Accumulation of the F Plasmid TraJ Protein in *cpx* Mutants of *Escherichia coli*

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We report here studies of the cellular control of F plasmid TraJ protein levels, focusing on the effects of chromosomal *cpx* mutations. The principal conclusion from our results is that the *cpx* mutations impair accumulation of the TraJ protein, thereby reducing *tra* gene expression. We measured TraJ activity in vivo by expression of a *traY'-'lacZ* fusion gene and TraJ protein by immuno-overlay blot. In strains with normal TraJ levels, *traY* expression and donor-related functions were reduced in cells carrying any of four *cpxA* mutations. In the strain background used to isolate *cpx* mutants, these reductions were especially evident in cells grown to high density, when *traY* expression and donor activity both increased in *cpx⁺* cells. In each of the four *cpxA* mutants tested, TraJ levels were lower than in the otherwise isogenic *cpxA⁺* strain. In cells grown to high density, the differences ranged from 4-fold in the *cpxA6* strain to >10-fold in the *cpxA2*, *cpxA5*, and *cpxA9* strains. The *cpxA2* mutation had little or no effect on *traY* expression or on donor-related functions when TraJ was present in excess of its limiting level in F' or Hfr cells or on a mutant *traY* promoter whose expression in vivo was independent of TraJ.

Cells of the gram-negative bacterium *Escherichia coli* receiving the F plasmid via conjugation undergo a surface morphogenesis culminating in the acquisition of conjugal DNA donor activity. Morphogenesis requires expression of the plasmid DNA transfer (*tra*) genes; presumably, the *tra* gene products assemble at the cell surface to form the organelle(s) that mediates DNA transfer (22).

F *tra* gene expression is controlled by a surprisingly complex network of plasmid- and host- (*E. coli*)-encoded proteins. Among the former is the *traJ* gene product, and among the latter are the *sfrA/arcA* and *cpx* gene products. The double mnemonic for *arcA/sfrA* recognizes the apparently independent functions of the corresponding protein (9, 12, 23): SfrA activity is required at the *traY* promoter along with TraJ activity to open expression of the 30-kb *traY-Z* gene block (25, 26); ArcA activity is required to regulate chromosomal gene expression as cells become anaerobic (11). Chromosomal gene *cpxA* is also involved in *tra* gene expression (21), but its locus of action and host cell functions are not clear. One possibility is that CpxA controls SfrA activity (9, 12). CpxA belongs to a family of membrane histidine kinases (4, 30), each of which catalyzes the phosphorylation of a genetic regulatory protein related to ArcA/SfrA (27). Since both *cpx* and *arcA/sfrA* mutations alter conjugal DNA transfer, CpxA could catalyze phosphorylation of SfrA, eliciting its regulatory activity at the *traY* promoter (9).

Several observations have suggested that this model be regarded with caution. First, *cpx* mutations affect cellular functions that *arcA/sfrA* mutations do not (14, 17, 18). Second, a *cpxA* deletion strain was quasi-wild type rather than Cpx⁻ (20); this fact is especially difficult to reconcile with the model. Third, while accumulating evidence suggests that ArcA activity is regulated by phosphorylation (10, 12), no such evidence exists for SfrA activity. In fact, the two activities appear to be independently regulated functions of

the same protein (23). Finally, the TraJ protein level was greatly reduced in one *cpx* mutant strain (6) but only moderately reduced, if at all, in SfrA⁻ mutants (6, 25). This last result suggests that the *cpx* mutations might reduce *tra* gene expression by reducing TraJ rather than SfrA activity.

Two recent results have allowed us to explore this hypothesis in more detail. The first was a simple selection for *cpxA* mutants based on low-level amikacin resistance (20). The second was the construction and characterization of plasmids containing a Φ(*traY'-'lacZ*)(Hyb) gene whose expression is linearly related to the TraJ level; these plasmids provide an in vivo assay for TraJ activity (25). Combining these two lines of investigation, we present data supporting the hypothesis that the *cpx* mutations impair TraJ accumulation in vivo.

