JOURNAL OF BACTERIOLOGY, May 1990, p. 2456–2461
0021-9193/90/052456-06$02.00/0

Vol. 172, No. 5

The Cpx Proteins of *Escherichia coli* K-12: Evidence that *cpxA*, *ecfB*, *ssd*, and *eup* Mutations All Identify the Same Gene

SUSAN RAINWATER AND PHILIP M. SILVERMAN*

Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, Oklahoma 73104

Received 21 November 1989/Accepted 12 February 1990

An existing *cpxA*(Ts) mutant was resistant to amikacin at levels that inhibited completely the growth of a *cpxA*$^+$ and a *cpxA* deletion strain and failed to grow as efficiently on exogenous proline. These properties are similar to those of mutants altered in a gene mapped to the *cpxA* locus and variously designated as *ecfB*, *ssd*, and *eup*. The amikacin resistance phenotype of the *cpxA* mutant was inseparable by recombination from the *cpxA* mutant phenotype (inability to grow at 41°C without exogenous isoleucine and valine) and was recessive to the *cpxA*$^+$ allele of a recombinant plasmid. Using methods that ensured independent mutations in the *cpxA* region of the chromosome, we isolated six new amikacin-resistant mutants following nitrosoguanidine mutagenesis. Three-factor crosses mapped the mutations to the *cpxA* locus. When transferred by P1 transduction to a *cpxB11* Hfr strain, each of the mutations conferred the Tra$^-$ and Ilv$^-$ phenotypes characteristic of earlier *cpxA* mutants. Two of the new mutations led to a significantly impaired ability to utilize exogenous proline, and four led to partial resistance to colicin A. Two of the new *cpxA* alleles were recessive to the *cpxA*$^+$ allele, and four were dominant, albeit to different degrees. On the basis of these data, we argue that *cpxA*, *ecfB*, *eup*, and *ssd* are all the same gene. We discuss the cellular function of the *cpxA* gene product in that light.

The *cpx* genes of *Escherichia coli* K-12 were defined by chromosomal mutations causing a defect in F plasmid-dependent DNA donor activity (6, 18, 19). The mutations were subsequently shown to be quite pleiotropic, affecting, for example, branch-chain amino acid synthesis and the synthesis and incorporation of certain membrane proteins (20, 21, 33; for a review, see reference 31).

Genetic and molecular genetic analyses mapped *cpxA* to the 87- to 88-min interval of the *E. coli* chromosome, between *pfkA* and *sodA* (1, 2, 19, 30). Several laboratories have described pleiotropic mutations in this region. Among the phenotypes commonly associated with these mutations are resistance to aminoglycoside antibiotics, tolerance to colicins A and K, defects in proline and lactose uptake, reduced growth yield on glucose, and impaired growth on succinate; apparently, these mutations define a single gene, which has been designated *ecfB*, *eup*, and *ssd*, depending on the laboratory of origin (23, 26, 28, 35).

Since *cpxA* mutants grew well on succinate, we concluded that *cpxA* and the gene identified by other pleiotropic mutations in the 87- to 88-min interval were not identical (1). Subsequently, however, impaired growth on succinate was reported to depend on the mutant strain background (28). In addition, Plate et al. (27) reported that a deletion strain lacking the *eup* locus was quasi-wild type and suggested that Eup$^-$ mutants had altered Eup function, rather than no function at all. Our data suggested the same was true of Cpx$^-$ mutants (30).

These facts prompted us to reexamine the relationship between *cpxA* and the *eup*/*ecfB*/*ssd* gene. Our results indicate that in fact all of these genes are one and the same.

* Corresponding author.

