Cross-induction of *glc* and *ace* Operons of *Escherichia coli*

Attributable to Pathway Intersection

CHARACTERIZATION OF THE *glc* PROMOTER

Maria Teresa Pellicer‡, Carmen Fernandez, Josefa Badia, Juan Aguilar, Edmund C. C. Lin§, and Laura Baldomá¶

From the Department of Biochemistry, School of Pharmacy, University of Barcelona, Avenida Diagonal 643, 08028 Barcelona, Spain and the ¶Department of Microbiology and Molecular Genetics, Harvard Medical School, Boston, Massachusetts 02115

The metabolic pathways specified by the *glc* and *ace* operons in *Escherichia coli* yield glyoxylate as a common intermediate, which is acted on by two malate synthase isoenzymes: one encoded by *glcB* and the other by *aceB*. Null mutations in either gene exhibit no phenotype, because of cross-induction of the *ace* operon by glycolate and the *glc* operon by acetate. In this study, the regulation of the *glc* operon, comprising the structural genes *glcDEFGB*, was analyzed at the molecular level. This operon, activated by growth on glycolate, is transcribed as a single message and is under the positive control of GlcC encoded by a divergent gene. Expression of the *glc* operon is strongly dependent on the integration host factor (IHF) and is repressed by the global regulatory protein ArcA-P. In vitro gel-shift experiments demonstrated direct binding of the promoter DNA to IHF and ArcA-P. Mutant analysis indicated that cross-induction of the *glc* operon by acetate is mediated by the GlcC protein that recognizes the compound as an alternative effector. The similar pattern of regulation of the *Glc* and *Ace* systems by IHF and ArcA-P ensures their effective cross-induction.

Glyoxylate is an important intermediate of the central microbial metabolism in the “glyoxylate bypass,” required when acetate or fatty acids are the main carbon and energy source (1). Glyoxylate is also generated from glycolate or purine degradation in *Escherichia coli* (2, 3) and is subsequently converted into malate (Fig. 1). Nevertheless, a constitutive glyoxylate reductase activity has been reported to convert glyoxylate back to glycolate (2).

There are two isoenzymes of malate synthase in *Escherichia coli*, malate synthase A (MSA) for growth on acetate and malate synthase G (MSG) for growth on compounds metabolized via glycolate/glyoxylate (4, 5). These two enzymes are distinguishable by thermal stability and kinetic properties (5) and recently have been shown to have significantly different amino acid sequences (6). The genes of the glycolate pathway that encode glycolate oxidase (GOX) (*glc*DEF) and MSG (*glcB*) are located in a cluster at 64.5 min. Their expression is induced by growth on glycolate and controlled by the gene *glcC* (6, 7). Disruption of this gene resulted in a phenotype that indicated the product to be an activator protein (GenBank™ accession number L43490; Ref. 7). The genes of the acetate pathway that encode isocitrate lyase (ICL) (*aceA*), MSA (*aceB*), and isocitrate dehydrogenase kinase/phosphatase (*aceK*) are clustered and transcriptionally repressed by the products of *iclR* and *fadR* (8, 9). The formation of the complex between the IclR repressor and the operator/promoter region has been reported to be impeded by phosphoenolpyruvate (8).

Results from different experiments point to the cross-induction of *glc* and *ace* operons. On the one hand, growth on glycolate induced an ICL structurally and functionally indistinguishable from that induced by acetate (10). On the other hand, it appears that *aceB* mutants failed to grow on acetate only if MSG was not available as a back up enzyme in *glc* mutants (4). So far, however, the recruitment mechanism of MSG for the acetate pathway and the putative involvement of MSA in glycolate metabolism have not been documented. Here we provide direct experimental evidence for the cross-induction of these two systems as well as the molecular basis for the acetate-induced *glc* expression.

EXPERIMENTAL PROCEDURES

Bacterial Strains and Phages—All the strains used were *E. coli* K-12 derivatives. The genotype and sources of the bacterial strains are given in Table I. Strain JA159 was obtained by inserting a chloramphenicol acetyltransferase (*cat*) cassette in the Clai restriction site of gene *glcB* as described previously (7). Other strains were constructed by P1 transduction (11). Transductants that lost the *glc*-1 or *glc*-mutations were selected on glucose or glycolate, respectively, and transductants that incorporated the *arcA* mutation by their sensitivity to O-toluidine blue (12).

