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Gene *cpxA* Is a New Addition to the Linkage Map of *Escherichia coli* K-12

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The *cpxA* gene of *E. coli* K-12 lies between genes *glpK* and *tpi*, closely linked to the latter at 87.8 min on the linkage map. Since no other gene has been mapped in this interval, *cpxA* is a new addition to the linkage map.

At least four chromosomal genes of *Escherichia coli* K-12 are required for the expression of DNA donor and related cellular activities associated with the conjugative plasmid F (3, 6, 8, 11). Mutations in two of these genes, *cpxA* and *cpxB*, jointly affect not only DNA donor activity (6, 8), but apparently unrelated cellular functions as well (7). In particular, *cpxA cpxB* double mutants are isoleucine and valine auxotrophs at 41°C because they fail to maintain adequate levels of acetohydroxy acid synthase I (7). This auxotrophy provided an easily scored phenotype that we used to locate *cpxA* and *cpxB* between 87 and 88 min and 40 and 41 min, respectively, on the *E. coli* K-12 genetic map (6). However, we could not determine from these studies whether or not *cpxA* mutations are alleles of a gene whose protein product is already known. This communication shows that *cpxA* lies between the *glpK* and *tpi*, very closely linked to the latter at 87.8 min. Since no other gene has been mapped in this interval, *cpxA* is a new addition to the *E. coli* K-12 map.

The genetic region relevant to the present studies is shown in Fig. 1. All of the genes except *metB* lie within the 87- to 88-min interval, and all of the known genes between *glpK* and *rha* are shown (2). The *cpxA* gene is between *glpK* and *tpi* (Fig. 1); the evidence for this placement is described below. The bacterial strains employed for these studies are described in Table 1. Genetic methods are cited in the text or follow those previously described (6). All of the recipient strains for P1 transductions carried the *cpxB1* allele (6).

Two experiments showed that *cpxA* lies between *glpK* and *rha*. In the first, a three-factor cross with a P1 lysate of AE1128 as the donor and AE2038 as the recipient indicated the gene order *metB-glpK-cpxA* and that *cpxA* and *glpK* are substantially linked to each other (Table 2). In the second experiment, AE2119, a λ^+ lysogen of the *cpxA2 cpxB1* strain AE2038, was used as the recipient for a P1 lysate prepared on ET1248, a *rha*::λ*c*I857 insertion mutant (10). Of 225 Met^+ recombinants, 12 were $cpxA^+$ and all 12 of these were rha^+. Since the presence of λ DNA in the *rha* genes failed to unlink *metB* and *cpxA*, *cpxA* lies between *glpK* and *rha*. We attribute the low frequency of cotransduction between *metB* and *cpxA* (5% in this experiment, compared with the normal frequency of about 30%) to the presence of λ DNA in the donor. Newman and Levinthal (9) observed a quantitatively similar linkage distortion with a P1 donor that contained bacteriophage Mu DNA.

I used various deletion strains to determine the relationship between *cpxA* and other genes within the *glpK-rha* interval. Some of these deletions were generated among 41°C survivors of an *rha*::λ *c*I857 insertion mutant by Pahel et al. (10). Since I was also interested in deletions generated in a *cpxB* mutant background, I constructed strain AE2121, a $glpK^+$ $cpxA^+$ *cpxB1* *rha*::λ*c*I857 recombinant of AE2115 (see Table 1). Among 800 41°C survivors of this strain selected on Luria broth plates, I identified 19 (*rha-pfkA*) deletions, as indicated by their inability to grow on mannose as a carbon source (see ref. 10). None of these lacked either *tpi*, as indicated by their ability to grow on fructose (10), or *cpxA*, as indicated by their Ilv^+ phenotype.

To examine the *cpxA* allele of (*rha-pfkA*) deletion strains in more detail, P1 lysates of several such mutants were used to infect AE2038. Met^+ recombinants were selected, and the distribution of the unselected *cpxA* and *pfkA* genes was determined. I used as donors two deletion strains previously described, DF1008 and ET2039, and one generated from AE2121, designated AE2122 (Table 1). As expected, all three strains contained a $cpxA^+$ allele between *metB* and the deletion endpoint, as shown for two of them in Table 3. In strain AE2122, *cpxA* is 80% linked to the deletion endpoint, whereas in strain DF1008, it is 55% linked. Since neither

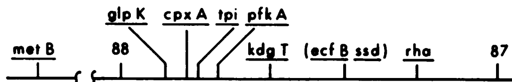

FIG. 1. The 87- to 88-min interval of the *Escherichia coli* K-12 linkage map. Gene symbols are as designated in reference 2.

deletion removed *tpi* or *cpxA*, these observations suggest that *tpi* and *cpxA* are closer to each other than either is to *pfkA*.

