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# The Gene *yjcG*, Cotranscribed with the Gene *acs*, Encodes an Acetate Permease in *Escherichia coli*

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**We isolated an *Escherichia coli* mutant strain that suppresses the glycolate-negative phenotype of a strain deficient in both GlcA and LldP transporters of this compound. This suppressing phenotype was assigned to *yjcG*, a gene whose function was previously unknown, which was found to encode a membrane protein able to transport glycolate. On the basis of sequence similarity, the *yjcG* gene product was classified as a member of the sodium:solute symporter family. Northern experiments revealed that *yjcG* is cotranscribed with its neighbor, *acs*, encoding acetyl coenzyme A synthetase, which is involved in the scavenging acetate. The fortuitous presence of an IS*2* element in *acs*, which impaired *yjcG* expression by polarity in our parental strain, allowed us to conclude that the alternative glycolate carrier became active after precise excision of IS*2* in the suppressed strain. The finding that *yjcG* encodes a putative membrane carrier for glycolate and the cotranscription of *yjcG* with *acs* suggested that the primary function of the *yjcG* gene product (proposed gene name, *actP*) could be acetate transport and allowed us to define an operon involved in acetate metabolism. The time course of [1,2-$^{14}$C]acetate uptake and the results of a concentration kinetics analysis performed with cells expressing ActP or cells deficient in ActP supported the the hypothesis that this carrier is an acetate transporter and suggested that there may be another transport system for this monocarboxylate.**

There are two alternative pathways for acetate utilization in *Escherichia coli*. One of these pathways is mediated by acetyl coenzyme A (acetyl-CoA) synthetase (EC 6.2.1.1), which catalyzes acetyl-CoA formation through an enzyme-bound acetyladenylate intermediate in an irreversible reaction (1). This enzyme is encoded by the gene *acs*, located at centisome 92.32 on the *E. coli* chromosome (14). The other pathway, mediated by the enzymes acetate kinase (EC 2.7.2.1) and phosphotransacetylase (EC 2.3.1.8), proceeds through a high-energy acetyl phosphate intermediate in two reversible reactions (23). These two enzymes are encoded by the *ackA* and *pta* genes, respectively, which are located in an operon at centisome 51.98 on the bacterial chromosome (11). *E. coli* cells use the Acs high-affinity pathway to scavenge small concentrations of acetate and the low-affinity AckA/Pta pathway to dissimilate large concentrations of acetate during glycolysis (4, 12). In a mixed-acid fermentation of carbohydrates cells synthesize acetyl-CoA through the reversible AckA/Pta pathway and excrete acetate in a process that generates ATP (8). Despite the large amount of information concerning acetate metabolism that has been obtained, no transport system for acetate has been described to date, although previous reports showing that acetate uptake is saturable seem to indicate that there is such a system (11).

The 2-hydroxymonocarboxylic acids L-lactate, D-lactate, and glycolate share the transporters encoded by two genes, *lldP* of the lactate operon and *glcA* of the glycolate operon (21). Both transporters accumulate their substrates against a concentration gradient driven by a proton motive force. Due to the functional similarity between these permeases, GlcA has been included in the LldP family of membrane carriers (24). The amino acid sequences of LldP and GlcA exhibit 65% identity and 80% similarity. Nonetheless, LldP is more effective in accumulating L-lactate, whereas GlcA is more effective in accumulating glycolate (20). Acetate, a two-carbon monocarboxylate, is not recognized by either of these permeases. Thus, during evolution the basic mechanisms of recognition seem to have been retained for the hydroxyl and carboxyl groups, even though L-lactate and D-lactate are three-carbon compounds and glycolate is a two-carbon compound.

The overlapping specificity of LldP and GlcA explains why a glycolate or L-lactate entry-negative phenotype requires inactivation of both transporters (21). In this study we analyzed the appearance in an *lldP glcA* double mutant of another transport system for the two-carbon carboxylate glycolate, which is also able to transport acetate. This transporter was identified as an acetate carrier encoded by a gene cotranscribed with the *acs* gene involved in acetate metabolism.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** All the strains used were *E. coli* K-12 derivatives. The genotypes and sources of the relevant bacterial strains and plasmids are shown in Table 1.

**Cell growth.** Cells were grown aerobically in Luria-Bertani broth (LB) or minimal medium (3). For growth on minimal medium, carbon sources were added at a carbon concentration of 60 mM unless otherwise specified. Casein acid hydrolysate was used at a concentration of 0.5%. The following antibiotic concentrations were used: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; chloramphenicol, 30 μg/ml; and tetracycline, 12.5 μg/ml. 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (X-Gal) and isopropyl-β-D-thiogalactoside (IPTG) were used at concentrations of 30 and 10 μg/ml, respectively.

