2001

Truncated Forms of the Dual Function Human ASCT2 Neutral Amino Acid Transporter/ Retroviral Receptor Are Translationally Initiated at Multiple Alternative CUG and GUG Codons

Chetankumar S. Tailor

Mariana Marin

Ali Nouri

Michael Kavanaugh

David Kabat

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 29, Issue of July 20, pp. 27221–27230, 2001

Printed in U.S.A.

Truncated Forms of the Dual Function Human ASCT2 Neutral Amino Acid Transporter/Retroviral Receptor Are Translationally Initiated at Multiple Alternative CUG and GUG Codons*

Received for publication, January 25, 2001, and in revised form, April 30, 2001
Published, JBC Papers in Press, May 11, 2001, DOI 10.1074/jbc.M100737200

Chetankumar S. Tailor‡§, Mariana Marin‡, Ali Nouri‡, Michael P. Kavanaugh¶, and David Kabat‡‖*

From the ‡Department of Biochemistry and Molecular Biology and the ¶Vollum Institute, Oregon Health Sciences University, Portland, Oregon 87201-3098

The sodium-dependent neutral amino acid transporter type 2 (ASCT2) was recently identified as a cell surface receptor for endogenously inherited retroviruses of cats, baboons, and humans as well as for horizontally transmitted type-D simian retroviruses. By functional cloning, we obtained 10 full-length 2.9-kilobase pair (kbp) cDNAs and two smaller identical 2.1-kbp cDNAs that conferred susceptibility to these viruses. Compared with the 2.9-kbp cDNA, the 2.1-kbp cDNA contains exonic deletions in its 3′ noncoding region and a 627-bp 5′ truncation that eliminates sequences encoding the amino-terminal portion of the full-length ASCT2 protein. Although expression of the truncated mRNA caused enhanced amino acid transport and viral receptor activities, the AUG codon nearest to its 5′ end is flanked by nucleotides that are incompatible with translational initiation and the next in-frame AUG codon is far downstream toward the end of the protein coding sequence. Interestingly, the 5′ region of the truncated ASCT2 mRNA contains a closely linked series of CUG(Leu) and GUG(Val) codons in optimal consensus contexts for translational initiation. By deletion and site-directed mutagenesis, cell-free translation, and analyses of epitope-tagged ASCT2 proteins synthesized intracellularly, we determined that the truncated mRNA encodes multiple ASCT2 isoforms with distinct amino termini that are translationally initiated by a leaky scanning mechanism at these CUG and GUG codons. Although the full-length ASCT2 mRNA contains a 5′-situated AUG initiation codon, a significant degree of leaky scanning also occurred in its translation. ASCT2 isoforms with relatively short truncations were active in both amino acid transport and viral reception, whereas an isoform with a 79-amino acid truncation that lacked the first transmembrane sequence was active only in viral reception. We conclude that ASCT2 isoforms with truncated amino termini are synthesized in mammalian cells by a leaky scanning mechanism that employs multiple alternative CUG and GUG initiation codons.

The broad specificity sodium-dependent neutral amino acid transporter type 2 (ASCT2)[1] was recently identified as a cell surface receptor for a large and widely dispersed group of retroviruses that includes endogenously inherited viruses of cats, baboons, and humans, and the horizontally transmitted avian reticuloendotheliosis viruses and type-D simian retroviruses (1–3). Furthermore, some of these viruses can promiscuously use the related transporter ASCT1 as an auxiliary receptor (4). Although ASCT1 and ASCT2 both function as exchangers for neutral amino acids, the specificity of ASCT2 is broader and it includes glutamine (5–8). These proteins are members of a large glutamate transporter superfamily, which are believed to have a common membrane topology (9) with a relatively large extracellular loop 2 (ECL2) that typically contains two consensus sites for *N*-linked glycosylation. Consistent with the hypothesis that it has been a battleground for virus-host co-evolution, the ECL2 of ASCT2 is critical for its retroviral receptor function and has been hypervariable throughout mammalian evolution (4).

According to the ribosome-scanning model for translation, protein synthesis in eukaryotes involves a process of 40 S ribosomal subunit attachment to the 5′ end of the mRNA, followed by ATP-dependent movement down the mRNA until an initiation codon is reached (10). Usually, but not always, this initiation site is the first AUG(Met) codon. However, initiation only proceeds efficiently if the AUG occurs within the Kozak consensus context (GCC(A/G)CCAUGG) in which the most important features are the G at position +4 adjacent to the AUG and the A/G at position −3 (11–16). Moreover, if the first initiation site in a mRNA is used inefficiently, some of the 40 S ribosomal subunits will move through the site by a "leaky scanning" process and may initiate translation at a downstream position (17). Interestingly, non-AUG codons such as CUG(Leu), ACG(Thr), and GUG(Val) can also serve as low efficiency initiation sites if they occur within the Kozak consensus context (10, 18–27). In these cases, however, the initial amino acid that is incorporated appears to be methionine, apparently because these non-AUG codons can still form weak base pairs with the initiator Met-tRNA^{Met} (26, 28). A clear illustration of these issues occurs with the Gag protein of murine leukemia viruses, which is inefficiently initiated at a CUG codon and then more efficiently by the leaked-through ribosomes at a downstream AUG codon (29). This produces a larger protein with an amino-terminal signal sequence that is

* This work was supported by National Institutes of Health Grants CA25810 and CA83835. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF334818.

§ Current address: The Hospital for Sick Children, Infection, Immunity and Injury Repair Program, Toronto, Ontario, Canada.

‖ To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, Oregon Health Sciences University, 3181 S.W. Sam Jackson Park Road, Mail Code L224, Portland, OR 97201-3098. Tel.: 503-494-8442; Fax: 503-494-8393; E-mail: kabat@ohsu.edu.

[1] The abbreviations used are: ASCT2, amino acid transporter type 2; ECL2, large extracellular loop 2; kbp, kilobase pair; kb, kilobase(s); CHO, Chinese hamster ovary; PCR, polymerase chain reaction; TM, transmembrane.

inserted into the endoplasmic reticulum and is then expressed as an *N*-glycosylated derivative on cell surfaces plus a smaller more abundant Gag protein that is synthesized in the cytosol (30). Another important example occurs with the Myc oncoprotein, which is translationally initiated at a non-AUG codon, followed by leaky scanning through a series of suboptimal AUG codons, resulting in production of multiple shorter Myc proteins that have diverse functions (31, 32). There is evidence that the efficiency of leaky scanning can be influenced by the proliferative status of cells (32, 33). In addition to this leaky scanning mechanism for producing protein isoforms with alternative amino termini, there are many examples in which differential RNA splicing or use of alternative promoters can produce a 5′ truncated mRNA that lacks the first AUG codon, resulting in initiation of a shorter protein from a downstream AUG (34–43).

In this study we describe the properties of two ASCT2 cDNAs that were reproducibly isolated from a human lymphocyte cDNA library. Both the 2.1- and 2.9-kbp cDNAs encode ASCT2 proteins that are functionally active in amino acid transport and in retroviral reception. Interestingly, the 2.1-kb mRNA lacks a functional AUG initiation codon and is translationally initiated by leaky scanning at multiple alternative in-frame CUG and GUG codons, thereby producing a family of shortened ASCT2 isoforms with diverse amino termini. Furthermore, the 2.9-kb mRNA also appears to be translationally initiated not only at the AUG codon nearest to its 5′ end but also to a significant extent at the downstream CUG and GUG codons. The leaky scanning of ASCT2 mRNAs was demonstrated not only in a cell-free system but also in cells cultured in physiological conditions. Surprisingly, truncated forms of ASCT2 that lack TM1 and as many as 79 amino acids are processed to cell surfaces in functionally active conformations.

MATERIALS AND METHODS

Cells and Viruses—Human TE671 and mouse NIH3T3 cells were maintained in Dulbecco's modified Eagle's medium with low glucose and 10% fetal bovine serum, Chinese hamster ovary (CHO) cells were maintained in Dulbecco's modified α-medium with 10% fetal bovine serum, and HEK293T cells were maintained in Dulbecco's modified Eagle's medium with high glucose and 10% fetal bovine serum.

LacZ(RD114), lacZ(BaEV), lacZ(SRV-1), and lacZ(SRV-2) pseudotype viruses were generated as previously described (3). LacZ(RD114) was produced by TELCeB6/RDF-7 helper-free packaging cells (44). LacZ(BaEV) was rescued by infection of mink Mv-1-Lu cells harboring a lacZ vector with a replication competent BaEV stock (45). LacZ(SRV-1) and lacZ(SRV-2) were produced from TELCeB6 cells infected with replication-competent SRV-1 and SRV-2.