MATERIALS AND METHODS

Strains and plasmids. All bacterial strains used in this study are derivatives of *E. coli* K-12 (Table 1). The Φ(*traY'-'lacZ*)(Hyb) plasmids pLW403 (*traJ⁺*) and pLW405 (*traJΔ15*), both low-copy-number pSC101 replicons, have been described before (25). Plasmid pRH1209 is identical to pLW405 except that the −10 and −35 hexamers of the *traY* promoter (25) were converted to the consensus sequences TATAAT and TTGACA, respectively (8). Plasmid pRH203 is a pUC19 derivative containing *tra* DNA from the *Bgl*II site in *traJ* to the *Bst*EII site near the *traY* promoter. The plasmid encodes a modified TraJ in which the N-terminal 15 amino acids were replaced by 10 amino acids encoded by the pUC19 polylinker. The altered protein complemented the *traJ90*(Am) mutation of JCFL90 (F' *lac*) for conjugal DNA donor activity and restored *traY* expression in pLW405 (TraJ⁻) cells (data not shown).

Cell growth. Cells were routinely grown aerobically in LB medium at 41°C (the nonpermissive temperature for *cpxA* mutants [15–17, 20]) and supplemented with antibiotics as appropriate. We have found it important to maintain constant conditions of aeration; for the experiments described

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TABLE 1. *E. coli* K-12 strains

Strain[a]	Relevant genotype	Source or reference
AE2074	F⁻ *cpxA⁺ cpxB⁺*	1
AE2072	F⁻ *cpxA2 cpxB11*	1
AE3192	JCFLO (F' *lac*) derivative of AE2074	L. Sambucetti
AE3189	JCFLO (F' *lac*) derivative of AE2072	L. Sambucetti
AE1031	Hfr (PO150) *cpxA⁺ cpxB⁺*	16
AE1019	Hfr (PO150) *cpxA2 cpxB11*	15
AE1184	Hfr (PO150) *cpxA⁺ cpxB⁺ recA1*	20
AE1183	Same as AE1184 except *cpxA2 cpxB11*	20
AE1187	Same as AE1183 except *cpxA5 cpxB11*	20
AE1188	Same as AE1183 except *cpxA6 cpxB11*	20
AE1189	Same as AE1183 except *cpxA9 cpxB11*	20
CT110	Hfr (PO3)	CGSC6049[b]
Hfr 61	Hfr (PO2A)	CGSC1877
KL96	Hfr (PO44)	CGSC4243
KL16-99	Hfr (PO45)	CGSC4245
AB312	Hfr (PO12)	CGSC312
KL228	Hfr (PO13)	CGSC4318
KL25	Hfr (PO46)	CGSC4244

[a] All of the strains designated AE are essentially isogenic. All are derived from the line JC355→JC411→JC1553→KL110 (3). The complete genotypes of these precursor strains can be found in references 3 and 16.

[b] CGSC designates strains from the *E. coli* Genetic Stock Center, Yale University (Barbara Bachmann, curator).

here, we used a New Brunswick G76 shaker at a speed setting of 6. Culture optical densities were measured at 600 nm. Samples with optical densities of >1 were diluted 10-fold before measurement. Low density denotes an optical density of between 0.5 and 1; high density denotes an optical density of >2. High-density cultures were never allowed to incubate indefinitely; samples were taken for analysis as the optical density reached the desired value.

Methods. β-Galactosidase assays were carried out as described before (19); activities were always expressed as Miller units (units of enzyme activity per optical density unit of culture [19]). Immuno-overlay (Western) blots were made and quantitated as described before (6) except that in one experiment, we used the ECL detection system (Amersham Life Science Products, Arlington Heights, Ill.) essentially according to the manufacturer's instructions (Fig. 1). The antibodies used in these experiments were also described in reference 6. For quantitation, exposed X-ray films were scanned with an LKB laser densitometer. Peak areas were determined by weighing paper fragments excised from the densitometer's printed output. For the experiment in Fig. 1, the TraJ level at each culture density was normalized to the amount of a protein of 40 kDa that apparently cross-reacts with the TraJ antibodies (6, 25). Conjugation experiments were carried out as described before (20).