MATERIALS AND METHODS

Bacterial strains, plasmids, colicin A, and bacteriophage. The bacterial strains used in this study are listed in Table 1. To construct the *cpxA*$^+$ plasmid pOK101, a 1.5-kilobase *cpxA*$^+$, *Bam*HI-*Eco*RI restriction fragment was cloned into the pINIII (*lpp5 lacPO*)A_3 *lacI*^{q} expression vector (17) digested with *Bam*HI and *Eco*RI. The *cpxA*$^+$ fragment consisted of the 1,508-base-pair *Dra*I-*Stu*I fragment previously described (37), along with surrounding polylinker DNA from the pUC19 cloning vector. The cloned fragment consists almost entirely (1,374 base pairs; 37) of the *cpxA* coding sequence. Expression of *cpxA* in pOK101 is nominally from the *lpp* and *lac* promoters of the vector. Immunooverlay (Western) blot analysis showed higher CpxA levels when transformants were induced, but CpxA protein accumulation over background could be detected even in the absence of an inducer (R. Harris, unpublished observation).

Colicin A was prepared from *Citrobacter freundii* CA31 induced with mitomycin C (0.1 μg/ml), essentially as previously described (36). After induction, cells were killed by the addition of 0.1 volume of chloroform. Debris was removed by sedimentation at 10,000 × *g* for 10 min. The supernatant fluid was used without further purification.

Bacteriophages R17 and P1 *vir* were from our laboratory stocks.

Bacterial media and growth. Luria broth (LB) medium and Vogel-Bonner minimal medium were as previously described (19). For determining amikacin resistance, we used either minimal medium or a medium consisting of LB in a Vogel-Bonner salts base; the resistance phenotype of some mutants was weak on LB medium itself. Solid media were prepared with 15 g of agar per liter. Minimal media were supplemented routinely with 0.2% glucose (or fructose for *pfkA* deletion strains) and 40 μg of required amino acids and thymidine per ml. Unless indicated otherwise, ampicillin and amikacin (Sigma Chemical Co., St. Louis, Mo.) were added at 100 μg/ml and 12 μg/ml, respectively. Bacteria were

TABLE 1. Bacterial strains[a]

Strain	Relevant genotype	Source and reference
AE2062	F⁻ *cpxA*⁺ *cpxB*⁺ *glpK*⁺ *metB1*	J. McEwen
AE2038	F⁻ *cpxA2 cpxB11*[b]	30
AE2072	F⁻ *cpxA2 cpxB11 recA1*	30
AE2115	F⁻ *cpxA*⁺ *cpxB11 glpK1 metB*⁺	30
AE2122[c]	F⁻ Δ(*rha-cpxA-pfkA*) *cpxB11*	1 and 30
AE1031	Hfr (PO150) *cpxA*⁺ *cpxB*⁺	19
AE1184	*recA1* derivative of AE1031	This study
AE1019	Hfr (PO150) *cpxA2 cpxB11*	18
AE1183	*recA1* derivative of AE1019	This study
AE1010	Hfr (PO150) *cpxA*⁺ *cpxB11 metB1*	18
AE2129	F⁻ *cpxA*⁺ *cpxB11* Δ(*lac-pro*)XIII	29
AE2132	F⁻ *cpxA2 cpxB11* Δ(*lac-pro*)XIII	29
AE2146	F⁻ *cpxA2 cpxB11* Δ(*lac-pro*)XIII *recA1*	From AE2132; 29
AE2144	F⁻ *cpxA*⁺ *cpxB11* Δ(*lac-pro*)XIII *recA1*	From AE2129; 29
AE2282	F⁻ Δ(*rha-cpxA-pfkA*) *cpxB11* Δ(*lac-pro*)XIII	From AE2122 as in 29
AE2289 through AE2294	F⁻ *metB*⁺ *glpK1 cpxA3*, *-4*, *-5*, *-6*, *-9*, *-10* derivatives of AE2062	This study
AE2295 through AE2306	F⁻ *cpxA3*, *-4*, *-5*, *-6*, *-9*, *-10* derivatives of AE2129 [Δ(*lac-pro*)XIII *cpxB11*]	This study
AE1177 through AE1182	Hfr *metB*⁺ *glpK1 cpxA3*, *-4*, *-5*, *-6*, *-9*, *-10* derivatives of AE1010	This study
AE1185 through AE1190	Hfr *recA1* derivatives of AE1177 through AE1182, respectively	This study
NK5148	F⁻ *thr-34*::Tn*10*	CGSC 6166[d]

[a] All strains except NK5148 were derived from *E. coli* K-12 strain AE2000 (18). This strain also carries the *arcB6* mutation (10).
[b] The *cpxB1* allele of previous publications has been redesignated *cpxB11* (B. Bachmann, personal communication).
[c] This strain was incorrectly designated *cpxA*⁺ (30).
[d] CGSC, *E. coli* Genetic Stock Center, Yale University, New Haven, Conn.

routinely grown with aeration at 37°C, except as noted. Bacterial growth was measured by culture optical density at 660 nm or, in mating experiments, by viable count.

