Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 7211–7215, October 1986
Biochemistry

Cloning and sequence analysis of cDNA for human argininosuccinate lyase

(urea cycle/genetic disorders/chromosome mapping)

WILLIAM E. O'BRIEN, RODERICK MCINNES*, KAREN KALUMUCK, AND MARK ADCOCK

Department of Pediatrics and Biochemistry, Baylor College of Medicine and the Howard Hughes Medical Institute, Houston, TX 77030

Communicated by Sarah Ratner, June 19, 1986

ABSTRACT **Using antibodies specific for argininosuccinate lyase (EC 4.3.2.1), we isolated two cDNA clones by screening a human liver cDNA library constructed in the λgt11 expression vector. The identity of these isolates was confirmed by *in vitro* translation of plasmid-selected mRNA. One of these isolates was used to rescreen the cDNA library and a 1565-base-pair (bp) clone was identified. The entire nucleotide sequence of this clone was determined. An open reading frame was identified which encoded a protein of 463 amino acids with a predicted molecular weight of 51,663. The clone included 115 bp of 5′ untranslated sequence and 46 bp of 3′ untranslated sequence. A canonical poly(A) addition site was present in the 3′ end, 16 bp from the beginning of the poly(A) tract. Comparison of the deduced amino acid sequence of the human enzyme with that of the yeast enzyme revealed a 56% homology, when conservative amino acid changes were taken into consideration. The yeast protein is also 463 amino acids long, with a molecular weight of 51,944. By use of a genomic DNA panel from human–Chinese hamster somatic cell hybrids, the human gene was mapped to chromosome 7. Another hybridizing region, corresponding to a portion of the 5′ end of the cDNA, was found on chromosome 22.**

Argininosuccinate lyase (EC 4.3.2.1) catalyzes the conversion of argininosuccinic acid into fumaric acid and arginine. The enzyme's primary physiological role is in the liver, where it functions in the urea cycle for the disposal of ingested nitrogen. The deficiency of the enzyme in human infants results in hyperammonemia, argininosuccinic aciduria, and mental retardation (1).

Argininosuccinate lyase has been purified from numerous mammalian sources (2, 3), including human liver (4, 5). In all cases, the enzyme is a homotetramer with a subunit molecular weight of approximately 50,000. The bovine enzyme has been extensively characterized by Ratner and colleagues and a review of their findings is available (6, 7).

Argininosuccinate lyase and argininosuccinate synthase (EC 6.3.4.5) are expressed in virtually all tissues. Outside of liver, these two enzymes are found in the highest concentrations in kidney and brain tissue. Since neither of these organs is capable of ureagenesis, it is presumed that they play an important role in the synthesis of arginine from citrulline. This ability to convert citrulline to arginine in cultured cells has been used as a selection system for the presence and expression of these genes (8, 9). Schimke (10) demonstrated that in Hela, KB, and L cells, both argininosuccinate lyase and argininosuccinate synthase are regulated by arginine content of the medium. In human lymphoblasts (11) and RPMI-2650 cells (12), argininosuccinate synthase is regulated by arginine but argininosuccinate lyase is not. The gene for human argininosuccinate synthase has been cloned and partially sequenced (13). The regulation of argininosuccinate synthase is mediated by a trans-acting molecule and the region of the gene required for the regulation is partially defined (14, 15).

The enzymes of the urea cycle, including argininosuccinate lyase, are coordinately regulated in response to both dietary (16, 17) and hormonal (18) changes. In rats, there is an abrupt induction of synthesis of the urea cycle enzymes just prior to birth (19). The enzymes of the urea cycle are present earlier in fetal liver development in the human, with detectable levels of all enzymes present by 8 weeks of gestation (19). This increase in activity is due to increased synthesis of stable mRNA for argininosuccinate synthase, carbamoyl phosphate synthase (20) and ornithine carbamoyltransferase (21). With this publication, cDNA clones are available for all of the urea cycle enzymes (20–27). In most instances, both human and rat cDNAs are available. These reagents will be invaluable molecular probes for delineating the molecular basis of the regulation of these genes during development. The cDNA for argininosuccinate lyase will allow the isolation and characterization of the human gene. Comparison of this gene with the human argininosuccinate synthase gene (13) might allow the delineation of regions responsible for tissue-specific expression and other regulatory regions common to these two coordinately expressed genes.