RESULTS

traY **promoter activity in** *cpx* **mutants.** Expression of the Φ(*traY'-'lacZ*)(Hyb) gene of pLW403 and related plasmids is an in vivo measure of *traY* promoter activity (25, 26). Using such plasmids, we established the TraJ and SfrA requirement for maximal (regulated) *traY* expression. Since the *cpx* mutations led to reduced *tra* mRNA levels (21), we expected that they would lead to reduced *traY* promoter activity as well. We examined the effect of *cpx* mutations by using isogenic F' *lac* and Hfr strains containing the *traJ* deletion plasmid pLW405; β-galactosidase activity in such strains is limited by the amount of TraJ protein, derived from the F
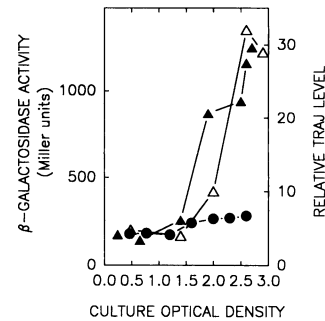

FIG. 1. *traY* expression as a function of culture density in *cpx⁺* and *cpx* mutant Hfr strains. pLW405 [*traJ*Δ15 Φ(*traY'-'lacZ*)(Hyb)] transformant cells were inoculated into LB containing kanamycin (50 μg/ml) and grown with aeration at 41°C. At the indicated times, samples were withdrawn for measurement of culture optical density and β-galactosidase activity. ▲, *cpxA⁺ cpxB⁺* Hfr strain AE1031; ●, *cpxA2 cpxB11* Hfr strain AE1019; △, relative TraJ protein level in the *cpx⁺* Hfr strain as a function of culture density. The data for TraJ levels were acquired in a separate experiment, as described in Materials and Methods.

traJ gene (25). Figure 1 shows the β-galactosidase activity of the *cpx⁺* Hfr strain AE1031/pLW405 as a function of culture density. The enzyme level was relatively low in growing cells at optical densities of <1; the value of 200 U was the lowest among eight Hfr strains we tested (Table 1; the range in pLW405 transformants of the seven other Hfr strains was 350 to 850 U). Enzyme activity began to increase as the culture optical density reached 1 and continued to increase as the cells entered the stationary phase. When net growth ceased, the enzyme level was 1,200 U, six times higher than during exponential growth; *traY* expression increased by about the same amount at high culture density of the F' *lac* strain AE3189, which is essentially isogenic with AE1031 (Fig. 2).

Importantly, Fig. 1 and 2 also show that *traY* expression in *cpxA2 cpxB11* mutant cells remained low throughout the culture growth cycle, in contrast to otherwise isogenic *cpx⁺* cells. Furthermore, while *cpx* mutant cells were poor conjugal DNA donors in low- and high-density cultures, the specific donor activity (recombinants per donor cell) of the *cpx⁺* Hfr strain increased 10-fold as the cells entered the stationary phase, whereas the specific donor activity of mutant cells remained constant or decreased somewhat (Table 2). Hence, β-galactosidase activity in these Hfr strains appears to reflect *tra* gene expression. Finally, the enzyme activity of Hfr strain AE1010 (*cpxA⁺ cpxB11*) was
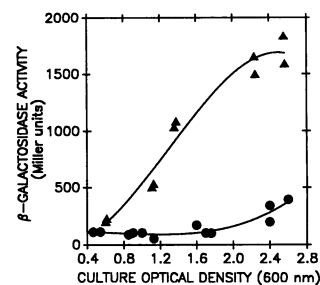

FIG. 2. *traY* expression in F' *lac* strains. The protocol was as described in the legend to Fig. 1. ▲, strain AE3192 (*cpx⁺*); ●, strain AE3189 (*cpxA2 cpxB11*).