Cell Growth, Preparation of Cell Extracts, and Enzyme Activities—Cells were grown and harvested as described previously (13). For aerobic growth, carbon sources were added to a basal inorganic medium (13) at 80 mM carbon concentration and for anaerobic growth at 120 mM. Casein acid hydrolyzate (Caa) was used at 0.5% for aerobic or at 1% for anaerobic cultures and for growth of transformed cells. Oleic acid was provided at 5 mM in the presence of Brij 58 (5 mg/ml). For anaerobic respiration nitrate was added to the cultures at 20 mM concentration. When required, the following antibiotics were used at the indicated concentrations: tetracycline, 25 μg/ml; ampicillin, 100 μg/ml; rifampicin, 10 μg/ml; spectinomycin, 75 μg/ml; kanamycin, 50 μg/ml; and carbenicillin, 100 μg/ml.

This paper is available on line at http://www.jbc.org
concentrations: ampicillin, 100 μg/ml; tetracycline, 12.5 μg/ml; chloramphenicol, 30 μg/ml; and kanamycin, 50 μg/ml. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside and isopropyl-β-D-thiogalactoside were used at 30 and 10 μg/ml, respectively. For β-galactosidase assays, the cells were allowed to double 5–6 times to an OD600 of 0.5 for aerobic cultures or 0.25 for anaerobic cultures.

Cell extracts were prepared as described previously (14). GOX and MSG activities were determined as described by Pellicer et al. (7), and β-galactosidase activity was assayed by hydrolysis of O-nitrophenyl-β-D-galactopyranoside and expressed as Miller units (11). Values reported are the mean of at least three independent experiments ± standard deviations.

Immunological Techniques—Antisera against MSG were raised in New Zealand White rabbits as described previously (16) using the purified enzyme as antigen (6). Quantitative immunoelectrophoresis was performed as described by Laurell (17). The agarose gel contained 1.3% specific globulins; 65 μg protein in 10 μl cell extract was applied to each of the wells. The specificity of this preparation was confirmed by the lack of immunoprecipitate in cell extracts of strains JA154 and JA159.

DNA Manipulation and Sequencing—Plasmid DNA was routinely prepared by the boiling method (18). For large scale preparation, a crude DNA sample was subjected to purification on a column (Qiagen GmbH, Düsseldorf, Germany). Other DNA manipulations were performed essentially as described by Sambrook et al. (19). The DNA sequence was determined by using the dideoxy-chain termination procedure of Sanger et al. (20), with double-stranded plasmid as the template. Sequencing gel compressions were resolved as described elsewhere (7).

Isolation of RNA, Northern Blot Hybridization, and Primer Extension—For preparation of total RNA, cells of a 25-ml culture grown to an A600 of 0.5 were collected by centrifugation at 5,000 × g and processed according to Belasco et al. (21). For primer extension analysis the RNA was prepared with a Qiagen RNeasy Total RNA kit. Northern blot hybridization was performed with each RNA sample (10 μg) by the procedure described by Moralezio et al. (22). For the determination of the 5'-end of the structural and the regulatory genes, the following oligonucleotides were used as primers: 5'-GGTCGACATCGGGTAAAGC-3' (complementary to an internal region within the glcD) and 5'-TGACCAGCCTTCAAGTACCCG-3' (complementary to a glcC internal sequence). The reaction was performed with 50 μg of total RNA at 37 °C for 30 min with 200 units of M-MLV reverse transcriptase (Life Technologies, Inc.) and [α-32P]Sthioph-dATP (>1,000 Ci/mmol; Amersham Pharmacia Biotech), and this was followed by a 30-min chase with all four nucleotides (at 1 μM each) (23). As a reference, double-strand sequence reactions were performed with the same primers.