Genetic methods. Nitrosoguanidine mutagenesis was as described by Miller (22). Survival was 5%, and the frequency of valine-resistant mutants increased from 5.6×10^{-7} per viable cell in the unmutagenized control to 7.2×10^{-4} per viable cell. Bacteriophage P1 transductions were carried out as previously described (19).

To transduce *cpxA* alleles by amikacin resistance, we adopted a procedure to minimize the probability of obtaining spontaneous mutations to aminoglycoside resistance, which can occur at several loci (35). Bacteriophage P1 lysates were prepared on *metB*⁺ *glpK1 cpxA* strains carrying the different *cpxA* mutations. Recipient strains were *metB1 glpK*⁺ *cpxA*⁺. Met⁺ transductants were screened for amikacin resistance and inability to grow on glycerol.

For mating experiments, overnight cultures of donor and NK5148 recipient cells grown at the appropriate temperature were diluted 20-fold in LB medium. The cultures were then incubated with aeration at 41 or 34°C for 90 or 120 min, respectively; donor cell densities ranged from 1.5×10^8 to 3.4×10^8 viable cells per ml at 41°C and from 2.0×10^8 to 6.4×10^8 viable cells per ml at 34°C. Donor (0.2 ml) and recipient (1.8 ml) cultures were mixed and incubated further without agitation for 20 min. The mating mixtures were then diluted, vortexed vigorously for 40 s, and scored for Thr⁺ recombinants on minimal plates containing leucine and dextrose. Counterselection was nutritional; donor cells required arginine, histidine, thymidine, and leucine, for all of which the recipient was prototrophic. Leucine was included in the plates since, owing to the proximity of *thr* and *leu*, Thr⁺ recombinants would frequently inherit the *leu-6* mutation of the donor.

Colicin A sensitivity. Cells to be tested were applied to a petri plate as an agar overlay. Twofold serial dilutions of colicin A, covering the dilution range of 10- to 160-fold, were applied to the surface in 2-μl portions. The plates were inspected after 18 h at 41°C.

Molecular biological methods. The molecular biological methods used were essentially as described by Maniatis et al. (16).

RESULTS

Additional properties of a *cpxA2* mutant. We had used the inability of *ecfB* and related mutants to grow aerobically on succinate to argue that *cpxA* and *ecfB* were different genes (1). However, that test appears to be variable among different mutant strains (28). A less variable property of such mutants is resistance to aminoglycoside antibiotics. The *cpxA2* strain AE2038 was resistant to levels of amikacin that abolished entirely the growth of the otherwise isogenic *cpxA*⁺ strain AE2062 (Fig. 1). Moreover, a *cpxA*⁺ plasmid similar to pRA330 (3) conferred amikacin sensitivity on the *recA1 cpxA2* strain AE2072 (data not shown). The amikacin resistance determinant must therefore be very close to *cpxA* or must be *cpxA* itself.

The *cpxA* (and *eup*) deletion strain AE2122 is amikacin sensitive, suggesting that resistance is the result of altered Eup function rather than the result of no function at all (Fig. 1) (28). Similarly, whereas the *cpxA2* strain AE2038 was unable to grow at 41°C in the absence of exogenous isoleucine and valine, strain AE2122 under the same conditions grew at a rate of about half that of the *cpx*⁺ strain AE2062 (Fig. 2). The difference between the *cpxA2* and the deletion strains was more apparent on solid media; there, the deletion strain formed colonies whereas the *cpxA2* strain did not (20, 30).