TABLE 2. Conjugal DNA donor activity of high- and
low-density cultures

Donor strain	*cpxA* allele	Donor activity[a] (no. of recombinants/donor cell)	
		Low-density cultures[b]	High-density cultures[c]
AE1084	*cpxA*$^+$	7×10^{-3} (3.0×10^{-3})	9.7×10^{-2}
AE1083	*cpxA2*	3×10^{-5} (4.5×10^{-5})	6.8×10^{-6}

[a] Donor cells were grown to the appropriate density and mixed with recipient strain NK5148 at a volume ratio of 1:1 (donor to recipient) for low-density cultures and 1:5 for high-density cultures. After 40 min of incubation at 41°C, the cells were washed, diluted, and plated on minimal dextrose plates containing leucine. Values in parentheses are donor activities reported in reference 20.

[b] AE1184 was at 8.5×10^7 cells per ml; AE1183 was at 5.5×10^7 cells per ml.

[c] AE1184 was at 4.4×10^8 cells per ml; AE1183 was at 7.0×10^8 cells per ml.
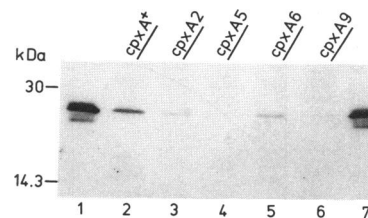

FIG. 3. Effect of *cpxA* mutations on TraJ accumulation in high-density cultures. Cultures of the indicated wild-type and mutant strains were grown in LB at 41°C to optical densities of 1.9 to 2.4. Extracts were prepared, and equal amounts of protein (40 µg) were analyzed by immuno-overlay blot developed with anti-rabbit immunoglobulin G ^{125}I-Fab fragment (6). The photograph shown was exposed for 117 h. Lanes 1 and 7 contain 3 µg of protein from a TraJ-overproducing strain.

indistinguishable from that of AE1031 (*cpxA*$^+$ *cpxB*$^+$) (data not shown), indicating that the low level of *traY* expression in high-density cultures is attributable to the *cpxA* mutation. This was also the case when donor activity itself was measured (16).

Additional *cpxA* alleles have been obtained by selecting for resistance to the aminoglycoside antibiotic amikacin (20). All the mutants had reduced DNA donor activity, especially at 41°C and in both high- and low-density cultures (see reference 20 for data on low-density cultures; specific donor activities of high-density cultures [not shown] were ≤0.02% of that of the *cpx*$^+$ control). The effects of three such mutations on *traY* expression in low- and high-density cultures are shown in Table 3. (Note that all the *cpxA* mutants also carry the *cpxB11* mutation.) For all three, *traY* expression in high-density cultures was reduced relative to that in the *cpx*$^+$ strain. In general, then, the low donor activity of *cpx* mutants can be attributed to reduced *tra* gene expression (21).

TraJ protein levels in *cpx* mutants. So far, two proteins are known to be required for a high level of *tra* gene expression in vivo; these are TraJ and ArcA/SfrA (13, 25, 26). The TraJ level in the wild-type Hfr strain grown to high density exceeded that in all four *cpxA* mutants, especially those carrying the *cpxA2*, *cpxA5*, or *cpxA9* allele (Fig. 3). Densitometric analysis indicated that these *cpxA* alleles led to reductions of at least 10-fold in TraJ accumulation in high-

TABLE 3. Effect of culture density on TraJ activity and conjugal DNA donor activity of *cpx*$^+$ and *cpx* mutant Hfr strains

Strain[a]	*cpxA* allele	TraY expression[b]	
		Low-density culture	High-density culture
AE1184	*cpxA*$^+$	152	766
AE1183	*cpxA2*	65	133
AE1187	*cpxA5*	42	106
AE1188	*cpxA6*	81	160
AE1189	*cpxA9*	36	70

[a] All strains were *recA1* and contained pLW405 [*traJ*Δ15 Φ(*traY*-'*lacZ*) (Hyb)]; all except AE1184 carried *cpxB11*.