Construction of lacZ Fusions and Deletions of the glcD Promoter Sequences—To create operon fusions, DNA fragments of the 5'-upstream region of each glc gene (Fig. 2) were cloned into plasmid pRS550 or pRS551 (24). These plasmids carried a cryptic lac operon and genes that confer resistance to both kanamycin and ampicillin. Recombinant plasmids were selected, after transformation of strain XL1Blue, as blue colonies on LB plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactoside, ampicillin, and kanamycin, and plasmid DNA was sequenced by using the M13 primer to ensure that the desired fragment was inserted into the correct orientation. Merodiploids were obtained by transferring the fusions as single copies into the trp operon of the E. coli strain TE2680 as described by Elliot (25). The transformants were selected for kanamycin resistance and screened for sensitivity to ampicillin and chloramphenicol. P1 vir lysates were made to transduce the fusions into strain MC4100.

TABLE I

Bacterial strains used in this work

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Source or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>XL1 Blue</td>
<td>recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1 (F proA8 lacI59lacZ ΔM15 Tn10)</td>
<td>Stratagene</td>
</tr>
<tr>
<td>MC4100</td>
<td>araD1 lac rpsL fliB deoC ptsF rbsR</td>
<td>40</td>
</tr>
<tr>
<td>TE2680</td>
<td>F' λ IN(rrnD-rrnE) Δlac X74 rpsL galK2 recD::Tn10d-tet trpDC700::putA13033::(Kan' Cam' lac)</td>
<td>25</td>
</tr>
<tr>
<td>M15</td>
<td>F' lac ara gal ntl</td>
<td>26</td>
</tr>
<tr>
<td>HN1491</td>
<td>stra galK2 suλΔhimA::cat</td>
<td>41</td>
</tr>
<tr>
<td>K2704</td>
<td>stra galK2 suλΔhimD3::cat</td>
<td>42</td>
</tr>
<tr>
<td>ECL1618</td>
<td>araC2 azi::Tn10</td>
<td>26</td>
</tr>
<tr>
<td>CH1827</td>
<td>araD139 Δ/oroBC-leu7687 Δlac X74 galU galK1 hsdR (rC, mC) rpsL160 thi 726::Tn10</td>
<td>43</td>
</tr>
<tr>
<td>CH1828</td>
<td>CH1827 met-50**</td>
<td>43</td>
</tr>
<tr>
<td>JA154</td>
<td>MC4100 gcE::cat</td>
<td>7</td>
</tr>
<tr>
<td>JA155</td>
<td>MC4100 glcD::cat</td>
<td>7</td>
</tr>
<tr>
<td>JA159</td>
<td>MC4100 glcB::cat</td>
<td>This work</td>
</tr>
<tr>
<td>JA162</td>
<td>MC4100 himA::cat</td>
<td>(HN1491) P1 × MC4100</td>
</tr>
<tr>
<td>JA163</td>
<td>MC4100 himD3::cat</td>
<td>(K2704) P1 × MC4100</td>
</tr>
<tr>
<td>JA164</td>
<td>MC4100 arcA</td>
<td>(ECL1618) P1 × MC4100</td>
</tr>
<tr>
<td>DV21A01</td>
<td>glu-1 aceA glc</td>
<td>2</td>
</tr>
<tr>
<td>DV21A05</td>
<td>glu-1 aceB glc</td>
<td>4</td>
</tr>
<tr>
<td>JA165</td>
<td>aceA</td>
<td>(MC4100) P1 × DV21A01</td>
</tr>
<tr>
<td>JA166</td>
<td>aceB</td>
<td>(MC4100) P1 × DV21A05</td>
</tr>
<tr>
<td>JA167</td>
<td>aceA gcE::cat</td>
<td>(JA159) P1 × JA165</td>
</tr>
<tr>
<td>JA168</td>
<td>aceB gcE::cat</td>
<td>(JA159) P1 × JA165</td>
</tr>
</tbody>
</table>

