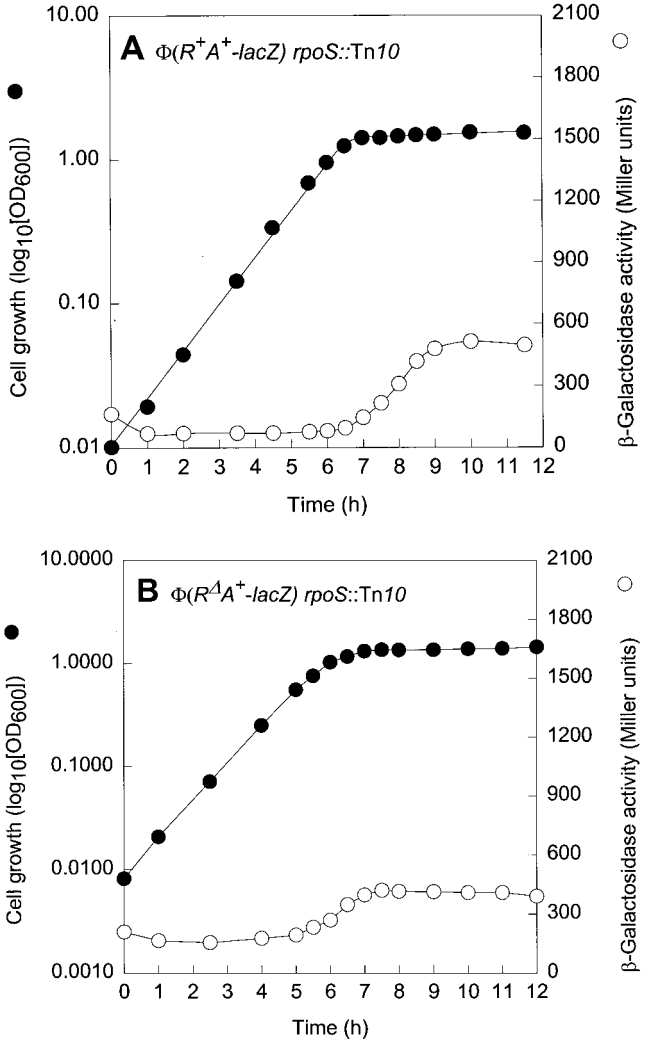

FIG. 4. Effect of RpoS on Φ(*cpxRA*$^+$-*lacZ*) expression. Growth curves and β-galactosidase activity profiles of strain ECL3506 [Φ(*cpxR*$^+$*A*$^+$-*lacZ*) *rpoS*::Tn*10*] (A) and strain ECL3507 [Φ(*cpxR*$^\Delta$*A*$^+$-*lacZ*) *rpoS*::Tn*10*] (B) are shown. The cells were grown in glucose minimal medium (pH 7.0) at 37°C.