[b] TraY expression is shown as β-galactosidase activity in Miller units. Strains lacking TraJ altogether express 30 to 70 U of activity. The level of expression in the control *cpxA*$^+$ strain appeared to be low in this experiment (compare with Fig. 1), but the cultures were sampled only at one density rather than throughout the culture growth cycle. Low-density cultures had optical densities of 0.6 to 0.7; high-density cultures had optical densities of 2.6 to 2.9.

density cultures; the reduction in the *cpxA6* mutant was 4-fold. These data generalize the results obtained with the *cpxA2* Hfr strain in exponential growth (6), indicating that the *cpx* mutations act to prevent TraJ accumulation, thereby reducing *traY* promoter activity.

In view of the data in Fig. 1 and 2, we also examined the relative TraJ levels in low- and high-density cultures. TraJ levels at several culture optical densities were quantitated by densitometry as described in Materials and Methods and in reference 6. The data were plotted in Fig. 1, where they can be compared with β-galactosidase activities. Both the magnitude and kinetics of the increase in TraJ level agree with those for enzyme activity (*traY* expression), given that the two measurements were from separate experiments. We therefore attribute the increase in *traY* expression in high-density cultures to the increase in TraJ protein.

Effect of the *cpxA2* mutation on a TraJ-independent *traY* promoter mutant. We constructed a TraJ-independent *traY* promoter mutant by changing the *traY* −10 and −35 hexamers to the consensus sequences for *E. coli* σ^{70} RNA polymerase promoters (8). The mutant promoter in a plasmid otherwise identical to pLW405, designated pRH1209, elicited the same level of β-galactosidase activity in strains containing and lacking TraJ and SfrA activity (8). Enzyme activities in the essentially isogenic strains AE3189 (F' *lac*/*cpxA*$^+$ *cpxB*$^+$), AE3192 (F' *lac*/*cpxA2* *cpxB11*), and AE2074 (F$^−$ *cpxA*$^+$ *cpxB*$^+$), each transformed with pRH1209, were indistinguishable over a range of culture optical densities (Fig. 4). Hence, the effect of the *cpxA2* allele on *traY* expression requires a promoter subject to normal TraJ/SfrA control.

***cpxA2* mutation does not reduce SfrA activity.** It remained possible that *cpxA* mutations alter the level or activity of SfrA as well as of TraJ, especially since SfrA and CpxA could constitute a two-component system (27). This appears not to be the case. In otherwise isogenic *cpxA2 cpxB11* and *cpx*$^+$ strains carrying pLW403, the *traJ*$^+$ ancestor of pLW405 (25), β-galactosidase levels were similar over a range of culture optical densities (Fig. 5). In repeated experiments, β-galactosidase activity in the mutant strain was occasionally as little as half of that in the *cpx*$^+$ strain, but if the *cpx* mutations were acting exclusively to reduce Sfr activity, then the β-galactosidase level in the *cpx* mutant strain should have been consistently much lower (25).

It is important to note that, as determined by immuno-overlay blot, the level of TraJ protein in the pLW403-carrying strain was much higher than in F' *lac* or Hfr strains,
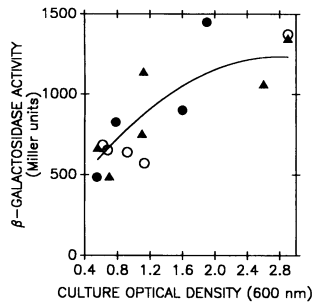
FIG. 4. Effect of *cpx* mutations on *traY* expression from a mutant promoter. The experiment was carried out as described in the legend to Fig. 1 and 2 except that all strains carried pRH1209, a derivative of pLW405 (*traJ*Δ15 [TraJ⁻]) in which the −10 and −35 hexamers of the *traY* promoter were converted to the consensus sequences (8). ▲, AE3192/pRH1209; ●, AE3189/pRH1209; ○, AE2074/pRH1209. The curve is a second-order regression line through the data for AE3192/pRH1209.

