Dye Sensitivity Correlated with Envelope Protein Changes in dye (sfrA) Mutants of Escherichia coli K12 Defective in the Expression of the Sex Factor F

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Mutations of the dye gene on the E. coli chromosome result in sensitivity to the dye toluidine blue and, in male cells, cause loss of F pili, producing sterility in conjugation. Compared with its dye+ parent, a strain deleted for dye (Δdye) showed an altered sensitivity to a wide range of dyes and antibiotics which affect different intracellular processes, and hence it appeared likely that the barrier properties of the cell envelope were impaired. Unlike mutants known to be defective in LPS structure, there appeared to be no correlation between the hydrophobicity of the compounds and the sensitivity of the Δdye strain. Moreover there was no difference between dye+ and Δdye strains in their sensitivity to LPS-specific phages, and chemical and GLC analysis of LPS components revealed no difference between the two strains. Examination of outer and inner membrane proteins from isogenic strains having the Δdye deletion and the dye+ gene cloned into the plasmid pACYC184, with or without insertional inactivation of dye by the transposon γ6, was performed by SDS-PAGE. This revealed a number of differences in the profile of proteins from both inner and outer membranes, correlated with mutation in the dye gene. The dye gene appears to be identical to the sfrA gene, which has been shown to be required for efficient transcription of the sex factor F. It is therefore proposed that the dye (sfrA) gene product may also control the expression of chromosomal genes coding for envelope proteins.

INTRODUCTION

Deletions of the Escherichia coli K12 chromosome between trpR and thr at 99-100 min result in sensitivity to the dye toluidine blue (Dye− phenotype) (Roeder & Somerville, 1979) and, in male cells (Hfr or F-prime), cause loss of F-pili and hence sterility in conjugation and resistance to male-specific phages (Fex− phenotype) (Buxton et al., 1978). A 6 kb SalI fragment which carries the dye+ gene, and which complements both the Dye− and Fex− phenotypes, has been cloned into the plasmids pBR322 and pACYC184. By insertional inactivation of dye with the transposon γ6 (Tn1000), we demonstrated that the Dye− and Fex− phenotypes are caused by mutation in a single cistron (Buxton & Drury, 1983). We have also shown that the dye mutation lies in the same complementation group as the previously isolated sfrA mutation, isolated as defective in sex factor F expression (Beutin & Achtman, 1979). The sfrA gene product has been shown to be necessary for efficient transcription of F-factor tra genes (Beutin et al., 1981).

Since increased sensitivity to dyes and antibiotics which cause inhibition of growth by intracellular processes is often correlated with changes in the permeability properties of the cell envelope, notably in the outer membrane of Gram-negative bacteria (see, for example, Nikaido, 1979 for a review), we have examined the effects of the dye mutation on the components of the cell envelope. The results presented below show that loss of the dye gene product causes alteration in the protein composition of the cell envelope. To explain why mutation of dye (sfrA) results in these two very different cellular changes, viz. alterations in cell envelope proteins and reduced F-factor gene transcription, we suggest that the dye gene product, besides being a
controlling element in the expression of the F-factor genes, also controls expression of genes coding for envelope proteins.

**METHODS**

**Bacterial strains and plasmids.** The strains of *E. coli* K12 used are listed in Table 1. Plasmid pRB38 is pACYC184 (Chang & Cohen, 1978) into which has been cloned a 6 kb SalI fragment carrying the dye+ gene. pRB40 has 2.7 kb of this cloned DNA cleaved out between two KpnI sites, but is still dye+. pRB51 has y0 (Tn1000) inserted into dye and hence is Dye-. pRB47 has y0 inserted into the promoter-distal end of dye and thus makes a truncated Dye protein, but is still phenotypically Dye+ (Buxton & Drury, unpublished results).

**Bacteriophage strains.** The LPS-specific phages U3 and C21 were obtained from Professors P. Reeves and A. D. Stocker, and Br2 and Br10 from Professor Stocker.

**Media.** Bacteria were grown in L-broth, which contained per litre of distilled water: Difco Bacto-tryptone, 10 g; Difco Bacto yeast extract, 5 g; NaCl, 10 g. It was solidified where necessary with 1.6% (w/v) Difco agar, or 0.8% (w/v) for soft top agar.

