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sfrA and *sfrB* Products of *Escherichia coli* K-12 Are Transcriptional Control Factors

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The mechanisms whereby mutations in *Escherichia coli* K-12 genes *sfrA* and *sfrB* reduce expression of the transfer functions of sex factor F have been examined by assaying the levels of *tra* messenger ribonucleic acid and of *tra* proteins. The *sfrA* product was necessary for efficient transcription of the control gene *traJ* and, directly or indirectly, for transcription of the *traY→Z* operon. In the absence of *sfrA*, reduced levels of the *traJ* and *traT* proteins were observed in the outer membrane. The *sfrB* product was needed to prevent premature transcription at one or more *rho*-dependent termination sites. *sfrB* mutations also reduced synthesis of full-length lipopolysaccharide molecules, of several chromosomally determined outer membrane proteins, and of functional flagella. Thus, the *sfrB* product may act as an antiterminator in transcription of several operons encoding cell envelope components.

The transfer of plasmid DNA by conjugation has been intensively investigated for the *Escherichia coli* K-12 sex factor F (12, 17). The series of stages required before DNA transfer can occur include recognition by the donor cell's F pilus of recipient cells, formation of stable mating aggregates, and triggering of specific DNA replication in the donor cells. DNA transfer occurs unidirectionally from donor to recipient because donor cells are prevented from acting as recipients by the surface exclusion proteins (3). Most of the various proteins responsible for these events are encoded by a series of transfer (*tra*) cistrons on the F sex factor and are located in the cell envelope (2). With a few exceptions, the *tra* cistrons are all included in a single 33-kilobase operon (8, 9, 14), now called the *traY→Z* operon (17; Fig. 1). The *traJ* cistron lies outside the *traY→Z* operon, and the *traJ* protein is somehow involved in the positive control of transcription of this operon (15). Consequently, *traJ* mutations are pleiotropic and result in the lack of synthesis of the various *tra* proteins (3). However, the *traJ* protein is located in the outer membrane of the cell envelope (2, 3), an unexpected location for a regulatory protein. Also, synthesis of *tra* operon proteins by chimeric plasmid pRS27 (pSC101 *traMJYALEKBV*) was apparently *traJ* independent in minicells or in vitro (10). This might reflect *traJ*-independent transcription from a nearby vector promoter, or it might indicate that transcription is only regulated by the *traJ* protein when other cell components, absent from minicells or the in vitro protein-synthesizing system, are present. It was therefore suggested (12) that the *traJ* protein may act only indirectly in cells to allow transcription of the *traY→Z* operon and that regulation depends on a currently unidentified, cytoplasmic regulatory protein. It is most unlikely that the F factor itself encodes any such regulatory protein (3), but two *E. coli* chromosomal genes (*sfrA* and *sfrB*) have recently been identified, whose products are also needed for maximal expression of the different *tra* proteins involved in F pilus synthesis and surface exclusion (7). *sfrB* mutants were also defective in certain other cell envelope-associated properties, namely, the synthesis of functional flagella and adsorption of the lipopolysaccharide-specific bacteriophages U3 and C21. All 12 *sfrA* or *sfrB* mutations examined were leaky, suggesting that more stringent mutations might be deleterious to cell growth or viability. The *sfrA* and *sfrB* products might directly affect interactions within the cell envelope or might regulate transcription of operons encoding *tra* and other cell envelope components. We have therefore analyzed the effects of *sfrA* and *sfrB* mutations in more detail. The results presented here show that the *sfrA* and *sfrB* products are novel transcriptional control factors.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* K-12 strains used have been described previously (7), with the exception of those carrying a *rho* mutation. Strain AD1704 carries a *rho* mutation called *rho-15*(Ts) and a closely

