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Biochemistry

Escherichia coli kgtP encodes an α-ketoglutarate transporter

(*witA*/membrane protein/complementation/*citA*)

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ABSTRACT **The *witA* gene located between *pss* and *rrnG* on the *Escherichia coli* chromosome encodes a 432-amino acid protein. It is homologous to a human hepatoma glucose transporter and to *E. coli* membrane proteins that transport citrate (CitA), arabinose (AraE), and xylose (XylE), and, like these carrier proteins, WitA also contains 12 highly hydrophobic putative membrane-spanning regions. Gene disruption mutants constructed in two *E. coli* strains grew slowly or not at all, depending on genetic background, in M9 minimal medium containing α-ketoglutarate. Growth on α-ketoglutarate and uptake of α-[^{14}C]ketoglutarate were restored by transformation with plasmids containing *witA*. These complementation studies indicate that WitA is an α-ketoglutarate transporter and should be renamed *kgtP*(α-*k*etoglu*t*arate *p*ermease).**

witA, discovered in a 9-kilobase (kb) fragment of *Escherichia coli* genomic DNA, maps at 56.5 min between *pss* (phosphatidylserine synthase) and the rRNA operon *rrnG* (1). The amino acid sequence deduced for WitA* is 32.5% identical to *E. coli* CitA (2), which is similar to the AraE, XylE, and tetracycline (TetA) transporters of *E. coli* and to glucose carriers of human and yeast (3, 4) as well as plant origin (5). Each of these proteins has 12 hydrophobic putative-membrane-spanning stretches with the 6th and 7th separated by a central hydrophilic region (3, 4). These features are also found in WitA, suggesting that it may be a membrane transporter.

α-Ketoglutarate (α-KG) plays an important role in *E. coli* carbon and nitrogen metabolism. It functions as a tricarboxylic acid cycle intermediate and a receptor for α-NH_2 transfer in aspartate transamination. It also stimulates uridylyltransferase that is related to activation of the nitrogen regulator (NR_I), which in turn activates transcription of nitrogen-regulated operons (6). Despite its importance, little is known about how α-KG enters the bacterial cell. We have identified α-KG as the substrate for WitA by incubating *witA*$^-$ mutants in minimal medium containing single carbon sources and measuring growth rates relative to the wild-type parental strains. Complementation by *witA* for mutant growth and for α-[^{14}C]KG uptake indicates that WitA transports α-KG.

MATERIALS AND METHODS

Bacterial Strains and Plasmids. The following *E. coli* K-12 strains were used for the construction of *witA*$^-$ mutants: JC7623 [*thr-1 ara-14 leuB6 Δ(gpt-proA)62 lacY1 tsx-33 supE44 galK2 rac*$^-$ *hisG4 rfbD1 mgl-51 rpsL31 kdgK51 xyl-5 mtl-1 argE3 thi-1 recB21 recC22 sbcC201 sbcB15*] (7) and MC1061 [*hsdR mcrB araD139 Δ(araABC-leu)7679 ΔlacX74 galU galK rpsL thi*] (8). Strain TG1 [*Δ(lac-proAB) supE thi hsdΔ5 F′ (traD36 proAB*$^+$ *lacI*^{q} *lacZΔM15)*] (9) was used for preparing the various plasmid subclones and strain K38 [HfrC(λ)] (10) was used for expressing the cloned *witA* gene. Strain JC7623, phages P1 and λ 3F10 and 21D7, and plasmids pUC4K and pCL1921 were obtained from M. Inouye (Robert Wood Johnson Medical School, Piscataway, NJ), and strain K38 and plasmids pGP1-2 and pT7-5 were from S. Tabor (Harvard Medical School, Boston).

Bacterial growth was measured as OD_{550} in M9 minimal medium (11) containing 22 mM glycerol or 20 mM of other carbon sources and, if needed, amino acids (leucine, threonine, histidine, arginine, and proline for JC7623 or leucine for MC1061) and thiamine at final concentrations of 40 and 10 μg/ml, respectively. Amino acid levels were minimal to avoid their utilization as a carbon source; under these conditions in glycerol, JC7623 grew to an OD_{550} of 1.2 within 24 hr and did not grow in the absence of glycerol.