**Transport and enzyme assays.** For transport assays, the cells were collected at the end of the exponential phase, washed twice, suspended in minimal medium (3) to a final density of 0.5 mg (dry weight)/ml, and placed at 25°C (20). The rate of uptake was assayed by diluting a radioactive substrate 10-fold with the cell suspension to a final concentration of 20 μM. The radioactive substrates assayed were [1,2-$^{14}$C]glycolate (50 mCi/mmol) purchased from ICN and [1,2-$^{14}$C]ac-

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TABLE 1. Bacterial strains and plasmids used in this study

| Strain or plasmid | Description | Source or reference |
|---|---|---|
| Strains | | |
| XL1Blue | *recA1 lac endA1 gyrA96 thi hsdR17 supE44 relA1* (F′ *proAB lacI*$^q$ *lacZ*ΔM15 Tn*10*) | Stratagene |
| TE2680 | F$^-$ *l*$^-$ IN(*rrnD-rrnE*) *Dlac X74 rplS galK2 recD*::Tn*10d*-Tet *trpDC700*::*putA13033*::(Kan$^r$ Cm$^r$ *lac*) | 7 |
| MC4100 | F$^-$ *araD* Δ(*argF-lac*) *rpsL relA flbB deoC ptsF rbsR acs*::IS2 | 6 |
| JA202 | MC4100 *glcA*::*cat lldP*::Tn*5* (Glc$^-$) | 21 |
| JA212 | Spontaneous Glc$^+$ mutant of JA202 (*acs*$^+$)[a] | This study |
| JA213 | JA212 *actP*::Tn*10* | This study |
| JA165 | *lacZ43 relA1 spoT1 thi1 aceA* | 22 |
| JA214 | JA165 *actP*::Tn*10* | This study |
| AJW803 | Δ*lacX74 thi-1 thr-1*(Am) *leuB6 metF159*(Am) *rpsL136* λ*lacY* Φ(Δ*acs*::*Km-1*) | 14 |
| JA215 | JA212 Φ(Δ*acs*::*Km-1*) | This study |
| Plasmids | | |
| pBR322 | Ap$^r$ Tc$^r$ | Boehringer Mannheim |
| pRS550 | Ap$^r$ Km$^r$, contains promoterless *lacZYA* | 26 |
| pFN12 | *glcA* in pBR322 | 21 |
| pFN41 | *actP* (*yjcG*) of strain MC4100 in pBR322 | This study |
| pFN42 | *actP* (*yjcG*) of strain JA212 in pBR322 | This study |

[a] IS2 was precisely excised from *acs*.

etate (57 mCi/mmol) purchased from Amersham. Samples (100 μl) were taken at different intervals and filtered through 0.45-μm-pore-size cellulose nitrate filters. The filters were washed with 5 ml of minimal medium, placed in plastic vials, and counted with a scintillator in the presence of Emulsifier-safe (Packard, Meriden, Conn.). To identify competitors, the assays were performed in the presence of 50-fold excesses of unlabeled candidate compounds.

To analyze the effect of sodium on acetate transport, cells were collected, washed up to three times with potassium phosphate buffer (pH 7.0) containing 10 mM $MgCl_2$, and suspended in the same buffer at a final cell density of 0.5 mg (dry weight)/ml. In this case, the cold acetate salt used to prepare the substrate mixture was the potassium salt. Transport of 20 μM [1,2-$^{14}$C]acetate was measured under standard conditions either in the absence or in the presence of 50 mM NaCl.

Specific β-galactosidase activity was assayed at 28°C in cells permeabilized with chloroform and sodium dodecyl sulfate by using *o*-nitrophenyl-β-D-galactopyranoside as the substrate and was expressed in Miller units (16). The protein concentration was determined by the method of Lowry et al. (15) by using bovine serum albumin as the standard.

The enzyme activities and transport rates reported below are the means of at least three separate experiments.

**DNA manipulation.** Bacterial genomic DNA was obtained by the method of Silhavy et al. (26). Plasmid DNA was routinely prepared by the boiling method (9). For large-scale preparation, a crude DNA sample was subjected to purification on a column (Qiagen Gmbh, Düsseldorf, Germany). DNA manipulations were performed essentially as described by Sambrook and Russell (25). DNA fragments were amplified by PCR by using *E. coli* chromosomal DNA as the template. When necessary, specific restriction sites were incorporated at the 5′ ends of the primers to facilitate cloning of the fragments in the appropriate vector. PCRs were performed with *Pfu* DNA polymerase under standard conditions.

DNA was sequenced by using an automated ABI 377 DNA sequencer and fluorescent dye termination methods.

**Mutagenesis and genetic techniques.** Tn*10* insertion mutagenesis was performed by infection with phage λNK1098 as described by Way et al. (30). Genetic crossing was performed by P1 *vir*-mediated transduction (16).

**Analysis of expression by Northern blotting.** Total RNA was prepared by using the SV total isolation system of Promega (Madison, Wis.) and was quantified by absorbance at 260 nm. Northern blot hybridization was performed with each RNA sample (10 μg) as described by Moralejo et al. (17).