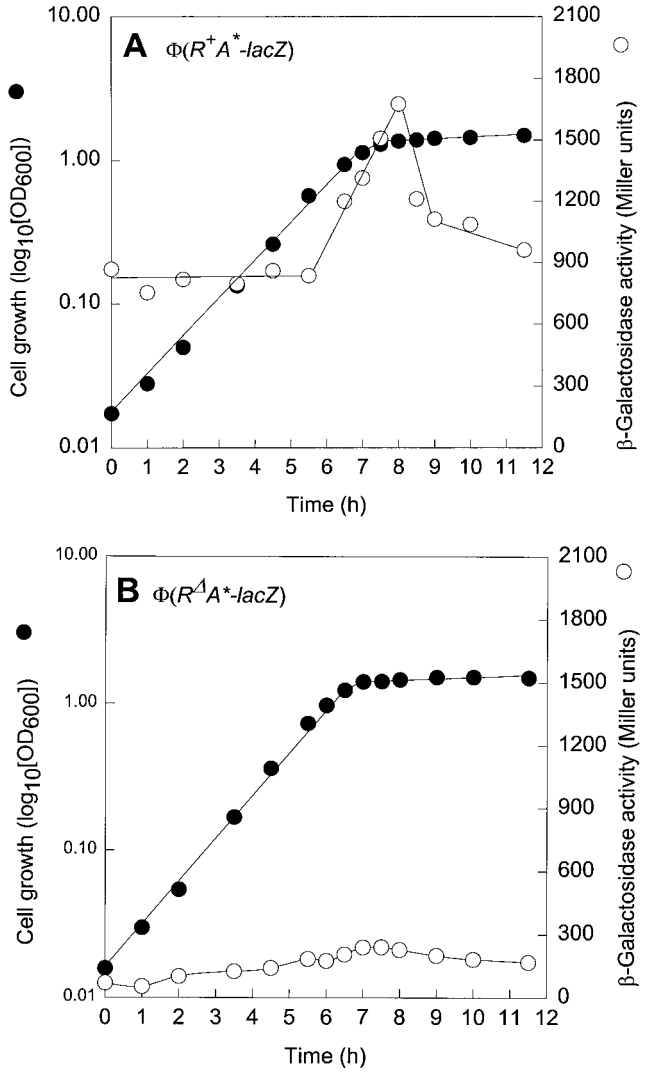

FIG. 5. Effect of CpxR-P on Φ(*cpxR*$^+$*A***-lacZ*) expression. Growth curves and β-galactosidase activity profiles of strain ECL3504 [Φ(*cpxR*$^+$*A***-lacZ*)] (A) and strain ECL3505 [Φ(*cpxR*$^\Delta$*A***-lacZ*)] (B) are shown. The cells were grown in glucose minimal medium (pH 7.0) at 37°C.

cpxP was shown to be activated by CpxR-P (5), but the possibility that *cpxRA* is also under the control of this regulator cannot be excluded. To examine this plausibility, we made two different *cpxRA-lacZ* constructs that express CpxR (intact or with an in-frame deletion), CpxA, and LacZ. These operon fusions were inserted at the *attB* site of strain ECL3501, yielding strains ECL3502 [Φ(*cpxR*$^+$*A*$^+$*-lacZ*)] and ECL3503 [Φ(*cpxR*$^\Delta$*A*$^+$*-lacZ*)]. In the latter fusion, *cpxR* sustained a 418-bp in-frame deletion (Fig. 2 and Table 1). In strain ECL3502 grown in glucose minimal medium, the Φ(*cpxR*$^+$*A*$^+$*-lacZ*) expression level (determined as specific β-galactosidase activity) rose dramatically at the onset of the stationary growth phase (typically at an OD_{600} of 1.0) (Fig. 3A). The in-frame deletion in *cpxR* (ECL3503) greatly diminished (ninefold) this increase (Fig. 3B). Thus, CpxR-P activates the expression of its own operon. Essentially the same results were obtained with cells grown in LB medium (data not shown).

RpoS activates *cpxRA* expression. The strong increase in Φ(*cpxR*$^+$*A*$^+$*-lacZ*) expression at the onset of stationary growth (Fig. 3A) suggested an involvement of RpoS in *cpxRA* expression. The participation of RpoS, direct or indirect, was confirmed by a fourfold-lower stationary-phase expression level of