DNA Analysis. Sequencing of both strands was done by the dideoxynucleotide chain-termination method (12) using oligonucleotide primers after subcloning restriction fragments of the 9-kb clone into *E. coli* expression vector pKK223-3 (13). Primers were synthesized according to presequenced regions and used for progressive sequencing. Sequence comparisons and hydropathy calculations were made with University of Wisconsin genetics computer group software (14). For Southern blot hybridization, genomic and λ DNAs were isolated as described (15, 16), digested with the indicated restriction endonucleases, fractionated by electrophoresis in 0.8% agarose gels, and transferred to membrane filters. Hybridization was done with DNA probes labeled with [α-^{32}P]dCTP by nick-translation or with $tRNA^{Glu}$ (Sigma) that was 5′-end-labeled using [γ-^{32}P]ATP and T4 polynucleotide kinase.

Construction of *witA*$^-$ Mutants by Gene Disruption. The kanamycin-resistance gene (kan^r) was excised from pUC4K (17) as a 1.2-kb *Hin*cII fragment and inserted into the *Nco* I site of *witA* at a position corresponding to amino acid 187. The linear 10.2-kb *Eco*RI fragment (kan^r in 9-kb DNA) was transformed into JC7623 (exonuclease V$^-$ and I$^-$; ref. 7), and kan^r colonies were selected. Transformants, resulting from double homologous recombination between the linear DNA and the corresponding genomic region, were confirmed to have kan^r in the middle of *witA* by restriction mapping and Southern hybridization. *witA*$^-$ MC1061 mutants were made by generalized transduction with P1 lysates (18) obtained from the JC7623 mutant and also confirmed by kanamycin resistance, restriction mapping, and hybridization.

Construction of *witA* in Low-Copy-Number Plasmids for Complementation Studies. A 2.8-kb *Afl* II–*Sal* I fragment including *witA* and its putative promoter was ligated to the 4.6-kb *Hin*dIII–*Sal* I fragment of low-copy-number pCL1921 (19). The resulting pCW28 contains *witA* under the control of *lac* in addition to its own promoter. To place *witA* under *lac* control only, a 6.5-kb *Sca* I–*Eco*RI fragment was ligated to the 4.3-kb *Acc* I–*Eco*RI pCL1921 fragment to produce pCW65, which encodes a WitA protein missing the first 4

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Abbreviation: α-KG, α-ketoglutarate.

*The sequence reported in this paper has been deposited in the GenBank data base (accession no. X53027).

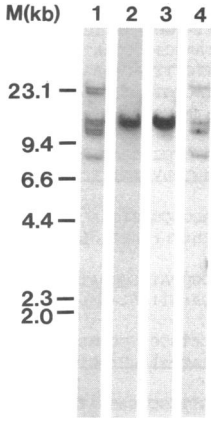

FIG. 1. Southern blot of *E. coli* K-12 genomic DNA. DNA was digested with *Bam*HI and transferred to nitrocellulose after agarose gel electrophoresis. The same filter (obtained from S. Inouye, Robert Wood Johnson Medical School, Piscataway, NJ) was hybridized successively [at 42°C in 50% (vol/vol) formamide solution with extensive intermediate washing] with nick-translated probes derived from the following regions of the 9-kb clone: left end (lane 1), middle region (lane 2), and right end (lane 3). The probe in lane 4 was 5′-end-labeled $tRNA^{Glu}$.

amino acids and containing 13 additional N-terminal amino acids encoded by part of the pCL1921 polycloning site.

Transport Assay. Cells were grown in M9 medium containing glycerol, amino acids, thiamine, and antibiotics, as necessary. Exponentially growing cells were harvested, washed extensively with 50 mM potassium phosphate (pH 7.5)/10 mM $MgSO_4$ (20), resuspended in the same buffer to an OD_{680} of 2.0 (cell dry mass, ≈1.1 mg/ml), and tested for α-[^{14}C]KG uptake. Cells (50 μl) were incubated at room temperature in 1.7 mM α-[U-^{14}C]KG (NEN; final specific activity, 1.1 Ci/mol; 1 Ci = 37 GBq). At the indicated times, cells were diluted and immediately filtered, and radioactivity was determined by liquid scintillation spectrometry (20).

