A Deletion Mutation Placing the Galactokinase Gene of Escherichia coli under Control of the Biotin Promoter

(operon fusion/transcription of bio operon)

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ABSTRACT A deletion has been isolated which fuses galK and presumably galT to the promoter and operator of the bioA operon. The deletion has endpoints in bioA and galE, and leaves the operator-proximal end of bioA intact and the regulatory sites themselves functional. In bacterial strains which carry the deletion, the expression of galK is regulated by biotin, although due to the inefficiency of the bioA promoter even the fully derepressed level of galactokinase is low. Electron micrographic examination of the mutation shows that it is a simple deletion, uncomplicated by other chromosomal abnormalities.

The bio gene cluster of Escherichia coli K12, near 17 min on the genetic map, comprises five structural genes whose products mediate the biosynthesis of the vitamin biotin (Fig. 1 and ref. 1). Transcription data (2) and complementation studies (3) show that bioB, bioF, bioC, and bioD constitute an operon transcribed rightward and repressible by biotin, with operator and promoter sites which fall between bioA and bioB. The transcription data also suggest that bioA lies in an operon repressible by biotin but transcribed leftward from a site also between bioA and bioB.

The gal operon, which lies to the left of the bio cluster and whose enzymes mediate galactose catabolism, is also transcribed leftward (4). If the presumed orientation of bioA transcription is correct, it should be possible, since no essential bacterial genes lie between gal and bio (5), to isolate deletions which allow transcription initiated at the bioA promoter to continue through one or more of the gal genes. This paper describes the isolation and properties of such a deletion.

MATERIALS AND METHODS

Strains are described in Table 1.

Media. Tryptone broth, tryptone broth agar, and tryptone broth soft agar were used to grow and titrate bacteria and phage; eson methylene blue galactose agar was used to select Gal+ transductants and to assay the Gal phenotype; and suitably supplemented synthetic agar was used to select prototrophic recombinants and transductants. These media have been described by Campbell (5, 7). Synthetic agar routinely contained 0.12 mM 2,3,5-triphenyl-2H-tetrazolium chloride (Baker), and 0.4 g/liter of vitamin-free casein amino acids (Difco). Dethiobiotin (DTB, K&K Chemicals) was used at a concentration of 47 nM (10 μg/liter) and biotin at either 41 nM (10 μg/liter), a concentration which represses the bio operons, or 1.6 nM (0.4 μg/liter), a concentration which substantially derepresses the bio operons while allowing normal growth of biotin auxotrophs. Synthetic soft agar was 0.65% agar in 0.01 M MgSO4.

Transductions. Transductions by phage Pλ were carried out according to Rothman (8). Recipient cells were plated 30 min after infection on selective plates spread with 0.1 ml of a 1.0 M sodium citrate solution to prevent the reinfection of transductants. Spot tests for transduction by λgal or λbio transducing phage were performed by placing a drop of lysate containing about 104 phage on a lawn of recipient cells in soft agar on a selective plate. Transductants gave rise to large, deeply colored colonies after several days of incubation.

Phage and Bacterial Crosses. Phage crosses were done as described by Parkinson (9).

To cross bacterial mutations in gal or bio into transducing phage carrying wild-type gal or bio operons, donor bacteria were infected with the transducing phage and lysogens were

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Source or reference</th>
</tr>
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<tbody>
<tr>
<td>C511</td>
<td>F&quot;bioA111 his str thi&quot;</td>
<td>(3)</td>
</tr>
<tr>
<td>C524</td>
<td>F&quot;bioA54 his str thi&quot;</td>
<td>(3)</td>
</tr>
<tr>
<td>C526</td>
<td>F&quot;bioA80 his str thi&quot;</td>
<td>(3)</td>
</tr>
<tr>
<td>C571</td>
<td>F&quot;bioA71 his str thi&quot;</td>
<td>(3)</td>
</tr>
<tr>
<td>C558</td>
<td>F&quot;bioB55 his str thi&quot;</td>
<td>(3)</td>
</tr>
<tr>
<td>C249</td>
<td>F&quot;bioI21 his str thi&quot;</td>
<td>(3)</td>
</tr>
<tr>
<td>W602</td>
<td>F&quot;bioA602 gal leu str thi&quot;</td>
<td>J. Rothman</td>
</tr>
<tr>
<td>W3805</td>
<td>F&quot;galE16&quot;</td>
<td>E. Lederberg</td>
</tr>
<tr>
<td>HS323</td>
<td>F&quot;W3110 (xaxis cI857) thy polAl&quot;</td>
<td>H. Shizuya</td>
</tr>
</tbody>
</table>