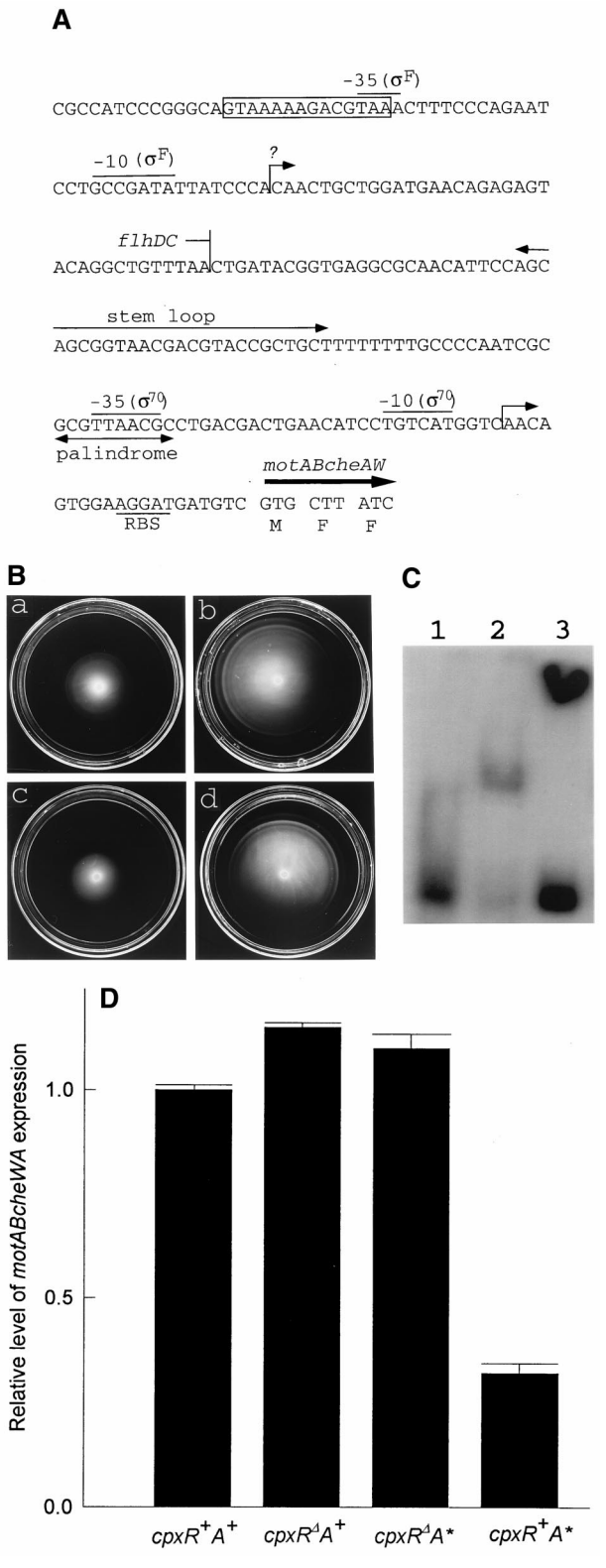

FIG. 6. Negative regulation of *motABcheAW* by the Cpx system. (A) Promoter region of *motABcheAW* (8, 14, 15). The CpxR-P recognition site is boxed. The Eσ^F promoter and transcriptional start site are assigned on the basis of the Eσ^F recognition consensus sequence (2, 15). RBS, ribosome binding site. (B) Swarm patterns. (a) ECL3502 [Φ(*cpxR*$^+$*A*$^+$*-lacZ*)]. (b) ECL3503 [Φ(*cpxR*$^\Delta$*A*$^+$*-lacZ*)]. (c) ECL3504 [Φ(*cpxR*$^+$*A***-lacZ*)]. (d) ECL3505 [Φ(*cpxR*$^\Delta$*A***-lacZ*)]. (C) Electrophoretic mobility shift analysis of the *motABcheAW* promoter DNA region with CpxR or CpxR-P. Lane 1, *motABcheAW* promoter DNA; lane 2, promoter DNA plus CpxR; lane 3, promoter DNA plus CpxR-P. (D) Profiles of expression of *motABcheAW* in the four Φ(*cpx-lacZ*) strains, as determined by Northern analysis. Error bars indicate standard deviations.

