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Original Research Communication

Molecular Cloning and Expression of Human xCT, the Light Chain of Amino Acid Transport System x_c^-

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ABSTRACT

Transport of system x_c^- is an exchange agency with high specificity for anionic form of cystine and glutamate. The protein mediating this transport is a disulfide-linked heterodimer of a light chain named xCT and a heavy chain previously known as 4F2hc. We have isolated two cDNAs encoding xCT from the human cDNA library. One clone coded for a protein of 501 amino acids with 12 putative transmembrane domains. When functionally expressed in *Xenopus* oocytes together with the human 4F2hc, human xCT induced the transport activity whose characteristics are similar to those of system x_c^-. Another clone seemed to contain a partial human xCT and a long 3′ untranslated region. The human xCT gene was localized at chromosome 4q28–31. Analysis of the 5′-flanking region of the human xCT gene revealed several sites for potentially binding of transcriptional factors, including NF-E2 and AP-1. Transport of cystine via system x_c^- has been known as a regulatory factor for the intracellular glutathione level, and its transport activity is induced in response to the oxygen tension in culture. Northern blot analysis demonstrated that the expression of both xCT and 4F2hc was significantly enhanced by oxygen. The results suggest that oxygen regulates the activity of system x_c^- by modulating the expression of both xCT and 4F2hc mRNAs. Antiox. Redox Signal. 2, 665–671.

INTRODUCTION

PLASMA MEMBRANE TRANSPORT of amino acids is mediated by several transport systems (Christensen, 1990), and recent studies have shown that some transport systems require two components for expressing their activities (Mastroberardino *et al.*, 1998; Torrents *et al.*, 1998; Pineda *et al.*, 1999; Segawa *et al.*, 1999). The heavy chain of 4F2 cell-surface antigen (4F2hc) has been shown to be the common component of these transporters. We have described in cultured mammalian cells a Na^+-independent anionic amino acid transport system highly specific for cystine and glutamate (Bannai, 1986). Cystine taken up via this system, designated system x_c^-, is rapidly reduced to cysteine, which is used for the synthesis of proteins and glutathione (Bannai and Tateishi, 1986). Because cysteine is a rate-limiting precursor for glutathione synthesis, the intracellular glutathione is regulated by the system x_c^- activity. The activity of system x_c^- is induced by electrophilic agents and oxidative stress (Bannai, 1984; Bannai *et al.*, 1991). In mouse peritoneal macrophages, bacterial lipopolysaccharide drastically induces this activity (Sato *et al.*, 1995). In human fibroblast IMR-90 cells,

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which are derived from a fetal lung, the activity is significantly induced by oxygen (Bannai *et al.*, 1989). Recently, we have isolated cDNA encoding this transporter from the mouse cDNA library and demonstrated that the expression of system x_c^- requires two components, 4F2hc and a novel protein designated xCT (Sato *et al.*, 1999). 4F2hc is expressed ubiquitously whereas the expression of xCT is detected in brain, but not heart, lung, liver, and kidney (Sato *et al.*, 1999). In the present study, we have constructed the cDNA library from the human fibroblast cell line $WI26Va_4$, and cloning and expression of the cDNA for human xCT in the combination of 4F2hc have been performed. We have also investigated the effect of oxygen on the expression of xCT and 4F2hc mRNAs.

MATERIALS AND METHODS

Cell culture

SV-40-transformed human fibroblast cell line ($WI26Va_4$) and normal human fibroblasts (IMR-90) were cultured routinely in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37°C in 5% CO_2 and 95% air. To investigate the effect of oxygen, cells were cultured in a gas-tight chamber flushed with 5% CO_2 and 95% O_2 and with 5% CO_2 and 95% N_2, and the concentration of O_2 was adjusted to 80% and 2%, respectively, monitoring by the oxygen meter.

cDNA cloning

$WI26Va_4$ cells were cultured for 8 hr with 100 μM diethyl maleate and mRNA was isolated. A unidirected cDNA library was constructed using λZAPII cloning system (Stratagene) and the cDNA library was screened using mouse xCT cDNA as a probe. Two plasmid clones, obtained by *in vivo* excision of the hybridizing λZAPII clones, were sequenced on both strands by dye terminator cycle sequencing method by PE Applied Biosystem.

Functional characterization by Xenopus *oocytes expression*

Oocytes were injected with 5 ng each of the cloned cRNA. Two days after injection, the rate of uptake of cystine by five oocytes was measured at 23°C for 10 min in 125 μl of uptake medium. The uptake medium contained [^{14}C] cystine (1 μCi/ml and at 20 μM) in modified Barth's saline (10 mM HEPES, pH 7.5, 88 mM NaCl, 1 mM KCl, 0.3 mM $Ca(NO_3)_2$, 0.4 mM $CaCl_2$, 0.8 mM $MgSO_4$, 50 units/ml penicillin, and 50 μg/ml streptomycin). The uptake was terminated by rapidly rinsing the oocytes three times with ice-cold modified Barth's saline, and then the radioactivity in oocytes was counted.