RESULTS AND DISCUSSION

Characterization of the 9-kb DNA. A 9-kb clone, obtained during screening of λgt11 fetal mouse cDNA libraries (1), surprisingly showed no homology to mouse L cell DNA or RNA but was strongly positive by Southern blot hybridization to DNA from *E. coli* K-12 and B (data not shown). Sequence analysis indicated that one end of the 9-kb clone is very similar to *rrlB* (one of the seven 23S rRNA genes) and that it contains an open reading frame that we named *witA*. The open reading frame corresponds in sequence to a very hydrophobic putative membrane protein, and we decided to investigate the nature and function of *witA*.

Fragments of the 9-kb DNA were used as probes to verify the origin of the clone from the *E. coli* genome. An *Eco*RI–*Sal* I, 0.5-kb end fragment containing the rRNA-like sequence and corresponding to a region upstream of *witA* yielded seven bands, a reasonable result because *E. coli* has seven highly conserved rRNA operons (*rrn*) (Fig. 1, lane 1). Middle (*Nco* I–*Sst* II 1.9 kb) and opposite-end (*Sac* I–*Eco*RI, 1.3 kb) fragments detected the same one of the seven bands, thus excluding the possibility that the 9-kb DNA is a λ–*E. coli* recombinant (Fig. 1, lanes 2 and 3). Since the flanking region of the *rrn* operons and the tRNA genes located within them are different (21), $tRNA^{Glu}$, which is contained in *rrnB*, *-C*, *-E*, and *-G* but not in *-A*, *-H*, and *-D* (21), was used to identify the *rrn* in the 9-kb clone and to help position the 9-kb DNA on the *E. coli* genome. As shown in Fig. 1, lane 4, the $tRNA^{Glu}$ probe detected four bands as expected, and one of the four was the same as detected with the three probes derived from the 9-kb DNA. The 3′ ends of *rrnB*, *-C*, and *-E* operons have been sequenced (22–24) and the corresponding region in the 9-kb fragment is different (25), indicating that the 9-kb clone contains part of *rrnG*.

This conclusion was confirmed by hybridization of λ phage 3F10 and 21D7 DNAs that include *rrnG* with a probe prepared from the 9-kb clone (ref. 26; Fig. 2 *A* and *B*). As shown in Fig. 2*C*, both phage DNAs yielded bands of the predicted sizes based on the restriction map of the 9-kb clone. In addition, part of the map of the 9-kb clone is identical to *pss* (27), which is located on the 3′ side of *rrnG* (W. Dowhan, personal communication). From these results, it is clear that the physical map of the 9-kb clone is identical to the region at ≈56.5 min on the *E. coli* chromosome (Fig. 2 *A* and *B*).

***witA* Sequence and Predicted Protein Structure.** The *witA* open reading frame consists of 1296 nucleotides coding for a protein of M_r 47,052 (Fig. 3). It is hydrophobic and basic, has a calculated pI value of 9.8–10.2, and is 32.5% identical to the