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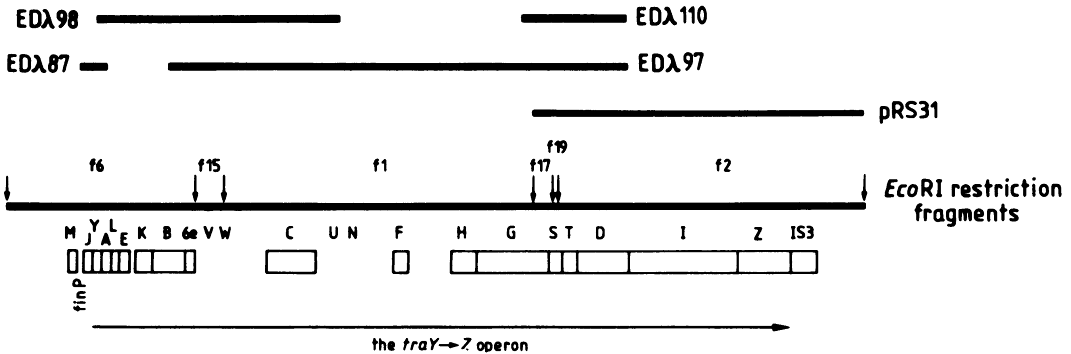

FIG. 1. *The F transfer region. The tra sizes are shown below the genetic map (12, 17), which is drawn to scale, and the lengths and locations of the tra segments carried by the λ tra transducing phages and pRS31 are shown above the map.*

linked mutation leading to valine resistance (6). This strain is temperature sensitive for growth, a property which was ascribed to the *rho* mutation. When a bacteriophage P1 lysate grown on AD1704 was used to transduce strain JC3272 to valine resistance, only half of the transductants were temperature sensitive, and most of the remainder carried a *rho* mutation but remained temperature insensitive for growth. Two mutants of F*lac* were separately introduced by conjugation into the latter transductants to demonstrate *rho* relief of transcriptional polarity: (i) F*lac* carrying the polar IS*1* insertion mutation MS319 in *lacZ* (11) expressed the *lacY* cistron, as manifested by growth on melibiose at 42°C; and (ii) the F*lac* mutant JCFL4 carrying the strongly polar *traK4* mutation (3, 5) expressed the *traS* and *traT* cistrons, as manifested by regained surface exclusion. We conclude therefore that strain AD1704 carries two closely linked mutations, one resulting in temperature sensitivity, and the other, *rho-15*, accounting for relief of polarity. Phage P1 grown on a temperature-insensitive *rho-15* derivative of strain JC3272 was used to transduce *rho-15* to other strains, selecting for valine resistance.

Bacterial plasmids and phages. Plasmid pRS31 is pSC101, carrying *Eco*RI fragments f17, f19, and f2 of F (4; Fig. 1). Phage EDλ4 (λ *c*I857 *S*am7 *b*515 *b*519) and the λ *tra* phages derived from it have been described previously (13, 15, 16; D. Johnson and N. S. Willetts, manuscript in preparation; Fig. 1). Bacteriophages U3 and C21 were from the Berlin laboratory collection.

Hybridization analysis. The preparation of λ phages and phage DNA and the hybridization methodology were as described previously (15). Hybridizations with RNA isolated from cells pulse-labeled with [^{3}H]uridine were to 0-, 5-, 7.5-, and 10-μg amounts of λ *tra* DNA immobilized on membrane filters. The plateau values were read from the resultant curves drawn after subtracting the background level of radioactivity on the filters without DNA.

Visualization of cell envelope proteins. Bacterial cell envelopes were isolated by centrifugation after disruption by sonication (1). Triton X-100-insoluble and -soluble fractions of the cell envelope were prepared and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, using 11 to 20% gradients of acrylamide (1). Gels were fixed in 50% (wt/vol) trichloroacetic acid for 30 min, stained overnight in 0.06% (wt/vol) Coomassie brilliant blue G250 in 3.5% (wt/vol) perchloric acid, and destained in 5% (vol/vol) acetic acid. To demonstrate lipopolysaccharide trapping of the dye, the gels were photographed within the first 2 h after destaining was begun.

RESULTS

Transcriptional analysis of *sfrA* mutants. mRNA from *sfr*$^+$, *sfrA*, or *sfrB* cells carrying an F prime plasmid was pulse-labeled with [^{3}H]-uridine and hybridized to DNA prepared from a set of λ *tra* transducing phages covering the transfer region from *traJ* to *traD* (Fig. 1). The levels of mRNA corresponding to these *tra* segments were determined as percentages of the total amounts of pulse-labeled RNA in the cells (Table 1).