Northern blot analysis

The cDNA probes for human xCT, human 4F2hc, and mouse β-actin were labeled using [α-^{32}P]dCTP and Rediprime™ II random prime labeling system (Amersham Pharmacia Biotech). RNA was electrophoresed on a 1% agarose gel

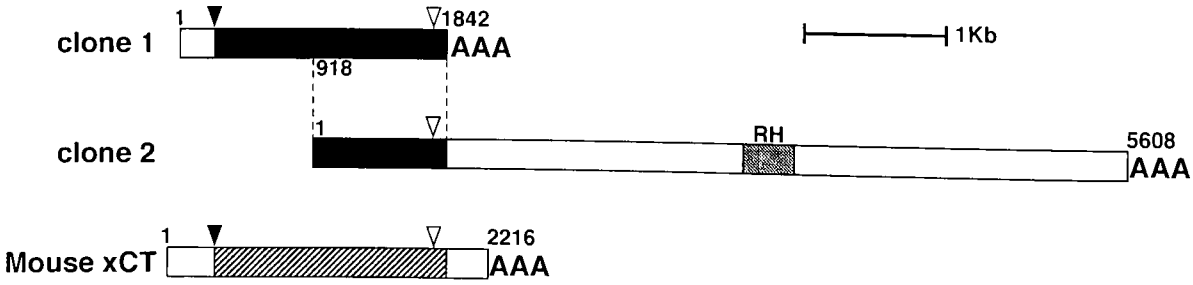

FIG. 1. Structure of human cDNA clones for xCT. Clone 1 contains a single open reading frame for human xCT. The region from 918 to 1,842 of clone 1 is identical to that from 1 to 925 of clone 2. The translation initiation codon and the stop codon are shown as black arrows and white arrows, respectively. The regions of the filled bar in human clones and the striped bar in mouse clone are highly conserved. RH represents the region of the PCR product for the radiation hybrid mapping. AAA represents poly(A)$^+$

in the presence of 2.2 *M* formaldehyde, transferred to a Hybond-N^+ membrane (Amersham Pharmacia Biotech), and hybridized in a solution containing 50% formamide for 16 hr at 42°C. The membranes were washed twice for 15 min at room temperature with 1 × SSC, 0.1% sodium dodecyl sulfate (SDS) and then washed twice for 15 min at 68°C with 0.1 × SSC, 0.1% SDS.

Chromosome mapping

Chromosome mapping was performed by Research Genetics, Inc. using the Genebridge 4 Radiation Hybrid Mapping panel. PCR screening was done using primers 5′-CTCTCCCTATGATATGTTGCTGGAGG-3′ and 5′-CGTGAAATGGCATTACAATGGCAGGG-3′, which are located at the 3′-untranslated region of the cDNA, clone 2.

Rapid amplification of cDNA ends (5′ RACE)

Total RNA was isolated from $WI26Va_4$ cells, and 5′ RACE was performed using the 5′ RACE system for rapid amplification of cDNA ends, version 2.0 (Life Technologies), following the manufacturer's protocol.

RESULTS

A cDNA library was constructed from $WI26Va_4$ cells that had been treated with diethyl maleate for 8 hr to augment the activity of system x_c^-. This library was screened using mouse xCT cDNA as a probe, and two clones were isolated. The insert of one clone (clone 1) was composed of 1,861 bp and contained a single open reading frame that encoded a putative protein. We decided the transcription initiation site by 5′ RACE and found that the predicted transcription initiation site existed at 43 bp 5′ upstream of clone 1. Another clone (clone 2) was composed of 5,626 bp and contained a single open reading frame. As shown in Fig. 1, the 5′-terminal sequence of this clone was identical with the sequence of 918 to 1,842 of clone 1, indicating that the 5′ region of clone 2 was partially deleted. However, clone 2 contained a long 3′-untranslated region. We have performed Northern blot analysis of total RNA isolated from human fibroblasts using clone 1 or the 3′-untranslated region of clone 2 as probes. Those probes recognized the same transcript, which was approximately 12 kb (data not shown). Clone 1 cDNA encoded a putative protein of 501 amino acids (Fig. 2). Comparison of the sequence of this protein against protein data bases revealed that this protein has 89% homology to mouse xCT and probably a human homologue, *i.e.*, human xCT. Analysis of the amino acid sequence according to the algorithm of Kyte and Doolittle (1982) predicts