in which TraJ activity is limiting for *traY* expression (6, 25). TraJ overproduction could explain why the *cpxA2* mutation had no effect on *traY* expression in cells with pLW403. In fact, whereas the plating efficiency of donor-specific bacteriophage R17 at 41°C was 8×10^{-4} on Hfr *cpxA2 cpxB11* strain AE1183 relative to the otherwise isogenic *cpx⁺* strain AE1184, it was 0.6 when the strains carried plasmid pRH203, which encodes TraJ that is dramatically overproduced under *lac* promoter control (see Materials and Methods). This result indicates that TraJ overproduction is epistatic to the effects of the *cpxA2* mutation. We have not tested the other *cpxA* mutants.

DISCUSSION

The principal conclusion from these studies, summarized in Fig. 6, is that expression of the F plasmid *tra* genes is controlled by a network organized into two interrelated circuits. These are the TraJ/SfrA circuit, acting positively at the *traY* promoter (25), and the Cpx circuit, acting negatively on TraJ accumulation. A general assumption has been that the CpxA and ArcA/SfrA proteins function in concert as elements of a two-component system. This now appears unlikely, at least as regards *tra* gene expression. Specifically, we observed that *cpx* mutations have little or no effect on *tra* gene expression in cells with pLW403, in which *arcA/sfrA* mutations reduce expression more than 10-fold (25). This
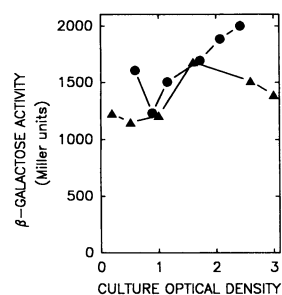

FIG. 5. Effect of *cpxA* mutations on *traY'-lacZ* expression at high TraJ levels. The protocol was as described in the legend to Fig. 1 except that the strains carried pLW403, the *traJ⁺* ancestor of pLW405 (25). ▲, AE2074 (*cpx⁺*); ●, AE2072 (*cpxA2 cpxB11*).
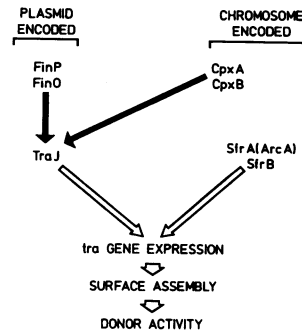

FIG. 6. Cell-plasmid network regulating F plasmid *tra* gene expression. Solid arrows indicate negative regulation; open arrows indicate positive regulation. See the text for discussion.

result is very difficult to reconcile with the idea that Sfr activity is reduced in *cpx* mutants. Instead, our results indicate that the *cpx* mutations impair accumulation of the F plasmid TraJ protein, and we believe that this is their primary effect on *tra* gene expression (21). For the *cpxA2* allele, this effect is manifested only when TraJ is limiting for *traY* promoter activity, the physiologically normal state (25). This result can be interpreted to suggest that the *cpxA2* mutation affects the TraJ protein itself rather than *traJ* expression, which is not reduced in *cpxA2* mutant cells (21, 28).

CpxA is an integral membrane protein with strong homologies to histidine kinases (27, 30). Like those enzymes, CpxA catalyzes an autophosphorylation reaction (4), and it is reasonable to suppose that transphosphorylation of other proteins by phospho-CpxA is involved in CpxA function. Whether or not those proteins are canonical response regulators remains to be determined. If, for example, CpxA directly catalyzes phosphotransfer to TraJ, it must function outside the histidine kinase/response regulator motif, because we could find no primary structural homologies between TraJ and response regulators. Otherwise, there must be at least one response regulator that functions in concert with CpxA and which has yet to be discovered. Conceivably, *cpxB* encodes such a protein.