Cross-induction of glc Promoter by Acetate

Fig. 1. Metabolic map of the pathways converging on glyoxylate. Glycolate, acetate, and purine degradation pathways are shown only with the enzymes relevant to this work.
Fig. 2. Transcriptional organization of \( \text{glc} \) genes. A, Northern blot of total RNA from wild-type strain CH1827 and its isogenic derivative mutant strain CH1828 with temperature-sensitive RNase E. Cells were grown at 30 °C to an \( A_{600} \) of 0.5. A sample of each culture was withdrawn before shifting the growth temperature to 45 °C. A second sample of each culture was taken after 45 min. Hybridization was performed with a 810-bp \( \text{glcD} \) specific probe (SalI internal fragment). B, the DNA region encompassing the \( \text{glc} \) operon is represented by the open bar where the relevant restriction sites are marked as follows: A, AvaII; B, BamHI; C, ClaI; D, NdeI; N, NruI; P, AgaI; S, SalI; V, EcoRV. The extension and direction of the \( \text{glc} \) genes are indicated by open arrows; \( \text{glcB} \) (encoding malate synthase); \( \text{glcG} \) (function unknown); \( \text{glcDF} \) (encoding glycolate oxidase subunits); \( \text{glcC} \) (encoding the activator protein). The thick black lines under \( \text{glcD} \) and \( \text{glcC} \) correspond to the fragments used as probes in the Northern experiments. Small black arrows represent the fragments fused to lacZ for testing \( \text{glc} \) promoter function labeled by numbers that indicate the length in nucleotides upstream of the ATG. The corresponding transcriptional fusions are shown on the left. kb, kilobase pairs.

- 103 to −86, −88 to −71, −54 to −37, and −27 to −10, all bearing additional nucleotides at the 5′-end to generate a BamHI site at the other end of the PCR product. After digestion with BamHI and EcoRI, the PCR products were cloned into the pRS550, and the corresponding recombinant plasmids were used to construct single copy fusions in strain MC4100 as described above. Among the fragments described above, the three obtained with the partner primers, −247 to −230, −192 to −177, and −170 to −153, were also cloned into pRS551 yielding sequences of the \( \text{glcC} \) promoter deleted at the 3′-end and fused to lacZ. Single copy fusions of these constructs were also obtained in strain MC4100.

Purification and Phosphorylation of His\(_6\)-ArcA Protein—Purification of the His-tagged ArcA protein from isopropyl-1-thio-\( \beta \)-galactosidase-induced \( \text{E. coli} \) M15 cells transformed with pREP4 and pQE30ArcA, by nickel chelate affinity chromatography using the nickel-nitriilotriacetic acid resin (Qiagen), was performed as described previously (26). Purified His\(_6\)-ArcA (50 \( \mu \)g/ml) was phosphorylated with 50 \( \mu \)M disodium carbamyl phosphate (Sigma) at 30 °C for 1 h.

DNA Binding Studies—For the binding studies with ArcA-P or I-I, the 360-bp DNA fragment containing the \( \text{glcD} \) promoter sequence (−247 to −194) was obtained by PCR with primers GLCBam (5′-CGCGGATCCGATATCCTCTGCGAACC-3′) and GLCEco (5′-GGGGAATTCCTGCGT-3′) using plasmid pTP25 (7) as a template. For binding studies with GlcC, the 113-bp DNA fragment containing the \( \text{glcC} \) promoter sequence (−27 to −10) was obtained by PCR with primers GLCBam and GLC P (5′-GGGGAATTCCTGCGT-3′). PCR-amplified products were purified from acrylamide gels and labeled by using T4 polynucleotide kinase and \( \gamma \)\(^{32}\)P\(-\)ATP (3,000 Ci/mmol, NEN Life Science Products). End-labeled fragments for DNase I footprinting assays were generated by digestion of the labeled DNA fragments with a restriction endonuclease that cleaves (uniquely) close to either of the termini. The short \( \gamma \)\(^{32}\)P-labeled, double-stranded DNA fragments generated by restriction enzyme digestion and non-incorporated nucleotides were removed by sequential ethanol precipitation.