A

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hxCT  MVRKPVVSTISKGGYLQGNVNGRLPSLGNKEPPGQEKVQLKRKVTLLRGVSIIIGTIIGA  60
mxCT  MVRKPVVATISKGGYLQGNMSGRLPSMGDQEPPGQEKVVLKKKITLLRGVSIIIGTVIGS  60

hxCT  GIFISPKGVLQNTGSVGMSLTIWTVCGVLSLFGALSYAELGTTIKKSGGHYTYILEVFGP 120
mxCT  GIFISPKGILQNTGSVGMSLVFWSACGVLSLFGALSYAELGTSIKKSGGHYTYILEVFGP 120

hxCT  LPAFVRVWVELLIIRPAATAVISLAFGRYILEPFFIQCEIPELAIKLITAVGITVVMVLN 180
mxCT  LLAFVRVWVELLVIRPGATAVISLAFGRYILEPFFIQCEIPELAIKLVTAVGITVVMVLN 180

hxCT  SMSVSWSARIQIFLTFCKLTAILIIIVPGVMQLIKGQTQNFKDAFSGRDSSITRLPLAFY 240
mxCT  STSVSWSARIQIFLTFCKLTAILIIIVPGVIQLIKGQTHHFKDAFSGRDTSLMGLPLAFY 240

hxCT  YGMYAYAGWFYLNFVTEEVENPEKTIPLAICISMAIVTIGYVLTNVAYFTTINAEELLLS 300
mxCT  YGMYAYAGWFYLNFITEEVDNPEKTIPLAICISMAIITVGYVLTNVAYFTTISAEELLQS 300

hxCT  NAVAVTFSERLLGNFSLAVPIFVALSCFGSMNGGVFAVSRLFYVASREGHLPEILSMIHV 360
mxCT  SAVAVTFSERLLGKFSLAVPIFVALSCFGSMNGGVFAVSRLFYVASREGHLPEILSMIHV 360

hxCT  RKHTPLPAVIVLHPLTMIMLFSGDLDSLLNFLSFARWLFIGLAVAGLIYLRYKCPDMHRP 420
mxCT  HKHTPLPAVIVLHPLTMVMLFSGDLYSLLNFLSFARWLFMGLAVAGLIYLRYKRPDMHRP 420

hxCT  FKVPLFIPALFSFTCLFMVALSLYSDPFSTGIGFVITLTGVPAYYLFIIWDKKPRWFRIM 480
mxCT  FKVPLFIPALFSFTCLFMVVLSLYSDPFSTGVGFLITLTGVPAYYLFIVWDKKPKWFRRL 480

hxCT  SEKITRTLQIILEVVPEEDKL 501
mxCT  SDRITRTLQIILEVVPEDSKEL 502
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B

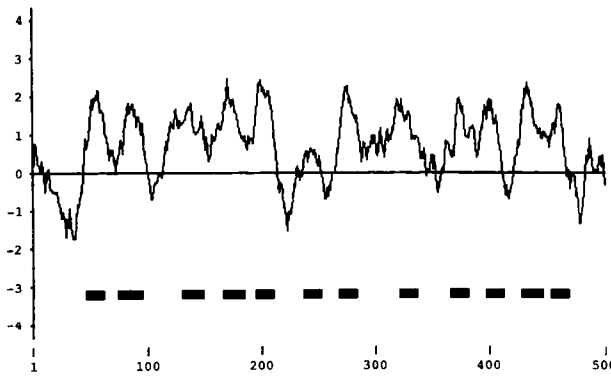

FIG. 2. (A) Sequence alignment of xCT. The deduced amino acid sequences of human xCT (hxCT) and mouse xCT (mxCT) were aligned. Identical and homologous amino acids are indicated by an asterisk and a dot, respectively. (**B**) Hydropathy plot of human xCT. Kyte-Doolittle hydropathy analysis was done using a window of 17 amino acids. The abscissa indicates the amino acid number. The solid bars indicate the putative transmembrane domains.

an extremely hydrophobic protein that may contain as many as 12 membrane-spanning domains.

When the cRNA from clone 1 was injected into *Xenopus* oocytes in combination with the cRNA of human 4F2hc, the uptake of cystine by the oocytes significantly increased (Fig. 3A). The cystine uptake did not increase with the injection of the cRNA of clone 1 or 4F2hc alone. The increased cystine uptake was inhibited by glutamate, but not by aspartate, arginine, serine, or leucine (Fig. 3B). The results confirm that