As selected, Cpx⁻ mutants have not lost CpxA function; in fact, a *cpxA* deletion strain proved to be quasi-wild type (20). We attribute the defects associated with *cpxA* mutations to altered CpxA protein function rather than loss of function. Ongoing studies support this view. Sequence analyses have revealed single point mutations in the 5′ segments of *cpxA* amplified from the *cpxA2* and *cpxA9* strains (24). The corresponding amino acid changes both occur in the periplasmic domain of the protein (30). This location suggests that the two alleles lead to a "signal-on" or "locked" configuration of the CpxA protein, analogous to the effect of some mutations in the periplasmic domain of the Tar chemosensory transducer (2). According to this interpretation, mutant CpxA proteins function continuously to phosphorylate substrate proteins. If this is so, the CpxA regulatory circuit must normally function negatively on *tra* gene expression, in contrast to the circuit that includes the SfrA protein (Fig. 6), and is presumably activated by one or more signals, as yet unknown. Except for the well-known F⁻ phenocopy phenomenon, the conjugal DNA donor activity of F⁺ strains appears to be largely constitutive (7).

The density-dependent increases in TraJ protein level and *tra* gene expression that we observed were unexpected and

deserve comment. These phenomena were unique to AE1031 and its derivatives among eight Hfr strains examined. Importantly, however, the Cpx⁻ phenotype is not restricted to strains of the AE1031 background; the *cpxA2* and *cpxB11* alleles in other strain backgrounds were accompanied by at least some and perhaps all components of the Cpx⁻ phenotype (14, 18). As to the origin of the phenomenon, we note that a donor strain isolated by A. J. Clark and designated JC12 was shown to express donor functions maximally as the cells entered the stationary phase (29). JC355, which provided the genetic background for the *cpx* mutants (15, 16, 20), was itself derived from a conjugal cross involving JC12 (3). Accordingly, we suspect that JC12 contains a mutation that leads to elevated *tra* gene expression in cells entering the stationary phase. Possibly, the mutation alters physiologically normal changes in DNA topology to which *tra* gene expression is sensitive (8).

