Use of Operon Fusions to Examine the Regulation of the \( \beta \)-1,2-Propanediol Oxidoreductase Gene of the Fucose System in *Escherichia coli* K12

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*Escherichia coli* K12 growing anaerobically on L-fucose excretes L-1,2-propanediol as a fermentation product whose formation is catalysed by an inducible NAD-linked oxidoreductase. The activity of this enzyme is highly induced only during anaerobic growth. Three bacterial strains bearing a hybrid operon with the structural genes for lactose utilization (lacZYA) fused to the promoter of the propanediol oxidoreductase gene (fucO) were constructed to test whether or not transcriptional control was involved. In contrast to propanediol oxidoreductase of wild-type cells, \( \beta \)-galactosidase in the \( \Phi (fuc–lac) \) strains was induced by fucose to high levels both aerobically and anaerobically. Data from this work are in accord with the previous report that the enzyme protein (assayed by specific antibodies) was induced both aerobically and anaerobically, but that only in anaerobically grown cells was the oxidoreductase catalytically active. In the present study, we found that the oxidoreductase induced anaerobically in wild-type cells remained enzymically active during aerobic growth in the absence of fucose. On the other hand, wild-type cells grown aerobically in the presence of fucose and then allowed limited anaerobic growth on glucose did not gain any oxidoreductase activity. The mechanism of this post-transcriptional control remains to be discovered.

INTRODUCTION

*Escherichia coli* K12 can grow on L-fucose as the sole source of carbon and energy via an inducible system (Fig. 1) consisting of a trunk pathway mediated by L-fucose permease (Hacking & Lin, 1976), L-fucose isomerase (Green & Cohen, 1956), L-fuculose kinase (Heath & Ghalambor, 1962), and L-fuculose-1-phosphate aldolase (Ghalambor & Heath, 1962). The aldolase cleaves the six-carbon substrate into dihydroxyacetone phosphate and L-lactaldehyde. The metabolic fate of the latter depends upon the respiratory conditions of growth. Aerobically, L-lactaldehyde is oxidized by an NAD\(^+\)-linked dehydrogenase to L-lactate (Sridhara *et al.*, 1969), which is converted by a flavoprotein dehydrogenase to pyruvate (Cocks *et al.*, 1974), thereby channelling all of the carbons from fucose into general metabolism. Anaerobically, lactaldehyde is reduced by an L-1,2-propanediol : NAD\(^+\) 1-oxidoreductase (hereafter referred to as propanediol oxidoreductase), and the product is excreted into the medium (Sridhara *et al.*, 1969; Cocks *et al.*, 1974; Boronat & Aguilar, 1979, 1981) via an inner membrane that facilitates diffusion (Hacking *et al.*, 1978). For each mole of fucose fermented, two reducing equivalents are disposed of by eliminating one mole of propanediol. The disposal of reducing equivalents in this manner reduces the need of the cell to excrete other fermentation end products, and thus allows more dihydroxyacetone phosphate to be utilized as a carbon source. Propanediol lost into

**Abbreviations:** Ap, ampicillin; Km, kanamycin; CAA, casein acid hydrolysate; XG, 5-bromo-4-chloro-3-indolyl-\( \beta \)-D-galactopyranoside.

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Fig. 1. Scheme for L-fucose utilization by E. coli.

the medium, however, is irretrievable even when molecular oxygen becomes available (Sridhara et al., 1969).

The structural genes of the fucose system appear to be partitioned among at least four operons under a common positive regulatory protein (Hacking & Lin, 1977). The fucose enzymes in the trunk pathway are inducible irrespective of the respiratory conditions of growth. In contrast, lactaldehyde dehydrogenase is highly inducible only aerobically, whereas propanediol oxidoreductase appears to be highly inducible only anaerobically (Hacking & Lin, 1976). An unexpected finding was then made with an immunoochemical assay that the oxidoreductase protein is actually induced both aerobically and anaerobically. This suggests that a post-transcriptional control mechanism is responsible for the difference between the enzyme activity in aerobic and anaerobic cells (Boronat & Aguilar, 1981b). The present study was undertaken to examine transcriptional control of the propanediol oxidoreductase gene by using operon fusions to the lacZYA genes.