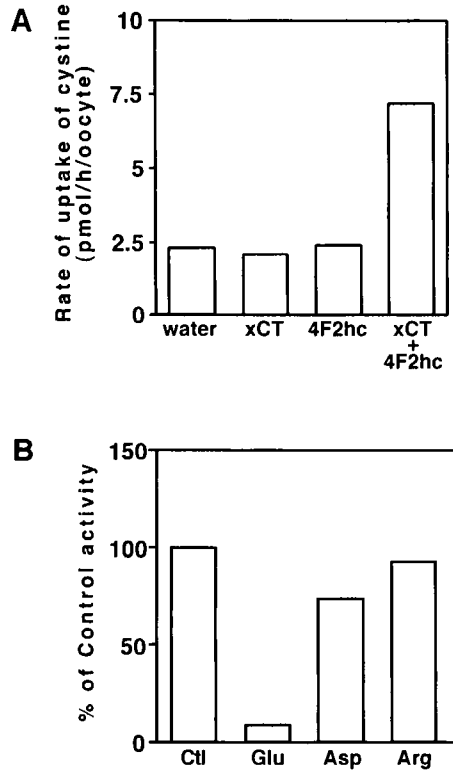

FIG. 3. (A) The rate of uptake of L-cystine in *Xenopus* oocytes. Oocytes were injected with water, 5 ng each of xCT cRNA (labeled as xCT), 4F2hc cRNA (labeled as 4F2hc), or both (labeled as xCT + 4F2hc), and 2 days after injection the rates of uptake of 20 μM L-[^{14}C]cystine were measured. Data represent the means of duplicate determinations from one experiment typical of three. **(B)** Comparison of the inhibitory potential of various amino acids on the uptake of L-cystine. Oocytes were injected with 5 ng each of xCT cRNA and 4F2hc cRNA, and 2 days after injection the rates of uptake of 20 μM L-[^{14}C]cystine were measured in the absence (Ctl) or presence of the various L-amino acids (2 mM) indicated. Each data point was calculated by subtracting the uptake value obtained in water-injected oocytes from those in cRNA-injected oocytes, and the uptake value in the absence of inhibitors is designated as 100%. Data represent the means of duplicate determinations from one experiment typical of three.

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-1120  tcactgaatccaaaaccacatgtaactatagccaaaaatgatatctaggatttcttttct
-1060  tttcatatttgtttttttcttgacacttggttgagaaagttagaagggtttaaaaatgttt
-1000  tgccttttttgactattgcaaagtaaatatttttaatttagcttacatttttaatgcatttt
 -940  aaatttataataataacaatagtttatataatgtggaaaaaagtcatacataatatcatt
 -880  actttctataactaccattctgttttcctttacaggcttttgctatatacatatttttat
 -820  gtaattgtaattgtgtaattttgaacttagaaatttttttttggtaattcaaaggaacaac
 -760  atgacaatgaaggatatgtatctttacatagttcaaaaaaatcacctagtgctaatgag
 -700  aatcagaaaatgactattttctggagtcatggtgaattttgtattagtcacactcggatg
 -640  gtgatttaaaatactggtttattatgagtagtaagaaataattttatctttttaatgttga
 -580  ggaaggcttatagttgtgtgtatgtgacagaagtataaagtgtagaatctttaattttgt
 -520  aaatattggatttgactgtattgccttataaaaaactcaaaataaaaaaatataaaagcat
 -460  tacacaaattatgaaattcaaactgtgttgttataaacaatgtagctttaggatacattc
 -400  tactcacaaaacagtcgcatgtaaaatgtttttatacacgcattaaagaaaaagaaactc
 -340  cttaaaatgaagtaactatttcctgtttcattttgtttgaacagcttttgttgctcacta
 -280  cgagttgctttaaatctctgggaaggtctgttccgaatttactacttctggattggctaa
 -220  aatctctttaaagtgtgtgctttgttctctaaaaagcttaggtcagttgagcaacaagct
 -160  cctcctgttttttttttttttaaaaaaaagaggctgagtaatgctggaggcttctcatgt
 -100  ggctgatgcaaacctggagaatttgcatcatcatttagctgtagtaagttggtgtgacag
  -40  gcaggcgcttgaatgcaagcccatgaggaagctgagctggTTTGTAATGATAGGGCGGCA
  +21  GCAGCAGCAGCAGCAGTGGTGGAACGAGGAGGTGGAGAATTGAGAGCACGATGCATA
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FIG. 4. Sequence of 5′-flanking region of human xCT gene. The predicted transcription initiation site is indicated by +1. The potential AP-1 binding sites are shown by underline and the potential NF-E2 binding site is boxed. The TATA-like box is shown by double underline.

the increased uptake is mediated by system x_c^- and that the protein encoded by clone 1 cDNA is human xCT.

We have performed database searches (BLAST programs, NCBI) using the sequence of clone 1 and found that three bacterial artificial chromosome (BAC) clones contained at least a part of clone 1 sequence. Two of these BAC clones were obtained and their sequences were analyzed. One BAC clone (CIT-HSP-2283E8.TF) contained the first exon and the 5′-flanking region of human xCT. Another BAC clone (RPCI11-60B17.TJ) contained the downstream of 761 of clone 1. A search of the 5′-flanking region, using MatInspector V2.2 based on TRANSFAC 4.0 (Quandt *et al.*, 1995), revealed the presence of one potential binding site of NF-E2 and 12 sequences that are homologous to the consensus AP-1-binding sequence (Fig. 4).

The human xCT gene was chromosome mapped by using a radiation hybrid panel (Genebridge 4 panel) with primers corresponding to the 3′-untranslated region of clone 2 (Fig. 1). The PCR reaction with these primers generated a single product of 355 nucleotides using the BAC clone (RPCI11-60B17.TJ) or the

genome DNA derived from human placenta as the templates. Chromosome mapping data demonstrated that the xCT gene was linked to a distance of 0.1 cR (27 kb) from the marker WI-5179 located at chromosome 4q28–31.