Expression Vectors—hASCT2 and ΔhASCT2 cDNA expression vectors were generated as follows. The hASCT2 and ΔhASCT2 cDNAs were isolated by PCR (upstream primer, 5′-GATCCCAGTGTGCTGGAAAG-3′; downstream primer, 5′-GGTGGGGTCTTTCATTCC-3′) (see Ref. 46 for PCR conditions) using genomic DNA isolated from lacZ(RD114) positive NIH 3T3 cellular clones that had been previously transduced with a retroviral cDNA library (3). The PCR products were subsequently cloned into the pcDNA3.1V5His-TOPO mammalian expression vector (Invitrogen). ΔhASCT2 cDNAs with various truncations at the 5′ end were generated by PCR using ΔhASCT2 cDNA as template, with a downstream primer (5′-CCGGGGTTTACATGACTGATT-3′) and the following upstream primers: Δ35, 5′-CAGCAGGCGGCTACTGCGGTT; Δ48, 5′-CTGCCTTCGAGCCAACCTGCT-3′; Δ67, 5′-GCGCTGGGACTGGGGGTGTCGG-3′; Δ96, 5′-CTGCTGCGTCTGCTGCGGATGATCA-3′; Δ106, 5′-CCGCTGGTGGTGTGCAGCTTG-3′. Mutant truncated ΔhASCT2 cDNAs were generated using the downstream primer described above and the following upstream primers: M102A, 5′-CTGCTGCGGGCGATCATCTTG-3′; LA1Δ67, 5′-GCGGCGGGAGCGGGGGTGTCG-3′; LA2Δ67, 5′-GGGGGTGCGGCGGCGTTGGGCCCG-3′; L23 mΔh,5′-CAGCGGCGGAGCCCACCGCCAACGGGGGCATGGCGCTGGCC3′;LA1Δ67(AUG), 5′-GGGGGTGCGATGGCGTTGGGC-3′. The PCR products were cloned into pcDNA3.1V5His-TOPO vector. For constructing the expression vectors of hASCT2- and ΔhASCT2-Myc tag fusion protein, the cDNAs were isolated by PCR using the same downstream primer (5′-TTGCGGCCGCCATGACTGATTCCTTCTC-3′ containing a *Not*I restriction site (underlined sequence)) and the following upstream primers: hASCT2, 5′-TAAAGCTTATGGTGGCCGATCCTCC-T-3′ containing a *Hin*dIII restriction site (underlined sequence); ΔhASCT2, 5′-CAGCGGCGGAGCCCACCGCCAACG-3′. The PCR products were‘ cloned into pcDNA3.1-MycHis-version C mammalian expression vector (Invitogen). All cDNAs were sequenced by the Microbiology and Molecular Immunology core facility, and by the Vollum Institute core facility on the PE/ABD 377 sequencer using dye terminator cycle-sequencing chemistry (Applied Biosystems, Foster, CA).

Transfection—Stable expression of hASCT2 and ΔhASCT2 in CHO cells was achieved by transfection of the corresponding expression vectors using the SuperFect transfection reagent (Qiagen). Transfected cells were selected with G418 sulfate (1 mg/ml) and resistant colonies of cells were pooled 1–2 weeks after addition of selection. Resistant cells were then analyzed for susceptibility to the lacZ pseudotype viruses outlined above. NIH 3T3 cells transiently expressing hASCT2, ΔhASCT2, or truncated hASCT2 proteins were generated by transient transfection of the corresponding cDNA expression vectors using SuperFect transfection reagent. Transfection of target cells was carried out in 24-well tissue culture plates. The transfected cells were subsequently tested for susceptibility to lacZ pseudotype viruses 24 h post-transfection. Normalization of transfection efficiencies was analyzed by co-transfecting the pLIB-EGFP (CLONTECH) reporter vector for the green fluorescent protein together with expression vectors for wild-type and mutant forms of hASCT2 and ΔhASCT2. The results suggested that the transfection efficiencies were the same for the vectors being compared in each assay.

Infection—Target cells transiently transfected with hASCT2, ΔhASCT2, or truncated ΔhASCT2 expression vectors were incubated overnight with serial dilutions of lacZ(RD114), lacZ(BaEV), lacZ(SRV-1), or lacZ(SRV-2). Infected cells were analyzed by staining with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) using a previously described protocol (47). LacZ pseudotype titers were expressed as the number of blue colony forming units obtained per milliliter of viral supernatant. The titers reported were averages of three infection studies.

Amino Acid Transport—The amino acid transport function of hASCT2, ΔhASCT2, and truncated ΔhASCT2 were analyzed in HEK293T cells. Briefly, HEK293T cells were transiently transfected with the corresponding cDNA expression vectors using the SuperFect Reagent. The initial rate of L-[^{3}H]alanine uptake was analyzed 24 h post-transfection using a previously described procedure (48). Alanine uptake values reported are average of 3 uptake studies.

In Vitro Translation—A rabbit reticulocyte lysate *in vitro* translation kit (Promega) was used to generate proteins encoded by hASCT2, ΔhASCT2, and truncated ΔhASCT2 mRNAs. The corresponding cDNA expression vectors (see above) were used as templates together with [^{35}S]methionine radiolabel, and reagents provided by the rabbit reticulocyte lysate *in vitro* translation kit, to generate [^{35}S]methionine-labeled receptor proteins encoded the hASCT2, ΔhASCT2, Δ35, Δ48, Δ67, Δ96 and Δ106 mRNAs. The xenotropic and polytropic murine leukemia virus receptor (X-receptor) (46, 49, 50) mRNA was used as a control. Protein samples were analyzed by electrophoresis in 10% polyacrylamide gels in the presence of 0.1% SDS. The gels were subsequently exposed overnight to a Kodak Scientific Imaging film.

Immunoblot Analyses—HEK293T cells expressing ASCT2 receptors were generated by transient transfection of the corresponding cDNAs using SuperFect transfection reagent (Qiagen). Cell lysates of transfected cells were prepared 48 h post-transfection. For total cell extracts, the cells were scraped off the culture dishes in cold phosphate-buffered saline and centrifuged at 200 × *g* at 4 °C for 5 min. The cell pellet was then resuspended in lysis buffer (50 mM Tris-HCl (pH 8.8), 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, protease inhibitor mixture), and incubated on ice for 30 min. The cell debris and nuclei were removed by centrifugation of the samples at 15,000 × *g* at 4 °C for 10 min. Three micrograms of total protein cell lysate were either treated with or untreated with *N*-glycosidase F (2 h; 37 °C) and subsequently analyzed by electrophoresis in 10% polyacrylamide gels in the presence of 0.1% SDS. For studies of surface ASCT2 receptors expression HEK293T transiently transfected with the corresponding cDNAs were surface biotinylated by the addition of 2 mM sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) to 2 × 10^7 cells for 1 h at 4 °C. The reaction was quenched with 20 mM glycine for 15 min. Washed cells were lysed in lysis buffer as described for total cell extracts. The biotinylated molecules were precipitated with streptavidin-agarose beads (Life Technologies, Inc.) at 4 °C. Beads were washed three times with lysis buffer and resuspended in 20 μl of lysis buffer. 10 μl of beads were

TABLE I

Susceptibility of CHO cells expressing hASCT2 and ΔhASCT2 to RD114, BaEV, SRV-1, and -2

CHO cells transfected with hASCT2 and ΔhASCT2 (CHO/hASCT2 and CHO/ΔhASCT2, respectively) cDNA expression vectors were tested for susceptibilities to lacZ(RD114), lacZ(BaEV), lacZ(SRV-1), and lacZ(SRV-2) pseudotype viruses. Human TE671 cells served as a positive control for all of these viruses. The titers are averages of three independent infection studies.

Target cell	Titer of lacZ pseudotype (CFU/ml)			
	RD114	BaEV	SRV-1	SRV-2
TE671	3.5×10^6	3.7×10^5	3.1×10^4	2.3×10^6
CHO	<3	0	<3	0
CHO/hASCT2	5.9×10^5	8.0×10^4	1.1×10^5	1.9×10^5
CHO/ΔhASCT2	9.5×10^4	1.5×10^4	6.5×10^4	3.3×10^4

treated with *N*-glycosidase F (2 h; 37 °C). The treated and untreated beads were boiled with an equal volume of 2 × Lammeli sample buffer and subsequently analyzed by electrophoresis in 10% polyacrylamide gels in the presence of 0.1% SDS. The proteins were then transferred to nitrocellulose filters, which were then treated with 5% milk powder in phosphate-buffered saline. The nitrocellulose blots were probed with anti-*myc* tag monoclonal antibody 9E10 (Sigma) and developed by using a horseradish peroxidase-conjugated goat anti-mouse antibody (Southern Biotechnology Associates, Inc.) and an enhanced chemiluminescence kit (NEN Life Research Products, Boston, MA).