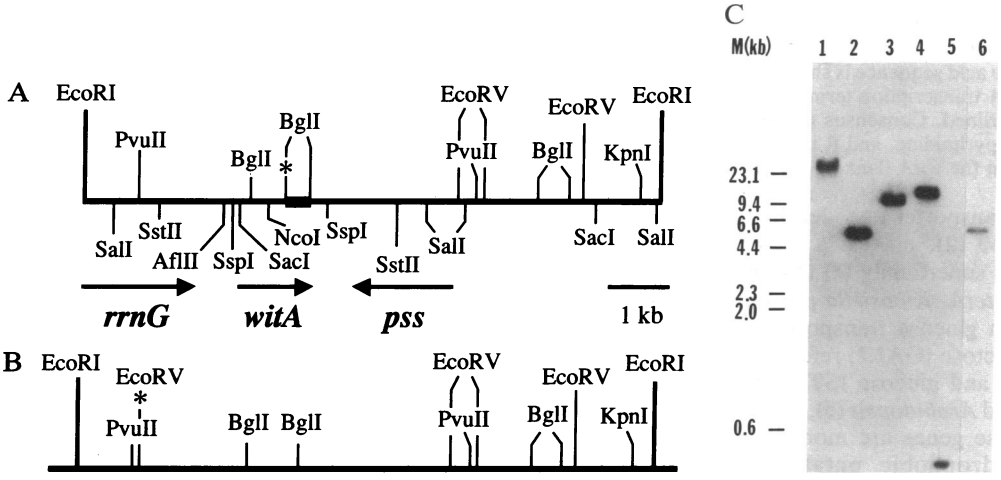

FIG. 2. Correspondence of the physical maps of the 9-kb clone (*A*) and the region at 56.5 min on the *E. coli* chromosome (26) (*B*). An additional *Bgl* I site and absence of a previously reported *Eco*RV site (ref. 26; marked by *) were confirmed by sizing the hybridized *Bgl* I and *Eco*RV fragments and by Y. Kohara (personal communication). Three additional *Ssp* I sites detected in the 9-kb clone downstream of *witA* are not indicated; note the absence of *Bam*HI, *Hin*dIII, and *Pst* I sites. (*C*) Southern blot of λ phage DNAs 3F10 (which contains the entire 9-kb region) and 21D7 (which includes approximately the left two-thirds of the 9-kb region) with a nick-translated *Bgl* I–*Bgl* I (thicker line in *A*) 0.4-kb *witA* DNA fragment. Lane 1 is undigested 3F10 DNA and lanes 2–5 are the same DNA digested with *Pvu* II, *Eco*RI, *Eco*RV, and *Bgl* I, respectively. Lane 6 is 21D7 DNA digested with *Pvu* II.

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-370  ATGGTAGTGTGGGGTCTCCCCATGCGAGAGTAGGGAACTGCCAGGCATCAAATTATGCGAAAGGCCATCCTGACGGATGG
-290  CCTTTTTGCATTGGCGCAGAAAAAAATGCCTGATGCGACGCTGCGCGTCTTATACTCCCACATATGCCAGATTCAGCAAC
-210  GGATACGGCTTCCCCAACTTGCCCACTTCCATACGTGTCCTCCTTACCAGAAATTTATCCTTAAGCTCCTCAATAACCAT
-130  TTTCCTGCTAACTAAATTCATGGTTAAGGTTGCATAATGATATGCAACAAATGTATAATATTTCCTTTACAAAAAAAATA
 -50  AACAAAAGCGACCGACAAAAGCATCGGATTACGGCAGGAGACATAATGGCATGGCTGAAAGTACTGTAACGGCAGACAGC
   1                                                    MetAlaGluSerThrValThrAlaAspSer

  31  AAACTGACAAGTAGTGATACTCGTCGCCGCATTTGGGCGATTGTGGGGGCCTCTTCAGGTAATCTGGTCGAGTGGTTCGA
  12  LysLeuThrSerSerAspThrArgArgArgIleTrpAlaIleValGlyAlaSerSerGlyAsnLeuValGluTrpPheAs

 111  TTTCTATGTCTACTCGTTCTGTTCACTCTACTTTGCCCACATCTTCTTCCCTTCCGGGAACACGACGACTCAACTACTAC
  38  pPheTyrValTyrSerPheCysSerLeuTyrPheAlaHisIlePhePheProSerGlyAsnThrThrThrGlnLeuLeuG

 191  AAACAGCAGGTGTTTTTGCTGCGGGATTCCTGATGCGCCCAATAGGCGGTTGGCTATTTGGCCGCATAGCCGATAAACAT
  65  lnThrAlaGlyValPheAlaAlaGlyPheLeuMetArgProIleGlyGlyTrpLeuPheGlyArgIleAlaAspLysHis

 271  GGTCGCAAAAAATCGATGCTGTTATCGGTGTGTATGATGTGTTTCGGATCGCTGGTTATCGCCTGCCTCCCAGGTTATGA
  91  GlyArgLysLysSerMetLeuLeuSerValCysMetMetCysPheGlySerLeuValIleAlaCysLeuProGlyTyrGl