**Construction of *lacZ* fusions to analyze promoter function.** Transcriptional fusions to *lacZ* were constructed by inserting DNA fragments into plasmid pRS550 (27). This plasmid carried a promoterless *lac* operon and genes that confer resistance to kanamycin and ampicillin. To construct the transcriptional *acs-yjcH-yjcG-lacZ* fusion, primers Acs3 and YjcG7 (Table 2) were used to amplify a 2,810-bp fragment of the 5′ upstream region of the *yjcG* gene. This fragment contained the *acsP1* and *acsP2* promoters (13) and the coding regions of the *yjcH* and *acs* genes. Using PCR, we also prepared two additional constructs in which *acsP1* and *acsP2* were deleted. Fusion ϕΔ1(*acs-yjcH-yjcG-lacZ*) was obtained with primers Acsdel-1 and YjcG7, and fusion ϕΔ2(*acs-yjcH-yjcG-lacZ*) was obtained with primers Acsdel-2 and YjcG7. Fragments were digested with *Sma*I and *Bam*HI and cloned into the *Eco*RI (blunt ended with the Klenow fragment) and *Bam*HI restriction sites of plasmid pRS550. After transformation of strain XL1Blue, recombinant plasmids were selected as blue colonies on LB plates containing X-Gal, ampicillin, and kanamycin. Plasmid DNA was se-

TABLE 2. Oligonucleotides used in this study

| Primer | Sequence[a] | Experiment |
|---|---|---|
| miniTn10-A | 5′-CCAAAATCATTAGGGGATTCATCAG-3′ | Mini-Tn*10* location |
| miniTn10-C | 5′-TTTTATTGGCTGGTGGTGGGATCGC-3′ | Mini-Tn*10* location |
| YjcG1 | 5′-TTTCCCGGGTTACTGATCGCCTTCGCG-3′ | *yjcG* cloning |
| YjcG2 | 5′-CAAGGATCCTATGGGAAAAGGTGATGC-3′ | *yjcG* cloning |
| YjcG3 | 5′-CGTAGCGACTACTACACC-3′ | *yjcG* probe for Northern blotting |
| YjcG4 | 5′-GATGTCGACACCTTTCG-3′ | *yjcG* probe for Northern blotting |
| Acs1 | 5′-GGTGATATCTACTGGTGC-3′ | *acs* probe for Northern blotting |
| Acs2 | 5′-TCGTGATCGCCAAACAGC-3′ | *acs* probe for Northern blotting |
| Acs3 | 5′-GGTGGATCCTTTCACGACAGTAACCGC-3′ | *acs* operon promoter fusion |
| YjcG7 | 5′-TAACCCGGGCATGATGCTTGTACCTCATG-3′ | *acs* operon promoter fusion |
| Acsdel-1 | 5′-ATCGGATCCGGAGAACAAAAGCATGAGC-3′ | *acs* operon promoter deletion |
| Acsdel-2 | 5′-ATCGGATCCCACAACACACCATTCCTG-3′ | *acs* operon promoter deletion |
| YjcF1 | 5′-CTCTGTCACAAGTCCATCAC-3′ | *yjcF* probe for Northern blotting |
| YjcF2 | 5′-TACCTCAGCCATGTATGCCA-3′ | *yjcF* probe for Northern blotting |

[a] The underlined sequences are the incorporated *Sma*I or *Bam*HI restriction sites.

quenced by using an M13 primer to ensure that the desired fragment was inserted in the correct orientation and that no mutations had been introduced by the PCR amplification. Merodiploids were obtained by transferring the fusions as single copies into the *trp* operon of *E. coli* strain TE2680 as described by Elliot (7). The transformants were selected on the basis of kanamycin resistance and were screened for sensitivity to ampicillin and chloramphenicol. P1 *vir* lysates were prepared to transduce the fusions into various genetic backgrounds.

## RESULTS

**Alternative transport for glycolate.** Cells of strain JA202 carrying mutations in the *glcA* and *lldP* genes encoding the glycolate and lactate transporters, respectively, do not grow on glycolate (21). However, spontaneous mutants of strain JA202 able to grow on glycolate were isolated. As these mutants did not grow on D-lactate, which is recognized by GlcA or LldP, the new glycolate-positive phenotype could not have been due to a reversion mutation restoring either of these transporters. In these mutants, which were isolated as efficient growers on glycolate plates, a suppressing mutation may have occurred. We selected one of them, strain JA212, for further study.

To locate the gene responsible for the suppressor phenotype, we obtained random Tn*10* insertion mutants from strain JA212 as described in Materials and Methods and selected them on tetracycline plates. Replica plating of the colonies on glycolate allowed us to search for clones that had lost the ability to grow on this carbon source. To avoid multiple transposon insertions, we transduced the mutations into strain JA212 and again selected transductants by using resistance to tetracycline and the glycolate-negative phenotype. In order to discard insertion mutations in other genes of the glycolate pathway, only mutants complemented by plasmid pFN12 (Table 1), which expresses the glycolate permease encoded by *glcA*, were selected. In this way, we isolated strain JA213 and used it to locate the gene encoding the new transporter. In this strain, the persistence of *glcA*::*cat* and *lldP*::Tn*5* mutations was shown by PCR.

To map the Tn*10* insertion in strain JA213, we digested the genomic DNA of this strain with *Hpa*II and ligated the resulting fragments, which were subjected to inverse PCR with oligonucleotides miniTn10-A and miniTn10-C (19). Sequencing of the amplified fragment and in silico analysis of the sequence allowed us to locate the insertion in gene *yjcG*. This gene, whose function is unknown, is located between positions 3356 and 5005 of the *E. coli* sequence deposited under accession number AE000480, which corresponds to centisome 92.28 of the bacterial chromosome (2).