Oocyte Transport Assays—Capped cRNAs were synthesized *in vitro* as previously described (5, 51) using pOG1-hASCT2 and pOG1-ΔhASCT2 plasmids as templates. 50 ng of the cRNAs were injected into stage V *Xenopus laevis* oocytes 3–5 days before analysis. Uptake of radiolabeled amino acid was measured by incubating oocytes in wells containing 10 μM L-[^{3}H]alanine (1 mCi/mmol; Amersham Pharmacia Biotech) for 10 min. Oocytes were then rapidly washed with Ringer's solution, transferred into a scintillation vial, lysed in 1% SDS, and radioactivity was measured. Specific transport was calculated by subtracting the mean uptake measured in uninfected oocytes from the uptake measured in injected cells.

RESULTS

Cloning of a Truncated Retroviral Receptor, ΔhASCT2—Previously, we isolated 12 human cDNAs that caused rodent cells to become susceptible to infections by the RD114 feline endogenous retrovirus (3). As we reported, 10 of these cDNAs had sizes of 2.9 kbp and encoded the full-length human ASCT2 (hASCT2) protein (7). Furthermore, another laboratory independently identified hASCT2 as the receptor for these same retroviruses (2). The other two cDNA clones that we isolated, which we term ΔhASCT2, had sizes of 2.1 kbp and identical sequences that will be further described below. These larger and smaller cDNAs are compatible with the sizes of major and minor mRNA components that were detected by Northern blot analyses of RNAs from different human tissues (3, 7). As shown in Table I, stable expression of the 2.1-kbp ΔhASCT2 cDNA in CHO cells caused susceptibility to infections by lacZ pseudotypes of RD114, baboon endogenous virus (BaEV), and type-D simian retroviruses (SRV-1 and SRV-2). These results strongly suggest that the 2.1-kbp ΔhASCT2 cDNA encodes a functional receptor for RD114, BaEV, and SRVs.

General Implications of the hASCT2 and ΔhASCT2 cDNA Sequences—Fig. 1*A* shows a diagrammatic comparison of the 2.9-kb hASCT2 and 2.1-kb ΔhASCT2 mRNAs based on their cDNA sequences. These mRNAs are closely related except for a truncation of 627 bases in the 5′ region and smaller deletions of 41 and 70 bases in the 3′-untranslated region of the ΔhASCT2 mRNA. In addition, there is a G to C base substitution in the coding region that would cause a Val to Leu substitution near the carboxyl-terminal end of the ΔhASCT2 protein. As indicated in Fig. 1*A*, the predicted 2.9-kb hASCT2 mRNA contains a large 5′-untranslated sequence of 590 bases and would be expected to encode a full-length hASCT2 protein of 541 amino acids. On the contrary, as further indicated below, the sequence of the 2.1-kb ΔhASCT2 mRNA does not provide an unambiguous indication about the encoded protein.

Fig. 1*B* shows the sequence of the full-length hASCT2 protein, which indicates the amino acid numbering system that we will use below to describe the hASCT2 and ΔhASCT2 proteins. The sequence has been annotated to show the ΔhASCT2 open reading frame and the site of the Val to Leu substitution described above. In addition, we have indicated the locations of the 10 hydrophobic potential TM sequences and the two consensus sites for *N*-linked glycosylation that occur in the putative ECL2 region between TM3 and TM4. Consistent with the absence of an amino-terminal signal sequence and with the transmembrane topology implied in Fig. 1*B*, previous evidence has indicated that the hASCT2 amino terminus is in the cytosol, that the presumptive ECL2 sequence between TM3 and -4 is *N*-glycosylated at both positions and that it is exposed on the cell surfaces where it comprises a critical site for virus attachment, and that the carboxyl terminus is also cytosolic (4).

The AUG(Met-102) Codon Is Not the Initiation Site for ΔhASCT2 Synthesis—Although the data in Table I clearly indicates that the ΔhASCT2 protein is a functional retroviral receptor, suggesting that it must contain the critical ECL2 residues described above, the sequence of the ΔhASCT2 mRNA does not provide an unambiguous indication about the encoded protein. Fig. 2*A* shows the initial 299-nucleotide sequence of the ΔhASCT2 mRNA. As can be seen by comparison of Figs. 1 and 2, the ΔhASCT2 open reading frame begins with the AA-EPT . . . sequence corresponding to amino acid position 14. The first AUG codon in the ΔhASCT2 mRNA corresponds to AUG-(Met-102), which is boxed in Fig. 2*A*. However, as indicated in Fig. 3, the nucleotides flanking AUG(Met-102) do not conform to the Kozak consensus sequence required for translational initiation. Moreover, the next downstream AUG corresponds to AUG(Met-229), which also lacks a Kozak consensus sequence and is too far downstream to include the ECL2 region that is critical for viral reception.

To further analyze this issue, we first generated 5′ deletions in the ΔhASCT2 cDNA. As indicated in Fig. 2*A*, these deletions eliminated portions of the ΔhASCT2 open reading frame from the encoded mRNAs. These deletion mutants and other site-directed mutants that we employed are diagrammed in Fig. 2*B*.

As shown in Fig. 4, NIH 3T3 fibroblasts that were transiently transfected with the Δ35, Δ48, and Δ67 expression constructs became highly susceptible to infections with the lacZ(RD114) virus, suggesting that the deleted mRNAs encode functional receptors and that an initiation site for ΔhASCT2 receptor synthesis is located downstream of nucleotide 162 (start of Δ67). In contrast, the Δ96 construct did not confer susceptibility to this virus, despite the presence of the AUG-(Met-102) in this mRNA, and the Δ106 mRNA was similarly inactive. Furthermore, we mutated the AUG(Met-102) codon to GCG(Ala) (Fig. 2*B*), and found that the M102A mutant was fully active as a viral receptor (Fig. 4). These results strongly suggest that AUG(Met-102) is not an initiation codon for protein synthesis. Furthermore, synthesis of a functional ΔhASCT2 receptor must initiate at a site between nucleotides 162 (start of Δ67 mRNA) and 249 (start of Δ96 mRNA) that is