A second property of *ecfB* and related mutants is a defect in proline uptake by washed cells or membrane vesicles (26, 28). Such a defect should also be manifested physiologically as the inability or reduced ability of mutant cells to utilize exogenous proline biosynthetically. At 41°C, the *cpxA2*(Ts) Δ(*lac-pro*) strain AE2146 in fact grew poorly in media containing up to 40 μg of L-proline per ml, more than enough to sustain the otherwise isogenic *cpxA*⁺ strain AE2144 at 41°C and both strains at 34°C (Fig. 3). That this property

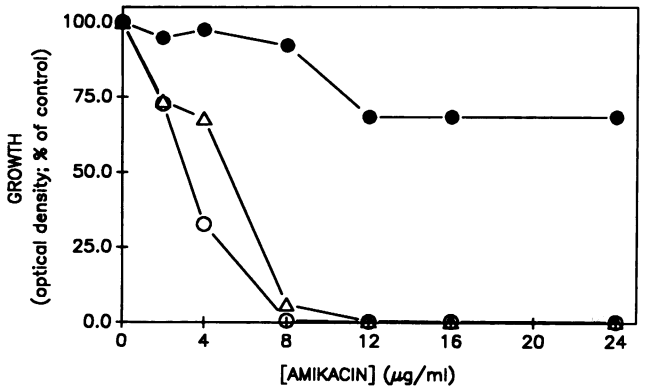

FIG. 1. Amikacin resistance of the *cpxA2 cpxB11* strain AE2038. LB medium (5 ml) containing the indicated amounts of amikacin was inoculated with AE2038 (●) (700 viable cells), AE2062 (○) (*cpxA*$^+$ *cpxB*$^+$; 1,800 viable cells), or AE2122 (△) [Δ(*cpxA*) *cpxB11*; 1,300 viable cells]. Cultures were incubated overnight at 37°C with aeration. Optical densities were then determined. Control values (no amikacin) were, in optical density units, 5.5 (AE2062), 3.8 (AE2038), and 5.3 (AE2122).

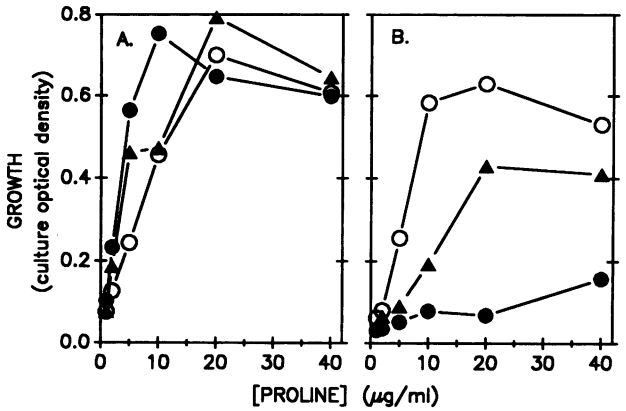

FIG. 3. Inability of a *cpxA2* mutant to utilize exogenous proline. Minimal medium (2 ml) containing the indicated amounts of L-proline was inoculated with 5 × 10^6 cells of the appropriate strain. Cultures were incubated for 24 h, at which time their optical densities were measured. (A) Incubation at 32°C; (B) incubation at 41°C. Symbols: ○, AE2144 (*cpxA*$^+$ *cpxB11*); ●, AE2146 (*cpxA2 cpxB11*); ▲, AE2282 [Δ(*cpxA*) *cpxB11*]. All three strains carried the Δ(*lac-pro*)XIII mutation.

reflects a defect in proline uptake is suggested by the fact that mutant cells at 41°C were able to grow on exogenous glycylproline (100 μg/ml) (data not shown).

Strain AE2282, from which the *proAB* and *cpxA* genes were deleted, while manifesting reduced growth at 41°C, resembled more nearly the *cpxA*$^+$ strain than the *cpxA2* mutant strain in its ability to utilize exogenous proline (Fig. 3). Thus, as with amikacin resistance and isoleucine-valine synthesis, CpxA$^-$ (Eup$^-$) mutants appear to have altered CpxA function rather than loss of function.