METHODS

Chemicals. L-Fuculose 1-phosphate was prepared enzymically from L-fuculose and purified (Heath & Ghalambor, 1962). L-Lactaldehyde was synthesized by the method of Zagalak et al. (1966). Ampicillin (Ap), kanamycin (Km) sulphate, chloramphenicol and O-nitrophenyl-β-D-galactopyranoside were from Sigma; vitamin-free casein acid hydrolysate (CAA) was from ICN Nutritional Biochemicals, Cleveland, Ohio, U.S.A.; 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (XG) was from Bachem Inc., Torrance, Calif., U.S.A.; and MacConkey agar base (without lactose) was from Difco. All other chemicals used were commercial products of reagent grade.

Bacteria and phage. Escherichia coli ECL116 was used as the parental strain. The structural gene for propanediol oxidoreductase is designated as fucO (O for oxidoreductase) and that for fuculose-1-phosphate aldolase as fucA (A for aldolase). The method of Casadaban & Cohen (1979) was used for the construction of fuc0-Mu d1 fusion strains. Escherichia coli strain MAL103 was used to prepare lysates of bacteriophage Mu d1. Transduction with phage P1(wt) was carried out by the method of Luria et al. (1960). Pertinent genetic characteristics and sources of the E. coli K12 strains and phages used are given in Table 1.
Control of E. coli propanediol oxidoreductase

Table 1. Strains of E. coli K12 and bacteriophage

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECL116</td>
<td>$\Delta lacU169$ thi endA hsdR</td>
<td>B. Magasanik</td>
</tr>
<tr>
<td>ECL325</td>
<td>$\Delta lacU169$ thi endA hsdR $\Phi[fucO-lac::\lambda p1(209)]$</td>
<td>This study</td>
</tr>
<tr>
<td>ECL326</td>
<td>as ECL325</td>
<td>This study</td>
</tr>
<tr>
<td>ECL327</td>
<td>as ECL325</td>
<td>This study</td>
</tr>
<tr>
<td>ECL355</td>
<td>argA21 galT23</td>
<td>B. Bachmann</td>
</tr>
<tr>
<td>ECL475</td>
<td>$fucA::Tn5$</td>
<td>T. Chakrabarti*</td>
</tr>
<tr>
<td>MAL103</td>
<td>$\lambda cts$ $\lambda d$ $\lambda$</td>
<td>M. Casadaban</td>
</tr>
<tr>
<td>Phage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1(cir)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mu d1</td>
<td>$\lambda cts$ $\lambda$ lac</td>
<td>From MAL103</td>
</tr>
<tr>
<td>$\lambda p1(209)$</td>
<td>$lac YZO -$ $AW209-$ $trp$ $AB' :: (+ Mu)$</td>
<td>M. Casadaban</td>
</tr>
<tr>
<td>$\lambda irr$</td>
<td></td>
<td>J. R. Beckwith</td>
</tr>
<tr>
<td>$\lambda c1h80$</td>
<td></td>
<td>J. R. Beckwith</td>
</tr>
</tbody>
</table>

* Isolated from strain 56 (Hacking & Lin, 1976) by T. Chakrabarti.

Growth of cells. The mineral medium consisted of 0.034 m-Na$_2$HPO$_4$, 0.064 m-K$_2$HPO$_4$, 0.02 m-(NH$_4$)$_2$SO$_4$, 1 x $10^{-4}$ m-FeSO$_4$, 3 x $10^{-4}$ m-MgSO$_4$, 1 x $10^{-4}$ m-ZnCl$_2$, and 1 x $10^{-4}$ m-CaCl$_2$, titrated to a final pH of 7.0 by HCl. Inducing medium for physiological studies contained fucose (0-2% for aerobic growth and 0-4% for anaerobic growth), CAA (0-1%), pyruvate (30 mm) and thiamine (2 $\mu$g ml$^{-1}$). Fucose was omitted from the non-inducing medium. Since lactaldehyde dehydrogenase was not significantly induced by fucose in the presence of 1% CAA (Hacking & Lin, 1976), only fucose was used as the carbon and energy source in experiments when induction of this enzyme was tested.