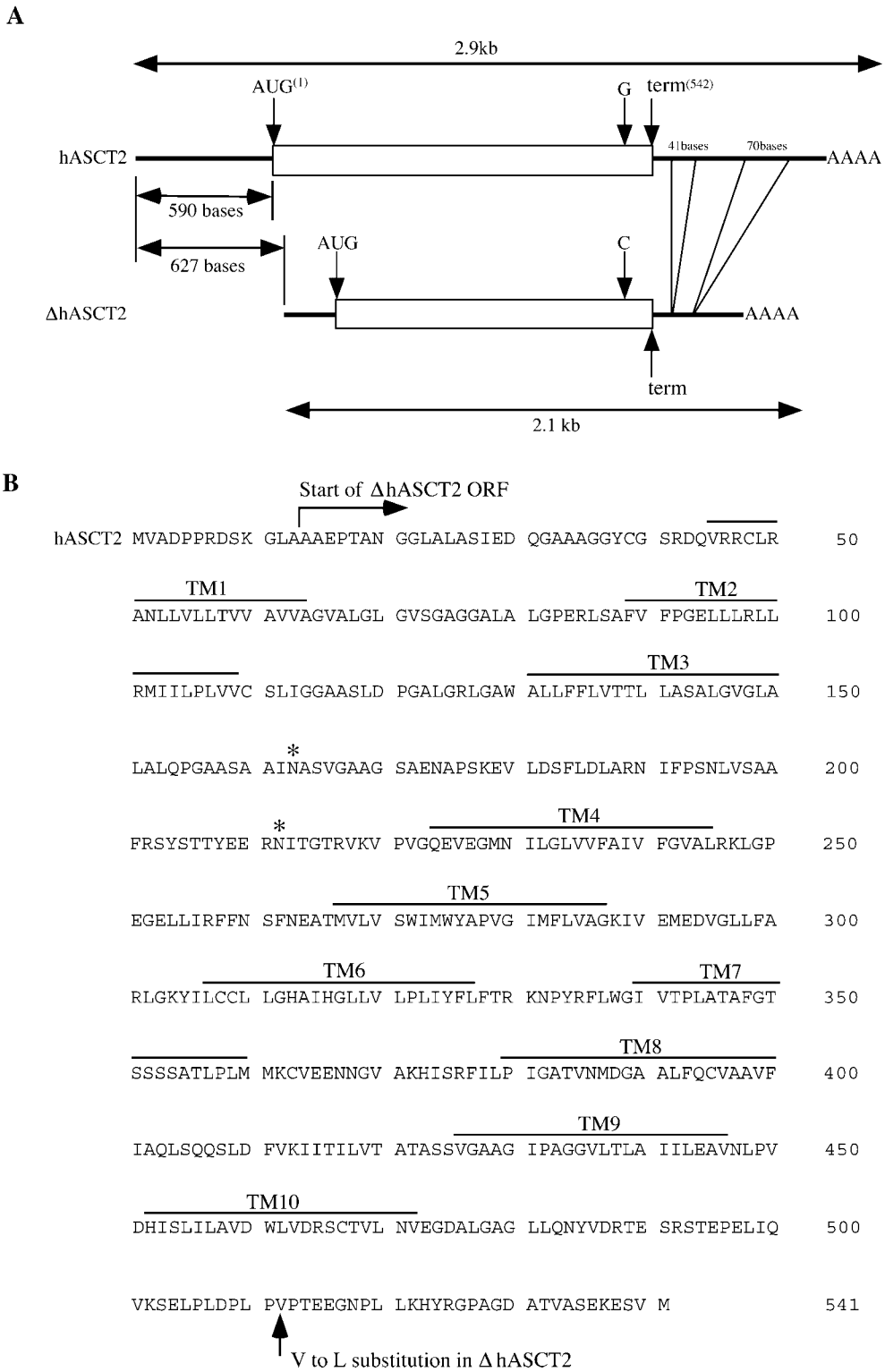

FIG. 1. **Comparison of the full-length and truncated ASCT2 mRNAs and proteins.** *A,* a diagrammatic comparison of the 2.9-kb hASCT2 mRNA with the truncated 2.1-kb ΔhASCT2 mRNA. The ΔhASCT2 mRNA shows high degree of sequence identity to hASCT2 mRNA except for a deletion of 627 bases in the 5′-untranslated region and two deletions of 41 and 70 bases in the 3′- untranslated region. In addition, there is a G to C substitution in the potential coding region (*open box*). *B,* amino acid sequences of hASCT2 and putative ΔhASCT2 proteins. The potential coding region of ΔhASCT2 is nearly identical to hASCT2 except for the following sequences. The ΔhASCT2 open reading frame (*ORF*) begins with the AAEPT . . . sequence, which corresponds to amino acid 14 of hASCT2. The ΔhASCT2 protein contains a valine to leucine substitution in the COOH terminus corresponding to amino acid 512 of hASCT2. A *line above* the amino acid sequence indicates the potential TM segments whereas *asterisks* indicate the potential *N*-linked glycosylation sites.

in-frame with the hASCT2 protein coding sequence. Accordingly, the upstream amino-terminal region of hASCT2 including TM1 must be unnecessary for folding and processing of a functional receptor protein.

Synthesis of ΔhASCT2 Initiates at Several CUG(Leu) Codons—Interestingly, the sequence of ΔhASCT2 mRNA between nucleotides 162 and 249 contains four CUG(Leu) codons that are all in the open reading frame (see Fig. 2*A*). Moreover,

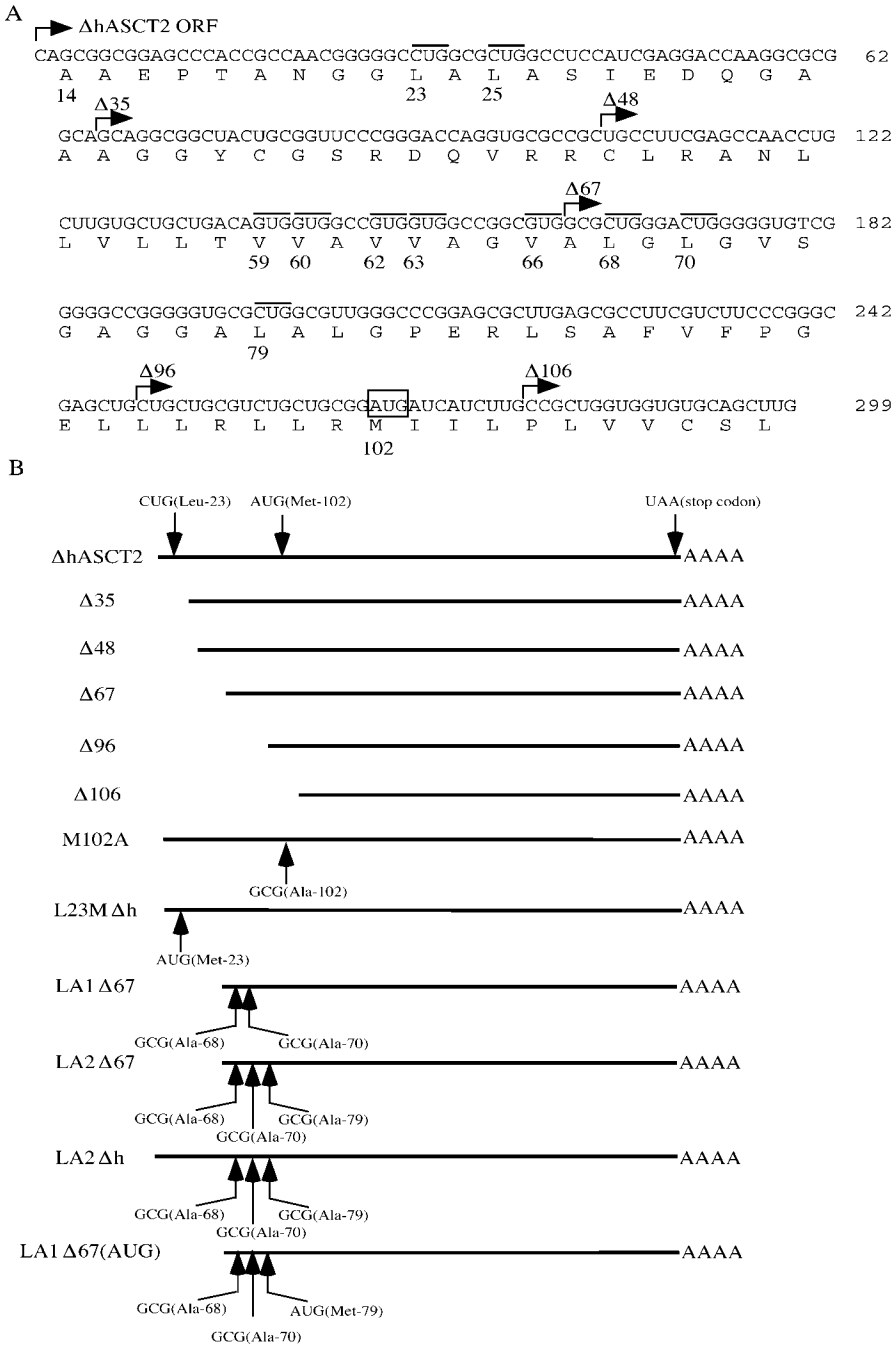

FIG. 2. **Sequence and mutagenesis of the 5′ region of the ΔhASCT2 mRNA.** *A,* sequence of the initial 299 nucleotides of the ΔhASCT2 mRNA. The encoded amino acid sequence is indicated *below* the mRNA sequence. The amino acid positions, as indicated *below* the residue, correspond to the numbering system for the full-length hASCT2 protein (see also Fig. 1). The *arrows* indicate the start of the 5′ deleted ΔhASCT2 mRNAs and the nomenclature of the deleted mRNAs corresponds to the position of the amino acid encoded by the first codon in the sequence. The potential initiator codons are indicated by a *line above* the mRNA sequence. The predicted AUG (Met-102) initiator codon is outlined by a *box. B,* diagram of the open reading frame of ΔhASCT2 mRNA and the 5′-deleted mRNAs. The 3′-untranslated region for all mRNAs were removed (see "Materials and Methods"). The mutant mRNAs that we employed are also shown.

three of these codons, CUG(Leu-68), CUG(Leu-70), and CUG(Leu-79) are flanked by nucleotides that conform to the Kozak consensus requirement as shown in Fig. 3. Since CUG codons can be used as initiation sites, we simultaneously mutated both the CUG(Leu-68) and CUG(Leu-70) codons to GCG(Ala) in the context of the Δ67 mRNA to generate the double mutant LA1Δ67 (see Fig. 2*B*). As shown in Fig. 4, NIH 3T3 cells transiently expressing LA1Δ67 were approximately as susceptible to lacZ(RD114) infections as cells expressing the unmutated Δ67 construct. This suggested that CUG(Leu-79) might be an initiation codon for ΔhASCT2 receptor synthesis. Consequently, we mutated the CUG(Leu-79) codon to GCG(Ala) in the context of the LA1Δ67 mutant to generate the LA2Δ67 mutant, and we found that this eliminated receptor activity (see Fig. 4), confirming that CUG(Leu-79) is an initiation codon for the ΔhASCT2 receptor. To further analyze the CUG(Leu-68), CUG(Leu-70), and CUG(Leu-79) codons, we simultaneously mutated these codons to GCG(Ala) in the context of the ΔhASCT2 mRNA to generate the LA2Δh mutant. Interestingly, as shown in Fig. 4, the LA2Δh mutant was active as a viral receptor. This strongly suggests that sequences upstream of nucleotide 162 in ΔhASCT2 mRNA can also function as initiation sites for protein synthesis. Accordingly, this upstream region of the ΔhASCT2 mRNA contains two additional in-frame CUG(Leu) codons (at positions 23 and 25) and five tightly clustered GUG(Val) codons (at positions 59, 60, 62, 63, and 66) that all have excellent Kozak consensus sequence contexts (see Figs. 2 and 3). We believe that at least one of these GUG(Val) codons is likely to be important for initiation because the Δ35 and Δ48 mutants are reproducibly several times more active as receptors than the Δ67 mutant (see Fig. 4). Further support for the idea that CUG(Leu) and GUG(Val) codons occur in sequence contexts compatible with translational initiation was obtained by mutating the CUG(Leu-23) codon to