Strains DF443 and ET2036 both contain the same (*rha-tpi*) deletion (1). The *cpxA* genotype of these strains was examined by P1 transduction, as described above. One hundred Met$^+$ transductants of AE2038 from each deletion mutant as donor were examined; none of these was Ilv$^+$. An additional experiment of this kind set an upper limit to the ratio of Met$^+$ Ilv$^+$ recombinants to total Met$^+$ recombinants of $\leq 10^{-3}$. Hence, the (*rha-tpi*) deletion in DF443 and ET2036 may include at least part of *cpxA*. I therefore used a P1 lysate of DF443 to obtain Met$^+$ transductants of the *cpxA*$^+$ *cpxB1* strain AE2000. Among these, Ilv$^+$ and Ilv$^-$ recombinants could clearly be distinguished. However, the Ilv$^-$ phenotype of these recombinants was leakier than those of strains containing the revertible *cpxA1* or *cpxA2* alleles (6, 8), suggesting either that the deletion does not extend through the entire *cpxA* gene, or that DF443 carries a point mutation in *cpxA* that in a *cpxB1* background produces a leaky Ilv$^-$ phenotype. In any case, there was essentially complete linkage between the *cpxA*, *tpi*, and *pfkA* alleles of the donor (Table 4). The one exception noted in the

TABLE 1. Bacterial strains[a]

Strain	Relevant genotype	Source or comment
AE2038	*metB1 cpxA2 cpxB1*	*cpxA2* derivative of AE2000 (8); J. McEwen, this laboratory
AE2072	*metB1 cpxA2 cpxB1 recA1*	From AE2038 by conjugation; J. McEwen, this laboratory
AE2115	*metB*$^+$ *glpK1 cpxA*$^+$ *cpxB1*	This study; from AE2038 by P1 transduction with AE1128 as donor
AE2121	*metB*$^+$ *glpK*$^+$ *cpxA*$^+$ *rha*::λcI857 *cpxB1*	This study; from AE2115 by P1 transduction with ET1248 as donor
AE2122	*metB*$^+$ *glpK*$^+$ *cpxA*$^+$ Δ(*rha-pfkA*) *cpxB1*	This study; 41°C survivor of AE2121
AE2111	*metB*$^+$ *glpK1 cpxA2 cpxB1*	This study; from AE2038 by P1 transduction with AE1128 as donor
AE2119	*metB1 cpxA2 cpxB1* λ$^+$	Mal$^+$ λ lysogen of AE2038; L. Sambucetti, this laboratory
AE2123	*metB*$^+$ Δ(*rha-tpi*) *cpxB1*	This study; from AE2000 by P1 transduction with DF443 as donor
AE1128	Hfr *metB*$^+$ *glpK1*	CGCS 1877[b]; (5)
ET1248	F$^-$ *rha*::λcI857	D. MacNeil; (10)
ET2036	F$^-$ *metB*$^+$ Δ(*rha-tpi*)	D. Fraenkel, (10, 12)
ET2039	F$^-$ *metB*$^+$ Δ(*rha-pfkA*)	D. Fraenkel, (10, 12)
DF443	F$^-$ *metB*$^+$ Δ(*rha-tpi*)	D. Fraenkel; same Δ(*rha-tpi*) allele as ET2036; (1)
DF1008	Hfr DE200 *metB*$^+$ Δ(*rha-pfkA*)	CGSC 6193
DF456	F$^-$ *recA1 pfkA300*::Mu	CGSC 5964; (12)

[a] All strains are derivatives of *E. coli* K-12.
[b] Strains obtained from Barbara Bachmann of the *E. coli* Genetic Stock Center at Yale University are indicated by their CGSC strain numbers.

TABLE 2. P1 transduction of the *metB*, *glpK*, and *cpxA* genes

Expt[a]	Selected donor allele	Transductant genotype[b]		% of transductants[c]
		glpK	*cpxA*	
Donor: *metB*$^+$ *glpK1 cpxA*$^+$	*metB*$^+$	D	D	28.5
Recipient: *metB1 glpK*$^+$ *cpxA2*		D	R	28.5
		R	D	1.0
		R	R	42.0

[a] The donor strain was AE1128, and the recipient strain was AE2038 (see Table 1).
[b] The symbol D refers to the donor allele of the indicated gene, and the symbol R refers to the recipient allele.
[c] A total of 193 transductants were analyzed.