The amikacin resistance determinant and *cpxA* are inseparable by recombination. We used a P1 lysate of strain NK5148 to transduce the *metB1 cpxA2*(Ts) strain AE2038 to methionine prototrophy; we chose NK5148 because it appears never to have been heavily mutagenized. A total of 400 Met$^+$ transductants were screened for amikacin resistance and for an Ilv^{ts} phenotype, the latter being characteristic of existing *cpxA* mutants (19, 20). Of the total, 119 recombinants were Cpx$^+$ Ami^s and 274 were Cpx$^-$ Ami^r. The remaining seven recombinants were all replated and retested; five proved to have contained a mixture of colonies, and two retested as parental types.

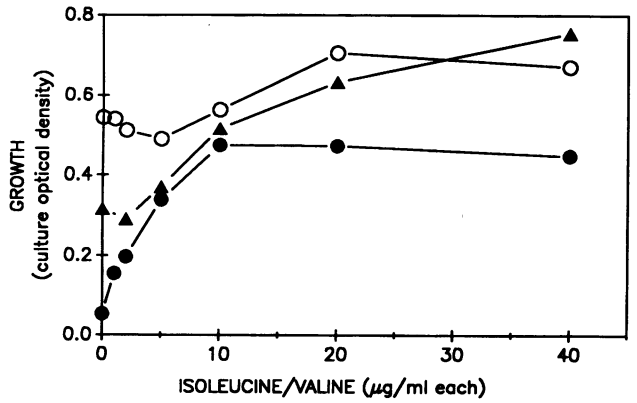

FIG. 2. Growth of *cpxA*$^+$, *cpxA2*, and *cpxA* deletion strains as functions of added isoleucine and valine levels. Minimal medium (2 ml) containing the indicated amounts of L-isoleucine and L-valine was inoculated with 5 × 10^6 cells of the appropriate strain. Cultures were incubated for 24 h at 41°C, at which time their optical densities were measured. Symbols: ○, AE2062 (*cpxA*$^+$ *cpxB*$^+$); ●, AE2038 (*cpxA2 cpxB11*); ▲, AE2122 [Δ(*cpxA*) *cpxB11*]. All three strains grew well at 34°C in the absence of exogenous isoleucine and valine (optical densities after 24 h were 0.72 for AE2062, 0.71 for AE2038, and 0.72 for AE2122).

We also carried out the converse experiment, transducing the *cpxA*$^+$ strain AE2062 with a P1 lysate of AE2038. We found no evidence for recombination separating the CpxA$^-$ (Ilv^{ts}) and amikacin resistance phenotypes among 160 Met$^+$ transductants tested (data not shown).

Isolation of new *cpxA* alleles by amikacin resistance. The *metB*$^+$ *glpK1 cpxA2*$^+$ strain AE2115 was mutagenized as described in Materials and Methods. The cells were divided immediately after mutagenesis into 10 samples, each of which was grown out and used to prepare a P1 lysate. The lysates were then used to transduce the *metB1 glpK*$^+$ *cpxA*$^+$ strain AE2062, with selection either for methionine prototrophy or for methionine prototrophy and amikacin resistance, both at 34°C. Overall, the ratio of Met$^+$ Ami^r transductants to Met$^+$ transductants was 10^{-3}. A single Met$^+$ Ami^r transductant from each transduction was repurified and retested. Six of these independently isolated Met$^+$ Ami^r strains were chosen for further analysis. None of the six could use glycerol as carbon and energy source, indicating that all received the *glpK1* allele of AE2115 as well as the *metB*$^+$ allele. All of the new mutations to amikacin resistance must therefore be closely linked to *glpK* and *cpxA*, which are only 10 to 11 kilobases apart (1). As we show below, the new mutations are very likely in *cpxA*, and we have therefore designated them as *cpxA* alleles (Table 1).