In this report we describe the identification of a cDNA clone for human argininosuccinate lyase and its complete nucleotide and amino acid sequence.

METHODS

Antibody. Purified human argininosuccinate lyase (4.5 mg) was bound to 1.0 g of CNBr-activated Sepharose 4B (Pharmacia) in 3.5 ml of 0.1 M $NaHCO_3$/0.5 M NaCl. Coupling was allowed to proceed for 18 hr at 4°C. The coupled resin was washed three times with 50 ml of 0.1 M HOAc/1 M NaCl, pH 4.0, followed by three washes with 50 ml of 0.1 M sodium borate/1 M NaCl, pH 8.0. A column (0.9 × 3 cm) was prepared with the resin and equilibrated in phosphate-buffered saline (PBS: 0.15 M NaCl/10 mM Na_2HPO_4, pH 7.2). Five milliliters of the immune serum (4) was applied to the column and recirculated at 0.8 ml/min for 2 hr. Unbound protein was removed with PBS and 100 ml of 0.2 M KSCN was applied to the column. Specifically bound antibody was removed by washing with 0.17 M HOAc in 1-ml aliquots; effluent samples were immediately neutralized with 5 M NH_4OH. The integrity of the antiserum was tested as described (4).

Library Screening. A human liver cDNA library prepared in the λgt11 expression vector was kindly supplied by S. Woo (Baylor College of Medicine). Phage were plated at approximately 10,000 plaques per 150-mm plate on host *Escherichia*

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Abbreviations: bp, base pair(s); kb, kilobase(s).

*Present address: Department of Genetics, Hospital for Sick Children, Toronto, ON M5G 1X8, Canada.

coli Y1090 as described by Young and Davis (28, 29). Transfer and hybridization procedures were essentially as described by Young and Davis (28), with only slight modifications. Positive recombinants were identified by their reaction with antibody and ^{125}I-labeled protein A. The labeled protein A was prepared with Bolton and Hunter reagent (30) as described by the supplier (Amersham).

DNA Sequencing. All sequencing was performed by the chain-termination method of Sanger (31), using [α-[^{35}S]thio]dATP and buffer-gradient gels (32). All positive recombinants were cloned in the *Eco*RI site of bacteriophage M13mp8 and sequenced with either the universal primer or custom primers specific for sites within the cloned argininosuccinate lyase sequence. These primers were supplied by K. Beattie (Baylor College of Medicine).

***In Vitro* Translation of Plasmid-Selected mRNA.** Procedures were as described (20) except that rabbit reticulocyte lysate was purchased from Promega Biotec (Madison, WI). Enzymes were supplied by either Boehringer-Mannheim or International Biotechnologies and were used as described by the supplier. Other techniques were as described in Maniatis *et al.* (33) unless otherwise stated.

Chromosomal Localization of the Human Argininosuccinate Lyase Gene. Chromosomal mapping was done using DNA prepared from a panel of 18 human–Chinese hamster somatic cell hybrids that has been described previously (34). Routine cytogenetic analysis was performed at the time of DNA preparation for all hybrids. Trypsin G-banding analysis of 20 cells was performed on all hybrids, and G-11 staining, on selected hybrids. A chromosome was scored as present in the hybrid if observed more than 4 times in 20 cells analyzed. This panel has been used extensively by other investigators to make human gene assignments (34, 35).