***yjcG* gene encodes a transporter belonging to the sodium:solute symporter family responsible for glycolate transport in strain JA212.** The *yjcG* gene encodes a highly hydrophobic 549-amino-acid protein with 14 putative transmembrane segments and a molecular mass of 59,197 Da. Computational analysis of the YjcG amino acid sequence (P32705) revealed high levels of similarity to the proline permeases of several bacteria, especially PutP (proline permease) of *E. coli* (39% similarity over the entire length). On the basis of these characteristics the *yjcG* gene product was classified as a member of the sodium:solute symporter family.

To show that the *yjcG* gene product accounted for the transport of glycolate in strain JA212, we grew on 0.5% casein acid hydrolysate cells of this strain and strain JA213, in which *yjcG* was disrupted by insertion of transposon Tn*10*. The cells were collected at the end of the exponential phase and were processed to measure glycolate transport as described above. The time courses of label incorporation in the two strains showed that only strain JA212 accumulated glycolate (Fig. 1), thus indicating that *yjcG* is needed for expression of the new transporter activated in this strain. Since induction of the *glc* operon requires glycolate, we grew these cultures in the absence of glycolate to ensure that the cells could not metabolize it.

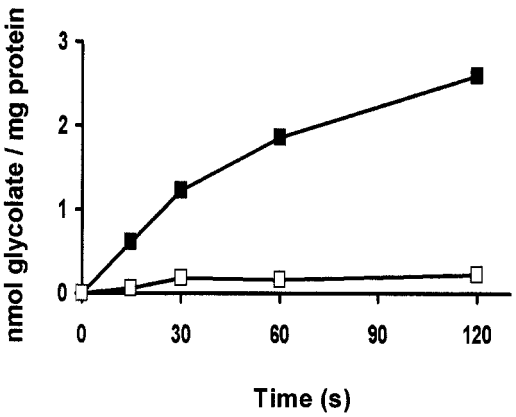


FIG. 1. Glycolate transport dependence on the YjcG function: time course of [1,2-$^{14}$C]glycolate uptake by whole cells of strain JA212 (■) and its derivative JA213 (□) grown on casein acid hydrolysate.

Furthermore, the *yjcG* gene was amplified by PCR from wild-type strain MC4100 and mutant strain JA212 by using oligonucleotides YjcG1 and YjcG2 (Table 2) and was cloned in pBR322, yielding plasmids pFN41 and pFN42, respectively. Sequencing of the genomic fragment of plasmid pFN42 revealed no differences from the wild-type gene present in pFN41. Both plasmids complemented the glycolate-negative phenotype of strain JA202 or JA213, indicating that wild-type YjcG recognizes glycolate as a substrate and that the suppressor phenotype was not a consequence of a gain of function in this protein.

***yjcG* gene belongs to an operon involved in acetate metabolism.** Searching for the suppressing mutation, we studied the expression of *yjcG* by comparing the patterns of transcription of this gene in wild-type and mutant strains by Northern blotting. Total RNAs were prepared from mid-exponential-phase cultures (optical density at 600 nm [$OD_{600}$], 0.5) of strains MC4100 and JA212 grown in LB or casein acid hydrolysate in the absence or presence of 30 mM glycolate. The probe used was a 575-bp internal fragment of the *yjcG* gene amplified by PCR with oligonucleotides YjcG3 and YjcG4 (Table 2). The results (Fig. 2) showed that the *yjcG*-specific transcript was not present in strain MC4100 grown in any of the conditions tested. In all these conditions, the RNA preparations of strain JA212 contained transcripts that ranged from 4.2 to 1.7 kb long; the latter size corresponds to the size of the *yjcG* gene. These results indicate that this gene is cotranscribed with flanking genes in strain JA212.

Upstream of *yjcG* is the small gene *yjcH*, whose function is unknown, and the *acs* gene, which is known to encode acetyl-CoA synthetase (14); all of the genes are in the same sense of transcription (Fig. 3). Downstream, and with the same sense of

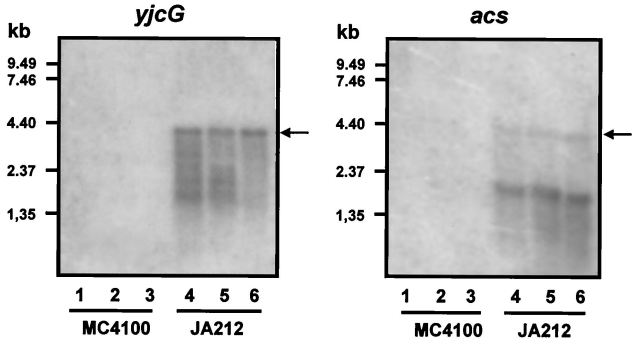


FIG. 2. Analysis of expression of *yjcG* and *acs* by Northern blotting. Total RNA was obtained from cultures of wild-type strain MC4100 and strain JA212 grown aerobically to an $OD_{600}$ of 0.5 in LB (lanes 1 and 4), casein acid hydrolysate (lanes 2 and 5), or casein acid hydrolysate in the presence of glycolate (lanes 3 and 6). Hybridization was performed with a 575-bp *yjcG* probe or with a 575-bp *acs* probe. The position of the full-length polycistronic mRNA is indicated by an arrow.

transcription, another gene whose function is unknown (*yjcF*) was separated by 178 bp from the stop codon of *yjcG*. Cotranscription of *yjcG* with the *acs* and *yjcH* genes was shown by Northern blot experiments performed with an internal 550-bp probe of the *acs* gene, which gave the same pattern of expression that was obtained for *yjcG*. In this case, a 2.0-kb band corresponding to the *acs* fragment of the transcript was accompanied by a 4.2-kb band with a lower intensity corresponding to the full-length undegraded transcript in the RNA preparations of strain JA212 (Fig. 2). In contrast, no transcripts were detected in any of these RNA preparations when an internal *yjcF* probe was used (data not shown), indicating that this gene is not cotranscribed with *acs*, *yjcH*, and *yjcG*.