Hybridization of mRNA from *sfrA4* or *sfrA5* bacteria was reduced approximately sixfold relative to *sfr*$^+$ bacteria for all three λ *tra* phages tested (except for *sfrA5* versus EDλ98 which gave even lower values). Thus, *sfrA* mutations on the bacterial chromosome resulted in lessened transcription of the F factor *tra* operon. The results with phage EDλ87 [λ *tra(Y)(A)J(Y)*; Table 1] allowed us to estimate how efficiently *traJ* was transcribed. Phage EDλ87 contains the 0.7-kilobase *traJ* cistron plus 0.9 kilobases of *traY→Z* operon DNA (Johnson and Willetts, manuscript in preparation). We assumed that the transcriptional rate of this 0.9-kilobase segment is the same as that for the 11 kilobases of *traY→Z* operon DNA present in phage EDλ98. Thus, the contribution of *traY→Z* DNA in phage EDλ87 could be subtracted from the total hybridization observed. These calculations revealed that *traJ* is transcribed in *sfr*$^+$ cells about fourfold more efficiently (per unit length) than the *traY→Z* operon. We noted that the translational rate of *traJ* protein in minicells was

TABLE 1. *Hybridization of tra mRNA to λ tra DNA*

DNA probe	*tra* Cistrons[b]	Plateau values[a]						
		sfr[+c]	*sfrA4*	*sfrB11*	%	*sfrA5*	*sfrB13*	%
EDλ4	None	0.010	0.007			0.010		
EDλ87	*tra(Y)(A)J(Y)*	0.107	0.024		18	0.038		29
EDλ98	*tra(Y)ALEKBVWCU*	0.303	0.049		14	0.025		5
EDλ97	*tra(B)VWCUNFHGSTD*	0.382	0.072		18	0.073		17
EDλ4	None	0.010		0.008			0.011	
EDλ87	*tra(Y)(A)J(Y)*	0.093		0.109	122		0.079	82
EDλ98	*tra(Y)ALEKBVWCU*	0.312		0.229	73		0.243	77
EDλ97	*tra(B)VWCUNFHGSTD*	0.405		0.165	40		0.188	45
EDλ110	*tra(G)STD*	0.098		ND[d]	ND		0.03	22

[a] The figures give the plateau values read from hybridization curves constructed by using different amounts of λ *tra* DNA, expressed as percentages of the total labeled RNA. The amounts of *tra* mRNA in the *sfr* mutants are also given as percentages of the amounts in sfr^+ cells after subtracting the EDλ4 background values. Labeled RNA (0.4×10^6 to 1.4×10^6 cpm) was added to each hybridization vial. Control filters with no DNA bound an average of 0.007% of the radioactivity added.

[b] Parentheses indicate that only a part of the *tra* gene is carried, i.e., EDλ98 carries only part of *traY*, EDλ97 carries only part of *traB*, etc. EDλ87 was derived from a lambda insertion into *traY* by an illegitimate recombination event between sites immediately to the left of *traJ* and within *traA*. Thus, *traY* is split into two segments within EDλ87.

[c] Labeled RNA was prepared from derivatives of the lambda-free strains M1986 (for *sfrA* mutations carrying F*lac*) or M3960 (for *sfrB* mutations carrying F*trp*).

[d] ND, Not done.

higher than that of most *tra* cistrons (10). Furthermore, the transcription of *traJ* was reduced on the average about threefold in the *sfrA* mutants relative to the sfr^+ cells. We concluded that the *sfrA* product is required for efficient transcription of the *traJ* gene. Since *traY→Z* operon transcription is *traJ* dependent (11), the sixfold-reduced level of operon transcription observed might result directly from the expected reduced level of the *traJ* protein. Alternatively or in addition, the *sfrA* product might be directly required for transcriptic f the *traY→Z* operon.