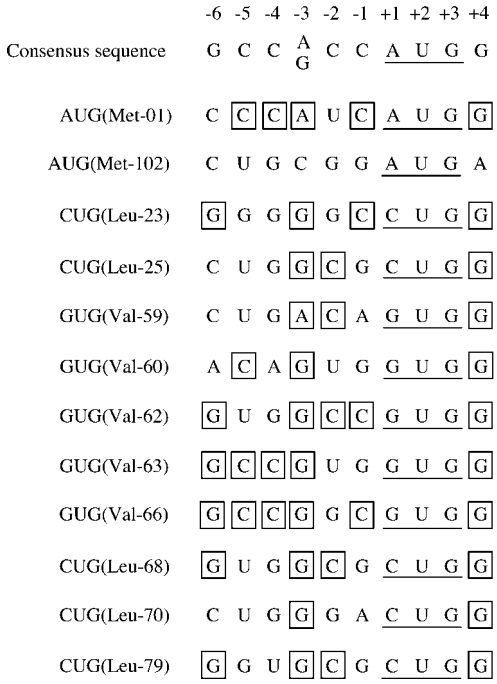

FIG. 3. **Comparison of the nucleotide sequence flanking the potential AUG(Met), CUG(Leu), and GUG(Val) initiator codons in ΔhASCT2 mRNA, with the eukaryotic consensus sequence (Kozak sequence) required for translational initiation (12, 13).** The numerical positions of the nucleotides in reference to the initiator codon are shown *above* the Kozak sequence. The potential initiator codons are *underlined* and nucleotides that match the consensus sequence are *boxed*. Efficient initiation of translation only proceeds when the initiator codon is flanked by a G at position +4 and A/G at position −3 (11–16). As observed in this figure, nucleotides flanking AUG(Met-102) codon do not conform to the Kozak sequence whereas nucleotides flanking AUG(Met-01), and the indicated CUG(Leu) and GUG(Val) codons are highly related to the Kozak sequence.

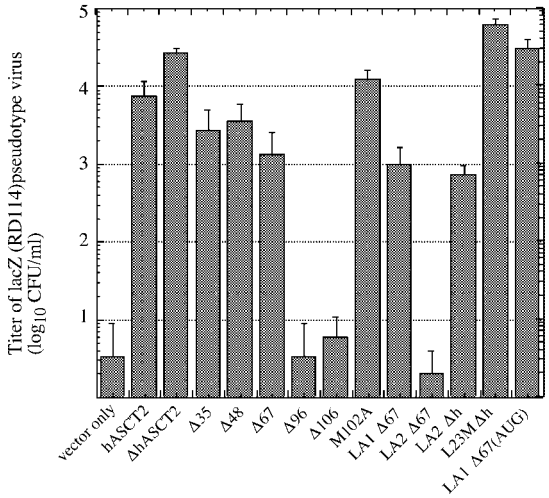

FIG. 4. **Susceptibility of NIH 3T3 cells expressing hASCT2, ΔhASCT2, and mutant ΔhASCT2 proteins to infection with the lacZ(RD114) virus.** NIH 3T3 cells were transiently transfected with the hASCT2, ΔhASCT2, or mutant ΔhASCT2 expression constructs and quantitatively tested for sensitivity to lacZ(RD114) 48 h post-transfection. The mutant ΔhASCT2 constructs were generated as described under "Materials and Methods." The titers of infection are averages of three infection assays.

AUG(Met-23). As shown in Fig. 4, the L23MΔh mutant was an enhanced receptor for lacZ(RD114) infections. Considered together, these results indicate that ΔhASCT2 synthesis initiates at several non-AUG codons, including CUG(Leu-79) and at least one of the other CUG or GUG codons that are highlighted in Fig. 2*A* and identified in Fig. 3.

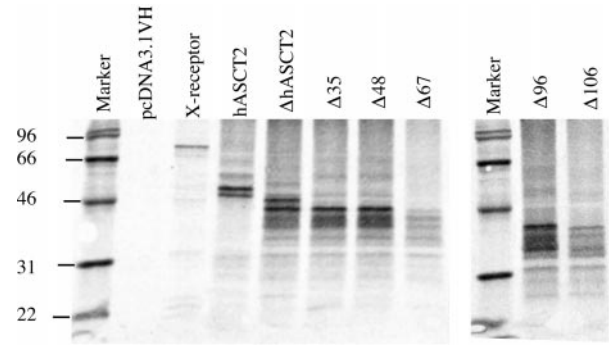

FIG. 5. **SDS-polyacrylamide gel electrophoresis of the proteins encoded by hASCT2, ΔhASCT2, and 5′-deleted ΔhASCT2 mRNAs.** The receptor proteins were synthesized by *in vitro* translation and labeled with L-[^{35}S]methionine. pcDNA3.1VH is the mammalian expression vector (Invitrogen); X-receptor is the receptor for xenotropic and polytropic murine leukemia viruses (46, 49, 50).

The clustering of these numerous CUG(Leu) and GUG(Leu) in-frame codons in Kozak consensus contexts into this 5′ region of the ΔhASCT2 mRNA as shown in Figs. 2 and 3 is highly significant. If these potential initiation sites were random, they would have been expected to occur in all three reading frames. Moreover, 10 of the 15 in-frame CUG(Leu) and GUG(Val) codons in this region occur in excellent Kozak consensus contexts (*i.e.* with A/G at the −3 end and G at +4), whereas such contexts occur elsewhere in the hASCT2 mRNA at frequencies of only ~0.2. This conclusion was supported by a codon preference analysis of hASCT2 mRNA. Codon usage was highly deviant from the expected *Homo sapiens* bias in this region but not in other regions of the hASCT2 mRNA (results not shown).

Δ*hASCT2 mRNA Encodes Truncated Protein Isoforms*—To analyze the proteins encoded by ΔhASCT2 mRNA, we initially used a rabbit reticulocyte lysate *in vitro* translation system (see "Materials and Methods"). Fig. 5 shows an electrophoretic analysis of L-[^{35}S]methionine-labeled receptor proteins encoded by ΔhASCT2 mRNA and by the 5′ deleted mRNAs Δ35, Δ48, Δ67, Δ96, and Δ106. We used the receptor protein for xenotropic and polytropic murine leukemia viruses (X-receptor) (46, 49, 50) as our control. This receptor has an apparent size of 80 kDa (Fig. 5), which is in agreement with its predicted size. We also analyzed the receptor encoded by hASCT2 mRNA. Unexpectedly, hASCT2 mRNA encoded two proteins, with a prominent protein product that had an apparent size of ~54–56 kDa, and a less prominent product of ~50 kDa. This result suggests that protein synthesis partially initiates at the predicted AUG codon and may also initiate at a downstream non-AUG codon. As observed in Fig. 5, the ΔhASCT2 mRNA encoded an array of proteins of various sizes with prominent protein products of ~46 and 40 kDa whereas the Δ35 and Δ48 mRNAs encoded a prominent protein product of only 40 kDa. Based on the sequence evidence in Fig. 2, these differences imply that CUG(Leu) codons at positions 23 and 25 are probably used for initiation by the ΔhASCT2 mRNA. Similarly, the Δ67 mRNA also encoded a prominent protein product of only 40 kDa. However, we consistently observed less receptor protein encoded by Δ67 mRNA compared with that encoded by Δ35 and Δ48 mRNAs. This result suggests that efficient *in vitro* translation at the CUG(Leu-79) codon might require sequences upstream of nucleotide 162 or that the GUG(Val) codons may be stronger initiation sites than CUG(Leu-79). Although, the Δ96 mRNA did not encode a functional receptor (Fig. 4), several smaller protein products were encoded by the mRNA suggesting that sequences further downstream of the AUG(Met-102)