Abbreviations: EDTA, ethylenediaminetetraacetate; DTB, dethiobiotin.

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selected either by their Gal or Bio phenotype or by their immunity of lambda. These lysogens were induced and the progeny phage screened for nontransducing recombinant particles by plating on a lawn of an appropriate indicator bacteria on a selective plate.

Mutations in gal or bio carried by transducing phage were transferred to wild-type recipient cells by lysogenization of the recipient strain with the marked phage and the subsequent screening of cultures of the lysogen for nonlysogenic gal− or bio− segregants.

Tests for Bacterial Genes. Tests for the bacterial genes pgl, chlD, aroG, uraB, and attB have been described (5, 10–13).

The bioB gene was scored by testing the ability of cells to grow on synthetic medium lacking biotin but supplemented with dethiobiotin.

Galactose Sensitivity. Cells which lack the products of any of the genes galT, galE, or galU, but which synthesize even small amounts of galactokinase (product of the galK gene), grow poorly in the presence of galactose due to the intracellular accumulation of galactose-1-phosphate (14). Galactose sensitivity was assayed on synthetic medium containing a 0.4% glycerol (wt/vol), 0.4% galactose, 0.4 g/liter of casein amino acids, and either 1.6 mM or 47 mM biotin.

Galactokinase Assay. Galactokinase activity was measured in crude extracts by a modification of the procedure of Sherman and Adler (15). Cells whose bio operons had been derepressed by starvation (3) or whose gal operons had been induced by growth in the presence of 10−3 M fucose were harvested by centrifugation and washed once in 20 mM phosphate buffer pH 7.4, containing 1 mM EDTA (ethylene-diaminetetraacetate) and 10 mM thioglycolic acid. The washed cells were resuspended in a small volume of buffer and sonicated, and the sonicate was clarified by centrifugation at 12,000 rpm in a Sorvall SS-34 rotor for 20 min. The reaction mixture contained final concentrations of 50 mM Tris-Cl (pH 7.8), 2 mM MgCl2, 1.5 mM NaF, 5 mM thioglycolic acid, and 5 mM ATP. Between 1 and 100 µg of protein were added to each assay and the reactions were begun by the addition of [14C]galactose (specific activity 0.5 mCi/mumole, New England Nuclear Corp.) to a final concentration of 0.5 mM. The total volume of each assay was 0.1 ml. Reactions were run at 37°C for 15 min in the wells of a Cooke microtiter plate, and were ended by chilling in an ice bath.

The amount of galactose converted to galactose-1-phosphate was measured by ion exchange chromatography. The entire reaction mixture was placed on a small (0.7 × 4.0 cm) Dowex AG1-X4 (chloride) column and the uncharged galactose was eluted with three 3-ml portions of distilled water. The negatively charged galactose phosphate was then washed into scintillation vials containing 15 ml of Aquasol (New England Nuclear Corp.) with two 3-ml volumes of 1 M LiCl, and the radioactivity of each sample was determined by scintillation spectrometry. Input radioactivity was determined by counting 1 µl of the [14C]galactose substrate in 15 ml of Aquasol containing 6 ml of 1 M LiCl.