We previously demonstrated that oxygen induced the activity of system x_c^- in human fibroblast, IMR-90 cells (Bannai *et al.*, 1989). To investigate the effect of oxygen on the expression of xCT and 4F2hc mRNAs, the cells were cultured at 2, 20, or 80% O_2 for 3 days, and total RNA was isolated. Northern blot analysis of these RNAs showed that the expression of xCT was significantly increased in an O_2 concentration-dependent manner (Fig. 5). It is noteworthy that the expression of 4F2hc was also induced by oxygen. IMR-90 cells, which had been cultured at 2% O_2 for 3 days, were transferred to the atmospheric oxygen and after 8 hr total RNA was isolated. Northern blot analysis of these RNAs revealed that both xCT and 4F2hc mRNAs were significantly induced by the change of oxygen tension (Fig. 6).

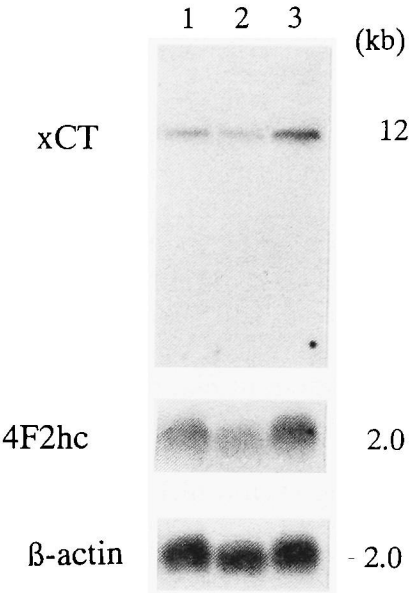

FIG. 6. Induction of xCT mRNA by oxygen. IMR-90 cells were cultured for 3 days at 2% O_2 and total RNA was isolated (lane 1). Then the cells were further cultured at 2% O_2 (lane 2) or 20% O_2 (lane 3) for 8 hr, and total RNA was isolated. Fifteen micrograms each of total RNA was loaded per lane. The hybridization was performed as described in Materials and Methods.

DISCUSSION

In the present study, we isolated two cDNA clones for human xCT. Clone 1 contained a complete reading frame encoding a protein of 501 amino acids. Clone 2 encodes a part of human xCT and has a long 3′-untranslated region. Northern blot analysis of total RNA isolated from human fibroblasts revealed that the major band that hybridized with the probe of human xCT cDNA was approximately 12 kb (Figs. 5 and 6). In Northern blot analysis of total RNA isolated from the mouse peritoneal macrophages, the band of similar size is detected with the probe of mouse xCT cDNA, although some bands of smaller sizes were also detected by the probe (Sato *et al.*, 1999). These results suggest that mRNAs for xCT have 3′-untranslated regions of different lengths, which probably represents alternative splicing, alternative polyadenylation sites, or a combination of both. It is not clear whether the major band mRNA (12 kb) functions as a mature mRNA for translation of xCT in the cell. Recently, mRNA for the cationic amino acid transporter-1, which has a long 3′-untranslated region, is post-transcriptionally modulated, and the 3′-untranslated region plays an important role to stabilize the mRNA (Aulak *et al.*, 1999). It is likely that 3′-untranslated region of xCT mRNA is in-

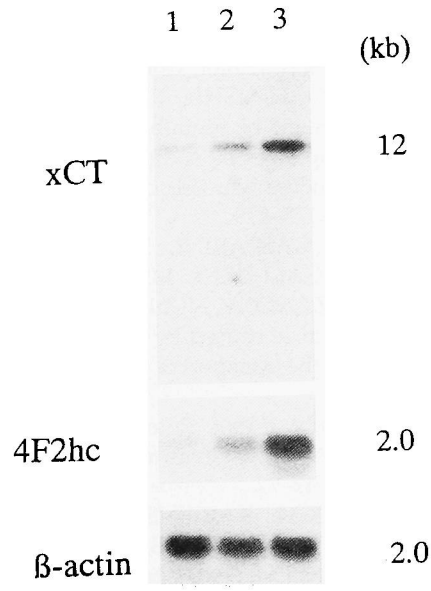

FIG. 5. Effect of oxygen on the expression of xCT mRNA in human fibroblasts. Total RNA was isolated from human fibroblast IMR-90 cells cultured at 2% (lane 1), 20% (lane 2), or 80% (lane 3) O_2 for 3 days. Fifteen micrograms each of total RNA was loaded per lane. The hybridization was performed as described in Materials and Methods.

volved in regulating the expression of xCT post-transcriptionally.