**Dye, antibiotic and detergent sensitivity tests.** These were performed on overnight L-broth cultures grown at 32 °C without aeration and diluted to the same OD₆₀₀ (~0.2). A 0.1 ml volume was overlayed in L-broth soft agar on a fresh L-agar plate, and discs impregnated with the appropriate compound were placed on the solidified overlay and incubated overnight at 35 °C. The zone of inhibition was then measured (without subtracting the diameter of the disc). Some discs were purchased from Oxoid (9 mm diam.), others from Mast Laboratories, Bootle, U.K. (6-5 mm diam.), and others were prepared by spotting the compounds on 5-5 mm diameter discs of Whatman no. 1 filter paper. In certain cases, e.g. with acriflavine, there was no inhibition of growth, due to low concentration on the disc, although there was increased sensitivity as measured by the MIC. MIC values were obtained by streaking out the strains on L-agar plates impregnated with the appropriate concentration of chemical, and incubating overnight at 35 °C.

**LPS analysis.** Cells grown to mid-exponential phase in L-broth supplemented with 0.25% (w/v) glucose and 0.25% (w/v) galactose were washed with distilled water and lyophilized. LPS was prepared from the lyophilized cells, following de-lipidation, by the method of Galanos et al. (1969) as modified by Boman & Monner (1975). Carbohydrate analyses were performed by GLC as described by Holme et al. (1968) and Monner et al. (1971). Mild acid hydrolysis of the LPS was as described by Schmidt et al. (1970a). Determination of phosphate concentration of polysaccharide and lipid A fractions of LPS was performed as described by Coleman & Leive (1979), using the assay method of Ames (1966). The aqueous phase after CHCl₃ extraction was also used for the determination of 2-keto-3-deoxyoctulosonic acid (KDO) concentration by the method of Weissbach & Hurwitz (1959) as modified by Osborn (1963). Glucosamine was determined as described by Levvy & McAllen (1959) after hydrolysis in 6 M-HCl at 100 °C for 4 h, and removal of HCl by lyophilization.

**Fatty acid analysis on isolated envelopes.** Lyophilized cells (0.6 g) were broken by sonication and the membrane fraction hydrolysed in 1:7 m-HCl in dry methanol for approximately 2 h. The fatty acids were separated as methyl esters by GLC on 15% (w/w) polyethylene glycol succinate on Chromasorb W (100–120 mesh).

**Envelope protein analysis.** Cells were grown in L-broth to an OD₆₀₀ of 0.5 at 35 °C, harvested by centrifugation, and envelope proteins prepared essentially as described by Churchward & Holland (1976) and Boyd & Holland (1979). The separation of outer and inner membrane proteins was achieved using Sarkosyl NL97. The outer membrane, which remains insoluble after this procedure (Filip et al., 1973) was recovered by centrifugation at 100000 g for 2 h in the 50Ti rotor. The pellet (outer membrane) was dissolved in 500 μl lysis buffer [0.05 M-

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**Table 1. Strains of E. coli K12 used**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Origin/Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB85</td>
<td>F⁻ thr leu lacY supF44 rpsL tonA (λ-)&lt;br&gt;sup deoD::(KcmR5757)&lt;br&gt;upp deoD::(KcmR5757)</td>
<td>Buxton &amp; Holland (1973)</td>
</tr>
<tr>
<td>RB400</td>
<td>HfrH thi tyrT&lt;sup&gt;+&lt;/sup&gt; Δ(gal·attl·bio·wrb·deoR)&lt;br&gt;upa deoD::(KcmR5757)</td>
<td>Buxton et al. (1978)</td>
</tr>
<tr>
<td>RB978</td>
<td>F&lt;sup&gt;+&lt;/sup&gt; leu lacY supF44 rpsL tonA deoD::&lt;br&gt;(KcmR5757)</td>
<td>RB400 x RB85 → Thr&lt;sup&gt;+&lt;/sup&gt; Str&lt;sup&gt;+&lt;/sup&gt; &lt;br&gt;(Buxton &amp; Drury, 1983)</td>
</tr>
<tr>
<td>RB979</td>
<td>F&lt;sup&gt;+&lt;/sup&gt; leu lacY supF44 rpsL tonA Δ(deoD-serB-trpR·dye-thr)</td>
<td>Heat-resistant derivative of RB978 &lt;br&gt;(Buxton &amp; Drury, 1983)</td>
</tr>
<tr>
<td>RB2123</td>
<td>RB979, pRB38 (dye&lt;sup&gt;-&lt;/sup&gt;) = pACYC184 Ω(2kb::dye)</td>
<td>See Methods</td>
</tr>
<tr>
<td>RB2132</td>
<td>RB979, pRB40 (dye&lt;sup&gt;-&lt;/sup&gt;)</td>
<td></td>
</tr>
<tr>
<td>RB2220</td>
<td>RB979, pRB51 (dye::Tn1000)</td>
<td></td>
</tr>
<tr>
<td>RB2223</td>
<td>RB979, pRB47 (dye::Tn1000)</td>
<td></td>
</tr>
<tr>
<td>RB2232</td>
<td>RB979, pRB54 (dye&lt;sup&gt;-&lt;/sup&gt;, Tn1000)</td>
<td></td>
</tr>
</tbody>
</table>
**dye (sfrA) mutants of E. coli**