Based on cotranscription of the *acs*, *yjcH*, and *yjcG* genes, we propose an operon structure for this cluster, which was further supported by the finding that expression of *yjcG* was dependent

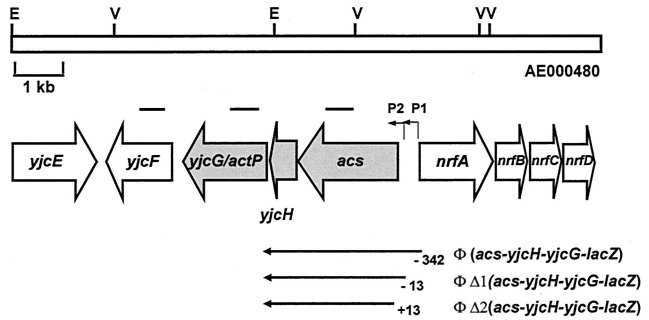


FIG. 3. Restriction map and gene organization of the *yjcG* genomic region. The open bar represents the genomic region (accession no. AE000480) encompassing *yjcG*, and relevant restriction sites are indicated as follows: E, *Eco*RI; and V, *Eco*RV. The large arrows indicate the positions and directions of the genes, and the genes belonging to the *acs-yjcH-actP* operon are indicated by grey arrows. The approximate locations of the *acs* promoters *acsP1* and *acsP2* are also indicated. The lines above the *yjcF*, *yjcG*, and *acs* genes indicate the fragments used as probes in the Northern experiments. The arrows at the bottom indicate the DNA fragments fused to *lacZ* for testing promoter function, and the numbers indicate the positions of their 5′ ends in relation to the ATG codon of the *acs* gene.

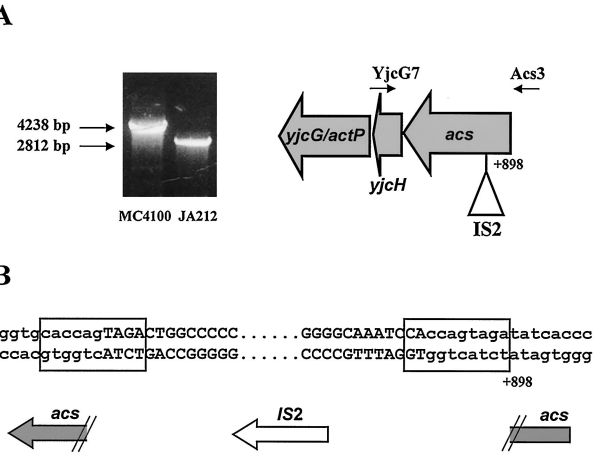


FIG. 4. Physical mapping of the IS*2* insertion in *acs* of strain MC4100. (A) Electrophoretic analysis of fragments obtained by PCR from chromosomal DNA of strains MC4100 and JA212 with primers Acs3 and YjcG7. The positions of the primers and of the IS*2* element in the *acs-yjcH-yjcG* operon are indicated in the diagram. (B) DNA sequence of the junction sites of the IS*2* insertion. The sequence corresponding to the IS*2* element is indicated by uppercase letters, while the sequence of *acs* is indicated by lowercase letters. The insertion-generated repeats are enclosed in boxes. The arrows at the bottom indicate the direction of transcription of the truncated *acs* gene and the IS*2* element.

on the *acs* promoter region and the finding that no other internal promoters were present in the proposed operon. To do this, we constructed an *acs-yjcH-yjcG* transcriptional fusion to *lacZ*, which extended from 342 bp upstream from the ATG of *acs* to the start codon of *yjcG*, including the two promoters (*acsP1* and *acsP2*) proposed for the *acs* gene (5, 13). We also prepared two additional fusions in which both the *acsP1* and *acsP2* promoters were deleted (Fig. 3). Expression of these fusions was analyzed at different times throughout growth of strain JA212 on casein acid hydrolysate. Only the fusion carrying the *acsP1* and *acsP2* promoters yielded β-galactosidase activity (575 ± 45 Miller units at an $OD_{600}$ of 0.6), indicating that the *acs-yjcH-yjcG* polycistronic mRNA was transcribed from the promoter region present 5′ of *acs*.