In either event, it would be expected that transcription of *traY→Z* operon genes from a different promoter which is independent of *traJ* should be insensitive to *sfrA* mutations. This was tested by using the chimeric plasmid pRS31 which carries the *tra* cistrons *S*, *T*, *D*, *I*, and *Z* cloned in plasmid pSC101 (Fig. 1) and transcribed (presumably) from a promoter in pSC101 (4, 10). The *traS* and *traT* cistrons together encode surface exclusion, a function which is dependent upon the gene copy number (1, 3). As predicted, plasmid pRS31 (unlike F*lac*) expressed surface exclusion as efficiently in *sfrA* mutants as in sfr^+ cells (Table 2).

TABLE 2. *Dependence of the SfrA⁻ and SfrB⁻ phenotypes on the traY→Z operon promoter*

Mutation	Surface exclusion index of cells carrying[a]:	
	F*lac*	pRS31
sfr^+	390	5,500
sfrA5	20	9,500
sfrA8	12	4,200
sfrB11	13	≥13,000
sfrB13	3.5	13,000

[a] Derivatives of JC3272 were tested in crosses with Hfr M2311 (7) to determine the surface exclusion indices (the reduction in the number of recombinants relative to the number obtained with JC3272 itself).

Transcriptional analysis of *sfrB* mutations. In contrast to *sfrA* mutants, the level of mRNA hybridizing to phage EDλ87 in *sfrB11* and *sfrB13* mutants was not reduced, showing that *traJ* is transcribed at normal levels. However, hybridization to DNA from λ *tra* phages carrying various segments of the *traY→Z* operon was reduced, and the extent of the reduction increased as the distance from the *traY→Z* operon promoter increased. Thus, transcription of the *tra(G)STD* region carried by phage EDλ110 was reduced most, to about one-fifth of the level observed in sfr^+ cells. We propose, therefore, that the $srfB^+$ product forms a part of an antitermination mechanism which normally ensures complete transcription of the *traY→Z* operon. In its absence, transcription is terminated prematurely at one or more relatively inefficient termination sites within the operon. Since surface exclusion encoded by plasmid pRS31 was unaffected in *sfrB* cells (Table 2), we concluded that either there are no termination sites promoter proximal to the *traS* and *traT* cistrons in the cloned *tra* DNA segment, or the putative

sfrB antitermination product is similar to the λ bacteriophage N protein in that its effect is promoter dependent.

If our explanation is correct, then transcriptional termination in the absence of the *sfrB* product might or might not be *rho* dependent (6). However, if the effects of *sfrB* mutations were reversed by *rho* mutations, this would supply strong evidence for the putative antitermination. Therefore, the *rho-15* mutation (see above) was introduced into all six *sfrB* mutants. Presence of the *rho-15* mutation was confirmed by demonstrating relief of polarity of the *lacZ*:: IS*1* mutation MS319 and continued presence of the *sfrB* mutations by the characteristic resistance to U3 and sensitivity to C21 bacteriophages that they determine. Although *sfrB* and *rho* are both separately cotransducible with *metE*, they were not cotransducible with each other because none (≤1%) of the *rho-15* transductants had become $sfrB^+$. In confirmation, *sfrB* mutations from selected *rho-15* transductants could be cotransduced with $metE^+$ at the usual frequency of 20% into a $MetE^-$ strain. The tests could now be performed to determine whether *rho-15* suppressed the effects of *sfrB* mutations on transfer functions by introducing F*lac* plasmids into the *rho-15* sfr^+ or *rho-15 sfrB* cells. In all cases, these transconjugants synthesized F pili (as measured by sensitivity to F-specific bacteriophages). They were as efficient donors of F*lac* as an sfr^+ rho^+ strain and had regained intermediate levels of surface exclusion (data not shown). Based on the hybridization data and on these results, it does indeed seem that the *sfrB* product causes antitermination. In the absence of the *sfrB* product, termination within the *tra* operon is at least partly *rho* dependent. In contrast, the effects of *sfrB* mutations on cell envelope lipopolysaccharides (as tested with the phages U3 and C21) were not *rho* dependent (see above). Finally, *sfrA* mutations were not suppressed by the *rho-15* mutation.