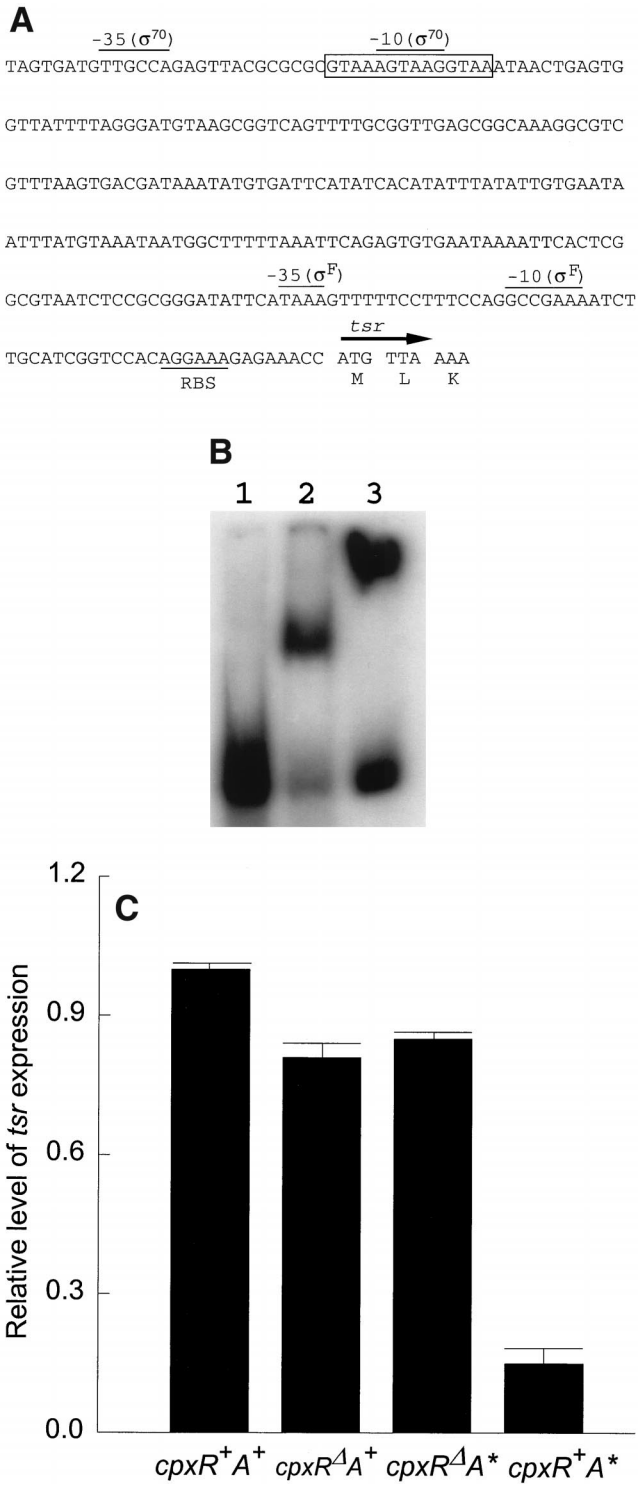

FIG. 7. Negative regulation of *tsr* by the Cpx system. (A) Promoter region of *tsr* (14). The CpxR-P recognition site is boxed. The Eσ^{70} promoter site is putative, but involvement of Eσ^{70} in *tsr* expression has been reported (14). RBS, ribosome binding site. (B) Electrophoretic mobility shift analysis of the *tsr* promoter DNA region with CpxR or CpxR-P. Lane 1, *tsr* promoter DNA; lane 2, promoter DNA plus CpxR; lane 3, promoter DNA plus CpxR-P. (C) Profiles of expression of *tsr* in the four Φ(*cpx-lacZ*) strains, as determined by Northern analysis. Error bars indicate standard deviations.

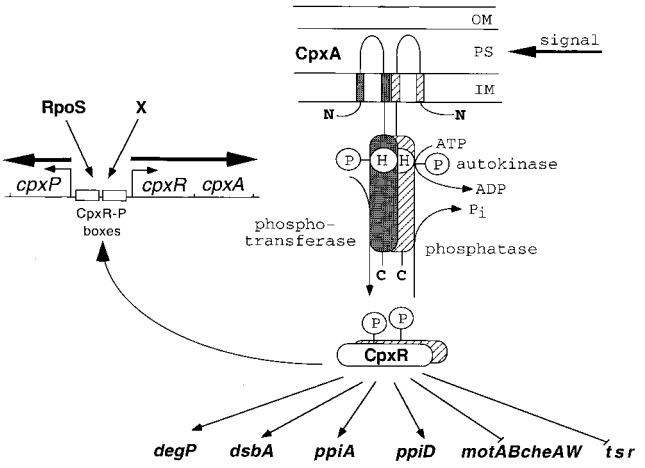

FIG. 8. Representation of CpxRA signal transduction. CpxA and CpxR are shown as dimers. OM, outer membrane; PS, periplasmic space; IM, inner membrane; X, unknown activator. For explanations, see the text.