Tris/HCl, pH 6.8, 1% (w/v) SDS, 0.002 m-EDTA, 1% (w/v) β-mercaptoethanol, 10% (v/v) glycerol, 0.05% (w/v) bromophenol blue, and the supernatant (inner membrane) was mixed with an equal volume of 2× lysis buffer. The samples were heated at 100 °C for 5 min and stored at −20 °C. Samples (40 μL, again heated at 100 °C for 5 min) were subjected to SDS-PAGE as described by Laemmli (1970) using a 12.5% (w/v) acrylamide separating gel and a 5% (w/v) stacking gel with a ratio of acrylamide (BDH; specially pure) to N,N'-methylene-bisacrylamide (Eastman) of 30:0.8. Gels were run overnight at 50 V constant voltage with a final 10 min or more at 150 V. Gels were stained in 50% (v/v) methanol, 10% (v/v) glacial acetic acid, 0.1% (w/v) Coomassie brilliant blue R (Sigma) for about 1 h, de-stained in this solution lacking stain for about 90 min, and then overnight in 5% (v/v) methanol, 7% (v/v) glacial acetic acid. Gels were then silver-stained using the Biorad kit as described in the Biorad bulletin on silver staining. Molecular weight markers were from BDH.

Outer and inner membrane proteins separated in this way have been reported (Boyd & Holland, 1979) to have identical profiles to those prepared by the less convenient density gradient procedure (Osborn et al., 1972). Extraction was performed with 5% (w/v) Sarkosyl as well as at the more usual 0-5% (w/v) concentration, the only significant difference being that more of the major outer membrane protein OmpA was extracted with the higher Sarkosyl concentration. The inner and outer membranes isolated in this way appeared free of contamination with cytoplasmic proteins. Thus the β and β′ subunits of RNA polymerase, elongation factor EF-Tu and, for the pACYC184-containing strains, chloramphenicol acetyltransferase, were not evident on gels of the inner and outer membranes, although easily seen on gels of the cytoplasmic fraction (unpublished data).

**RESULTS**

**Antibiotic, dye and detergent sensitivity**

Roeder & Somerville (1979) reported that strains with deletions of the chromosome between trpR and thr were sensitive to methylene blue (present in eosin methylene blue agar) and even more sensitive to the related compound, toluidine blue. We have confirmed this observation and in Tables 2 and 3 we present this data, together with the results of sensitivity tests for a wide range of other dyes, antibiotics and detergents. The Δdye strain showed increased sensitivity in particular to ampicillin, carbenicillin, chloramphenicol, nalidixic acid, methylene blue and especially toluidine blue, and slight sensitivity to a number of other compounds. To some, e.g. gentamicin, the Δdye strain was actually less sensitive than its dye+ parent.

The range of sensitivities did not appear to be particularly related to the hydrophobicity of the substance as exemplified by its partition coefficient (Nikaido, 1976). In this respect therefore, the Δdye strain differed from mutants known to be altered in LPS structure, in which increase in sensitivity was correlated with the hydrophobicity of the compounds tested (Nikaido, 1976; Coleman & Leive, 1979).