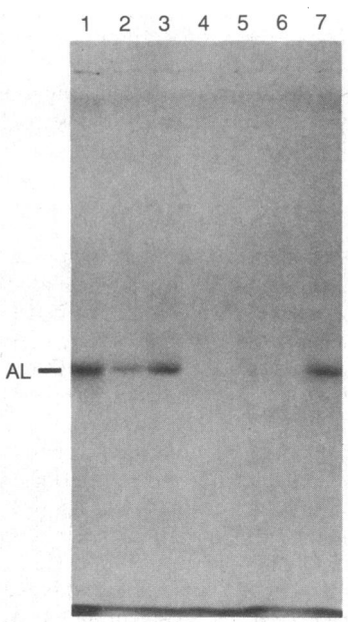

FIG. 1. *In vitro* translation of plasmid-selected mRNA. The [^{35}S]methionine-labeled proteins synthesized in a cell-free rabbit reticulocyte extract from plasmid-selected mRNA were immunoprecipitated and analyzed by $NaDodSO_4$/12.5% polyacrylamide gel electrophoresis followed by autoradiography. Lanes 1 and 7: products derived from the translation of unselected rat mRNA. Lanes 2–6: products synthesized from the mRNAs selected by clones P1–P5, respectively. The protein products of the reactions were immunoprecipitated using anti-human argininosuccinate lyase antibodies in all experiments. Migration position of authentic argininosuccinate lyase (AL) is indicated at left.

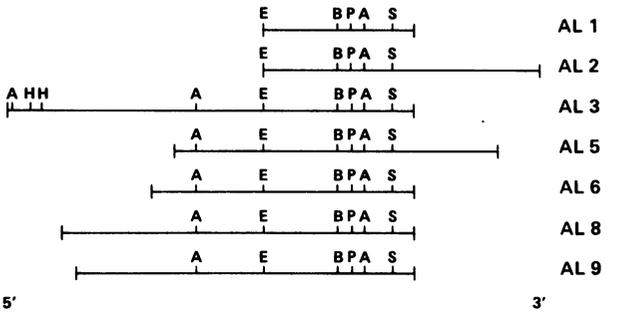

FIG. 2. Restriction map of the argininosuccinate lyase cDNA clones. Restriction sites: A, *Ava* I; B, *Bgl* I; E, *Eco*RI; H, *Hin*dIII; P, *Pvu* II; and S, *Sal* I.

RESULTS

Library Screening. Approximately 150,000 recombinant phage were screened. A total of 38 positive signals were detected in the primary screen. Of these, 5 remained after three additional rounds of plating and screening. These recombinants, designated P1–P5, had inserts of 600, 1100, <200, 1200, and 1100 base pairs (bp), respectively. Before any further analysis, all recombinants were moved into the pUC8 vector system. Restriction mapping of these recombinants revealed that P1 and P2 shared considerable homology, whereas the other three did not share any restriction sites with P1 or P2 or among themselves. In addition, the insert from each recombinant was labeled by nick-translation and hybridized to all other recombinants. This analysis also revealed that P1 and P2 shared sequence homology. No other combination of recombinants showed any evidence of homology. The recombinants also were analyzed to determine the size and abundance of their homologous mRNA species, by blot hybridization of poly(A^+) RNA from rat liver. P1 and P2 hybridized to an mRNA of ≈2000 bases, P4 did not give a positive signal under the conditions of the experiment, and P5 hybridized to a 6300-base mRNA (data not shown). Due to the small size of P3, it was not analyzed further. Given the estimated size of the protein, 49,000 daltons, a mRNA of 2000 bases would be sufficient to encode the protein.

As an initial test for the authenticity of these recombinants, we performed plasmid-selected mRNA translation. Only two of the five clones (P1 and P2) gave positive signals for argininosuccinate lyase (Fig. 1, lanes 2 and 3). The protein product that was immunoprecipitated in these experiments comigrated with pure human argininosuccinate lyase in $NaDodSO_4$/polyacrylamide gels. These data strongly support the conclusion that recombinants P1 and P2 contain sequences homologous to human argininosuccinate lyase. The insert from one of the original isolates, P1, was nick-

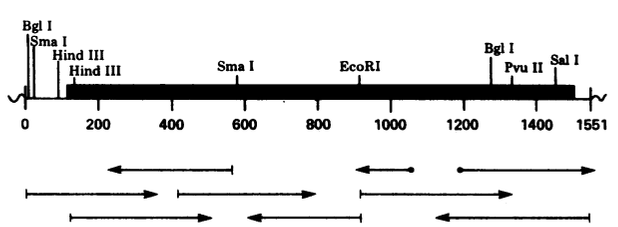

FIG. 3. Sequencing strategy and restriction map of human argininosuccinate lyase cDNA. The coding sequence is denoted by the black bar. Length is indicated in bp below the map. Horizontal arrows indicate the direction and extent of the sequence determination. Arrows that initiate with a vertical bar were sequenced with the M13 universal primer. Arrows that initiate with a filled circle were sequenced with synthetic 20-base oligomers.