P1 lysates of the six mutants were used in backcrosses to AE2062. Met$^+$ transductants were screened for growth on glycerol and for amikacin resistance. For all six mutant alleles, the gene order was *metB-glpK-cpxA*, as expected (1, 28). Moreover, linkage data for the new alleles were generally consistent with those previously reported for the *cpxA1* and *cpxA2* alleles (Table 2); the ratios of *cpxA-glpK* linkage to *cpxA-metB* linkage varied little among the six new *cpxA* alleles (average ± standard deviation = 1.42 ± 0.08) and were comparable to the corresponding ratio for the *cpxA2* mutation (1.35).

TABLE 2. Linkage data for *cpxA* alleles

Allele	Linkage[a]	
	cpxA-glpK	*cpxA-metB*
cpxA1[b]		40
cpxA2[b]	50	37
cpxA3	64	46
cpxA4	52	34
cpxA5	48	35
cpxA6	67	50
cpxA9	62	44
cpxA10[c]	21	14

[a] Data are expressed as cotransduction frequency per 100 Met^+ transductants. Fifty transductants were scored for *cpxA* alleles *cpxA3*, *cpxA4*, *cpxA6*, and *cpxA10*; 100 for *cpxA5*; and 200 for *cpxA9*.

[b] Data for *cpxA* alleles *cpxA1* and *cpxA2* are from references 19 and 30.

[c] The data for the *cpxA10* strain are consistent with a DNA insertion or duplication between *glpK* and *cpxA*, since the linkage between *glpK* and *metB* (data not shown) was normal.

We next tested the effect of the new mutations on conjugal DNA donor activity, which was the basis for the isolation of the original *cpxA* mutations (18). Each of the six new mutations was moved by P1 transduction to the *cpxB11* Hfr strain AE1010. Appropriate isolates were then tested as donors at 41 and 34°C. Every one of the new mutations reduced donor activity (Table 3). Moreover, five of the six mutants showed a greater effect at 41°C than at 34°C, as did the *cpxA2* mutation (Table 4; 18, 19). The amikacin resistance of existing *cpx* mutants and the Cpx^- phenotype of these new amikacin-resistant mutants argue that a single gene is involved.

The effects of the different alleles on donor activity were variable, ranging from less than 1 order of magnitude (*cpxA3*) to more than 3 orders of magnitude (*cpxA6*). This observation suggests that the new mutations are not only of independent origin but in fact reflect different sequence alterations of *cpxA*. The isolation of different alleles could also be inferred from other properties of the mutants. The *recA1* derivative of each Hfr mutant was transformed with the $cpxA^+$ plasmid pOK101. Three transformants, those of the *cpxA2*, *cpxA4*, and *cpxA5* strains, were amikacin sensitive even without isopropyl-β-D-thiogalactopyranoside induction (there is a low level of constitutive *cpxA* expression from pOK101 [R. Harris, unpublished observation]). These mutant alleles are therefore recessive to the $cpxA^+$ allele. Two transformants, those of the *cpxA3* and *cpxA10* strains, remained amikacin resistant even in the presence of isopropyl-β-D-thiogalactopyranoside; these alleles are therefore dominant. pOK101 transformants of the *cpxA6* and *cpxA9* strains were resistant in the absence of isopropyl-β-D-thiogalactopyranoside but were significantly more sensitive in its presence; these alleles appear to be incompletely dominant, the phenotype perhaps depending on the ratio of mutant and wild-type CpxA protein.

In the *cpxB11* background of AE1010, all six of the new mutations conferred an Ilv^- phenotype at 41°C, similar to that of the original *cpxA1* and *cpxA2* mutants (19, 20). The *cpxA5* and *cpxA9* mutants were Ilv^- at 34°C as well, at which temperature the other mutants were Ilv^+. The *cpxA4* and *cpxA5* mutants shared with the *cpxA2* mutant impaired ability to utilize exogenous proline. The impairment was not, however, as severe as that of the *cpxA2* strain.

We also tested the *cpxA2* mutants for resistance to colicin A (15). (Colicin K tolerance was initially attributed to *ecfB*-type mutants [26]. However, the colicinogenic strain used in those experiments may have produced colicin A instead [14]. In any event, that strain has been lost [J. Suit, personal communication]). We therefore used a known $colA^+$ strain for our experiments. All of the mutants were sensitive to the colicin, as shown by an obvious zone of inhibition at the highest colicin concentration tested (see Materials and Methods). However, the zones of inhibition were markedly turbid around the *cpxA3*, *cpxA5*, *cpxA9*, and *cpxA10* strains whereas the zone around the $cpxA^+$ control was clear with a corona of reduced growth. At lower colicin concentrations, differences between the other mutants and the $cpxA^+$ strain were also evident.