**LPS structure**

In order to determine whether there were any changes in LPS structure, the sensitivity of dye+ and Δdye strains towards the LPS-specific phages U3, C21, Br2 and Br10 was tested. The sensitivity pattern of these phages has been correlated with the presence or absence of certain LPS components (Kalckar et al., 1966; Watson & Paigen, 1971; Rapin et al., 1968; Sanderson et al., 1974; Schmidt et al., 1969, 1970b). There were, however, neither qualitative nor quantitative differences in the sensitivity patterns of strains RB85 (dye+) and RB979 (Δdye). Thus both were sensitive to U3 and Br10, and resistant to C21 and Br2, whereas an E. coli B strain was sensitive to C21 and resistant to Br2, Br10 and U3. There were, therefore, no apparent differences in the LPS when dye was deleted. However, in order to be quite sure that no qualitative differences in LPS structure existed, a chemical analysis on isolated LPS from RB85 and RB979 was performed (see Methods). No significant difference was observed in the amount of glucosamine, 2-keto-3-deoxyoctonate acid, polysaccharide phosphate or lipid A phosphate from LPS, and GLC analysis revealed no significant changes in the ratio of the sugars in the LPS between mutant and wild-type (Table 4).

**Envelope fatty acid composition**

GLC analysis of the fatty acids extracted from isolated envelopes of RB85 and RB979 revealed no differences between these two strains (C. A. M. Curtis, N. L. Hemmings & R. S. Buxton, unpublished data).
Increased sensitivity to:

- Ampicillin
- Carbenicillin
- Chloramphenicol
- Chlorotetracycline
- Methylene blue
- Mitomycin C
- Nalidixic acid
- Tetracycline
- Toluidine blue

No change in sensitivity to:

- Acriflavine
- Bacitracin
- Clindamycin
- Cloxacillin
- Crystal violet
- Erythromycin
- Novobiocin
- Rifamycin SV
- Sodium deoxycholate
- Sodium dodecyl-sulphate
- Vancomycin

Decreased sensitivity to:

- Gentamicin
- Kanamycin
- Neomycin

Table 2. Dye, antibiotic and detergent sensitivity tests

<table>
<thead>
<tr>
<th>Conc (µg per disc)</th>
<th>Disc diameter (mm)</th>
<th>Inhibition zone diameter (mm)</th>
<th>Partition coefficient (where known)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RB85 (dye+)</td>
<td>RB979 (Δdye)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>25</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100</td>
<td>6-5</td>
<td>30</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>10</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Chlorotetracycline</td>
<td>25</td>
<td>6-5</td>
<td>17</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>2</td>
<td>5-5</td>
<td>NI</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>20</td>
<td>5-5</td>
<td>17</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>50</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>Toluidine blue</td>
<td>2</td>
<td>5-5</td>
<td>NI</td>
</tr>
</tbody>
</table>

Table 3. Minimal inhibitory concentrations of various compounds

The MIC is the lowest concentration that completely inhibits growth on L-agar plates. In liquid cultures, although both strains grew in acriflavine and sodium deoxycholate, it was noted that whereas RB85 had normal rod-shaped cells, those of RB979 were often lysed or of abnormal shapes. Although the MIC was not reached for crystal violet and methylene blue, RB979 was in fact slightly more sensitive than RB85.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Acriflavine</th>
<th>Chloramphenicol</th>
<th>Crystal violet</th>
<th>Methylene blue</th>
<th>Nalidixic acid</th>
<th>Novobiocin</th>
<th>Sodium deoxycholate</th>
<th>SDS</th>
<th>Toluidine blue</th>
</tr>
</thead>
<tbody>
<tr>
<td>RB85</td>
<td>dye+</td>
<td>&gt; 320</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>&gt; 10</td>
<td>&gt; 100</td>
<td>&gt; 80000</td>
<td>&gt; 40000</td>
<td>&gt; 200</td>
<td></td>
</tr>
<tr>
<td>RB979</td>
<td>Δdye</td>
<td>&gt; 320</td>
<td>2-5</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
<td>5</td>
<td>100</td>
<td>&gt; 80000</td>
<td>&gt; 4000</td>
<td>200</td>
</tr>
</tbody>
</table>