In the *acs-yjcH-yjcG* transcriptional fusion the fragment obtained by PCR from strain MC4100 was 1 kb longer than the fragment amplified from strain JA212 (Fig. 4A), which matched the size expected from analysis of this region of the *E. coli* genome. This result suggested that there was an insertion mutation in strain MC4100. Subsequent sequencing of this region revealed an IS*2* element at position 898 of the *acs* gene in this strain (Fig. 4B). This inserted element had the same orientation with respect to the direction of transcription of the *acs* gene, hence causing polar effects on expression of downstream genes (28). This explained the lack of the *yjcG* transcript in the Northern experiments with strain MC4100 and is consistent with the operon structure proposed above. In addition, no *yjcG* transcript was seen with RNA preparations of Δ*acs*::Km mutant strains AJW803 and JA215 (data not shown). In strain JA212, the IS*2* element was precisely excised, which restored the wild-type sequence of the *acs* gene and expression of the downstream genes. Consistent with these results, strain MC4100 displayed an acetate utilization pheno-

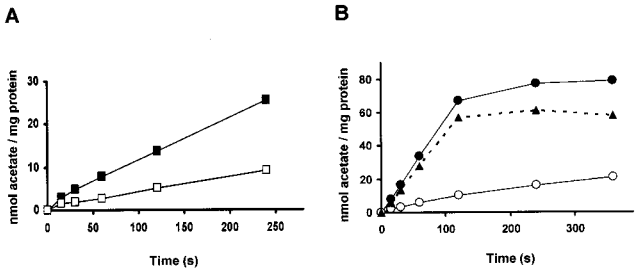


FIG. 5. Acetate transport dependence on the YjcG (ActP) function in various genetic backgrounds. (A) Time course of 20 μM [1,2-$^{14}$C]acetate uptake at 15°C by whole cells of strain JA212 (■) and its *actP*::Tn*10* derivative JA213 (□) grown on casein acid hydrolysate. (B) Time course of 20 μM [1,2-$^{14}$C]acetate uptake at 25°C by whole cells of the isocitrate lyase-deficient strain JA165 (■) and its *actP*::Tn*10* derivative JA214 (□) grown on casein acid hydrolysate. The dotted line indicates the theoretical acetate transport mediated by ActP calculated by subtracting the rates obtained for the two strains.

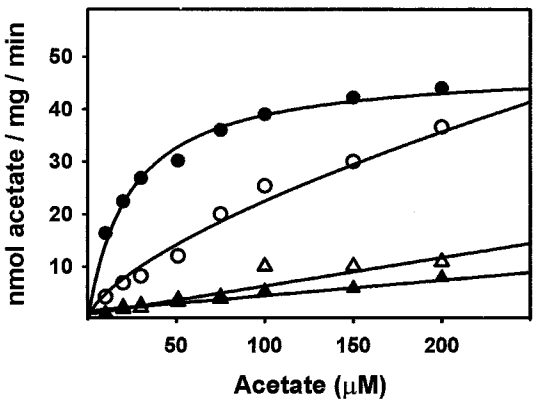


FIG. 6. Effect of CCCP on the concentration kinetics of acetate uptake. Transport of [1,2-$^{14}$C]acetate was measured in the absence (circles) or in the presence (triangles) of 5 μM CCCP in strain JA165 (solid symbols) and in its derivative, ActP-deficient mutant strain JA214 (open symbols). The effect of CCCP was analyzed in cells that were preincubated with the uncoupler for 30 s before the radioactive substrate was added.

type similar to that of an *acs*-deficient mutant, which grew poorly on acetate at concentrations below 5 mM (14), whereas strain JA212 grew well on acetate even at concentrations as low as 2.5 mM.

**Acetate transport by the *yjcG* gene product.** The transcription of *yjcG* in a polycistronic messenger including the *acs* gene encoding acetyl-CoA synthetase, together with the fact that the encoded protein transported glycolate, a two-carbon carboxylate, suggested that the primary function of the protein could be transport of acetate. To determine whether acetate is recognized and transported by YjcG, we assayed [1,2-$^{14}$C]acetate uptake by cells of strains JA212 and JA213. Cells were grown on 0.5% casein acid hydrolysate, collected, and processed to measure acetate transport as described above, except that they were incubated at 15°C to reduce metabolism. The time course of incorporation of radioactivity showed that strain JA212 displayed a good rate of acetate transport, while strain JA213 displayed a significantly lower, but not null, rate (Fig. 5A). These results indicate that the *yjcG* gene encodes a protein that mediates the transport of acetate and suggest there may be another less efficient acetate transporter. We thus propose the designation *actP* for *yjcG*. The possibility that these conclusions could be affected by the presence of acetate metabolism in the strains used prompted us to carry out acetate transport experiments with mutant strains unable to metabolize acetate. We used strain JA165 (22) lacking isocitrate lyase, the product of the *aceA* gene, and prepared the corresponding transport-negative organism (strain JA214) by transducing the mutation *actP*::Tn*10* into this genetic background. The time course obtained (Fig. 5B) showed that there was saturation of intracellular acetate accumulation after 2 to 3 min and confirmed that the initial rate of acetate transport was 10-fold higher than that of the ActP-deficient cells. These experiments allowed us to determine the rate of transport due to ActP, which could theoretically be estimated by subtracting the rate found in strain JA214 from the total rate measured in strain JA165 (Fig. 5B).