Aerobic growth was carried out in a 2000 ml flask that contained 150 ml medium and was agitated on a rotary shaker. Anaerobic growth was carried out in 150 ml flasks filled with the top with medium, tightly capped, and gently stirred by a magnet. Anaerobic incubation of cells on solid medium was carried out in sealed jars under an H$_2$/CO$_2$ atmosphere (BBL GasPak, Becton Dickenson & Co., Cockeysville, Md., U.S.A.). All strains were grown at 37 °C except those carrying Mu d1 fusions which were grown at 30 °C. Drug sensitivity was tested using LB agar containing ampicillin or kanamycin sulphate each at 20 $\mu$g ml$^{-1}$.

Isolation of fucO : Mu d1 fusion strains. To a 2.5 ml suspension of 2 x $10^9$ overnight grown cells of strain ECL116 was added Mu d1 phage at an m.o.i. of 0-1. After infection, 0.5 ml of the suspension was incubated aerobically in 20 ml of mineral medium containing ampicillin (25 $\mu$g ml$^{-1}$) and glucose (0-4%) for 5 h at 30 °C. The cells were collected by centrifugation, washed, resuspended in fresh medium of the same composition, and grown anaerobically overnight. Samples of the selected cells were plated on MacConkey agar containing fucose (0-2%), ampicillin (25 $\mu$g ml$^{-1}$), and XG (40 $\mu$g ml$^{-1}$) and incubated anaerobically for 2 d. The plates were inspected immediately after removal from the sealed jars and colonies that were colourless (possibly blocked in the fucose trunk pathway) or pink (possibly lacking propanediol oxidoreductase) were marked. The plates were then kept in refrigerator for at least 2 h to allow spontaneous oxidation of 5-bromo-4-chloro-3-indole to the blue indigoid product. Colonies originally noted to be pale and which subsequently turned blue or purple were picked as presumptive fucO : Mu d1 fusion strains. Three clones with different inducibilities of $\beta$-galactosidase were identified.

Conversion of the Mu d1 fusion strains to $\lambda$ lysogens. To stabilize genetically the hybrid operon, Mu d1 fusion strains were lysogenized with $\lambda p1(209)$ by the method of Komeda (1979). The $\lambda$ lysogen without the Mu d1 prophage was isolated by growth of the cells at 42 °C. They were shown to be sensitive to ampicillin, to possess the $\Phi[fucO-lac]$, and to be sensitive to $\lambda$ irr and immune to $\lambda c1h80$. Strains ECL325, ECL326 and ECL327 were thus obtained.

Preparation of cell extracts. Cells were harvested from exponentially growing cultures at 100–150 Klett units (no. 42 filter) and washed once with 0-1 m-potassium phosphate (pH 7-0). The pellet was weighed and dispersed in 4 volumes of 2-5 mm-glutathione and 0-1 m-potassium phosphate (pH 7-0). The dispersed cells in the tube were disrupted (for 1 min per ml of suspension) in a model 60 W ultrasonic disintegrator (MSE) while being chilled in a -10 °C bath. The resultant mixture was centrifuged 100000 g for 2 h at 4 °C, and the supernatant fraction was used for enzyme assays.

Enzyme assays. Fucose permease activity was determined by the initial rate of cellular uptake of L-[1-3H]fucose (Hacking & Lin, 1976). Fucose isomerase activity was determined from the initial rate of formation of dihydroxyacetone phosphate (Ghalambor & Heath, 1962). Propanediol oxidoreductase activity was measured by the rate of decrease in $A_{440}$ in an assay mixture consisting of 0-5 mm-
Table 2. Activities of the enzymes necessary for aerobic growth on L-fucose

Units are defined in Methods. Dashes indicate not determined.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth medium</th>
<th>Fucose permease</th>
<th>Fucose isomerase</th>
<th>Fuculose-1-phosphate aldolase</th>
<th>Lactaldehyde dehydrogenase</th>
<th>Isomerase/Aldolase</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECL116</td>
<td>Non-inducing</td>
<td>0.16</td>
<td>250</td>
<td>16</td>
<td>60</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>17</td>
<td>1000</td>
<td>110</td>
<td>100</td>
<td>9.1</td>
</tr>
<tr>
<td>ECL325</td>
<td>Non-inducing</td>
<td>0.04</td>
<td>120</td>
<td>4</td>
<td>58</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>16</td>
<td>800</td>
<td>58</td>
<td>130</td>
<td>16</td>
</tr>
<tr>
<td>ECL326</td>
<td>Non-inducing</td>
<td>0.1</td>
<td>230</td>
<td>12</td>
<td>48</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>23</td>
<td>1200</td>
<td>82</td>
<td>76</td>
<td>15</td>
</tr>
<tr>
<td>ECL327</td>
<td>Non-inducing</td>
<td>0.02</td>
<td>170</td>
<td>4</td>
<td>58</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>26</td>
<td>810</td>
<td>42</td>
<td>120</td>
<td>19</td>
</tr>
</tbody>
</table>