ACKNOWLEDGMENTS

REFERENCES

1. **Albin, R., and P. Silverman.** 1984. Physical and genetic structure of the *glpK-cpxA* interval of the *Escherichia coli* K-12 chromosome. Mol. Gen. Genet. **197**:261–267.
2. **Ames, P., and J. Parkinson.** 1988. Transmembrane signaling by bacterial chemoreceptors: *E. coli* transducers with locked signal output. Cell **55**:817–826.
3. **Bachmann, B.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
4. **Britt, M., and P. Silverman.** Unpublished data.
5. **Broda, P.** 1975. Transience of the donor state in an *Escherichia coli* K12 strain carrying a repressed R factor. Mol. Gen. Genet. **138**:65–69.
6. **Cuozzo, M., and P. Silverman.** 1986. Characterization of the F plasmid TraJ protein synthesized in F' and Hfr strains of *Escherichia coli* K12. J. Biol. Chem. **261**:5175–5179.
7. **Curtiss, R., III, L. Caro, D. Allison, and D. Stallions.** 1969. Early stages of conjugation in *Escherichia coli*. J. Bacteriol. **100**:1091–1104.
8. **Gaudin, H., and P. Silverman.** Contributions of context and structure to regulated expression of the F plasmid *traY* promoter in *Escherichia coli* K12. Mol. Microbiol., in press.
9. **Iuchi, S., D. Furlong, and E. Lin.** 1989. Differentiation of *arcA*, *arcB*, and *cpxA* mutant phenotypes of *Escherichia coli* by sex pilus formation and enzyme regulation. J. Bacteriol. **171**:2889–2893.
10. **Iuchi, S., and E. Lin.** 1992. Purification and phosphorylation of the Arc regulatory components of *Escherichia coli*. J. Bacteriol. **174**:5617–5623.
11. **Iuchi, S., and E. Lin.** 1988. *arcA* (*dye*), a global regulatory gene in *Escherichia coli* mediating repression of enzymes in aerobic pathways. Proc. Natl. Acad. Sci. USA **85**:1888–1892.
12. **Iuchi, S., Z. Matsuda, T. Fujiwara, and E. Lin.** 1990. The *arcB* gene of *Escherichia coli* encodes a sensor-regulator protein for anaerobic repression of the *arc* modulon. Mol. Microbiol. **4**:715–727.
13. **Maneewannakul, K., S. Maneewannakul, and K. Ippen-Ihler.** 1992. Sequence alterations affecting F plasmid transfer gene expression: a conjugation system dependent on transcription by the RNA polymerase of phage T7. Mol. Microbiol. **6**:2961–2973.
14. **McEwen, J., L. Sambucetti, and P. Silverman.** 1983. Synthesis and translocation of outer membrane proteins in *cpxA* and *cpxB* mutants of *Escherichia coli* K12. J. Bacteriol. **154**:375–382.
15. **McEwen, J., and P. Silverman.** 1980. Chromosomal mutations of *Escherichia coli* K12 alter expression of conjugative plasmid functions. Proc. Natl. Acad. Sci. USA **77**:513–517.
16. **McEwen, J., and P. Silverman.** 1980. Genetic analysis of *Escherichia coli* K-12 chromosomal mutants defective in the expression of F plasmid functions: identification of genes *cpxA* and *cpxB*. J. Bacteriol. **144**:60–67.
17. **McEwen, J., and P. Silverman.** 1980. Mutations in genes *cpxA* and *cpxB* of *Escherichia coli* K-12 cause a defect in isoleucine and valine synthesis. J. Bacteriol. **144**:68–73.
18. **McEwen, J., and P. Silverman.** 1982. Mutations in genes *cpxA* and *cpxB* alter the protein composition of the *Escherichia coli* K-12 inner and outer membranes. J. Bacteriol. **144**:1553–1559.
19. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
20. **Rainwater, S., and P. Silverman.** 1990. The Cpx proteins of *Escherichia coli* K-12: evidence that *cpxA*, *ecfB*, *ssd*, and *eup* all identify the same gene. J. Bacteriol. **172**:2456–2461.
21. **Sambucetti, L., L. Eoyang, and P. Silverman.** 1982. Cellular control of conjugation in *Escherichia coli* K12: effect of chromosomal mutations on F plasmid gene expression. J. Mol. Biol. **161**:13–31.
22. **Silverman, P.** 1987. The structural basis of prokaryotic DNA transfer, p. 277–310. *In* M. Inouye (ed.), Bacterial outer membranes as model systems. John Wiley & Sons, Inc., New York.
23. **Silverman, P., S. Rother, and H. Gaudin.** 1991. Arc and Sfr functions of the *Escherichia coli* K-12 *arcA* gene product are genetically and physiologically separable. J. Bacteriol. **173**:5648–5652.
24. **Silverman, P., and E. Wickersham.** Unpublished data.
25. **Silverman, P., E. Wickersham, and R. Harris.** 1991. Regulation of the F plasmid *traY* promoter by host and plasmid factors. J. Mol. Biol. **218**:119–128.
26. **Silverman, P., E. Wickersham, S. Rainwater, and R. Harris.** 1991. Regulation of the F plasmid *traY* promoter in *Escherichia coli* K12 as a function of sequence context. J. Mol. Biol. **220**:271–279.
27. **Stock, J., A. Ninfa, and A. Stock.** 1989. Protein phosphorylation and regulation of adaptive responses in bacteria. Microbiol. Rev. **53**:450–490.
28. **Tran, L., and P. Silverman.** Unpublished observations.
29. **Valentine, R., P. Silverman, K. Ippen, and H. Mobach.** 1969. The F-pilus of *Escherichia coli*. Adv. Microb. Physiol. **3**:1–52.
30. **Weber, R., and P. Silverman.** 1988. The Cpx proteins of *Escherichia coli* K12: structure of the CpxA polypeptide as an inner membrane component. J. Mol. Biol. **203**:467–478.
31. **Willetts, N., and R. Skurray.** 1980. The conjugative system of F-like plasmids. Annu. Rev. Genet. **14**:41–76.