translated and used to screen the λgt11 library again. From this screening, an additional five clones were identified, ranging in size from 1100 bp to 1650 bp. The seven cDNA clones are shown in Fig. 2. The original isolates, P1 and P2, were renamed pAL1 and pAL2, respectively. The largest of the group, designated pAL3, was used for all subsequent work. The additional clones proved especially useful because they were of varying length at the 5′ end and provided numerous sites from which to sequence in areas where no useful restriction sites were present.

Sequence of the pAL3 Clone. The largest clone, pAL3, was sequenced in its entirety. This clone was 1565 bp long. The restriction map of this clone and the sequencing strategy employed are shown in Fig. 3. The complete nucleotide

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AGAACTCGGAGCCAGCCCGGCCCGGGGGACCCTGCTGGCCAAGGAGGTCGTCAGTCCGGTCTTGTCTTCCAGACCCGGAGACCGAAGCTTCCGGACGACGAGG

AACCGCCCAAC ATG GCC TCG GAG AGT GGG AAG CTT TGG GGT GGC CGG TTT GTG GGT GCA GTG GAC CCC ATC ATG GAG AAG TTC  186
human       met ala ser glu ser gly lys leu trp gly gly arg phe val gly ala val asp pro ile met glu lys phe   24
yeast        -  ser asp gly thr gln  -   -   -   -   -   -   -  thr  -  glu thr  -   -  leu  -  his leu tyr

AAC GCG TCC ATT GCC TAC GAC CGG CAC CTT TGG GAG GTG GAT GTT CAA GGC AGC AAA GCC TAC AGC AGG GGC CTG GAG AAG  267
asn ala ser ile ala tyr asp arg his leu trp glu val asp val gln gly ser lys ala tyr ser arg gly leu glu lys   51
 -   -   -  leu pro  -   -  tyr lys met tyr lys ala  -  leu glu  -  thr  -  val  -  thr ala  -   -  gln  -

GCA GGG CTC CTC ACC AAG GCC GAG ATG GAC CAG ATA CTC CAT GGC CTA GAC AAG GTG GCT GAG GAG TGG GCC CAG GGC ACC  348
ala gly leu leu thr lys ala glu met asp gln ile leu his gly leu asp lys val ala glu glu trp ala gln gly thr   78
leu  -   -   -   -  glu thr  -  leu ala lys  -  his glu  -   -  ala glu ile lys lys  -   -  asp ala asp lys

TTC AAA CTG AAC TCC AAT GAT GAG GAC ATC CAC ACA GCC AAT GAG CGC CGC CTG AAG GAG CTC ATT GGT     GCA ACG GCA  426
phe lys leu asn ser asn asp glu asp ile his thr ala asn glu arg arg leu lys glu leu ile gly     ala thr ala  104
 -  val arg his pro  -   -   -   -   -   -   -   -   -   -   -   -   -   -  gly  -   -   -   -  arg glu ile  -

GGG AAG CTG CAC ACG GGA CGG AGC CGG AAT GAC CAG GTG GTC ACA GAC CTC AGG CTG TGG ATG CGG CAG ACC TGC TCC      504
gly lys leu his thr gly arg ser arg asn asp gln val val thr asp leu arg leu trp met arg gln thr cys ser      130
 -   -  val  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -  ile tyr cys  -  asp ile val asn asp

ACG CTC TCG GGC CTC CTC TGG GAG CTC ATT AGG ACC ATG GTG GAT CGG GCA GAG GCG GAA CGT GAT GTT CTC TTC CCG GGG  585
thr leu ser gly leu leu trp glu leu ile arg thr met val asp arg ala glu ala glu arg asp val leu phe pro gly  157
 -   -  phe pro ala  -  lys gly  -  val glu val leu ile lys  -   -   -  gly  -  ile  -   -   -  met  -   -