TABLE 3. P1 transduction of the *metB*, *cpxA*, and *pfkA* genes

Expt[a]	Selected donor allele	Transductant genotype[b]		% of transductants[c]
		cpxA	*pfkA*	
1. Donor: *metB*$^+$ *cpxA*$^+$ Δ(*pfkA-rha*) Recipient: *metB1 cpxA2 pfkA*$^+$ *rha*$^+$	*metB*$^+$	D	D	20.5
		D	R	17.0
		R	D	0.5
		R	R	62.0
2. Donor: *metB*$^+$ *cpxA*$^+$ Δ(*pfkA-rha*) Recipient: *metB1 cpxA2 pfkA*$^+$ *rha*$^+$	*metB*$^+$	D	D	33
		D	R	8
		R	D	<1
		R	R	59

[a] The donors were DF1008 and AE2122 in experiments 1 and 2, respectively. The recipient in both experiments was AE2038.
[b] The symbol D refers to the donor allele of the indicated gene, and the symbol R refers to the recipient allele.
[c] In experiment 1, 200 transductants were analyzed; in experiment 2, 100 transductants were analyzed.

TABLE 4. P1 transduction of the *metB*, *cpxA tpi*, and *pfkA* genes

Expt[a]	Selected donor allele	Transductant genotype[b]			No. of transductants[c]
		cpxA	*tpi*	*pfkA*	
Donor: *metB*$^+$ Δ(*tpi-rha*) Recipient: *metB1 cpxA*$^+$ *tpi*$^+$ *pfkA*$^+$ *rha*$^+$	*metB*$^+$	D	D	D	51
		R	D	D	1[d]
		R	R	R	148

[a] The donor was strain DF443, and the recipient was strain AE2089.
[b] The symbol D refers to the donor allele of the indicated gene, and the symbol R refers to the recipient allele.
[c] A total of 200 transductants were examined; transductant genotypes not observed were not included in the Table.
[d] See text for discussion.

Table may be the result of a suppressor mutation, as we previously described (6), or a rare recombination event separating a *cpxA* point mutant in the donor from the deletion endpoint. Either interpretation is compatible with the gene order *glpK-cpxA-tpi*. I confirmed this order by complementation analysis, as described below.

The plasmid pLC16-4 from the Clarke and Carbon *E. coli* K-12 gene bank (4) contains DNA from the *tpi-pfkA* interval (12). When transferred by F-mediated mobilization, this plasmid complements both *tpi* and *pfkA* mutations (ref. 12 and Table 5). However, the plasmid failed to complement the *cpxA2* mutation in AE2072.

TABLE 5. Complementation pattern of pLC16-4

Recipient	Selected gene	Complementation by pLC16-4[a]
AE2072	*cpxA*$^+$	0/26
AE2123	*tpi*$^+$	26/26
DF456	*pkfA*$^+$	13/14

[a] Complementation was measured by patch matings, as described (12). The donor strain was JA200/pLC16-4 (12). The ratios show the number of positive complementation responses over the number of donor colonies tested.

Since the *cpxA2* mutation is recessive (6), this experiment shows the *cpxA* is not in the *pfkA-tpi* interval and confirms the gene order *glpK-cpxA-tpi*.

The results of this study are summarized in Fig. 2. They establish *cpxA* as a new addition to

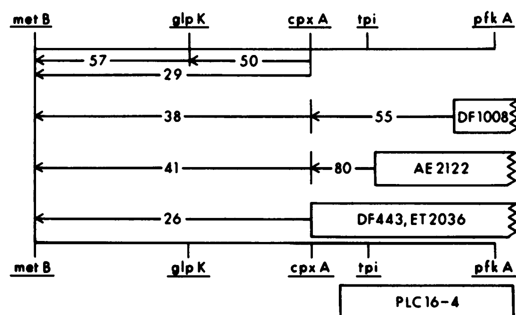

FIG. 2. Genetic localization of *cpxA*. The numbers are the percent P1 cotransduction frequencies for the genes connected by the arrows; the arrowheads point to the selected genes (see Tables 2 through 4). The serrated boxes in the middle of the figure indicate the approximate endpoints of the deletions in the strains indicated within each box. The box at the bottom of the figure indicates the approximate location of the DNA segment contained in the plasmid pLC16-4 (see Table 5).

the *E. coli* genetic map between *glpK* and *tpi*, probably very close to the latter.

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