Electrophoretic mobility shift assays for GlcC or I-I were performed using crude extracts obtained as described by Nunohiba et al. (27). Acrylamide gels containing 10% glycerol and 0.5× TBE buffer were run at 4 °C (28). Protein samples were mixed with \( \gamma \)\(^{32}\)P-end-labeled DNA substrates (−2.5 \( \mu \)M final concentration, −10,000 to 25,000 cpm) in a 20-\( \mu \)l reaction volume containing 10 \( \mu \)M Tris-HCl (pH 7.5), 75 \( \mu \)M KCl, 10% glycerol, and 2 \( \mu \)M dithiothreitol. Poly(dI-dC) was used as nuclease-Sensitive competitor. After incubation for 15 min at 25 °C, the reaction was continued for 1 min at room temperature. Then 3 \( \mu \)l of 200 \( \mu \)M CaCl\(_2\), 10 \( \mu \)M MgCl\(_2\) was added, and the incubation was continued for 1 min at room temperature. Then 1 \( \mu \)l (0.15 units) of RNase-free RNase I (Promega) was added, and following a further minute at room temperature, the nucleolytic reaction was terminated by addition of 90 \( \mu \)l of 200 \( \mu \)M NaCl, 30 \( \mu \)M N\(_2\)EDTA, 1% SDS, 100 \( \mu \)g of yeast RNA per ml. After the phenol/chloroform extraction, DNA products were recovered by ethanol precipitation, and resuspended in 0.1 M NaOH/formamide (1:2 v/v), 0.1% xylene cyanol, 0.1% bromphenol blue. Reactions were heated at 95 °C for 2 min prior to loading on 5% polyacrylamide wedge-shaped sequencing gels.

Results

Experimental Evidence of the Cross-induction of ace and \( \text{glc} \) Operons—Retention of growth abilities on acetate and glycolate by mutants defective in either aceB (strain JA166) or \( \text{glcB} \) (strain JA159) first suggested the cross-induction of these two systems. Consistent with this notion, a double aceB-glcb mutant (strain JA168) failed to grow on both acetate and glycolate. On the other hand, mutations abolishing ICL activity (strains JA165 and JA167) only prevented growth on acetate, and mutations abolishing GOX activity (strain JA155) prevented only growth on glycolate, despite the presence of both MSA and MSG.

Further evidence for the cross-induction was obtained by assaying MSA, MSG, and GOX activities on crude extracts of the wild-type strain MC4100 grown on acetate or glycolate. Basal levels of these activities in casein acid hydrolysate were found to be in the range of 20 milliunits/mg for MSA and MSG and <5 milliunits/mg for GOX. Glycerol induced MSA activity at a level of 80 milliunits/mg, one-half of the 170 milliunits/mg obtained when the cell grew on acetate. MSG and GOX were coordinately induced to levels of 450 and 40 milliunits/mg, respectively, on cultures grown on glycolate, whereas growth on acetate induced MSG levels to significantly lower values (70...
milliliters/mg). The 6-fold lower level of glc induction in this carbon source, as indicated by MSG activities, brings GOX activity below limits of detection (<5 milliliters/mg). Nevertheless, acetate-induced expression of genes encoding GOX subunits was evidenced by Northern experiments using RNA preparations obtained from strain MC4100 grown on acetate. Levels of MSG induction in the different conditions were confirmed by immunochemical detection of the MSG protein (not shown).

Transcriptional Organization of the glc Gene Cluster—To understand better the mechanisms of the ace and glc cross-induction, we proceeded to determine the transcriptional units of the glc gene cluster. Previous Northern blot experiments with the wild-type strain MC4100 failed to detect a polycistronic mRNA (7), possibly because of a message decay. To circumvent such a possibility, we grew an RNase E temperature-sensitive mutant strain CH1828 and its isogenic parent CH1827 on Caa plus glycolate to compare the results of Northern blots. Only RNA preparations of mutant strain CH1828 grown at restrictive temperature showed an mRNA of 7.5 kb corresponding to the full-length transcript of the glc system (Fig. 2A). The same polycistronic mRNA was detected using either a glcD or glcB probe. Transcription of the genes glcDEFGB as a single unit is supported by the properties of five lacZ fusions corresponding to each of the glc genes (Fig. 2B). These operon fusions were transferred to strain MC4100. Of the five resulting merodiploids grown on Caa either in the presence or in the absence of glycolate, only the one bearing the glcD-lacZ fusion expressed a glycolate-inducible β-galactosidase (Fig. 3A). By contrast, the other four fusions exhibited neither significant basal nor inducible activity (not shown). Furthermore, Φ(glcEFGB), containing a 3′-fragment of glcD (Fig. 2B), also exhibited no activation of transcription by glycolate. The same pattern of expression was obtained in acetategrown cells. These observations indicated that the only functional promoter for the glc structural genes is located upstream of glcD.