Lactaldehyde, 0.1 mM-NADH and 100 mM-sodium phosphate (pH 7.0) in a final volume of 1 ml. Lactaldehyde dehydrogenase activity was measured by the rate of increase in A₄₅₀ in an assay mixture consisting of 2 mM-lactaldehyde, 20 mM-Na₂CO₃ (pH 10.5), 1 mM-glutathione and 0.66 mM-NAD in a final volume of 1 ml. Specific activities of the fucose enzymes are given in nmol product formed per min per mg protein at 25 °C. Fucose permease activity is expressed in nmol substrate retained per min per mg dry mass at 37 °C. β-Galactosidase activity was measured and expressed in units according to Miller (1972).

Immunoechemical assay. Quantitative immunoelectrophoresis for the determination of the propanediol oxidoreductase protein was performed as described by Laurell (1966).

RESULTS

Growth and activities of the fucose enzymes in the fusion strains

All three fusion strains isolated as showing fucose-inducible β-galactosidase activity grew aerobically on fucose. Strains ECL325 and ECL327 showed no anaerobic growth on fucose, whereas strain ECL326 showed poor growth. The anaerobic growth defects of the mutants suggest that the fusion affected the synthesis of propanediol oxidoreductase, which was confirmed by assay of extracts of cells grown anaerobically in the inducing medium. Table 2 shows that among the tested gene products which are necessary for aerobic fucose utilization, all remained inducible in the fusion strains. The aldolase activity level in all three fusion strains, however, was lower than expected.

Absence of the propanediol oxidoreductase protein in the fusion strains

To see if the propanediol oxidoreductase protein was also abolished by the insertion of lac genes, parental and fusion strains were grown anaerobically in the inducing medium. Immuno-electrophoretic assays for the presence of the enzyme protein were carried out with the cell extracts. Figure 2 shows that whereas the expected amount of the protein antigen was present in the parental strain, no cross-reacting material was detectable in the fusion mutants.

Mapping of the operon fusions

The positions of the Mu d1 phage (Ap⁶ lac) in the fusion strains were mapped by transduction. P1 phage grown on strain ECL475, which has a Tn5 (KmR) insertion in the fuculose-1-phosphate aldolase gene (T. Chakrabarti, personal communication) were used to infect strains ECL325–327. Transductants were selected for kanamycin resistance. If, as previously suggested, all the structural genes of the fucose system, which include fucose permease, fucose isomerase, fucose kinase, fuculose-1-phosphate aldolase, propanediol oxidoreductase and lactaldehyde dehydrogenase, were linked (Hacking & Lin, 1976, 1977), then incorporation of Tn5 into the recipient chromosome should result in the elimination of the Mu d1 phage at a high frequency. This was indeed the case. Of the 96 transductants of each fusion strain which were Fuc⁻, all were found to be Ap⁶ and Lac⁻ on L-broth/fucose/XG agar. Displacement of the Φ(fucO–lac) by fucA::Tn5 indicated also that only a single copy of Mu d1 was present in the chromosome of strains ECL325–327. In another transduction, an expected linkage of 50%
Fig. 2. Immunoelectrophoretic quantification of propanediol oxidoreductase in anaerobically grown cells induced with fucose. The Laurell rockets were obtained by applying to a gel containing 1.5% antiserum (Boronat, 1981), 25 μg of protein in 8 μl cell extract of strain: (1) ECL116; (2) ECL325; (3) ECL326; and (4) ECL327.