The activity of system x_c^- is induced by electrophilic agents such as diethyl maleate in various mammalian cultured cells (Bannai, 1984; Bannai *et al.*, 1991). This induction is probably mediated by the transcription factor NF-E2 and the electrophile response element (Wasserman and Fahl, 1997), which exists in the 5′-flanking region of human xCT gene (Fig. 4). As shown in Fig. 5, the expression of xCT and 4F2hc mRNAs significantly depended on the O_2 concentration in culture. In the 5′-flanking region of human 4F2hc, there are several AP-1 binding sites (Gottesdiener *et al.*, 1988). Similar sites exist in the 5′-flanking region of the human xCT gene (Fig. 4). AP-1 is a heterodimer of the protein products of individual members of the Fos and Jun immediate-early response gene families, or a homodimer of Jun proteins. AP-1 is activated by various stimuli, including superoxide anion and H_2O_2. This transcription factor may be involved in the regulation of the expression of xCT and 4F2hc by oxygen through these potential AP-1 binding sites.

The gene for human xCT was assigned by linkage analysis to 4q28–31. There are some disorders that are linked in the similar region. However, the link between those disorders and xCT is unknown. Recently, it has been shown that the mutations in the genes for the components of amino acid transporters, rBAT (also named NBAT, D2, or NAA-Tr) and y^+LAT-1, cause disorders, cystinuria (Calonge *et al.*, 1994), and lysinuric protein intolerance (Torrents *et al.*, 1999), respectively. Isolation of human xCT cDNA will enable us to investigate further the genetic disorders and the mutation of xCT gene.

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The nucleotide sequences reported in this paper have been submitted to the GenBank™/EBI Data Bank with accession number AB026891 and AB042201.

ABBREVIATIONS

BAC, bacterial artificial chromosome; DMEM, Dulbecco's modified Eagle medium; 4F2hc, 4F2 heavy chain; FBS, fetal bovine serum; kb, kilobase(s); 5′ RACE, rapid amplification of cDNA ends; SDS, sodium dodecyl sulfate.

REFERENCES

AULAK, K.S., MISHRA, R., ZHOU, L., HYATT, S.L., JONGE, W.D., LAMERS, W., SNIDER, M., and HATZOGLOU, M. (1999). Post-transcriptional regulation of the arginine transporter Cat-1 by amino acid availability. J. Biol. Chem. **274,** 30424–30432.

BANNAI, S. (1984). Induction of cystine and glutamate transport activity in human fibroblasts by diethyl maleate and other electrophilic agents. J. Biol. Chem. **259,** 2435–2440.

BANNAI, S. (1986). Exchange of cystine and glutamate across plasma membrane of human fibroblasts. J. Biol. Chem. **261,** 2256–2263.

BANNAI, S., and TATEISHI, N. (1986). Role of membrane transport in metabolism and function of glutathione in mammals. J. Membrane Biol. **89,** 1–8.

BANNAI, S., SATO, H., ISHII, T., and SUGITA, Y. (1989). Induction of cystine transport activity in human fibroblasts by oxygen. J. Biol. Chem. **264,** 18481–18484.

BANNAI, S., SATO, H., ISHII, T., and TAKETANI, S. (1991). Enhancement of glutathione levels in mouse peritoneal macrophages by sodium arsenite, cadmium chloride, and glucose/glucose oxidase. Biochim. Biophys. Acta **1092,** 175–179.

CALONGE, M.J., GASPARINI, P., CHILLARON, J., CHILLON, M., GALLUCCI, M., BARCELO', P., ESTIVILL, X., ZORZANO, A., NUNES, V., and PALACÍN, M. (1994). Cystinuria caused by mutations in rBAT, a gene involved in the transport of cystine. Nature Genet. **6,** 420–426.

CHRISTENSEN, H.N. (1990). Role of amino acid transport and countertransport in nutrition and metabolism. Physiol. Rev. **70,** 43–77.

GOTTESDIENER, K.M., KARPINSKI, B.A., LINDSTEN, T., STROMINGER, J.L., JONES, N.H., THOMPSON, C.B., and LEIDEN, J.M. (1988). Isolation and structural characterization of the human 4F2 heavy-chain gene, an inducible gene involved in T-lymphocyte activation. Mol. Cell. Biol. **8,** 3809–3819.

KYTE, J., and DOOLITTLE, R.F. (1982). A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157,** 105–132.

MASTROBERARDINO, L., SPINDLER, B., PFEITTER, R., SKELLY, P.J., LOFFING, J., SHOEMAKER, C.B., and VERREY, F. (1998). Amino-acid transport by heterodimers of 4F2hc/CD98 and members of a permease family. Nature **395,** 288–291.

PINEDA, M., FERNÁNDEZ, E., TORRENTS, D., ESTÉVEZ, R., LÓPEZ, C., CAMPS, M., LLOBERAS, J., ZORZANO, A., and PALACÍN, M. (1999). Identification of a membrane protein, LAT-2, that co-expresses with 4F2 heavy chain, an L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. J. Biol. Chem. **274,** 19738–19744.

QUANDT, K., FRECH, K., KARAS, H., WINGENDER, E., and WERNER, T. (1995). MatInd and MatInspector-New fast and versatile tools for detection of consensus matches in nucleotide sequence data. Nucleic Acids Res. **23,** 4878–4884.