Φ(*cpxR*$^+$*A*$^+$-*lacZ*) in strain ECL3506, bearing *rpoS*::Tn*10* (Fig. 4A and 3A). However, in the absence of RpoS, the expression of Φ(*cpxR*$^+$*A*$^+$-*lacZ*) still rose moderately at the start of the stationary growth phase (Fig. 4A). Moreover, even in the absence of both RpoS and CpxR, a slight rise in Φ(*cpxR*$^\Delta$*A*$^+$-*lacZ*) expression was observed toward the end of exponential growth, suggesting the involvement of (an)other regulatory element(s) (Fig. 4B).

Expression of *cpxRA is disproportionately enhanced during growth.** In view of the autogenous activation of *cpxRA* and the report that CpxA* sensor kinases are locked in the net phosphorylating mode (32), the basal level of expression of *cpxRA* can be expected to be elevated, possibly even during exponential growth. To test this hypothesis, we inserted two different *cpxRA*-lacZ* constructs that express CpxR (intact or with an in-frame deletion), CpxA* (with the *cpxA** allele specifying a Leu38→Phe substitution), and LacZ. These operon fusions were inserted at the *attB* site in a Δ*cpxRA* strain (ECL3501). The resulting strains, ECL3504 [Φ(*cpxR*$^+$*A**-*lacZ*)] and ECL3505 [Φ(*cpxR*$^\Delta$*A*-lacZ*), in which the *R* allele bears a 418-bp in-frame deletion] were then compared for their β-galactosidase activity levels during growth (Fig. 2 and Table 1). The exponential-phase expression of Φ(*cpxR*$^+$*A*-lacZ*) (Fig. 5A) was found to be about fourfold higher than that of Φ(*cpxR*$^+$*A*$^+$-*lacZ*) (Fig. 3A). The level of Φ(*cpxR*$^+$*A*-lacZ*) expression rose further before the end of exponential growth (typically at an OD_{600} of 0.6). It is unclear why the level of stationary-phase expression of Φ(*cpxR*$^+$*A*-lacZ*) was lower than that observed in the wild-type strain. Nonetheless, the results as a whole support the notion that CpxA* causes excessive levels of CpxR-P, which may be responsible for most, if not all, of the *cpxRA** phenotypes.

Genomic screening for CpxR-P-controlled operons. To identify more putative CpxR-P-controlled target operons, we performed genomic scanning (Genetics Computer Group version 9.1 software) with the CpxR-P binding consensus sequence. Successful identification of additional target operons could provide more specific clues about the individual CpxA* phenotypes. About 50 consensus hits that lie within 450 bp upstream of start codons were found. The activities of some of the encoded proteins can be related to particular CpxA* phenotypes. First, the regulation of *rpoH* (encoding heat shock transcription factor σ^{32}; CpxR-P box between bp 257 and 271

upstream of the *rpoH* start codon) can be linked to the temperature-sensitive growth condition of *cpxA** mutants (16). This association is consistent with CpxR-P control of operons involved in protein rescue and/or clearance during heat stress (4, 6, 7, 30). Second, the gene product of *psd* (phosphatidylserine decarboxylase; CpxR-P box between bp 120 and 134 upstream of the *psd* start codon) catalyzes the synthesis of phosphatidylethanolamine. Lack of this phospholipid was shown to make a wild-type Cpx system hyperactive (21). A response at the level of *psd* expression might be a homeostatic strategy to restore a healthy membrane protein/phospholipid ratio. Third, direct regulation of the gene with accession no. U58330 (encoding a probable copper-transporting ATPase; CpxR-P box between bp 91 and 105 upstream of the U58330 start codon) may underlie the elevated resistance to $CuCl_2$ with increasing CpxR-P levels (e.g., the $CuCl_2$ resistance level is ECL3503 = ECL3505 < ECL3502 < ECL3504; data not shown). The functional diversity of the consensus sequence-identified operons further hints at a broad role for the Cpx system.