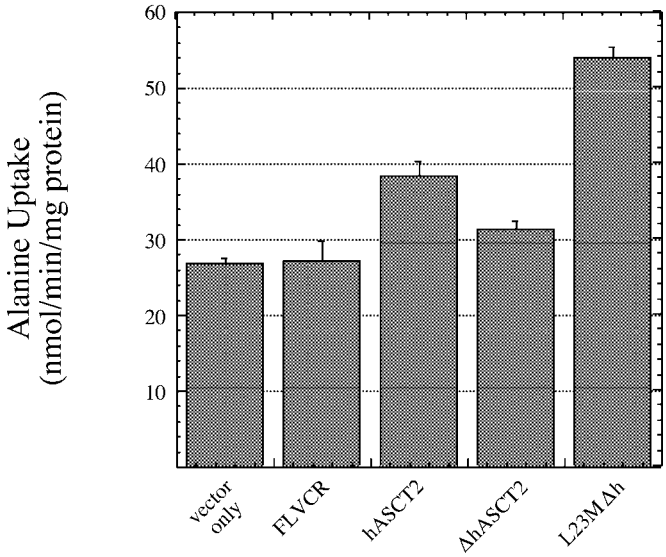

FIG. 6. **Uptake of L-[^{3}H]alanine by HEK293T (293T) cells and HEK293T cells expressing hASCT2, ΔhASCT2 or the L23 MΔh mutant.** 293T cells were transiently transfected with pcDNA3.1V5His (vector only), or with expression vectors for FLVCR, hASCT2, ΔhASCT2, or L23 MΔh, and uptake of L-[^{3}H]alanine was measured 24 h post-transfection. The transfections of these expression vectors were equally efficient as shown by co-transfection studies using the pLIB-EGFP reporter vector (see "Materials and Methods"). Each expression vector was assayed for amino acid uptake in three separate culture wells.

codon can function as weak initiator codons in this cell-free system. Together, these results suggest that the ΔhASCT2 mRNA encodes several proteins.

Truncated Forms of hASCT2 Are Active Amino Acid Transporters—Because the truncated proteins encoded by the ΔhASCT2 mRNA were retroviral receptors, we inferred that they must fold into functionally active proteins that are at least partially processed to cell surfaces. Consequently, we determined whether they can also function as transporters for neutral amino acids. Initially, we addressed this issue by transiently transfecting the hASCT2 and ΔhASCT2 cDNA expression vectors into HEK293T cells (see "Materials and Methods") for subsequent analyses of initial rates of L-[^{3}H]alanine uptake. As shown in Fig. 6, cells transfected with the full-length hASCT2 expression vector reproducibly had an elevated level of L-[^{3}H]alanine uptake compared with the control HEK293T cells that were transfected with the vector alone or with the human feline leukemia virus subgroup C receptor (hFLVCR). In contrast, cells that expressed ΔhASCT2 had only a small elevation in the uptake of the L-[^{3}H]alanine in this assay system (see Fig. 6) (n = 3).

Although the above results would be consistent with the idea that the ΔhASCT2 receptor might be only weakly active as an amino acid transporter, an alternative interpretation would be that the ΔhASCT2 proteins are fully active transporters but are expressed on cell surfaces at lower levels than the full-length hASCT2 protein. The latter interpretation would be compatible with previous evidence that non-AUG initiation codons are generally used at substantially lower efficiencies than standard AUG initiation codons. Moreover, it is conceivable that some of the ΔhASCT2 isoforms might be processed to cell surfaces with relatively low efficiencies. Indeed, the latter possibility is supported by evidence described below. To address these quantitative issues more directly, we mutated the first CUG(Leu-23) codon that our previous data had implicated as an initiation site for ΔhASCT2 (see above) into an AUG codon to generate the L23 MΔh mutant (see Fig. 2*B*). As described above, the L23MΔh mutant was highly active as a viral receptor, consistent with the improved expression of this protein on cell surfaces (see Fig. 4). Expression of this L23MΔh mutant in HEK293T cells substantially enhanced L-[^{3}H]alanine uptake compared with control cells or to cells expressing ΔhASCT2 (see Fig. 6). This result suggests that a low translational efficiency at the non-AUG initiation codons must be at least partly responsible for the reduced level of L-[^{3}H]alanine uptake activity of the ΔhASCT2 mRNA as seen in Fig. 6. Furthermore, the L23MΔh protein, which lacks 23 amino acids at the amino-terminal end is an active amino acid transporter.

To obtain additional evidence pertinent to this matter, we used the LA1Δ67 mutant which contains the Δ67 truncation with additional mutations of CUG(Leu-68) and CUG(Leu-70) to GCG(Ala) codons. As described above, the only initiation codon in this LA1Δ67 mRNA that encodes a functional viral receptor is the CUG(Leu-79) site. Consequently, we mutated this CUG(Leu-79) codon to form an AUG(Met-79) mutant in the context of LA1Δ67, thereby generating the LA1Δ67(AUG) mutant (Fig. 2*B*). Expression of the LA1Δ67(AUG) protein in NIH 3T3 fibroblasts caused the cells to become highly susceptible to infection with the lacZ(RD114) virus (Fig. 4). Infection titers were ~30-fold higher than the titers on LA1Δ67-expressing cells, implying an enhanced expression of the LA1Δ67(AUG) protein, consistent with the leaky scanning model for protein synthesis. However, expression of the LA1Δ67(AUG) mRNA in HEK293T cells did not increase L-[^{3}H]alanine uptake compared with the background in negative control cells (data not shown). These results suggest that the NH_2-terminal residues between amino acids 23 and 79 are critical for the transport function of ASCT2 but not for its receptor function.

Additional evidence concerning the potential transporter activity of proteins encoded by ΔhASCT2 mRNA was obtained by expressing this mRNA in *X. laevis* oocytes. This system is advantageous for studies of ASCT2-dependent transport because it has a low background of endogenous transport activity. Using previously described methods (5, 51), we expressed ASCT2 and ΔhASCT2 cRNAs in *X. laevis* oocytes and subsequently measured transport by incubating them for 10 min with 10 μM L-[^{3}H]alanine (1 mCi/mmol). Reproducibly, the oocytes injected with these cRNAs had similar rates of L-[^{3}H]alanine uptake that were at least 10 times greater than the control oocytes. For example, in one experiment the oocytes injected with hASCT2 cRNA had a specific uptake rate above background of 7.6 + 0.4 pmol/min (n = 5), whereas those injected with the ΔhASCT2 cRNA had a specific uptake rate of 7.7 + 0.4 pmol/min (n = 5). Similar results were obtained by measuring uptake by two-electrode voltage clamp methods (5, 51). These results strongly support our conclusion that the ΔhASCT2 mRNA encodes at least one active form of the transporter.

Synthesis of hASCT2 and ΔhASCT2 Proteins in Mammalian Cells—To identify the hASCT2 and ΔhASCT2 proteins synthesized intracellularly, we added a Myc epitope coding sequence onto the 3′ ends of the open reading frames and we then expressed these cDNAs in HEK293T cells. Since hASCT2 contains *N*-linked but not *O*-linked oligosaccharides (4) the extracted cellular proteins were incubated in the presence or absence of protein *N*-glycanase before electrophoresis and Western immunoblotting with the Myc-specific monoclonal antibody. As shown in Fig. 7 the proteins encoded by both mRNAs were heterogeneously *N*-glycosylated as indicated by their increased electrophoretic mobilities after digestions with protein *N*-glycanase. Moreover, the glycoprotein(s) encoded by the hASCT2 mRNA were more extensively processed to higher M_r forms than those encoded by the ΔhASCT2 mRNA, implying that the full-length hASCT2 protein may be more efficiently

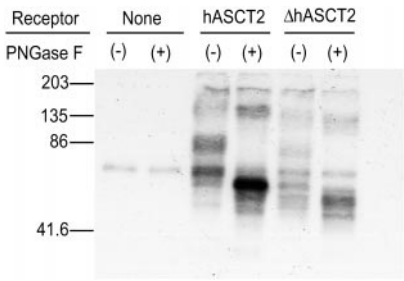

FIG. 7. **Western blot analysis of total cell lysates, with and without *N*-glycosidase F, prepared from HEK293T cells transiently expressing *myc*-tagged hASCT2 and ΔhASCT2.** The cell lysates were prepared 48 h post-transfection (see "Materials and Methods"). Three micrograms of total cell lysate protein treated with (+) or without (−) *N*-glycosidase F (PNGase F) were analyzed by SDS-polyacrylamide gel electrophoresis.