 351  AACTATAGGTACGTGGGCTCCGGCATTATTGCTTCTCGCTCGTTTATTTCAGGGATTATCTGTTGGCGGAGAATATGGCA
 118  uThrIleGlyThrTrpAlaProAlaLeuLeuLeuLeuAlaArgLeuPheGlnGlyLeuSerValGlyGlyGluTyrGlyT

 431  CCAGCGCCACCTATATGAGTGAAGTTGCCGTTGAAGGGCGCAAAGGTTTTTACGCATCATTTCAGTATGTGACGTTGATC
 145  hrSerAlaThrTyrMetSerGluValAlaValGluGlyArgLysGlyPheTyrAlaSerPheGlnTyrValThrLeuIle

 511  GGCGGACAACTGCTAGCCCTACTGGTTGTCGTGGTTTTACAACACACCATGGAAGACGCTGCACTCAGAGAGTGGGGATG
 171  GlyGlyGlnLeuLeuAlaLeuLeuValValValValLeuGlnHisThrMetGluAspAlaAlaLeuArgGluTrpGlyTr

 591  GCGTATTCCTTTCGCGTTAGGAGCTGTGTTAGCTGTTGTGGCGTTGTGGTTACGTCGTCAGTTAGATGAAACTTCGCAAC
 198  pArgIleProPheAlaLeuGlyAlaValLeuAlaValValAlaLeuTrpLeuArgArgGlnLeuAspGluThrSerGlnG

 671  AAGAAACGCGCGCTTTAAAAGAAGCTGGATCTCTGAAAGGATTATGGCGCAATCGCCGTGCATTCATCATGGTTCTCGGT
 224  lnGluThrArgAlaLeuLysGluAlaGlySerLeuLysGlyLeuTrpArgAsnArgArgAlaPheIleMetValLeuGly

 751  TTTACCGCTGCGGGCTCCCTTTGTTTCTATACCTTCACTACTTATATGCAGAAGTATCTGGTAAATACTGCGGGAATGCA
 251  PheThrAlaAlaGlySerLeuCysPheTyrThrPheThrThrTyrMetGlnLysTyrLeuValAsnThrAlaGlyMetHi

 831  TGCCAACGTGGCGAGTGGCATTATGACTGCCGCATTGTTTGTATTCATGCTTATTCAACCACTCATTGGCGCGCTGTCGG
 278  sAlaAsnValAlaSerGlyIleMetThrAlaAlaLeuPheValPheMetLeuIleGlnProLeuIleGlyAlaLeuSerA

 911  ATAAGATTGGTCGCCGTACCTCAATGTTATGTTTCGGTTCGCTGGCAGCCATTTTTACCGTTCCTATTCTCTCAGCATTG
 305  spLysIleGlyArgArgThrSerMetLeuCysPheGlySerLeuAlaAlaIlePheThrValProIleLeuSerAlaLeu

 991  CAAAACGTTTCCTCGCCTTATGCCGCTTTTGGTCTGGTGATGTGTGCCCTGCTGATAGTGAGTTTTTATACATCAATCAG
 331  GlnAsnValSerSerProTyrAlaAlaPheGlyLeuValMetCysAlaLeuLeuIleValSerPheTyrThrSerIleSe

1071  TGGAATACTGAAGGCTGAGATGTTCCCGGCACAGGTTCGCGCATTAGGCGTTGGTCTGTCATATGCGGTCGCTAATGCTA
 358  rGlyIleLeuLysAlaGluMetPheProAlaGlnValArgAlaLeuGlyValGlyLeuSerTyrAlaValAlaAsnAlaI

1151  TATTTGGTGGTTCGGCGGAGTACGTAGCGTTGTCGCTGAAATCAATAGGAATGGAAACAGCCTTCTTCTGGTATGTGACC
 385  lePheGlyGlySerAlaGluTyrValAlaLeuSerLeuLysSerIleGlyMetGluThrAlaPhePheTrpTyrValThr

1231  TTGATGGCCGTGGTGGCGTTTCTGGTTTCTTTGATGCTACATCGCAAAGGGAAGGGGATGCGTCTTTAGTGACGGGTCAG
 411  LeuMetAlaValValAlaPheLeuValSerLeuMetLeuHisArgLysGlyLysGlyMetArgLeu