CpxR-P represses motility and chemotaxis genes. To test the usefulness of the consensus sequence screening, we analyzed Cpx control of two unanticipated target operons identified by the screening. The first was *motABcheAW* (specifying motility and chemotaxis). The location of the CpxR-P recognition box (Fig. 6A), overlapping the −35 site of a perfect consensus sequence for $E\sigma^F$ (2, 14), made the regulatory involvement of CpxR-P quite plausible. We therefore compared the swarming abilities of the four Φ(*cpx-lacZ*) strains. Figure 6B shows that motility was negatively affected by CpxR-P. Electrophoretic mobility shift analysis showed retardation of the *motABcheAW* promoter DNA by CpxR in the presence of competitor DNA and protein. The CpxR regulator was more effective in the phosphorylated form (Fig. 6C), as was shown for the known target *degP* (32). Northern analysis supported the negative control of *motABcheAW* by CpxR-P (Fig. 6D). Whereas the deletion of CpxR dramatically increased the swarming rate, the effect of this deletion on the *motABcheAW* mRNA level was relatively small. Apparently, additional controlling elements are involved in the control of cell motility under the experimental conditions used.

Next, the involvement of the Cpx system in the regulation of *tsr* (encoding the serine chemoreceptor) was examined. The *tsr* operon is transcribed by both $E\sigma^F$ and $E\sigma^{70}$ (Fig. 7A) (14). The $E\sigma^{70}$ promoter site has not yet been defined, but a best-fitting $E\sigma^{70}$ promoter sequence overlaps the CpxR-P recognition consensus sequence at the −10 site (Fig. 7A). Electrophoretic mobility shift analysis showed retardation of the *tsr* promoter DNA by CpxR in the presence of competitor DNA and protein. The CpxR regulator was again more effective in the phosphorylated form (Fig. 7B). Northern analysis showed the negative control of *tsr* by CpxR-P (Fig. 7C). Why the level of expression of *tsr* in the *cpxR* deletion strain did not exceed that in the wild-type strain (average of three experiments) remains unclear. It is possible that the *cpxR* deletion affected the synthesis or properties of other proteins involved in *tsr* expression. The *motABcheAW* and *tsr* operons are the first operons shown to be under the direct negative control of the Cpx regulatory pathway.

CpxRA has a function beyond the management of periplasmic protein distress. It is known that CpxR-P and σ^E jointly activate the expression of *degP* (6) and that CpxR-P and σ^{32} jointly activate the expression of *ppiD* (7). Since under conditions of heat shock the expression of *rpoH* (encoding σ^{32}) is activated by σ^E (9, 41), an intricate regulatory circuit seems to have evolved. Our finding that CpxR-P and σ^S act synergistically on *cpxRA* transcription expands the function of the Cpx signal transduction system into stationary-phase adaptation and suggests that starvation or energy depletion amplifies the Cpx signaling capacity. It is noteworthy that stationary-phase cells are more resistant to oxidative damage and heat shock (10). If *rpoH* is found to be Cpx controlled, the importance of the CpxRA system in the expression of the stress response network will become even more extensive. The fact that deleting *cpxR* and *rpoE* independently results in copper sensitivity (10a) further fuses the CpxRA and σ^E pathways. In this context, it would be interesting to see whether the expression of the gene with accession no. U58330 (encoding a probable copper transporter) is also under the joint control of CpxR-P and σ^E.

Although with the present knowledge it is difficult to integrate the control of motility and chemotaxis into a response network dealing with protein distress (σ^E and σ^{32}), the suppression of movement may be an energy-saving strategy during starvation. From this point of view, it is of interest to note that a recent study of *Rhizobium meliloti* showed that motility and chemotactic behavior are down-regulated during starvation. In that study, both flagellar maintenance and motor activity were found to be affected, but upon addition of a carbon source or chemoattractant, swarming and chemotaxis were partially restored (42).

In sum, evidence from this study suggests that the Cpx signal transduction system (Fig. 8), in conjunction with σ^E and σ^{32}, responds to a broad spectrum of adverse environmental conditions. These include heat shock, high pH (activates the Cpx pathway [5]), oxidative stress, and nutritional deprivation.

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ADDENDUM

During the preparation of this manuscript, the independent discovery of the autogenous regulation of the *cpxRA* operon was presented by T. J. Silhavy at the 99th General Meeting of the American Society for Microbiology.

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