Envelope protein composition

Comparisons involving dye+ and Δdye strains suffer from the disadvantage that genes other than dye are deleted and so many phenotypic differences between dye+ and Δdye may not be ascribed to mutation solely in the dye gene. In order to circumvent this problem we have isolated a series of strains which differ only in the dye gene (R. S. Buxton & L. S. Drury, unpublished...
dye (sfrA) mutants of E. coli

Table 4. Analysis of LPS components

<table>
<thead>
<tr>
<th>Composition [μmol (mg LPS)^{-1}]</th>
<th>Strain</th>
<th>Genotype</th>
<th>Glucosamine</th>
<th>KDO*</th>
<th>Total phosphate</th>
<th>Polysaccharide phosphate</th>
<th>Lipid A phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Polysaccharide Lipid A</td>
<td>RB85</td>
<td>dye^+</td>
<td>0.495</td>
<td>0.156</td>
<td>1.25</td>
<td>1.91</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>RB979</td>
<td>Δdye</td>
<td>0.448</td>
<td>0.145</td>
<td>1.19</td>
<td>0.87</td>
<td>0.32</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ratio of sugars by GLC^†</th>
<th>Strain</th>
<th>Genotype</th>
<th>Galactose</th>
<th>Glucose</th>
<th>Unknown</th>
<th>Heptose</th>
<th>Rhamnose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RB85</td>
<td>dye^+</td>
<td>1.0 (0-84)</td>
<td>2.96 (1.0)</td>
<td>0.34 (1.25)</td>
<td>2.77 (2.41)</td>
<td>&lt;0.1 (0.16)</td>
</tr>
<tr>
<td></td>
<td>RB979</td>
<td>Δdye</td>
<td>1.0 (0-84)</td>
<td>3.13 (1.0)</td>
<td>0.41 (1.25)</td>
<td>2.87 (2.43)</td>
<td>&lt;0.1 (0.16)</td>
</tr>
</tbody>
</table>

* 2-Keto-3-deoxyoctulosonic acid.
† Numbers in parentheses are retention times relative to that for glucose.

results). These strains are all derivatives of RB979 and thus carry the deoD-serB-trpR-dye-thr deletion. Strain RB2123, in addition, carries the plasmid pRB38, which is plasmid pACYC184 into which has been cloned a 6 kb SalI fragment carrying dye^+. In strain RB2132, approximately half of this cloned DNA, lying between two KpnI sites, has been removed, although it is still Dye^+; strain RB2220 has the transposon y6 inserted into the dye gene, and hence this strain is Dye^-; and strain RB2223 has y6 inserted into the promoter-distal part of the dye gene, which does not, however, result in a Dye^- phenotype, although a slightly shorter dye gene product is synthesized. The plasmids were retained by growth in the presence of chloramphenicol. It should be noted that these strains are all F^-.

The outer and inner membrane proteins of these strains were examined by SDS-PAGE after extraction of cell envelopes with the ionic detergent sodium lauroyl sarcosinate (Sarkosyl), which has been reported to preferentially extract inner membrane proteins (Filip et al., 1973).

Two major changes, b and e (Fig. 1), in the protein profiles associated with the Dye^- phenotype were evident and also four minor changes (a, c, d and f, Fig. 1; d is very faint). These were all increases in abundance of proteins, three being in the inner membrane (b, c and e) and three in the outer membrane. We cannot yet be certain which are primary defects of the dye lesion.

In addition, one inner membrane protein band, c, appeared to be missing in strain RB2132, which lacks part of the 6 kb SalI cloned DNA, although this was not associated with the Dye^- phenotype.

DISCUSSION

Mutation of the dye (sfrA) gene has been shown to selectively alter the protein composition of the cell envelope, both in the outer and in the inner membrane.

The growth rate of isogenic dye and dye^+ strains was not significantly different and this, therefore, cannot be the reason for the protein changes. Since the analysis of the membrane proteins was performed on isogenic strains, there can be no doubt that these protein changes are due to mutation at the dye locus. The dye mutation did not significantly alter the pattern of soluble (cytoplasmic plus periplasmic) proteins resolved by either one- or two-dimensional gel electrophoresis (R. S. Buxton & L. S. Drury, unpublished data). The dye gene product has a subunit molecular weight of 29000 (R. S. Buxton & L. S. Drury, unpublished). We could not, however, detect a change in a protein band with this molecular weight in either the envelope or soluble fraction, suggesting that this gene product is a minor component of the cell.