RNA-DNA Hybridization. (1) Labeling of cultures: 10-ml cultures of the strains to be labeled were grown from an inoculum of about 5 × 106 cells per ml to a density of 5 × 108 cells per ml at 37°C in synthetic medium containing 41 mM biotin. Cells were harvested by centrifugation, washed twice with cold 0.9% saline and resuspended in the original volume of prewarmed medium containing 410 mM biotin to repress the bio operons, 0.01 units/ml of avidine (Nutritional Biochemicals) to starve the cells of biotin and derepress the bio operons, or 10−3 M D-fucose to induce the gal operon. The cells were shaken at 37°C for 30 min, at which time the bio operons are fully derepressed in starved cultures, and were then labeled.
TABLE 2. Transduction of SK61 by Pl grown on bio point mutants*

<table>
<thead>
<tr>
<th>Type of transductant</th>
<th>bioA</th>
<th>bioB</th>
</tr>
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<tbody>
<tr>
<td>bio602</td>
<td></td>
<td></td>
</tr>
<tr>
<td>bio71(bioA)</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>bio26</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>bio11</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>bio58</td>
<td></td>
<td>1.8</td>
</tr>
<tr>
<td>Gal+</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>Bio+</td>
<td>&lt;0.025</td>
<td>0.4</td>
</tr>
<tr>
<td>Bio+/Gal+</td>
<td>&lt;0.0076</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* SK61 was infected at a multiplicity of one with Plc grown on gal+ donor strains carrying the indicated bio point mutations, and bio+ or gal+ recombinants were selected. Frequencies of transduction are expressed as recombinants per input phage particle, and have been multiplied by 10^4. The bio alleles are listed in their proper map order; the order of bio71 and bio24 is not known (12). NT: not tested.

for two minutes with 200 μCi per culture of [3H]uridine (New England Nuclear Corp.). The pulse was ended by pouring cultures over 10 ml of crushed, frozen killing buffer (16).

(2) Preparation of RNA was according to Rose et al. (16).

(3) Hybridization: The hybridization method used was that of Gillespie and Spiegelman (17) as modified by Morse and Yanofsky (18).

For the measurement of bio message, each hybridization vial contained 20–100 μg (10^4 to 3 × 10^5 cpm @ 40% efficiency) of [3H]RNA, one 13-mm nitrocellulose filter loaded with 2 μg of either the r or l strand of λbio602c1857sam7 DNA, and one control filter loaded with the corresponding λc1857sam7 strand. Since bioA operon message is synthesized from right to left, and bioB–bioD operon message is synthesized from left to right, bioA message hybridizes to the l strand of λbio609 DNA and bioB–bioC message hybridizes to the r strand of λbio69 DNA (2).

For the measurement of gal message, experimental and control filters were loaded with 4 μg of denatured λgal8c1857sam7 or λc1857sam7 DNA, respectively.

DNA was in excess in all experiments. All data presented are corrected for the nonspecific binding of [3H]RNA to the control filters. Bio specific message levels are low, and control values were typically 30% of experimental values for λbio609 l filters and 10%, for λbio69 r or λgal5 filters.

(4) Preparation of DNA: The separated strands of phage chromosomes were prepared by the method of Hardrec and Szybalski (19). The separated strands were self-anneled for 6 hr at 67°C in 0.15 M NaCl 0.015 M Na citrate, pH 7.

Dethiobiotin Synthetase was assayed by the procedure of Krell et al. (20), as described by Cleary et al. (3).

EDTA and Pyrophosphate Treatment of Phage Particles. The size of phage chromosomes was estimated by the rate of inactivation of phage particles by EDTA or pyrophosphate as described by Parkinson and Huskey (21).

Electron Microscopy was according to Davis et al. (22).

RESULTS AND DISCUSSION

(a) Isolation of Deletions. A deletion fusing the galactose genes to the bioA promoter should have its left endpoint in the galactose operon and its right endpoint in bioA, and should eliminate the genes which fall between the gal and bio operons.

Deletions in the gal–bio region can be conveniently selected using strains which harbor a λc1857 prophage at attO, located between gal and bio. The λc1857 prophage synthesizes a thermolabile phage repressor, and cl857 lysogens die when heated to 42°C due to prophage induction. Many of the survivors of heating have suffered deletions of the lethal prophage genes and of bacterial DNA adjacent to the attachment site (12). The proportion of deletion mutants among the survivors can be increased by the inclusion of an att mutation on the prophage, which blocks spontaneous curing by the prophage site-specific excision system (23), and by the presence of polA mutation in the host genome, which increases the frequency of deletion mutations in at least some regions of the E. coli chromosome (24).