Table 3. Propanediol oxidoreductase and β-galactosidase activities in parental and fusion strains

Units are defined in Methods. Dashes indicate not determined.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth medium</th>
<th>Propanediol oxidoreductase</th>
<th>β-Galactosidase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aerobic (I)</td>
<td>Anaerobic (II)</td>
</tr>
<tr>
<td>ECL116</td>
<td>Non-inducing</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>17</td>
<td>900</td>
</tr>
<tr>
<td>ECL325</td>
<td>Non-inducing</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>19</td>
<td>44</td>
</tr>
<tr>
<td>ECL326</td>
<td>Non-inducing</td>
<td>—</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>20</td>
<td>47</td>
</tr>
<tr>
<td>ECL327</td>
<td>Non-inducing</td>
<td>—</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Inducing</td>
<td>23</td>
<td>56</td>
</tr>
</tbody>
</table>

between the Φ(fucO–lac) and argA was found. The same mapping results were obtained with Φ(fucO–lac) strains in which the Mu1 prophage was replaced by λp1(209) prophage.

Activities of propanediol oxidoreductase and β-galactosidase

Whereas the propanediol oxidoreductase activity of the parental strain was over 50-fold higher in anaerobically induced than in aerobically induced cells, less than twofold respiratory effects were seen on the β-galactosidase activity of the fusion strains (Table 3). Extracts of the
Table 4. Effects of glucose and cAMP on the induction of fucose enzymes and β-galactosidase

All cultures were grown anaerobically. All media contained 1% CAA, and, where indicated, glucose was added to 0-4% and cAMP to a concentration of 2 mM. Dashes indicate not determined.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Addition to CAA medium</th>
<th>Fucose isomerase activity</th>
<th>Propanediol oxidoreductase activity</th>
<th>β-Galactosidase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECL116</td>
<td>None</td>
<td>250</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Fucose</td>
<td>2100</td>
<td>900</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Fucose and glucose</td>
<td>50</td>
<td>24</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Fucose, glucose and cAMP</td>
<td>2500</td>
<td>750</td>
<td>—</td>
</tr>
<tr>
<td>ECL325</td>
<td>None</td>
<td>120</td>
<td>—</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Fucose</td>
<td>1600</td>
<td>—</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td>Fucose and glucose</td>
<td>500</td>
<td>—</td>
<td>1900</td>
</tr>
<tr>
<td></td>
<td>Fucose, glucose and cAMP</td>
<td>2900</td>
<td>—</td>
<td>1100</td>
</tr>
<tr>
<td>ECL326</td>
<td>None</td>
<td>200</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Fucose</td>
<td>2700</td>
<td>—</td>
<td>890</td>
</tr>
<tr>
<td></td>
<td>Fucose and glucose</td>
<td>200</td>
<td>—</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Fucose, glucose and cAMP</td>
<td>3200</td>
<td>—</td>
<td>860</td>
</tr>
<tr>
<td>ECL327</td>
<td>None</td>
<td>250</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
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<td>Fucose</td>
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<td>1900</td>
</tr>
<tr>
<td></td>
<td>Fucose and glucose</td>
<td>70</td>
<td>23</td>
<td>380</td>
</tr>
<tr>
<td></td>
<td>Fucose, glucose and cAMP</td>
<td>2700</td>
<td>44</td>
<td>1800</td>
</tr>
</tbody>
</table>

fusion strains retained detectable propanediol oxidoreductase activities (less than 5% of the wild-type value) which were increased appreciably by fucose, especially anaerobically. These residual activities might be attributable to another protein whose synthesis was weakly inducible by fucose.

Catabolite repression

Joining the lac genes to the fucO promoter evidently did not affect the catabolite repressibility. This conclusion was reached by analysing cells of parental and mutant strains grown anaerobically in the non-inducing medium, the inducing medium, the inducing medium with glucose, and the inducing medium with glucose plus cAMP. Table 4 shows that anaerobic induction of the oxidoreductase in the parental strain and β-galactosidase in the fusion strains were inhibited alike by glucose, and the glucose effect was counteracted by cAMP. In the absence of catabolite repression, the isomerase was induced anaerobically to a level about twice that observed in aerobically induced cells (compare with Table 2).