1311  TTGCCAGACGGTATAGCCGGTGCTTGCACCGGCGACATCCCAGGCCAAATCCTTCCAGCTCCAGCCGCTCCCTTCGGGGC
1391  GGCTATCCCAAAGCTCTTTTGACGCCCCCAAACTGACAGAGAACATCAATCCAAACATGGCACTGCGATCCCGGCTCATC
1471  CCCTGATGCTGTGAATATTCATTTCCGGCGGCG
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FIG. 3. Nucleotide sequence of *witA* and flanking regions, including the 3′ end of the *rrnG* 5S RNA gene (underlined positions −370 to −323). The deduced amino acid sequence is shown beneath. Predicted sites are underlined for RNA polymerase and ribosome binding in the 5′ upstream region and for *witA* transcription termination in the 3′ region. The 12 hydrophobic protein domains calculated by the Kyte–Doolittle method (28) are also underlined. Consensus upstream nucleotide sequences AANTGTGAN$_2$TN$_4$CA and CTGGYAYRN$_4$TTGCA (where N is any nucleotide, Y is a pyrimidine, and R is a purine) observed in some *E. coli* genes related to carbon and nitrogen metabolism, respectively (6), were not evident in the *witA* flanking region.

E. coli citrate transporter encoded by *citA* in the naturally occurring pWR60 (2). *citA* is a member of a membrane transporter gene superfamily (4) that includes *E. coli araE*, *xylE*, *galP*, and *tetA*, *Klebsiella pneumoniae* citrate carrier (29), mammalian glucose transporters (3), glucose (SNF3; ref. 30) and galactose (GAL2; ref. 31) carriers in yeast, and hexose (HUP1) and glucose (STP1) transporters found in *Chlorella* (32) and *Arabidopsis* (5), respectively. The proteins encoded by these genes are more than 20% identical and contain 12 hydrophobic putative-membrane-spanning stretches, like the *E. coli* lactose transport protein (LacY; ref. 33).

Alignment of WitA, CitA, AraE, and the hepatoma glucose transporter demonstrates the presence of a duplicated motif, (Arg/Lys)-Xaa-Gly-Arg-(Arg/Lys), between hydrophobic segments 2 and 3 and segments 8 and 9 (Fig. 4). The presence of this motif in several transporters suggests duplication of an ancestral transporter gene during evolution (4). The duplication in WitA is somewhat longer [i.e., Asp-Lys-Xaa-Gly-Arg-(Arg/Lys)-Xaa-Ser-Met-Leu] and it is of interest that in the TetA first motif, Asp-Arg-Phe-Gly-Arg-Arg, the aspartate is essential for tetracycline transport (34). Also potentially significant for WitA and CitA function are the highly homologous sequences, Gly-Phe-Leu-Met-Arg-Pro-Ile-Gly and Glu-Trp-Gly-Trp-Arg-Ile-Pro-Phe, which are present in the 2nd and 6th hydrophobic domains, respectively, of both proteins.

As predicted from the WitA hydropathy plot that shows 12 hydrophobic domains connected by hydrophilic loops, *witA* expressed in a T7-based system (35) yielded a protein that migrated by PAGE as a broad 35-kDa band and aggregated at the top of the gel if boiled before loading (data not shown), phenomena also reported for other hydrophobic bacterial proteins (33, 36). These similarities in sequence and structure