SATO, H., FUJIWARA, K., SAGARA, J., and BANNAI, S. (1995). Induction of cystine transport activity in mouse peritoneal macrophages by bacterial lipopolysaccharide. Biochem. J. **310,** 547–551.

SATO, H., TAMBA, M., ISHII, T., and BANNAI, S. (1999). Cloning and expression of a plasma membrane cystine/glutamate exchange transporter composed of two distinct proteins. J. Biol. Chem. **274,** 11455–11458.

SEGAWA, H., FUKASAWA, Y., MIYAMOTO, K., TAKEDA, E., ENDOU, H., and KANAI, Y. (1999). Identification and functional characterization of a Na^+-independent neutral amino acid transporter with broad substrate selectivity. J Biol. Chem. **278,** 19745–19751.

TORRENTS, D., ESTÉVEZ, R., PINEDA, M., FERNANDEX, E., LLOBERAS, J., SHI, Y.B., ZORZANO, A., and PALACÍN, M. (1998). Identification and characterization of a membrane protein (y^+L amino acid transporter-1) that associates with 4F2hc to encode the amino acid transport activity y^+L. J. Biol. Chem. **273,** 32437–32445.

TORRENTS, D., MYKKÄNEN, J., PINEDA, M., FELIUBADALÓ, L., ESTÉVEZ, R., CID, R.D., SANJURJO, P., ZORZANO, A., NUNES, V., HOUPONEN, K., REINIKAINEN, A., SIMELL, O., SAVONTAUS, M.L., AULA, P., and PALACÍN, M. (1999). Identification of SLC7A7, encoding y^+LAT-1, as the lysinuric protein intolerance gene. Nature Genet. **21,** 293–296.

WASSERMAN, W.W., and FAHL, W.E. (1997). Functional antioxidant responsive elements. Proc. Natl. Acad. Sci. USA. **94,** 5361–5366.

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1. Jan Lewerenz , Sandra J. Hewett , Ying Huang , Maria Lambros , Peter W. Gout , Peter W. Kalivas , Ann Massie , Ilse Smolders , Axel Methner , Mathias Pergande , Sylvia B. Smith , Vadivel Ganapathy , Pamela Maher . The Cystine/Glutamate Antiporter System xc- in Health and Disease: From Molecular Mechanisms to Novel Therapeutic Opportunities. *Antioxidants & Redox Signaling*, ahead of print. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links] [Supplemental material]
2. Cesar Valdovinos-Flores, María E. Gonsebatt. 2012. The role of amino acid transporters in GSH synthesis in the blood–brain barrier and central nervous system. *Neurochemistry International* **61**:3, 405-414. [CrossRef]
3. Xin He, Hongtao Li, Zhiyong Zhou, Zongsheng Zhao, Wei Li. 2012. Production of Brown/Yellow Patches in the SLC7A11 Transgenic Sheep via Testicular Injection of Transgene. *Journal of Genetics and Genomics* **39**:6, 281-285. [CrossRef]
4. Takeo Nakanishi, Ikumi Tamai. 2011. Solute carrier transporters as targets for drug delivery and pharmacological intervention for chemotherapy. *Journal of Pharmaceutical Sciences* **100**:9, 3731-3750. [CrossRef]
5. Myoung Shim, Jin Kim, Kwang Lee, Hee Jung, Bradley Carlson, Xue-Ming Xu, Dolph Hatfield, Byeong Lee. 2011. l(2)01810 is a novel type of glutamate transporter that is responsible for megamitochondrial formation. *Biochemical Journal* . [CrossRef]
6. Marcus Conrad, Hideyo Sato. 2011. The oxidative stress-inducible cystine/glutamate antiporter, system x c - : cystine supplier and beyond. *Amino Acids* . [CrossRef]
7. Jan Lewerenz, Pamela Maher, Axel Methner. 2011. Regulation of xCT expression and system $$ x_{\text{c}}^{ - } $$ function in neuronal cells. *Amino Acids* . [CrossRef]
8. Gethin J. McBean. 2011. The transsulfuration pathway: a source of cysteine for glutathione in astrocytes. *Amino Acids* . [CrossRef]
9. Anita Kinne, Ralf Schülein, Gerd Krause. 2011. Primary and secondary thyroid hormone transporters. *Thyroid Research* **4**:Suppl 1, S7. [CrossRef]
10. Yan Zeng, Yan Li, Ri#Sheng Chen, Xin He, Lei Yang, Wei Li. 2010. Overexpression of xCT induces up-regulation of 14-3-3# in Kaposi's sarcoma. *Bioscience Reports* **30**:4, 277-283. [CrossRef]
11. Brian Sims, Melinda Clarke, Wilfred Njah, E'lana Shuford Hopkins, Harald Sontheimer. 2010. Erythropoietin-induced neuroprotection requires cystine glutamate exchanger activity. *Brain Research* **1321**, 88-95. [CrossRef]
12. Naoto Shikano, Masato Ogura, Hiroyuki Okudaira, Syuichi Nakajima, Takashi Kotani, Masato Kobayashi, Shinya Nakazawa, Takeshi Baba, Naoto Yamaguchi, Nobuo Kubota. 2010. Uptake of 3-[125I]iodo-#-methyl-l-tyrosine into colon cancer DLD-1 cells: characterization and inhibitory effect of natural amino acids and amino acid-like drugs#. *Nuclear Medicine and Biology* **37**:2, 197-204. [CrossRef]
13. R-S Chen, Y-M Song, Z-Y Zhou, T Tong, Y Li, M Fu, X-L Guo, L-J Dong, X He, H-X Qiao. 2009. Disruption of xCT inhibits cancer cell metastasis via the caveolin-1/#-catenin pathway. *Oncogene* **28**:4, 599. [CrossRef]
14. Takayoshi Nishiya, Kazuhiko Mori, Chiharu Hattori, Kiyonori Kai, Hiroko Kataoka, Noriko Masubuchi, Toshimasa Jindo, Sunao Manabe. 2008. The crucial protective role of glutathione against tienilic acid hepatotoxicity in rats. *Toxicology and Applied Pharmacology* **232**:2, 280-291. [CrossRef]
15. Maisie Lo, Yu-Zhuo Wang, Peter W. Gout. 2008. The xc- cystine/glutamate antiporter: A potential target for therapy of cancer and other diseases. *Journal of Cellular Physiology* **215**:3, 593-602. [CrossRef]
16. Hirofumi Fujita, Masahiko Shiosaka, Tetsuya Ogino, Yuya Okimura, Toshihiko Utsumi, Eisuke F. Sato, Reiko Akagi, Masayasu Inoue, Kozo Utsumi, Junzo Sasaki. 2008. #-Lipoic acid suppresses 6-hydroxydopamine-induced ROS generation and apoptosis through the stimulation of glutathione synthesis but not by the expression of heme oxygenase-1. *Brain Research* **1206**, 1-12. [CrossRef]
17. Ying Huang, Wolfgang Sadée. 2006. Membrane transporters and channels in chemoresistance and -sensitivity of tumor cells. *Cancer Letters* **239**:2, 168-182. [CrossRef]
18. CHRISTY C BRIDGES, RUDOLFS K ZALUPS. 2005. Cystine and glutamate transport in renal epithelial cells transfected with human system x-c. *Kidney International* **68**:2, 653-664. [CrossRef]
19. Judy Aschner, Tore Syversen, Gouri Shanker, Michael AschnerAstroglia and Methylmercury Neurotoxicity 379-393. [CrossRef]