Propanediol oxidoreductase activity in cells shifted to non-inducing media under different respiratory conditions

Wild-type cells from a culture grown anaerobically on fucose to mid-exponential phase were collected and reincultivated at a density of 20 Klett units in a medium containing glucose and CAA. Aerobic growth was allowed to take place and samples were removed at zero time and at three 1-h intervals for assay of propanediol oxidoreductase. Even after 3 h (sixfold increase in cell number), the total activity units of the enzyme in the culture remained the same. Thus, the low level of propanediol oxidoreductase activity in aerobically grown cells could not be explained by inactivation of the mature enzyme by molecular oxygen.

A reciprocal experiment was carried out in which cells from a culture grown aerobically on fucose, CAA and pyruvate to mid-exponential phase were collected and reincultivated at a density of 40 Klett units in a medium containing glucose, CAA and pyruvate. Anaerobic growth was allowed to take place until the cell mass doubled or tripled. No increase in the basal specific oxidoreductase activity occurred. No increase in specific activity of the enzyme occurred in another experiment in which the aerobically induced cells were incubated anaerobically for 4 h in a medium containing glucose (or fucose), CAA, pyruvate and chloramphenicol (100 μg ml⁻¹).
DISCUSSION

Extracts of wild-type *E. coli* K12 cells grown anaerobically on fucose exhibit much higher specific activity of propanediol oxidoreductase than those of aerobically grown cells (Hacking & Lin, 1976). In contrast, the enzyme protein level is only slightly higher in anaerobically than in aerobically grown cells, which strongly suggests that the enzyme activity is controlled post-transcriptionally (Boronat & Aguilar, 1981b). The induction pattern of β-galactosidase in the $\Phi(fucO-lac)$ strains under aerobic and anaerobic conditions was consistent with this interpretation. The higher level of this enzyme in anaerobically than in aerobically induced cells probably reflected a general effect, since a twofold difference was found with the isomerase.

In view of the absence of specific respiratory control of β-galactosidase synthesis encoded by the $\Phi(fucO-lacZ)$, it was reassuring that the catabolite repressibility by glucose was retained by the hybrid operons in all three independent fusion strains. This would suggest that the insertion of extraneous base sequences did not radically disturb the property of the promoter. Although it could not be excluded that the failure to synthesize active or inactive oxidoreductase proteins was the result of the lac insertion into a regulator gene encoding a specific activator protein for the oxidoreductase structural gene, we consider fusion to the structural gene itself more likely because of the strength of the fucO promoter, which is atypical for regulator genes. The induced β-galactosidase activities in the three strains, which varied from 890 to 1900 units, were respectable in comparison to an activity of 5000 units (50000 monomers per cell) induced by isopropyl-$\beta$-D-thiogalactoside in cells bearing the wild-type lac operon and grown on glycerol (Casadaban, 1976). In contrast, a fusion of lacZ to the promoter of araC, encoding the activator of the L-arabinose operon, produced 55 β-galactosidase units (Casadaban, 1976), and among 11 protein fusion strains of lacZ to malT, encoding the activator of the maltose regulon, the one most productive of the enzyme activity gave 80 units (Débarbouillé & Schwartz, 1979).

A physiological question on anaerobic growth is posed by strains ECL325 and ECL327. Failure to reduce lactaldehyde should still allow some growth on the dihydroxyacetone phosphate generated from fucose. It is possible that lactaldehyde is not readily excretable and that its accumulation is bacteriostatic. This would explain the failure of strains ECL325 and ECL327 to grow anaerobically on fucose. The immunity of strain ECL326 from this effect might be conferred by a subsequent mutation that allowed either more rapid exit of lactaldehyde or an increased tolerance for high internal concentrations of the compound. The screening procedure involving anaerobic growth in the presence of fucose might have enriched the double mutant and favoured its isolation.

The nature of the enzyme modification that gives a catalytically active protein during anaerobiosis is yet to be understood. But clearly the mechanism involved is different from the one that controls the activity of the NAD$^+$-linked dehydrogenase responsible for anaerobic glycerol dissimilation by *Klebsiella pneumoniae* strain 1033. In the latter case, a high level of enzyme activity is induced anaerobically, but the protein is rapidly inactivated by a process dependent upon aerobic metabolism. Enzyme inactivation is not associated with concomitant disappearance of the immunochemically cross-reacting material (Lin et al., 1960; Ruch et al., 1980).

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