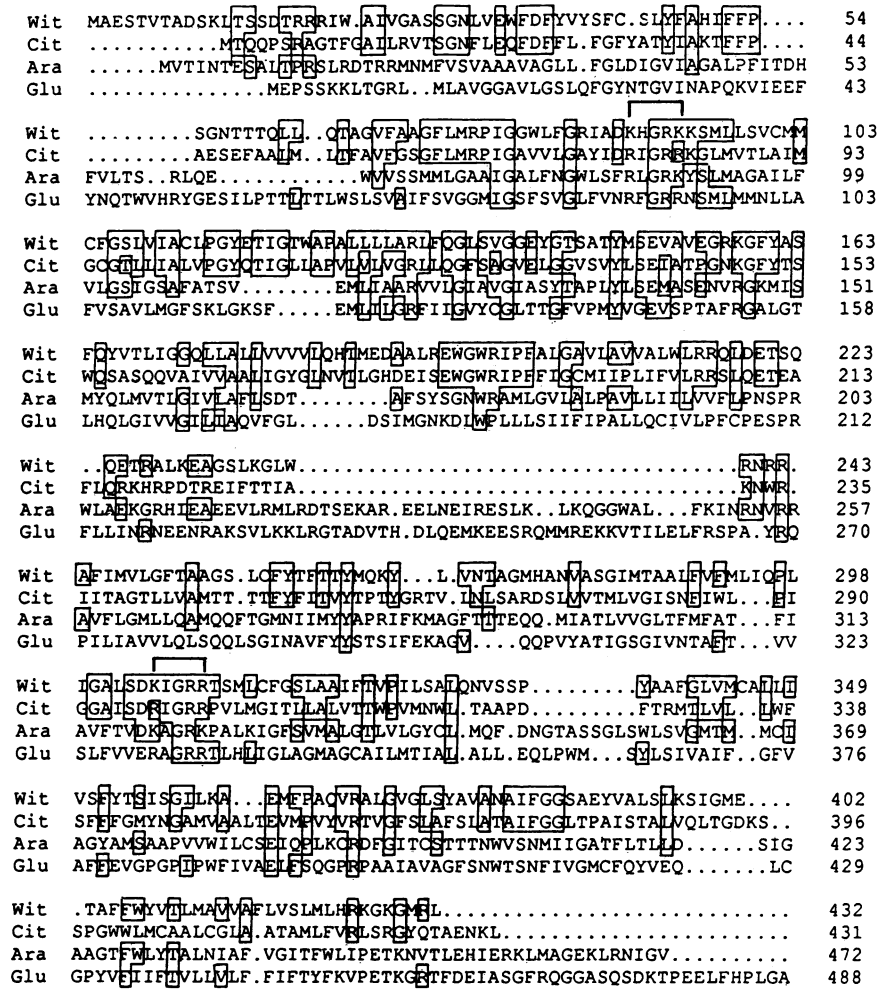

FIG. 4. Sequence and structural homology of *E. coli* WitA, CitA, and AraE transporters and human hepatoma glucose carrier (Glu). The numbers on the right show the position in the protein of the last amino acid in each row. Amino acids identical to WitA are boxed and the common duplicated motifs are bracketed above the consensus sequence. WitA is 32.5% identical to CitA and 25% identical to both AraE and the human hepatoma glucose carrier.

suggested that WitA is a membrane transporter. Results in the following sections suggest "*w*hat *i*s *t*ransported" by WitA.

Growth Analysis of *witA*$^-$ Mutants. Among the many and various carbon sources tested including all the tricarboxylic acid cycle intermediates, the most significant differences in growth between the wild-type parental strain and *witA*$^-$ mutant cells were obtained with α-KG, although mutants also grew less well on fumarate, aspartate, and glutamate. As shown in Fig. 5, the MC1061 *witA*$^-$ mutant failed to grow in α-KG and the JC mutant started to grow only after 24 hr, presumably due to induction of another compensatory α-KG transporter since *E. coli* often has at least two transport systems per substrate (36). The parental wild-type MC and JC strains, by contrast, initiated growth on α-KG within 10 hr or less (Fig. 5).

Complementation by Plasmids Containing *witA*. pCW28-transformed JC7623 and pCW65-transformed MC1061 *witA*$^-$ mutant strains grew essentially as well as wild-type cells in α-KG (Fig. 5). pCW65-transformed JC strain grew to an