TAC ACC CAT TTG CAG AGG GCC CAG CCC ATC CGC TGG AGC CAC TGG ATT CTG AGC CAC GCC GTG GCA CTG ACC CGA GAC TCT  666
tyr thr his leu gln arg ala gln pro ile arg trp ser his trp ile leu ser his ala val ala leu thr arg asp ser  184
 -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -  leu ser  -  tyr  -  thr tyr phe  -  glu  -  tyr

GAG CGG CTG CTG GAG GTG CGG AAG CGG ATC AAT GTC CTG CCC CTG GGG AGT GGG GCC ATT GCA GGC AAT CCC CTG GGT GTG  747
glu arg leu leu glu val arg lys arg ile asn val leu pro leu gly ser gly ala ile ala gly asn pro leu gly val  211
lys  -   -  gly gln ile leu his  -  leu  -  gln ser  -   -   -  ala  -   -  leu  -   -  his  -  tyr  -  ile

GAC CGA GAG CTG CTC CGA GCA GAA CTC AAC TTT GGG GCC ATC ACT CTC AAC AGC ATG GAT GCC ACT AGT GAG CGG GAC TTT  828
asp arg glu leu leu arg ala glu leu asn phe gly ala ile thr leu asn ser met asp ala thr ser glu arg asp phe  238
 -   -   -  phe  -  ala glu gly  -  gly  -  asn ser val ile gly  -   -  leu val  -  val  -  asp  -   -   -

GTG GCC GAG TTC CTG TTC TGG CGT TCG CTG TGC ATG ACC CAT CTC AGC AGG ATG GCC GAG GAC CTC ATC CTC TAC TGC ACC  909
val ala glu phe leu phe trp arg ser leu cys met thr his leu ser arg met ala glu asp leu ile leu tyr cys thr  265
ile val  -  leu met  -   -  gly thr  -  phe  -  asn  -  ile  -   -  phe  -   -   -   -   -  ile  -   -   -

AAG GAA TTC AGC TTC GTG CAG CTC TCA GAT GCC TAC AGC ACG GGA AGC AGC CTG ATG CCC CAG AAG AAA AAC CCC GAC AGT  990
lys glu phe ser phe val gln leu ser asp ala tyr ser thr gly ser ser leu met pro gln lys lys asn pro asp ser  292
ala  -   -  gly  -  ile  -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -   -  ala  -   -

TTG GAG CTG ATC CGG AGC AAG GCT GGG CGT GTG TTT GGG CGG TGT GCC GGG CTC CTG ATG ACC CTC AAG GGA CTT CCC AGC 1071
leu glu leu ile arg ser lys ala gly arg val phe gly arg cys ala gly leu leu met thr leu lys gly leu pro ser  319
 -   -   -  arg  -  gly  -  ser  -   -   -   -   -  asp leu thr  -  phe  -   -  ser  -   -   -  ile  -   -

ACC TAC AAC AAA GAC TTA CAG GAG GAC AAG GAA GCT GTG TTT GAA GTG TCA GAC ACT ATG AGT GCC GTG CTC CAG GTG GCC 1152
thr tyr asn lys asp leu gln glu asp lys glu ala val phe glu val ser asp thr met ser ala val leu gln val ala  346
 -   -  asp  -   -  met  -   -   -   -   -  pro leu  -  asp cys leu thr  -  val glu his ser met leu ile  -

ACT GGC GTC ATC TCT ACG CTG CAG ATT CAC CAA GAG AAC ATG GGA CAG GCT CTC AGC CCC GAC ATG CTG GCC ACT GAC CTT 1233
thr gly val ile ser thr leu gln ile his gln glu asn met gly gln ala leu ser pro asp met leu ala thr asp leu  373
 -   -   -   -   -   -   -  thr val asn lys  -  lys  -  glu ala  -   -  thr met  -   -   -   -   -   -   -