The expression of Φ(glcC-lacZ) corresponding to the regulatory gene, in contrast to that of Φ(glcD-lacZ), was repressed by the presence of glycolate (Fig. 3A).

Mapping of the mRNA 5′-End for the Structural and Regulator Transcriptional Units—The 5′-end of the structural genes was determined by primer extension analysis. Total mRNA from strain MC4100 grown in the presence of glycolate was obtained. For the primer extension reaction a primer complementary to a region within glcD (positions +52 to +33 of the coding region) was used, and a single putative 5′-end was determined (Fig. 4B). The 5′-end was thus located 55 bp upstream of the ATG codon. The putative 5′-end, position +1 in Fig. 4A, is preceded by a promoter sequence (~35, TAGAGC; −10, TAATAA, with a spacing of 17 bp) that conforms relatively well to the σ70 consensus sequence.
does not change the $\Psi_{\text{glcC-lacZ}}$ expression (data not shown). To verify this expectation, an arcA null mutation was introduced in strain MC4100, yielding strain JA164. The expression of $\Phi_{\text{glcD-lacZ}}$ in this genetic background was no longer repressed under fermentative conditions, indicating that ArcA acts as an anaerobic repressor of the glc operon (Fig. 3B).

To analyze the interaction of ArcA and ArcA-P with the glc promoter region, gel retardation assays were carried out using purified His$_6$-ArcA and His$_6$-ArcA-P generated by treatment of the former with carbamyl phosphate. The results indicated that the phosphorylation of ArcA significantly enhanced its DNA binding activity (Fig. 6A). Increasing the concentration of ArcA-P in the incubation mixture resulted in the appearance of further retarded species, indicating multiple binding and/or oligomerization of ArcA-P within the glc promoter region.

The binding of His$_6$-ArcA and His$_6$-ArcA-P to the glc promoter region was also analyzed by DNase I footprinting. No discrete regions of protection could be observed with His$_6$-ArcA, whereas clear DNase I footprints were seen with His$_6$-ArcA-P. It is noteworthy that within the protected regions there are highly protected segments as follows: from positions −45 to +31 (ArcA site I) and from −106 to −161 (ArcA site II) relative to the 5'-end of glcD gene (Fig. 6B). Protection of the two ArcA sites was observed on both strands. These large protected regions again suggested ArcA-P binding to multiple sites and/or oligomerization of the regulator protein molecule.

Deletion Analysis of the glcD Promoter—To locate the cis-acting elements required for regulation of the glcD promoter, different 5'-deletions were fused to the lacZ reporter gene and introduced as single copy fusions in MC4100 background.

Ten constructions labeled by the 5' terminus were analyzed by $\beta$-galactosidase expression in cultures grown aerobically on Caa, either in the presence or in the absence of glycolate. Results presented in Fig. 7 show that full induction of $\Phi_{\text{glcD-lacZ}}$ by glycolate required sequences up to position −184. The construct f-177 showed a 40% reduction in activity levels, whereas construct f-170 totally lost the ability for expression. These results therefore point to an upstream activator site (UAS) for GlcC binding (−2 to −170). Moreover, the location of this site between the 5'-end and the ATG start codon of glcC could also explain the reduced expression of the regulator gene glcC during growth in the presence of glycolate. This was further supported by the results obtained with three transcriptional fusions in the opposite orientation which expressed the glcC promoter activity (r-247, r-192, and r-170 in Fig. 7). Absence of the UAS element in these constructs caused enhanced activation of glcC transcription. Hence, while activating the transcription of the structural genes, GlcC exerts an autogenous repression on its own gene. Interaction of GlcC with the UAS element was assessed by gel retardation experiments.
performed with a 113-bp fragment of glcD promoter (positions −247 to −134) containing this element. Whereas retardation was observed with an extract of the wild-type strain, no effect was seen with that of the glcC mutant (Fig. 5B).