The inclusion of ActP in the sodium:solute symporter family led us to study the possible sodium dependence of ActP function. Although it is very difficult to remove sodium from assay mixtures, and taking into account that radioactive acetate is available only as a sodium salt, we performed acetate transport assays in a sodium-free buffer with and without 50 mM sodium chloride. The cold acetate added to these preparations was a potassium salt, and the resulting sodium concentration due to radioactive acetate was kept as low as 2 μM. In these experiments performed with strain JA165 addition of sodium had no effect on the uptake of acetate. These results do not rule out the possibility that ActP function is independent of sodium; however, if ActP were to use sodium, it could do it at concentrations below that used under our sodium-depleted experimental conditions.

To examine the possibility that the residual transport was due to passive diffusion, we measured acetate transport in strains JA165 and JA214 in the presence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) at a concentration of 5 μM, which blocks proton motive force-dependent uptake. It has also been reported that this protonophore can indirectly dissipate the sodium concentration gradient depending on the activity of $Na^+/H^+$ antiporters. This may result in the inhibition of $Na^+$-dependent transport systems, as has been reported for proline transport (31) or sodium-dependent GltS glutamate transport, both in *E. coli* (10). Our results showed that the two entry processes are sensitive to CCCP (Fig. 6), thus proving that they are both mediated by transporters driven by transmembrane electrochemical potential. In the absence of ActP function (strain JA214) and with the second transport activity inhibited by CCCP, the observed residual entry process, which was directly proportional to the acetate concentration in the medium, was compatible with a passive diffusion component for this solute.

**ActP substrate specificity.** To further characterize the acetate transport systems, we examined possible competitors at concentrations in the assay mixture that were 50-fold higher than that of acetate. The solute substrate analogues used as competitors were aliphatic carboxylates and dicarboxylates up to four carbons long, glycolate and other α-hydroxy acids up to four carbons long, and the amino acid proline due to the similarity of the ActP carrier to PutP. Figure 7 shows that in

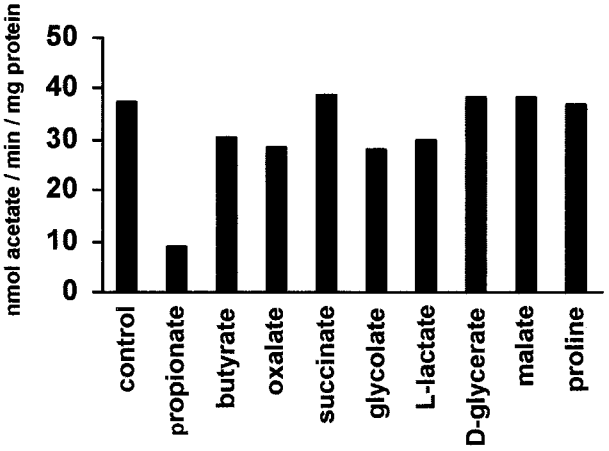


FIG. 7. Inhibition of acetate transport by other solutes. Uptake of 20 μM [1,2-$^{14}$C]acetate by cells of strain JA165 grown on casein acid hydrolysate was assayed in the presence of competing solutes, which were added to the assay mixture at a final concentration of 1 mM.

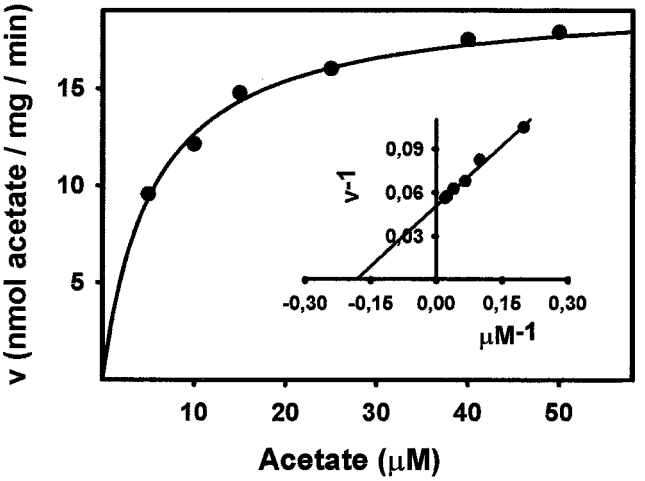


FIG. 8. Concentration kinetics of [1,2-$^{14}$C]acetate transport mediated by ActP: plot of the values calculated by subtracting the rates determined for strain JA214 from the values determined for strain JA165 against acetate concentrations. The inset shows a double-reciprocal plot of the data.

strain JA165 propionate caused significant inhibition (more than 70%), whereas butyrate caused only weak inhibition (<20%). Among the α-hydroxy acids, those containing two or three carbon atoms caused weak inhibition (around 25%). Malate, with four carbon atoms, had no effect. In contrast, in strain JA214, which has no ActP function and transports acetate through the secondary unidentified carrier, none of the compounds tested caused inhibition (data not shown).

**Kinetics of acetate uptake by ActP.** To analyze the efficiency and function of the ActP-mediated acetate transport, we performed concentration kinetics experiments with both strain JA165 and strain JA214, using acetate concentrations up to 50 μM. Again, the apparent specific values for ActP were calculated by subtracting the transport rates of strain JA214 from the total rates of strain JA165. The values obtained yielded an apparent $K_m$ of ActP for acetate of 5.4 μM and a $V_{max}$ of 19.6 nmol · mg$^{-1}$ · min$^{-1}$ (Fig. 8).