GCC TAT TAC CTG GTC CGC AAA GGG ATG CCA TTC CGC CAG CCC ACG AGG CTC CGG GAA AGC TGT GTT CAT GGC CGA GAC CAA 1314
ala tyr tyr leu val arg lys gly met pro phe arg gln pro thr arg leu arg glu ser cys val his gly arg asp gln  400
 -  asp  -   -   -   -   -   -  val  -   -   -  glu thr his his ile ser gly glu  -   -  ala thr ala glu arg

GGG GGT CGC CCT GAA CCA GCT GTC ACT GCA GGA GCT GCA GAC CAT CAG CCC CCT GTT CTC GGG CGA CGT GAT CTG CGT GTG 1395
gly gly arg pro gln pro ala val thr ala gly ala ala asp his gln pro pro val leu gly arg arg asp leu arg val  427
leu  -  leu ser gly ile asp lys leu thr leu glu gln tyr gln lys ile asp ser arg phe gly gln  -   -  phe glu

GGA CTA CGG GCA CAG TGT GGA GCA GTA TGG TGC CCT GGG CGG CAC TGC GCG CTC CAG CGT CGA CTG GCA GAT CGC CAG GTG 1476
gly leu arg ala gln cys gly ala val trp cys pro gly arg his cys ala leu gln arg arg leu ala asp arg gln val  454
thr phe asn phe glu gln ser val glu arg arg asp ala thr gly gly thr ala lys ser ala val leu lys gln leu asp

CGG GCG CTA CTG CAG GCA CAG CAG GCC TAG GTCCTCCCACACCTGCCCCCTAATAAAGTGGGCGCGAGAGGAGAAAAAAAAAAAAAAAAA      1550
arg ala leu leu gln ala gln gln ala ***                                                                    463
asn leu lys ser  -  leu asn
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FIG. 4. Nucleotide and deduced amino acid sequences of human argininosuccinate lyase cDNA. Amino acid sequence encoded by the yeast gene is included for comparison. Dashes represent homologous amino acids.

sequence and the deduced amino acid sequence are shown in Fig. 4. For comparison, the amino acid sequence specified by the yeast gene (36) is included. An open reading frame of 1503 bp was identified, starting at base 1 in the clone and terminating at base 1503. The first methionine codon in this open reading frame occurred at base 115. Both the human and yeast proteins contain 463 amino acids. Although the two enzymes have the same number of amino acid residues, we have introduced two spaces in the human amino acid sequence (positions 101 and 130) to maximize the homology.

The predicted molecular weight of the human enzyme is 51,663 and that of the yeast enzyme is 51,944. The human enzyme had been estimated to be approximately 49,000 daltons (4) by $NaDodSO_4$/polyacrylamide gel electrophoresis. There is 51% amino acid sequence homology between the two enzymes. The pAL3 clone contains 114 bp of 5′ untranslated sequence but only 46 bp of 3′ untranslated sequence. Although this appears to be a relatively short 3′ untranslated region, six of the seven clones we studied used this polyadenylylation site. A canonical poly(A) addition signal (AATAAA) (37) is present 16 bp upstream of the poly(A) tract. One clone, pAL5, contained an additional 356 bp at the 3′ end and terminated at a second polyadenylylation site. One of the initial isolates, pAL2, appeared to extend even further at the 3′ end, but sequence analysis revealed that this isolate contained a cloning artifact. It terminated at the first polyadenylylation signal and contained a poly(A) tract. An additional 400 bp of unknown origin was ligated to the clone 3′ to the poly(A) tract.

Chromosomal Localization of the Human Argininosuccinate Lyase Gene. Human argininosuccinate lyase has been mapped to human chromosome 7 by Naylor *et al.* (38), using somatic cell hybrids. To confirm this map position and also to provide further proof of the identity of this recombinant, we hybridized the pAL3 cDNA to a genomic DNA panel prepared from a series of human–Chinese hamster somatic cell hybrids. The Southern blots are presented in Fig. 5 and the data are summarized in Fig. 6. We observed two distinct patterns of hybridization in the Southern blots of the hybrid cell DNAs. When only the 3′ end of the pAL3 clone was used as probe, all hybridizing fragments were found to be present on chromosome 7. When the 5′ end of the clone was used as probe, one group of hybridizing bands showed the same pattern as with the 3′ probe (i.e., chromosome 7). One hybridizing fragment (≈4 kb) clearly was discordant with chromosome 7 and completely concordant with chromosome 22 (Fig. 6). Thus, a portion of the 5′ region of the argininosuccinate lyase cDNA is homologous to a site on chromosome 22. The presence of hybridizing fragments representing the entire cDNA sequence on chromosome 7 confirms the previous map assignment of this gene.