Since it is likely that the IHF effect is mediated by sequences downstream of position −170, a computer search for IHF sites (30) was undertaken. Probable consensus sequences were found between positions −128 to −93 in the glcD coding strand and −142 to −95 in the complementary strand (Fig. 4A). The construct f-54, lacking a A/T-rich segment likely to facilitate bending (positions −61 to −69) but retaining the −35 and −10 promoter sequences, allowed a \( \Phi(\text{glcD-lacZ}) \) expression at 10% of the normal level, and this expression was independent of GlcC. The same result was obtained when this construct was expressed in a himA mutant (not shown) indicating that the IHF effects are mediated by sequences upstream of position −54. This basal expression was lost in the construct f-27 missing the −35 sequence for RNA polymerase binding.

Induction of the glc Operon by Acetate Is Mediated by the Action of GlcC Protein on the glcD Promoter—The introduction of a cat insertion in glcC absolutely abolished induction of glc by acetate, indicating that the cross-induction was mediated by the glcC gene product (not shown). Moreover, when strain MC4100 bearing the \( \Phi(\text{glcD-lacZ}) \) was transformed with a pBS derivative plasmid containing the 400-bp SalI fragment flanking the glcD promoter, expression from the chromosomal fusion was reduced by about 80%. This diminution, produced either in the presence of glycolate or acetate as inducers, can be explained by GlcC titration by the high copy number of glcD promoter. Consistent with this interpretation, the induced activity levels were high (70% of the control level) when sequences upstream of position −170 were eliminated from the construct.

To analyze whether acetate induces the glc operon expression by itself or via glyoxylate formation, the \( \Phi(\text{glcD-lacZ}) \) was introduced in mutant strains JA165 (ICL-deficient), JA166 (MSA-deficient), and JA168 (lacking both MSA and MSG). β-Galactosidase assay and immunological quantification of MSG were performed on cultures of these strains growing on Caa 0.05% in the presence of acetate or glycolate, with strain MC4100 \( \text{glcD-lacZ} \) serving as control (Fig. 8). Acetate induction in an ICL-deficient mutant suggested that the effector molecule responsible for the cross-induction could be acetate itself or any derived metabolite leading to isocitrate formation (Fig. 8). To check if acetate itself acted in the cross-induction,
Fig. 7. Deletion analysis of glcD

**glcC** promoter region. A set of 5′-deletions of glcD promoter (labeled f) and three 3′-deletions of glcC promoter (labeled r) were used to generate transcriptional lacZ fusions on the MC4100 chromosome. At the top, the relevant regulatory elements of the glcD promoter are shown. Indicated positions correspond to the glcD-coding strand, whereas those corresponding to the complementary glcC-coding strand are in parentheses. The 5′-end is marked by an arrow labeled +1. Upstream of this position, the −10 and −35 consensus, high AT content region, potential IHF-binding site, and UAS are located by the corresponding boxes. Deleted promoter fragments are represented by lines, and the corresponding lacZ fusion is labeled on the right. Numbers on the left of each line indicate the extent of the deletion. Cells of strain MC4100 bearing each fusion were grown aerobically on Caa or Caa plus glycolate and β-galactosidase activities determined. Activity values are indicated in the table at the right.

Fig. 8. Induction analysis of the gle operon by acetate in different ace mutants. β-Galactosidase levels in cells of strains MC4100 (1), JA165 (2), JA166 (3), and JA168 (4) bearing the glcD-lacZ fusion grown on Caa 0.05% alone or in the presence of acetate (+Ace) or glycolate (+Glc). Immunoprecipitate quantification of MSG, performed in the corresponding cell extracts, is shown below.

Strain JA165 (φgldD-lacZ) was grown on 0.05% Caa in the presence of oleate whose metabolism provide acetyl-CoA but not acetate. Levels of induction in the presence of oleate (180 Miller units) were similar to those obtained in the absence of this fatty acid and were about less than 50% those obtained in the presence of acetate (450 Miller units). These results indicate that acetate but not acetyl-CoA is the effector molecule.