We have previously shown that mutation of the dye gene by deletion or insertional inactivation with the transposon y6 also results in severe male sterility in Hfr, F^+ or F-prime strains. The dye gene, in fact, has been shown by complementation test to be identical to the sfrA
Fig. 1. SDS-PAGE of (a) inner and outer membrane proteins, extracted with 0.5% Sarkosyl, silver-stained, and (b) outer membrane proteins extracted with 5% Sarkosyl, (1) Coomassie blue stained and (2) silver-stained. The strains were: track 1, RB85, dye+; track 2, RB979, Δdye; track 3, RB2123, Δdye pRB38 (dye+); track 4, RB2132, Δdye pRB40 (dye+); track 5, RB2220, Δdye pRB51 (dye::Tn1000); track 6, RB2223, Δdye pBR47 (dye::Tn1000); track 7, RB2234, Δdye pRB54. Note that only RB979 (track 2) and RB2220 (track 5) are phenotypically Dye- (see Methods). pRB54 has 6 inserted in a gene other than dye. Molecular weight markers are: cytochrome c (equine), 12300; myoglobin (equine), 17200; chymotrypsinogen A (bovine), 25700; ovalbumin (hen egg), 45000; albumin (bovine serum), 66200; ovotransferrin (hen egg), 76000–78000.
gene, mutation of which also results in male sterility (Beutin & Achtman, 1979) but no significant dye sensitivity. Deletion or insertion inactivation of dye results in a much more marked Fex− (F-expression-less) phenotype than that evident in the sfrA mutants isolated (a 7000-fold reduction in mating ability). This is apparently due to the difference between missense and deletion or insertion mutations (Buxton & Drury, 1983).

The sfrA, and hence dye, gene product has been shown to be a transcriptional control factor for the F-factor (Beutin et al., 1981). We therefore consider that this gene product may also be involved in the regulation of genes coding for envelope proteins.

A situation analogous to dye seems to exist in the case of the sfrB gene, also required for expression of the sex factor F, and which maps at 84 min on the E. coli chromosome (Beutin & Achtman, 1979). This gene, which appears from mapping data and phenotype to be analogous to rfaH in Salmonella typhimurium LT-2, has been proposed to be part of an anti-termination mechanism which normally ensures complete transcription of the traY→Z F-factor operon (Beutin et al., 1981). Mutations in sfrB also affect the efficient synthesis of full-length LPS, and rfaH in S. typhimurium has likewise been shown to affect LPS core structure (Sanderson & Stocker, 1981) resulting in increased sensitivity to several antibiotics (Stocker et al., 1980). Beutin et al. (1981) have suggested that the sfrB gene product is therefore required as an anti-terminator in transcription of operons encoding cell envelope components [which the work of Sanderson & Stocker (1981) indicates are the genes which encode the glycosyl transferases for LPS synthesis], whilst also affecting the transcription of the tra operon of the F-factor.

It therefore seems likely that a number of chromosomally-encoded gene products involved in regulating the expression of genes affecting envelope components are used by the F-factor to regulate expression of its own tra genes. Further investigations may therefore throw more light on the regulation of genes encoding cell envelope components.

Note added in proof. In a recent paper Gaffney et al. (1983) report that sfrA mutations do not affect the level of β-galactosidase synthesized by a traJ-lac2 fusion plasmid, although previous hybridization data (Beutin et al., 1981) suggested that traJ mRNA synthesis was reduced. To rationalize these two observations Gaffney and coworkers suggest that the sfrA product is needed to prevent premature termination of traJ transcription, rather than its initiation; such termination might result from the coupling of transcription to sfrA-dependent translocation of the TraJ protein to its outer membrane location. Thus the synthesis of β-galactosidase from a traJ-lacZ fusion, because of its cytoplasmic location, would not be inhibited. The results presented in the present paper, that mutation of dye primarily affects the protein composition of the envelope, would be compatible with the idea that the dye product is required for the translocation of other membrane proteins besides TraJ, although the exact relationship between the changes observed and the action of the dye gene product remains to be determined.

We thank Dr S. G. Sedgwick for teaching us how to run SDS-polyacrylamide gels, and Mr N. L. Hemmings for his expert assistance with the GLC.

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