DISCUSSION

We have used a λgt11 expression library to isolate a cDNA clone for human argininosuccinate lyase. From the initial screening of the library, five isolates were characterized and only two were found to be authentic. Both of these isolates were noted to be ligated into the phage at an authentic *Eco*RI site in the cDNA, as opposed to the other clones, which were ligated by means of synthetic *Eco*RI linkers used in the construction of the library. Subsequent sequencing of these isolates revealed that they were ligated in-frame with the β-galactosidase gene of the phage and in-frame with argininosuccinate lyase, thereby giving rise to a hybrid protein reactive to the anti-argininosuccinate lyase antibodies.

The cDNA clone was sequenced in its entirety and an open reading frame encoding a protein of 51,663 daltons was identified. $NaDodSO_4$/polyacrylamide gel analyses of human argininosuccinate lyase by ourselves and others have suggested a molecular mass of 49,000 daltons. This difference is well within the limits of accuracy of the electrophoretic technique. The yeast protein was also found to be 51,944 daltons, based on the sequence of the gene. Comparison of the weight percent of the individual amino acids reveals near identical values for the human, yeast, and bovine enzymes. A 56% homology was observed between the sequence of the human and yeast enzymes, when conservative changes were taken into consideration. There is an abrupt cessation of homology at amino acid 400 of the human sequence that continues to the carboxyl terminus. When this region is excluded from the calculations, the homology is 65%. Perhaps this carboxyl-terminal region is not important in catalysis and has undergone extensive change since the separation of these divergent species. Garrard *et al.* (39) have suggested that a histidine residue plays a critical role in the catalysis of the enzyme reaction. Both the yeast and the human enzyme have 12 histidine residues. Of these, 4 are within regions of high homology and 1 is in a region of partial homology.

The cDNA clone has 114 bp of noncoding sequence at the 5′ end and only 46 bp at the 3′ end. Although there is no amino acid sequence of the protein with which to compare this sequence, the homology with the yeast gene in this area strongly suggests that the indicated methionine residue is in fact the correct initiation codon. Translation of most eukary-