Analysis of cell envelope components. Based on the above results, *sfrA* cells carrying F*lac* were expected to possess less *traJ* and *traT* protein, whereas *srfB* cells carrying F*lac* were expected to possess less *traT* protein in rho^+, but not in *rho*, cells. For the analysis, cells were broken by sonication, and the cell envelope was separated from the cytoplasm by centrifugation. The envelope was separated into Triton X-100-soluble inner membrane proteins and Triton X-100-insoluble outer membrane proteins. All three fractions were analyzed by sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis, but the only differences noted were in the outer membrane fraction. Of the numerous *tra* proteins encoded by F DNA, those encoded by *traJ* and *traT* (both outer membrane proteins) can be readily detected by these techniques (1, 3).

As predicted, there were undetectable amounts of the *traJ* protein and reduced amounts of the *traT* protein in the outer membranes of *sfrA* mutants (Fig. 2). No other significant differences were noted. *sfrB* mutants possessed normal levels of the *traJ* protein but greatly reduced levels of the *traT* protein (Fig. 2). Furthermore, the *rho-15* mutation restored (at least in part) the level of the *traT* protein in *sfrB* (but not in *sfrA*) cells (Fig. 2). Minor changes in the concentration of a few chromosomally encoded outer membrane proteins were also produced by *sfrB* mutations. A dramatic change was seen in the region where lipopolysaccharide migrates (bracketed area in Fig. 2). Lipopolysaccharide traps Coomassie brilliant blue G250 and can be detected in polyacrylamide gels before destaining is complete. The lipopolysaccharide in *sfrB* mutants was probably shorter than normal because it migrated more quickly. This effect was not prevented by the *rho-15* mutation, in agreement with the unchanged abilities of bacteriophages C21 and U3 to plate (or not) on *rho-15 sfrB* hosts (see above).

DISCUSSION

These results allow the recognition of the *sfrA* and *sfrB* products as novel transcriptional con-

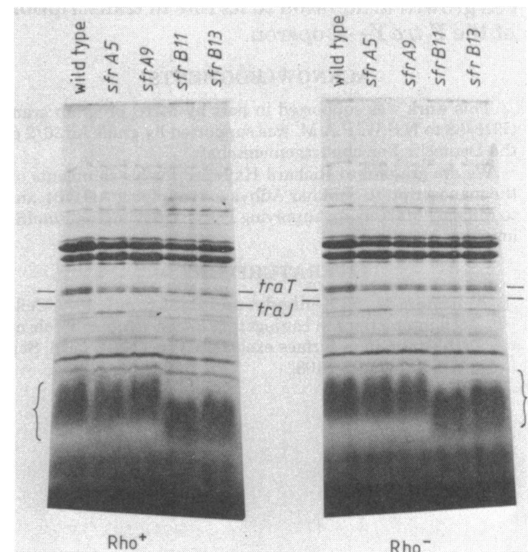

FIG. 2. *Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of Triton X-100-insoluble outer membranes isolated from JC3272 (Flac) derivatives that were his^+ and carried the sfr and rho mutations indicated.*

trol factors. The *sfrA* product is needed for efficient transcription of *traJ*. Because *traJ* mutations prevent transcription of the *traY→Z* operon (15), the reduced synthesis of *traJ* mRNA in *sfrA* cells can account for reduced transcription of the *traY→Z* operon. However, the possibility has not been excluded that the *sfrA* product is also directly needed for transcription of the main operon and forms a part of the control mechanism which starts its transcription when *traJ* protein is present. It is not clear how or why control of the plasmid *tra* gene by a chromosomal gene may have arisen or what other roles the *sfrA* product may play in the bacterial cell.

The *sfrB* product was needed to prevent premature *rho*-dependent transcription termination at one or more inefficient sites within the *traY→Z* operon. *sfrB* mutations affected the efficient synthesis of full-length lipopolysaccharide molecules, of several outer membrane proteins, and of functional flagella (7; Fig. 2). The latter effects could not be suppressed by the *rho-15* mutation. These observations suggest that if the *sfrB* product is a normal antiterminator for operons encoding cell envelope components, then in most cases the termination is *rho* independent. Cells carrying *sfrB* mutations grew more slowly than did sfr^+ cells, and all the *sfrB* mutations available were leaky (7). The *sfrB* product might therefore be required for transcription of cell envelope operons essential for cell growth in addition to its role in transcription of the F *traY→Z* operon.

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