In strain JA166 (φgldD-lacZ), lacking MSA, the pool of unmetabolized glyoxylate is expected to increase during growth on acetate. In this strain, glyoxylate itself or glycolate formed by glyoxylate reduction enhanced ψ(gldD-lacZ) expression and the level of MSG synthesis by 2.5-fold. In strain JA168 (φgldD-lacZ), lacking both MSG and MSA, the intracellular glyoxylate pool was even higher, and the expression of lacZ by acetate increased 5-fold. The increased promoter activity in Caa-grown cells suggests that glyoxylate may be formed from amino acid degradation. Results of immunoassay of MSG levels in all of the mutants grown under various conditions (Fig. 8) matched the results of ψ(gldD-lacZ) expression.

**DISCUSSION**

It is tempting to speculate that the presence of redundant malate synthases and the cross-induction of the gle and ace operons evolved as a safeguard against toxic accumulation of the chemically reactive glyoxylate. The hypothesis is consistent with the enhanced cross-induction resulting from glyoxylate accumulation in mutants lacking either of the malate synthase isoenzymes.

Molecular characterization of the cross-induction required fine structure analyses of these operons. The ace operon has been well studied (31, 32), and its inducer has been identified to be phosphoenolpyruvate (8). Thus, the generation of this inducer from glycolate via the δ-glycerate pathway (2) would explain cross-induction of the ace operon by glycolate. Here we report a fine structure study of gle operon and its regulation by glycolate and acetate. Promoter deletion analysis has permitted us to define a cis-acting element (labeled UAS) that has been shown to interact with glcC gene product in the presence of glycolate. This regulator protein functions simultaneously as glcDEFGB activator and glcC repressor. When the gle system is cross-induced it appears that acetate or a derivative serve as an alternative effector. The use of an ICL (aceA) mutant showed that glyoxylate formation from acetate was not required for induction. The inability of oleate, which yields acetyl-CoA but not acetate, to induce the gle system in this ICL mutant corroborates the role of acetate as inducer molecule.

In the context of the cross-induction, several features of the gle promoter are of interest. Anaerobic repression of gle by ArcA-P is consistent with the fact that both acetate and glycolate metabolisms take place aerobically (33). Acetate cannot be utilized as a carbon source without the aerobic Krebs cycle, and the ace operon has been suggested to be a target for the Arc modulon (34). On the other hand, glycolate oxidase is absolutely dependent on the aerobic electron transport chains of the...
bacterial membrane. ArcA mutants, footprinting, and gel-shift experiments indeed demonstrated a functional control of *glc* by the Arc system. In this regard it might be pointed out that the ArcA-P-protected regions contained three sequences with high similarity (7:10 agreement) to the consensus proposed by Lynch and Lin (26): site I from positions –7 to –16 and site II from positions –102 to –111 and from positions –110 to –119 (Fig. 4A). Although the proposed consensus was questioned, no amendment was offered (35). On the other hand, the two consensus sequences (10:10 agreement) in *aldA* promoter have been useful in predicting the *in vitro* site of ArcA-P binding and *in vivo* regulation as demonstrated by site-directed mutagenesis. The overlap of site I with the –10 promoter sequence would explain the transcriptional repression of *glc* operon in anaerobic conditions. Hypersensitivity to DNase I at several positions within the ArcA-P-protected regions suggests that ArcA-P promoted DNA bending.

The proposed hairpin structure for this promoter is supported by the absolute dependence of its activation on IHF. It has to be underscored that in this system the IHF effect is not mediated by a repression in the expression of the regulator gene *glcC* (see Fig. 3A). Binding of IHF to sequences between the distant specific activator site (UAS) and the promoter have been useful in predicting the functional control of the *ace* operon (39). Thus, the metabolic role of cross-induction is made possible by the fact that both *ace* and *glc* operons are similarly controlled by Arc and IHF.

Acknowledgments—We thank Simon Lynch for critical reading of the manuscript and Robin Rycroft for editorial assistance.

REFERENCES


---

Cross-induction of glc and ace Operons of Escherichia coli Attributable to Pathway Intersection: CHARACTERIZATION OF THE glc PROMOTER

Maria Teresa Pellicer, Carmen Fernandez, Josefa Badià, Juan Aguilar, Edmund C. C. Lin and Laura Baldomà

doi: 10.1074/jbc.274.3.1745

Access the most updated version of this article at http://www.jbc.org/content/274/3/1745

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 38 references, 21 of which can be accessed free at http://www.jbc.org/content/274/3/1745.full.html#ref-list-1