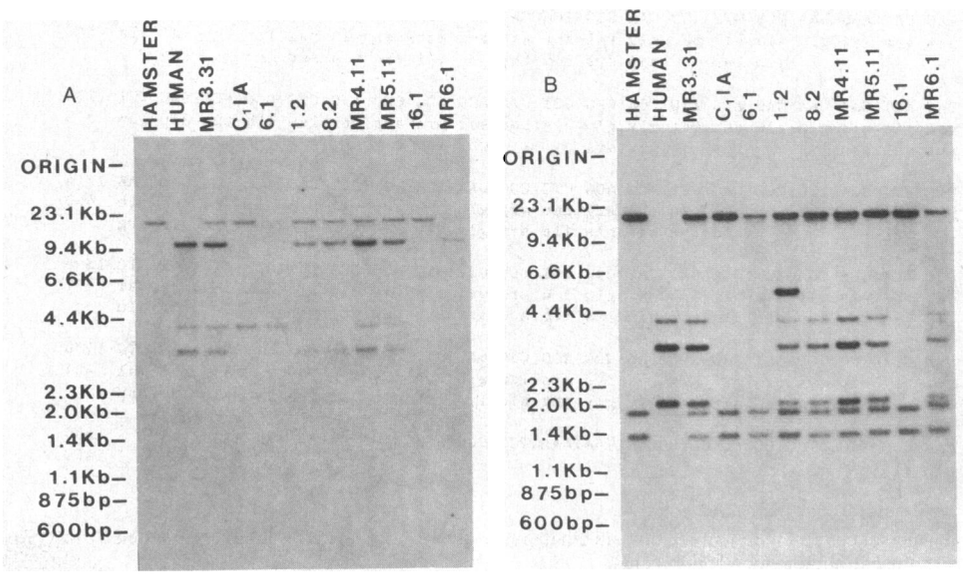

FIG. 5. Southern blots of DNA from Chinese hamster–human somatic cell hybrids probed with an *Eco*RI fragment containing bases 1–910 (*A*) or 911–1565 (*B*) of the cDNA. Lanes labeled hamster and human contain DNA from the parental cell lines. All other lanes contain DNA from the various hybrid cell lines. DNA from each cell line (7.5 μg) was digested with *Eco*RI and electrophoresed in a 0.8% agarose gel. The extra hybridizing band in hybrid 1.2 is due to plasmid contamination in this DNA sample. kb, Kilobases.

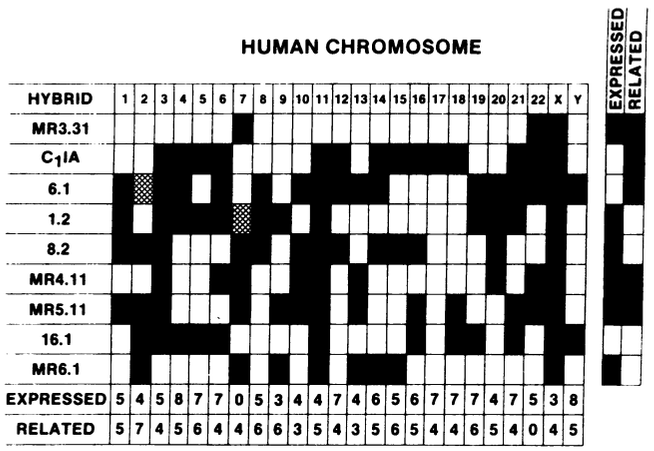

FIG. 6. Summary of chromosomal mapping of argininosuccinate lyase sequences. The presence of a particular human chromosome in each somatic cell hybrid is depicted by a filled-in space. The filled-in spaces in the columns on the far right of the figure (designated expressed and related) indicate the presence of a hybridizing fragment in the hybrid cell line as detected by the cDNA probe. The 4.0-kb fragment detected by the 5′ probe (Fig. 5*A*) is designated the "related" sequence. All of the other hybridizing fragments were scored as the "expressed" region. The numbers in the bottom two lines of the figure correspond to the number of discordances observed when the presence or absence of a set of DNA fragments was correlated with the chromosomal composition of the nine hybrid cell lines used. The two crosshatched regions indicated that only part of the chromosome was present in the hybrid cell line.

otic mRNAs is initiated at the first available methionine codon, but exceptions to this rule have been noted. In addition, the sequence surrounding the first AUG is nearly identical to Kozak's (40) consensus sequence, CCRCCAUGG, where R represents A or G. This correlation, combined with the corresponding sequence of the yeast protein, leaves little doubt that this is the correct initiation site for protein synthesis. At the 3′ end a canonical AATAAA is located 16 nucleotides prior to the polyadenylate tract. Some heterogeneity was noted in the 3′ end of the message. One of the cDNA clones we examined extended some 365 bp and terminated at another canonical AATAAA sequence.

Analysis using a somatic cell hybrid panel showed that the cDNA clone hybridized to two chromosomal sites. All hybridizing bands except one were consistent with the previous assignment of the structural gene to human chromosome 7. The 5′ end of the cDNA was noted to hybridize to a site on chromosome 22 in addition to the chromosome 7 site. At present, we assume that this represents a truncated, processed pseudogene, but the exact nature of this region must await the isolation of the region.

We wish to express our appreciation to Dr. Savio Woo and his collaborators for supplying the λgt11 library and for many helpful discussions on the screening techniques. We thank Lynn Loewenstein for expert secretarial assistance in the preparation of this manuscript. This work was supported by Grant AM31861 from the National Institutes of Health.

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