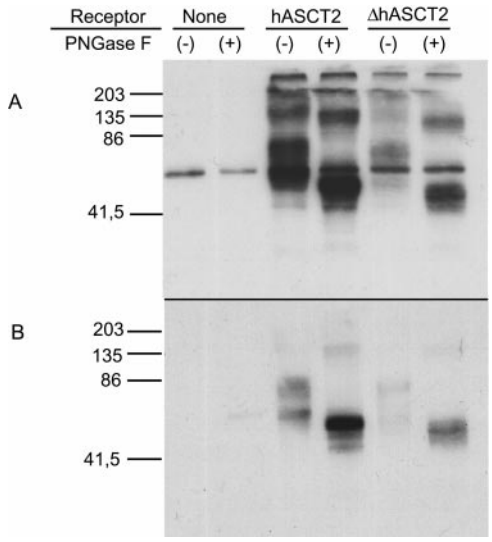

FIG. 8. **Identification of ASCT2 proteins on the surface of HEK293T cells.** HEK293T cells transiently expressing *myc*-tagged hASCT2 and ΔhASCT2 proteins were surface-biotinylated as described under "Materials and Methods." Samples of the total protein cell lysate were also used in a parallel analysis. The biotinylated proteins were then purified by affinity chromatography. The samples that were untreated (−) or treated (+) with *N*-glycosidase F (*PNGase F*) were analyzed by Western immunoblotting with anti-*myc* tag monoclonal antibody 9E10 (Sigma). *A,* total cellular *myc*-tagged hASCT2 and ΔhASCT2 proteins; *B, myc*-tagged hASCT2 and ΔhASCT2 proteins that were biotinylated on cell surfaces and then affinity purified prior to immunoblot analysis.

processed through the Golgi apparatus than the ΔhASCT2 proteins. In addition, higher M_r components compatible with oligomerization were also visible in these blots. From the perspective of this paper, however, we consider it most interesting that the ΔhASCT2 proteins are clearly highly heterogeneous even after exhaustive digestion with protein *N*-glycanase (see Fig. 7, ΔhASCT2 treated with and without PGNase F). Moreover, the protein *N*-glycanase-digested hASCT2 protein was also slightly heterogeneous (see Fig. 7). Although the majority of this hASCT2 protein has a uniform apparent size of 55–60 kDa, ~10% of the total appears to consist of smaller components that co-electrophorese with the deglycosylated isoforms encoded by the ΔhASCT2 mRNA. Since all of the detected proteins have carboxyl-terminal Myc tags, these results strongly suggest that the diverse hASCT2 and ΔhASCT2 proteins differ in the sizes of their amino termini. Similar results were observed in four independent repeats of this experiment.

We then analyzed the cell surface expression of hASCT2 and ΔhASCT2 proteins using a cell-membrane-impermeant biotinylation reagent. Extracts from surface-biotinylated HEK293T cells transiently expressing hASCT2 and ΔhASCT2 proteins were adsorbed onto streptavidin-agarose beads and the affinity purified proteins were then either treated or untreated with *N*-glycanase before electrophoresis and Western immunoblotting with the Myc-specific monoclonal antibody (see Fig. 8*B*). As a control for this analysis, we also examined the total protein extracts that had not been adsorbed onto streptavidin-agarose beads (Fig. 8*A*). The results confirm that truncated hASCT2 isoforms encoded by both the full-length and ΔhASCT2 mRNAs are expressed to substantial extents on cell surfaces. However, proteins encoded by the full-length hASCT2 mRNA are expressed several times more abundantly than proteins encoded by the ΔhASCT2 mRNA. These results also suggest the presence of hASCT2 and ΔhASCT2 oligomers in the cell extracts. Interestingly, several of the smallest truncated ASCT2 isoforms appear to have been processed to cell surfaces more efficiently in the presence of an excess of full-length hASCT2, implying that their processing might have been facilitated by hetereo-oligomer formation with the full-length protein.

DISCUSSION

This study suggests that the 2.1-kb ΔhASCT2 mRNA that we functionally cloned from a human lymphocyte cDNA library is translationally initiated by a leaky scanning mechanism (10) at multiple alternative in-frame CUG(Leu) and GUG(Val) codons, resulting in synthesis of an ensemble of ASCT2 protein isoforms with progressively more truncated amino-terminal ends. According to this leaky scanning model, the 40 S ribosomal subunits with their associated initiation factors would attach to the 5′ end of ΔhASCT2 mRNA and would move by an energy-dependent mechanism down the mRNA until they reach an initiation codon (10). If this initiation codon is an AUG in a poor Kozak consensus context or a non-AUG (*e.g.* CUG or GUG) in a strong Kozak context, then initiation may occur inefficiently, and many of the 40 S ribosomal subunits will scan through the site and proceed to the next potential initiation codon. The ΔhASCT2 mRNA provides an extreme example of this process because it lacks any in-frame AUG initiation site and appears to be entirely initiated at a series of non-AUG codons. Although each of these non-AUG codons appears to be used inefficiently, together they result in production of a substantial quantity of heterogeneous ΔhASCT2 proteins (*e.g.* see Fig. 7) that are processed to cell surfaces (Fig. 8) where they function to different extents in amino acid transport (*e.g.* see Figs. 6) and in retroviral reception (Fig. 4). These results could not be explained by alternative models for protein synthesis initiation that involve differential mRNA splicing or internal ribosome entry site mechanisms (10).

The structure of the available ΔhASCT2 cDNAs suggests that the mRNA might have been transcribed from an alternative TATA-less promotor in the hASCT2 gene. This hypothesis is consistent with the fact that the upstream sequences present in the full-length hASCT2 mRNA are highly GC-rich (2, 3, 7), and that a minor ASCT2 mRNA component of ~2.1 kb has been previously observed in Northern blot analyses of RNAs from some human tissues, with highest amounts apparent in pancreas and in the HT-2 and Caco-2 human intestinal cell lines (3, 7). The deletions in the 3′-untranslated region of the ΔhASCT2 mRNA also suggest that it was spliced differently than the full-length mRNA (see Fig. 1), supporting the conclusion that it represents a natural mRNA component. As mentioned above, the abundance of these in-frame CUG and GUG codons in optimal Kozak consensus contexts in this specific region of the hASCT2 mRNA is also inconsistent with a random model and strongly supports the idea that they perform an important

function in protein synthesis. In this context it is relevant that 5′ truncated mRNAs have been previously described for other members of the glutamate transporter family (52). Further studies of hASCT2 mRNAs are in progress to evaluate these issues.

Surprisingly, our results also imply that translation of the full-length hASCT2 mRNA results in synthesis of a small proportion of NH_2-terminal truncated isoforms. Because the full-length mRNA contains a 5′ situated AUG initiation codon at position 1 in an excellent Kozak consensus context (see Fig. 3), the scanning model would predict efficient initiation at this site, with minimal initiation at the downstream CUG and GUG codons. Although the results in Figs. 7 and 8 partially support this expectation, careful inspection of this data implied that ~10% of the intracellular and cell surface protein encoded by this mRNA may have been translationally initiated at these downstream non-AUG codons. Thus, in Western blots, smaller forms of this protein were detected, and these co-electrophoresed precisely with the proteins encoded by the ΔhASCT2 mRNA (see Fig. 7). Since all of these proteins contain the Myc-tag at their COOH termini and were fully deglycosylated, this suggests that they differ at their NH_2-terminal ends. Moreover, translation of the full-length hASCT2 mRNA in a reticulocyte cell-free system also reproducibly yielded both full-length and truncated protein products (*e.g.* see Fig. 5) and expression of this mRNA in *Xenopus* oocytes also has yielded several NH_2-terminal truncated isoforms (data not shown). These results imply that a significant degree of leaky scanning can occur in the translation of mRNAs that have 5′ situated AUG codons in suitable Kozak consensus contexts. Leaky scanning has also been reported to be dramatically increased in rapidly growing cells and to be sensitive to environmental conditions (10, 32). Presumably, such truncated isoforms could have physiologically important functions. For example, they might have different lifetimes or subcellular locations or regulatory properties, either alone or in hetero-oligomeric complexes with the full-length proteins.

We conclude that the cytosolic NH_2-terminal region of full-length hASCT2 at least to position 23 is not essential for amino acid transport or viral receptor functions, and that a truncated isoform lacking 79 amino acids including TM1 (*i.e.* mutant LA1Δ67(AUG)) is active in viral reception but not in transport (see Figs. 4, and 6). These conclusions are compatible with other evidence that the middle and carboxyl-terminal regions are most important for the amino acid selectivity of ASCT1 and ASCT2 and for the Na^+-dependent transporter functions of the glutamate transporters (9, 53). Previous evidence has also indicated that other members of this transporter family form oligomers (54) and have NH_2-terminal truncated isoforms (52). The evidence in Figs. 7 and 8 implies that the truncated ΔhASCT2 isoforms might also form hetero-oligomers with each other and possibly with the full-length ASCT2 protein.