To select gal–bio deletions, H5323, grown overnight in tryptone at 30°C, was plated at 42°C on eosin methylene blue galactose agar containing 410 nM biotin. Each plate was spread with 0.1 ml of anti-lambda serum, K = 100, to protect survivors from reinfection by phage liberated from neighboring cells after induction. The frequency of survivors was about 10^-4 per plated cell, of which 2–10% (in different independent cultures) were Gal-. Survivors were replica-plated onto an intermediate plate lacking biotin, and then onto three synthetic agar plates: one lacking biotin, one containing DTB, and one supplemented with biotin. Of 110 independent Gal- survivors, five were Bio- but able to grow on the DTB plate, and must have suffered deletions removing some or all of bioA, none of bioB, and some of the gal operon.

The desired gal–bio fusion would spare at least galK, and so the gal endpoints of the deletions in the five galbio biob+ survivors were checked by spot tests with the transducing phage λgal28. λgal28 is a plaque-forming phage which carries the gal operator and promoter, all of galE, and the right end of galT, but none of galK (27). It can give rise to Gal+ transductants only if the recipient cells contain the part of the gal operon which λgal28 lacks, that is, all of galK and the left end of galT. Of the five deletion strains tested, only one, SK58, could be transduced to Gal- by the phage; its deletion must enter gal from the right and end either in the right end of galT, or in galE.

TABLE 3. Bio and gal mRNAs and galactokinase activity in SK61

<table>
<thead>
<tr>
<th>Strain</th>
<th>Biotin concentration (nM)</th>
<th>Hybridization to phage DNAs*</th>
<th>Galactokinase activity (μoles/min per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>bio69 l strand (bioA)</td>
<td>bio69 r strand (bioB)</td>
<td>gal8 +fucose -fucose</td>
</tr>
<tr>
<td>C524</td>
<td>410 &lt;0.1 0.1 NT</td>
<td>7.3 0.09</td>
<td></td>
</tr>
<tr>
<td>SK61</td>
<td>0.35 7.2 2.6</td>
<td>0.28 0.35</td>
<td></td>
</tr>
<tr>
<td>C524E+</td>
<td>410 &lt;0.1 0.2 1.3</td>
<td>0.018 0.027</td>
<td></td>
</tr>
</tbody>
</table>

* Measurements were made as described in Materials and Methods. Hybridization figures are the averages of at least three measurements. The radioactivity is that measured in the trichloroacetic acid precipitable fraction.

† C524E is isogenic with C524 but carries the galE106 mutation. NT: not tested.
Although the cannot some amount of bioA is SK58 as SK58. The deletion in SK61, and presumably in SK58, has been named Δ61.

(b) Genetic Properties of SK61. SK61 was tested for the presence of the bacterial genes pgp, aroG, chlD, attA, and urrB. All of the genes between gal and bio (pgp, chlD, and attA) are missing from SK61, while the two genes flanking the gal–bio region, aroG and urrB, are intact.

The relative positions of the Δ61 bioA endpoint and several bio point mutations have been determined by PI transduction. PI lysates grown on one of several gal*bioA or gal*bioB donors were used to infect SK61 and Bio+ or Gal+ transductants were selected. The frequencies of transduction are presented in Table 2 along with the frequencies of Bio+ transductants normalized to the frequency of Gal+ transductants in the same experiment as a correction for the various efficiencies of transduction in different crosses. No Bio+ transductants arose in crosses in which the donor carried any of the three left-most bioA mutations (bioA62, bioA71, or bioA24), showing that the sites corresponding to these alleles are deleted in SK61. Donors carrying either of the two right-most bioA mutations (bioA26 or bioA11) or the left-most bioB mutation (bioB85) can yield Bio+ transductants; the right end of bioA must be present in SK61. Deletion 61 extends into bioA from the left, leaving intact the right, operator proximal (2) end of the bioA gene.