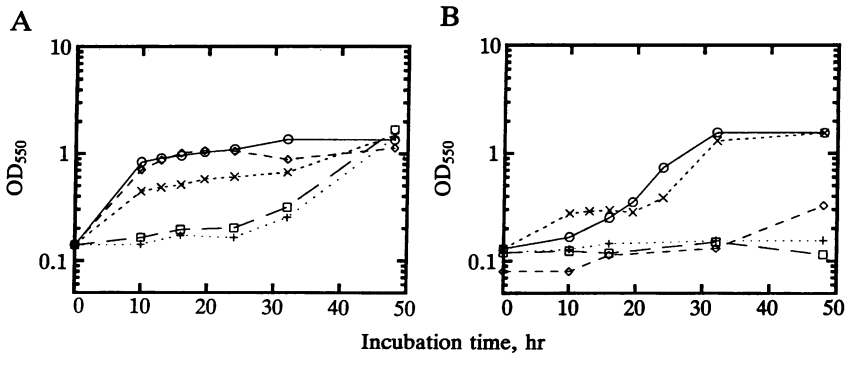

FIG. 5. Growth of wild-type cells and *witA*$^-$ mutants of JC7623 (*A*) and MC1061 (*B*) in α-KG. The pCW28-transformed MC1061 did not grow more, even after 1 week. ○, Wild type; □, mutant; ◇, pCW28-transformant; ×, pCW65-transformant; +, pCL1921-transformant.

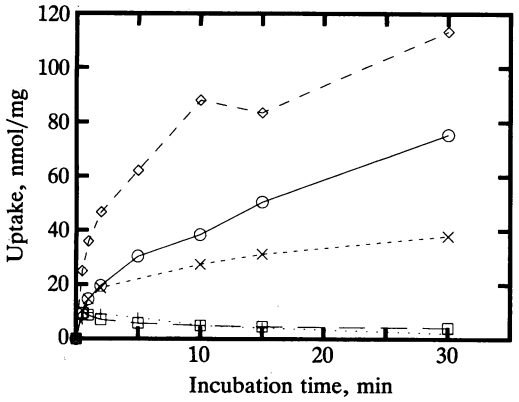

FIG. 6. Uptake of α-[U-^{14}C]KG. Each JC7623 culture was tested in triplicate using the same master cultures. Uptake was expressed on the basis of cell mass calculated from absorbance at the start of incubation since it did not change during the experiment. The uptake values measured with uniformly labeled substrate are probably higher than shown because in the tricarboxylic acid cycle C-1 of α-KG is quickly converted to CO_2 and not detected by scintillation counting. ○, Wild type; □, mutant; ◇, pCW28-transformant; ×, pCW65-transformant; +, pCL1921-transformant.

intermediate level, and pCW28 transformants of MC-derived mutant cells grew poorly on α-KG. By contrast to these low-copy-number plasmid transformants, high-copy-number plasmid pKK223-3 containing *witA* did not yield transformants of the JC-derived *witA*$^-$ mutant and could not complement MC1061 *witA*$^-$ transformants for growth on α-KG (data not shown). These differences may be due to different genetic backgrounds; e.g., the MC but not the JC strains may have a *witA* repressor that prevents synthesis of large toxic amounts of the highly hydrophobic WitA. Consistent with this suggestion, plasmid DNA reisolated from MC1061 *witA*$^-$ mutant transformed with pKK223-3 containing *witA* also failed to transform the JC7623 mutant (data not shown). Thus, the MC strain can survive even with high numbers of *witA* but is killed by toxic levels of WitA made in the presence of α-KG due to inactivation of the putative repressor. The very limited growth of the pCW28-transformed MC mutant in α-KG may be related to regulation mediated by its promoter region because the pCW65 transformant containing *witA* under control of the *lac* promoter grew as well or better than wild-type cells in α-KG.

Consistent with *witA* complementation for growth on α-KG, the JC-derived *witA*$^-$ mutant, which was unable to transport α-[U-^{14}C]KG effectively accumulated the substrate after transformation with pCW28 or pCW65 (Fig. 6). As a control, *witA*$^-$ mutants, with or without *witA* complementation, transported [^{3}H]proline equally well and to the level of wild-type cultures (data not shown). In addition, because of the results of the growth tests, inhibition analyses were done using aspartate, citrate, fumarate, and glutamate and the pCW28-transformed JC7623 *witA*$^-$ mutant. Only fumarate decreased uptake of radiolabeled α-KG significantly during a 5-min incubation, and the inhibition was <2-fold at molar concentrations ranging from 0.25- to 30-fold relative to α-KG.

Although the possibility remains that α-KG is not the only physiological substrate for WitA, the data indicate that *witA* is an *E. coli* gene that codes for an α-KG transporter. We suggest that *witA* be renamed *kgtP* (α-*k*etoglu*t*arate *p*ermease).

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