It is conceivable that the unusual diversity of hASCT2 amino termini might be related to the fact that this protein has played a critical role in retroviral infections and in host-retroviral co-evolution (2–4). Furthermore, infections by retroviruses generally cause severe down-modulation of receptor expression on cell surfaces, with potential pathogenic consequences (2). Indeed, it has been proposed that expression of the envelope glycoprotein of the human endogenous retrovirus HERV-W in placenta may be responsible for hASCT2-dependent cell-cell fusion to form syncytiatrophoblasts (1, 55). From these perspectives, it will be important to learn how infections by viruses that use hASCT2 may alter expression or processing of these hASCT2 isoforms and conversely how expression of these isoforms may modulate infections and pathogenesis. This is especially intriguing because changes in cellular growth rates and in physiological conditions can dramatically alter the efficiency of leaky scanning (10, 32).

Acknowledgments—We thank Alex Stein for generously performing the *Xenopus* oocyte analyses. We are also grateful to Navid Madani and Susan Kozak for helpful advice.

REFERENCES

1. Blond, J. L., Lavillette, D., Cheynet, V., Bouton, O., Oriol, G., Chapel-Fernandes, S., Mandrand, B., Mallet, F., and Cosset, F. L. (2000) *J. Virol.* **74,** 3321–3329
2. Rasko, J. E., Battini, J. L., Gottschalk, R. J., Mazo, I., and Miller, A. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 2129–2134
3. Tailor, C. S., Nouri, A., Zhao, Y., Takeuchi, Y., and Kabat, D. (1999) *J. Virol.* **73,** 4470–4474
4. Marin, M., Tailor, C. S., Nouri, A., and Kabat, D. (2000) *J. Virol.* **74,** 8085–8093
5. Arriza, J. L., Kavanaugh, M. P., Fairman, W. A., Wu, Y. N., Murdoch, G. H., North, R. A., and Amara, S. G. (1993) *J. Biol. Chem.* **268,** 15329–15332
6. Broer, A., Wagner, C., Lang, F., and Broer, S. (2000) *Biochem. J.* **346,** 705–710
7. Kekuda, R., Prasad, P. D., Fei, Y. J., Torres-Zamorano, V., Sinha, S., Yang-Feng, T. L., Leibach, F. H., and Ganapathy, V. (1996) *J. Biol. Chem.* **271,** 18657–18661
8. Utsunomiya-Tate, N., Endou, H., and Kanai, Y. (1996) *J. Biol. Chem.* **271,** 14883–14890
9. Slotboom, D. J., Konings, W. N., and Lolkema, J. S. (1999) *Microbiol. Mol. Biol. Rev.* **63,** 293–307
10. Kozak, M. (1999) *Gene* (*Amst.*) **234,** 187–208
11. Kozak, M. (1986) *Cell* **44,** 283–292
12. Kozak, M. (1987) *Nucleic Acids Res.* **15,** 8125–8148
13. Kozak, M. (1987) *J. Mol. Biol.* **196,** 947–950
14. Kozak, M. (1989) *Mol. Cell. Biol.* **9,** 5073–5080
15. Kozak, M. (1991) *J. Biol. Chem.* **266,** 19867–19870
16. Kozak, M. (1997) *EMBO J.* **16,** 2482–2492
17. Kozak, M. (1991) *J. Cell Biol.* **115,** 887–903
18. Acland, P., Dixon, M., Peters, G., and Dickson, C. (1990) *Nature* **343,** 662–665
19. Arnaud, E., Touriol, C., Boutonnet, C., Gensac, M. C., Vagner, S., Prats, H., and Prats, A. C. (1999) *Mol. Cell. Biol.* **19,** 505–514
20. Bugler, B., Amalric, F., and Prats, H. (1991) *Mol. Cell. Biol.* **11,** 573–577
21. Dickson, C., and Acland, P. (1990) *Enzyme* **44,** 225–234
22. Falvey, E., Fleury-Olela, F., and Schibler, U. (1995) *EMBO J.* **14,** 4307–4317
23. Imataka, H., Olsen, H. S., and Sonenberg, N. (1997) *EMBO J.* **16,** 817–825
24. Lock, P., Ralph, S., Stanley, E., Boulet, I., Ramsay, R., and Dunn, A. R. (1991) *Mol. Cell. Biol.* **11,** 4363–4370
25. Packham, G., Brimmell, M., and Cleveland, J. L. (1997) *Biochem. J.* **328,** 807–813
26. Peabody, D. S. (1989) *J. Biol. Chem.* **264,** 5031–5035
27. Taira, M., Iizasa, T., Shimada, H., Kudoh, J., Shimizu, N., and Tatibana, M. (1990) *J. Biol. Chem.* **265,** 16491–16497
28. RajBhandary, U. L. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 1325–1327
29. Prats, A. C., De Billy, G., Wang, P., and Darlix, J. L. (1989) *J. Mol. Biol.* **205,** 363–372
30. Pillemer, E. A., Kooistra, D. A., Witte, O. N., and Weissman, I. L. (1986) *J. Virol.* **57,** 413–421
31. Hann, S. R., King, M. W., Bentley, D. L., Anderson, C. W., and Eisenman, R. N. (1988) *Cell* **52,** 185–195
32. Spotts, G. D., Patel, S. V., Xiao, Q., and Hann, S. R. (1997) *Mol. Cell. Biol.* **17,** 1459–1468
33. Liu, Y., Garceau, N. Y., Loros, J. J., and Dunlap, J. C. (1997) *Cell* **89,** 477–486
34. Baklouti, F., Huang, S. C., Tang, T. K., Delaunay, J., Marchesi, V. T., and Benz, E. J., Jr. (1996) *Blood* **87,** 3934–3941
35. Bejanin, S., Cervini, R., Mallet, J., and Berrard, S. (1994) *J. Biol. Chem.* **269,** 21944–21947
36. Cunillera, N., Boronat, A., and Ferrer, A. (1997) *J. Biol. Chem.* **272,** 15381–15388
37. Gammie, A. E., Stewart, B. G., Scott, C. F., and Rose, M. D. (1999) *Mol. Cell. Biol.* **19,** 817–825
38. George, C. X., and Samuel, C. E. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 4621–4626
39. Lee, M. H., Reynisdottir, I., and Massague, J. (1995) *Genes Dev.* **9,** 639–649
40. Liu, L., Shao, G., Steele-Perkins, G., and Huang, S. (1997) *J. Biol. Chem.* **272,** 2984–2991
41. Philippe, J. (1995) *Mol. Endocrinol.* **9,** 368–374
42. Sun, Z., Sassone-Corsi, P., and Means, A. R. (1995) *Mol. Cell. Biol.* **15,** 561–571
43. Wimmer, B., Lottspeich, F., van der Klei, I., Veenhuis, M., and Gietl, C. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 13624–13629
44. Cosset, F. L., Takeuchi, Y., Battini, J. L., Weiss, R. A., and Collins, M. K. L. (1995) *J. Virol.* **69,** 7430–7436
45. Takeuchi, Y., Cosset, F. L., Lachmann, P. J., Okada, H., Weiss, R. A., and Collins, M. K. L. (1994) *J. Virol.* **68,** 8001–8007
46. Tailor, C. S., Nouri, A., Lee, C. G., Kozak, C., and Kabat, K. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 927–932
47. Tailor, C. S., and Kabat, D. (1997) *J. Virol.* **71,** 9383–9391
48. Wang, H., Kavanaugh, M. P., North, R. A., and Kabat, D. (1991) *Nature* **352,** 729–731

49. Battini, J. L., Rasko, J. E., and Miller, A. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 1385–1390
50. Yang, Y. L., Guo, L., Xu, S., Holland, C. A., Kitamura, T., Hunter, K., and Cunningham, J. M. (1999) *Nat. Genet.* **21,** 216–219
51. Zerangue, N., and Kavanaugh, M. P. (1996) *J. Biol. Chem.* **271,** 27991–27994
52. Matsumoto, Y., Enomoto, T., and Masuko, T. (1999) *Tohoku J. Exp. Med.* **187,** 173–182
53. Li, J., Fei, J., Huang, F., Guo, L. H., and Schwarz, W. (2000) *Biochim. Biophys. Acta* **1467,** 338–346
54. Eskandari, S., Kreman, M., Kavanaugh, M. P., Wright, E. M., and Zampighi, G. A. (2000) *Proc. Natl. Acad. Sci. U. S. A.* **97,** 8641–8646
55. Mi, S., Lee, X., Li, X., Veldman, G. M., Finnerty, H., Racie, L., LaVallie, E., Tang, X. Y., Edouard, P., Howes, S., Keith, J. C., Jr., and McCoy, J. M. (2000) *Nature* **403,** 785–789

Truncated Forms of the Dual Function Human ASCT2 Neutral Amino Acid Transporter/Retroviral Receptor Are Translationally Initiated at Multiple Alternative CUG and GUG Codons

Chetankumar S. Tailor, Mariana Marin, Ali Nouri, Michael P. Kavanaugh and David Kabat

J. Biol. Chem. 2001, 276:27221-27230.
doi: 10.1074/jbc.M100737200 originally published online May 11, 2001