A λgal25 lysogen of SK61 has been constructed, and among the phage released after its induction are found particles which, unlike the parental λgal25, do not complement the galE mutation present in W3805. These phage apparently carry a mutation present in a fragment of the galE gene which must remain in SK61; Δ61 must end within galE. The origin of this mutation (galE106) is unknown, although it is not present in HS323, which is gal–. It is neither a large deletion nor a large addition of DNA, since it alters neither the EDTA sensitivity nor the buoyant density of λgal25 particles, nor the appearance of λgal25 DNA in the electron microscope (data not shown). GalE106 has been crossed into an otherwise wild-type gal operon and does not affect the expression of galK (Table 3).

(c) Physiological Properties of SK61. SK61 is sensitive to galactose at low biotin concentrations but loses its sensitivity at high concentrations. Since Δ61 has endpoints appropriate for a deletion which would fuse galK to the bioA promoter, and since it lacks part of galE, its galactose sensitivity presumably reflects control of the expression of galK by biotin. Measurements of the activity of galactokinase in SK61 (Table 3) confirm this suggestion: galactokinase activity is regulated by biotin, although it is low even in cells whose bio operons are fully derepressed; and is insensitive to the gal operon inducer, p-fucose.

Measurements of bio-specific mRNAs also appear in Table 3. The essentially normal amount and repressibility of the bioB–bioC message indicate that the rightward promoter and operator are unaltered in SK61, and the normal repressibility of bioA message confirms the integrity of its operator. The amount of bioA message is reduced in SK61, probably because some of the bioA gene is included under the Δ61 deletion, although the possibility that the bioA promoter has been damaged cannot be excluded.

Some of the RNA hybridizable to λgal8 DNA is also repressible by biotin. If the length of the bioA gene and of the average gal gene are comparable, the amount of biotin-repressible message synthesized per length of gal DNA is about 1/4; that made from the same length of bio DNA. Deletion 61 is not, therefore, strongly polar on the gal operon. The nonrepressible RNA which hybridizes with λgal8 DNA is probably made on E. coli genes to the left of gal and not from gal itself, since galactokinase activity disappears almost entirely upon the repression of SK61 by biotin. The leftward extent of the bacterial DNA carried by λgal8 is unknown, although the phage does not transduce arnG. Guha et al. (25) have observed RNA capable of hybridizing with the antisense (for gal) strand of a λig phage which also carried all of gal and probably some bacterial DNA to its left. The amount of this RNA is similar to the amount of non-repressible gal RNA in SK61 and may have the same origin.

(d) Physical Properties of Deletion 61. The mutation in SK61 seems to be a deletion which places galK under the transcriptional control of the bioA operator and promoter. To rule out the possibility that SK61 carries some additional mutation, such as a duplication of the bioA operator–promoter region which might artificially divorce galK regulation from the bioB–bioD regulatory sites, the gal–bio region of SK61 has been examined with an electron microscope. The gal–bio region of SK61 was first crossed into the transducing phage λgal8bio69c1857 by lysogenizing SK61, inducing the lysogen, and using the lysate to make bioB+ transductants of C249. The transductants were tested for galactose sensitivity and a clone with the properties of SK61 was picked. Phage released by this clone were unable to complement the galE mutation of W3805 and must carry the Δ61 deletion. Heteroduplexes between the deletion phage and an imm34 derivative of the gal*bio+ parent were then prepared and examined. Fig. 2 shows the gal through immunity region of one heteroduplex. It contains...
reliance upon of Rose broadening the required for of. The addition) contains gene end abnormalities gene(s) contains a deletion rather than an addition is confirmed by its relatively high resistance to pyrophosphate (Fig. 3).

CONCLUSION

The isolation of the SK61 gal–bioA fusion shows that the sites required for the transcription and regulation of leftward bio message are located to the right of bioA, confirming the suggestion of Guha et al. (2) that the bioA gene is transcribed from right to left. The existence of a strain which expresses galK under bioA control also make it possible to select mutants with altered patterns of leftward bio transcription without reliance upon the function of genes in the right operon, thus broadening the spectrum of mutations available and allowing investigation of the genetic interdependence of the two operons (26).

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