TABLE 3. Donor activity of *cpxA* mutants

Strain[a]	*cpxA* allele	Donor activity[b] at:	
		41°C[c]	34°C[d]
AE1031	$cpxA^+$	100	100
AE1019	*cpxA2*	1.5	4.1
AE1177	*cpxA3*	14	14
AE1178	*cpxA4*	0.7	6.8
AE1179	*cpxA5*	0.2	1.6
AE1180	*cpxA6*	<0.1	0.9
AE1181	*cpxA9*	0.6	2.9
AE1182	*cpxA10*	3.8	317[e]

[a] All donor strains were derived from AE1010 (Table 1).

[b] Matings were carried out as described in Materials and Methods. The data are presented as: [(recombinants/donor cell)/(recombinants/AE1031 control donor cell)] × 100.

[c] Average of two experiments. The control values (AE1031 donor) were 2.0×10^{-3} and 3.9×10^{-3}.

[d] The control value was 4.1×10^{-4}.

[e] In a second experiment, this value was 50.

DISCUSSION

Mutations in gene *cpxA* have been difficult to isolate for want of a simple selection, and mutant characterization has been confined to a single allele, *cpxA2*(Ts). A second allele, *cpxA1*(Ts), exists; the properties of the *cpxA1* and *cpxA2* mutants, insofar as they were compared, appeared to be the same (18, 19).

The observation that *cpxA2* mutant strains are resistant to amikacin and the genetic evidence indicating that resistance is attributable to the *cpxA2* mutation suggested a means to obtain new mutant alleles. We were in fact able to isolate amikacin-resistant mutants that manifested defects that characterized the original *cpx* mutants. While DNA sequence determination is necessary to identify the new mutations unequivocally as being in gene *cpxA*, we believe the genetic and physiologic data in hand justify designating them as *cpxA* alleles.

Other mutations resulting in aminoglycoside resistance have been mapped to the *cpxA* region. The altered gene has been designated *ecfB*, *ssd*, or *eup*, depending on the laboratory of origin (23, 26, 29, 35). We propose that all of these mutations and the *cpxA* mutations define the same gene. We base this proposal on the fact that the *cpxA2* mutation, identified because of its effects on conjugal DNA transfer (18), also affected properties associated with *ecfB*/*ssd*/*eup* mutants (amikacin resistance and impaired proline uptake). Moreover, new mutants selected for amikacin resistance manifested properties associated with the original *cpx* mutants (defects in conjugal DNA transfer and branch chain amino acid synthesis). Genetic data placed these new mutations within the narrow interval of the chromosome that

includes the existing *cpxA*, *ecfB*, *eup*, and *ssd* mutations (19, 23, 28, 30, 35). Extensive complementation analysis is not possible because so many of the original mutants have been lost (C. Plate, personal communication). However, T. Rong-Tuan and E. B. Newman (personal communication) have shown that a $cpxA^+$ plasmid complements an *ssd* mutant for elevated *sdaA* expression (32). Finally, similar deletion strains are quasi-wild type with respect to Cpx and Eup properties (27, 30). While these data argue for a single gene, we note that *cpxA* is the 3′ gene of an operon (2) and polar mutations in the 5′ gene(s) may lead to phenotypes other than those associated with mutations in *cpxA* itself. Nevertheless, complementation data presented here and elsewhere (2, 3) indicate that many, if not all, of the mutant properties we describe can be attributed to *cpxA* mutations.