To define the role of ActP, we compared the acetate utilization phenotype of strain JA213, which lacks the ActP function, with that of strain JA212, which has a functional ActP. The colony sizes of the two strains were not different, even at concentrations as low as 2.5 mM, indicating that the absence of ActP did not limit growth at this acetate concentration.

## DISCUSSION

The presence of a transport system for acetate in *E. coli* was demonstrated here by identification of *actP*, formerly designated *yjcG*, as a gene encoding a membrane carrier of acetate. Two fortuitous facts led us to the discovery of this acetate carrier. First, the newly discovered acetate permease recognizes and transports glycolate with a rate high enough to support growth on this carbon source. Second, the presence of an IS*2* element in *acs* impaired ActP function in our parent strain. Indeed, the precise excision of this insertion element activated acetate (glycolate) transport in mutant JA212.

The mapping of *actP* in a cluster with *acs* involved in acetate dissimilation and the cotranscription of the two genes strongly suggested that *actP* is located in an operon for acetate dissimilation and that its product participates in acetate transport. Cotranscription was first shown by detection of a full-length transcript in strain JA212. The low concentration of this transcript observed when a Northern blot was hybridized with an *acs* probe may have been due to the presence of two repetitive extragenic palindromic sequences in the 199-bp *acs-yjcH* intercistronic region (accession number AE000480). It has been reported that these sequences can stabilize upstream RNA by protecting it from 3′-to-5′ nuclease degradation (18, 29), which in our case resulted in accumulation of the transcript fragment corresponding to the *acs* gene (Fig. 2).

The hypothesis that there is an operon that includes *acs*, *yjcH*, and *actP* is supported by (i) the absence of promoter activity downstream of the *acs* gene promoters *acsP1* and *acsP2* and (ii) the polar effects of an IS*2* insertion in *acs* on *actP* expression, as shown by the lack of transcripts in strain MC4100. Thus, in the proposed operon the *acs* gene, encoding the acetyl-CoA synthetase, is cotranscribed with the *yjcH* gene, whose function is unknown, and the *actP* gene, which is responsible for acetate transport. The *yjcH* gene codes for a small putative membrane protein consisting of 104 amino acid residues, which is highly conserved in many bacterial species but still has with no defined function.

The specificity of ActP is rather narrow, a common feature of most bacterial carboxylate permeases, such as GlcA and LldP; an exception is the monocarboxylate permease of *Rhizobium leguminosarum*, which displays wider specificity (10). In this context, it is of interest that although ActP, like the GlcA and LldP permeases, transports small carboxylates, there is no sequence similarity between these transporters and ActP. This is probably reflected in the structure of the substrate recognition site. Indeed, ActP is highly specific for short-chain aliphatic monocarboxylates, whereas GlcA and LldP recognize two- and three-carbon monocarboxylates with a hydroxyl

group in position 2. The presence of this hydroxyl group in short-chain monocarboxylates (e.g., glycolate) reduces the ability of these compounds to compete with acetate transport mediated by ActP and indicates a low affinity for ActP (Fig. 7). The ActP substrate specificity may have evolved from selective pressure created by the need to recover the carbons of acetate excreted during fermentation, perhaps an essential process in certain conditions.

Although ActP appears to be a transport system belonging to the sodium:solute symporter family, our experiments did not allow us to show dependence on sodium of acetate transport by ActP. It is known that it is difficult to completely deplete sodium in the reaction mixtures and buffers used in transport assays, especially under our conditions, under which sodium was present in the labeled source of acetate. The residual sodium levels might be sufficient to allow transport by systems with a high affinity for this cation (10). In this context, the inhibition of acetate transport by the uncoupler CCCP indicates that the driving force used by ActP is a transmembrane electrochemical potential. However, as reported by other authors (31), these experiments do not discriminate between $H^+$- and $Na^+$-coupled symporters.

The residual acetate uptake in the ActP-deficient mutants suggests that there is another so-far-undetermined transporter for this compound, which is partially inhibited by the uncoupler CCCP. Passive diffusion accounts for the acetate entry that is insensitive to CCCP, which represents, at an acetate concentration of 20 μM, less than 8% of the total uptake in cells of strain JA165 expressing both ActP and the undetermined transporter.

The test for acetate utilization at a wide range of concentrations (2.5 to 50 mM) showed that strain JA212 grew well at all acetate concentrations, while the *acs*-deficient parental strain MC4100 grew poorly at low concentrations. In contrast, no differences were detected between the growth at these low concentrations of an ActP-deficient mutant and the growth of cells with a functional ActP. This observation may be explained by the presence of the second acetate carrier and the passive diffusion component for acetate entry, which can substitute for ActP function. Even the lowest concentration used, 2.5 mM, is far greater than the saturating concentrations for these carriers. These findings do not invalidate the hypothesis that ActP plays a role as an efficient carrier able to recapture acetate at very low concentrations (in the micromolar range). In these conditions, the ActP function provides increasing intracellular concentrations of acetate to be activated by acetyl-CoA synthetase, which has a $K_m$ of 0.2 mM for this substrate (4). Taken together, these facts may indicate that the combined action of ActP and Acs facilitates scavenging of the traces of excreted acetate during mixed-acid fermentation or in aerobic conditions when the carbon flux exceeds the capacity of the central metabolic pathways.

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