20. H SATO, S NOMURA, K MAEBARA, K SATO, M TAMBA, S BANNAI. 2004. Transcriptional control of cystine/glutamate transporter gene by amino acid deprivation. *Biochemical and Biophysical Research Communications* **325**:1, 109-116. [CrossRef]
21. HASSANE IZZEDINE, VINCENT LAUNAY-VACHER, ALAIN BAUMELOU, GILBERT DERAY. 2004. Antiretroviral and immunosuppressive drug-drug interactions: An update. *Kidney International* **66**:2, 532-541. [CrossRef]
22. Emilio Ruiz, Richard C.M Siow, Simon R Bartlett, Andrew M Jenner, Hideyo Sato, Shiro Bannai, Giovanni E Mann. 2003. Vitamin C inhibits diethylmaleate-induced L-cystine transport in human vascular smooth muscle cells. *Free Radical Biology and Medicine* **34**:1, 103-110. [CrossRef]
23. Morihiro Ito, Shin-Ichiro Takebayashi, Katsuzumi Okumura, Takeshi Ohkubo, Machiko Nishio, Mitsuo Kawano, Hiroshi Komada, Yasuhiko Ito, Masato Tsurudome. 2002. Up-regulated expression of a novel gene in activated human peripheral blood mononuclear cells that is a truncated paralog of the human system l-amino acid transporter 1. *The International Journal of Biochemistry & Cell Biology* **34**:9, 1152-1163. [CrossRef]
24. Gethin J McBean. 2002. Cerebral cystine uptake: a tale of two transporters. *Trends in Pharmacological Sciences* **23**:7, 299-302. [CrossRef]
25. Ju Young Kim, Yoshikatsu Kanai, Arthit Chairoungdua, Seok Ho Cha, Hirotaka Matsuo, Do Kyung Kim, Jun Inatomi, Hiroki Sawa, Yoshiteru Ida, Hitoshi Endou. 2001. Human cystine/glutamate transporter: cDNA cloning and upregulation by oxidative stress in glioma cells. *Biochimica et Biophysica Acta (BBA) - Biomembranes* **1512**:2, 335-344. [CrossRef]
26. M Palacín. 2001. The molecular bases of cystinuria and lysinuric protein intolerance. *Current Opinion in Genetics & Development* **11**:3, 328-335. [CrossRef]