The pleiotropic effects of the *ecfB/ssd/eup* mutations were first attributed to the loss of a factor required to conduct energized protons derived from oxidative phosphorylation laterally to sites of active transport (7, 12, 26). However, the observations that deletion strains are quasi-wild type and that several of the new mutations described above are at least partly dominant argue against that interpretation. Furthermore, Krulwich and Guffanti (13) have pointed out the importance to such conduction models of high concentrations of proton-binding membrane components. The CpxA protein is not an abundant membrane component (37) and is therefore unlikely to participate directly in proton conduction.

Plate et al. (27) suggested that the *eup* gene product, under conditions of partial membrane deenergization, normally controls membrane proteins, such as the *lacY* and *putP* gene products, whose unregulated activities could further collapse the proton motive force. In this view, mutations would be analogous to dominant *cheD* alleles of the *tsr* chemosensory transducer, which apparently "lock" that protein into a signal-on conformation (25). The fact that some of the new mutations we describe above are at least partly dominant is consistent with the suggestion of Plate.

The resistance of *ecfB/ssd/eup/cpxA* mutants to aminoglycoside antibiotics can also be incorporated into a regulatory hypothesis. Resistance of *ecfB* mutants can be attributed to reduced uptake (35), which is believed to depend initially on the $\Delta\psi$ component of the proton motive force, on one or more nonsaturable redox carriers, probably quinones, functional in terminal electron transport, or on both (5, 34). Neither *eup* nor *ssd* mutations impaired the ability of cells to establish or maintain a respiration-linked proton motive force (7, 11, 26), suggesting that the effect of the mutations on aminoglycoside uptake (or on any other process) is not secondary to deficiencies in proton translocation or in aerobic electron transport. An altered regulatory mechanism is a reasonable alternative.

The structure and localization of the CpxA polypeptide are also consistent with a sensory-regulatory role. The CpxA polypeptide is a component of the inner membrane, where it is organized with intracellular and extracellular domains connected by two transmembrane segments (37). Hence, the protein is well positioned to sense and respond to an external stimulus or to changes in a property that is based on transmembrane differentials. Moreover, the CpxA protein is one in a family of bacterial sensory proteins (summarized in reference 4 and references therein). In concert with one or more cytoplasmic proteins, these sensors alter patterns of gene expression in response to environmental variability. The CpxA protein appears to be functionally paired with the cytoplasmic *sfrA* gene product (6), which is required for transcription initiation at the major *tra* promoter of the F plasmid (29; P. M. Silverman, E. Y. Wickersham, and R. L. Harris, submitted for publication). The *sfrA* gene product also represses, under anaerobic conditions, the expression of genes required only during aerobic growth; Iuchi and Lin designated this property as Arc (aerobic respiration control) (10). The Arc function of the *sfrA* gene product requires the *arcB* gene product, a membrane sensor distinct from CpxA (8). ArcB may respond directly to reduced components of the electron transport chain (S. Iuchi, Z. Matsuda, T. Fujiwara, and E. C. C. Lin, submitted for publication). CpxA affects SfrA function in response to a different, but perhaps related, signal (9; Silverman et al., submitted). The physiological basis for host control of plasmid *tra* gene expression by the *cpx* and *sfrA* gene products is unclear. However, indications that functions related to DNA donor activity transiently collapse the proton motive force (24, 38) may provide an important clue.

In summary, we propose that *cpxA* mutations are allelic with *ecfB/ssd/eup* mutations and that these mutations alter, as opposed to abolish, the function of the CpxA polypeptide as an inner membrane component. We proposed elsewhere that the CpxA polypeptide functions as a membrane sensor (37); if so, the combined data on the *cpxA* and *ecfB/ssd/eup* mutations, and on the relationship between Cpx and Arc functions (6, 9, 10), suggest that CpxA normally responds to changes in a property or process closely related to aerobic electron transport. However, we can not exclude the possibility that *cpx* mutations directly alter membrane function, conductance for example, in such a way as to disrupt a variety of cellular events. Experiments to test both possibilities are in progress.

ACKNOWLEDGMENTS

We are grateful to William Cramer, E. B. Newman, Charles Plate, and Joan Suit for essential information and materials.

This work was supported by Public Health Service grant GM38657 from the National Institutes of Health. P.M.S. acknowledges support from the Marjorie Nichlos Chair in Medical Research.

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