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Transcriptional control of cystine/glutamate transporter gene by amino acid deprivation☆

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Abstract

Recent studies have demonstrated that depletion of amino acids results in the induction of several genes and that a genomic *cis*-element termed amino acid response element (AARE) is required for the induction. System x_c^- is an anionic amino acid transport system highly specific for cystine and glutamate, and its activity is known to be induced by cystine deprivation. This transporter is composed of two protein components, xCT and 4F2 heavy chain, and xCT is thought to mediate the transport activity. In the present study, the molecular mechanism for the induction of xCT by amino acid deprivation has been investigated. In mouse NIH3T3 cells, the activity of system x_c^- and xCT mRNA is induced not only by deprivation of cystine but also by deprivation of other amino acids. Two AAREs, each located in the opposite direction with an intervening sequence, were found in the 5′-flanking region of the mouse xCT gene. Promoter analysis revealed that both AAREs were necessary for the maximal induction of xCT mRNA in response to the amino acid deprivation. Glucose deprivation had no effect on the induction of the activity of system x_c^-. Electrophoretic mobility shift assay showed that ATF4, but not ATF2, is involved in the amino acid control of xCT expression. These results demonstrate that xCT is a new member of the proteins whose transcriptional control by the amino acid deprivation is mediated by AARE.

Keywords: Cystine; xCT; Amino acid response element; System x_c^-; Amino acid transporter; Amino acid deprivation

Regulation of gene expression by amino acids is an important mechanism in the adaptation of mammalian cells to their environment. Amino acid deprivation is known to activate the expression of several genes involved in amino acid biosynthesis and other cellular processes. The molecular mechanisms involved in the control of gene expression by amino acid deprivation have been obtained mainly by the studies on the expression of CCAAT/enhancer-binding protein homologous protein (CHOP) and asparagine synthetase (AS) genes (reviewed in [1]). The promoter analysis of these genes has revealed that the *cis*-element, designated amino acid response element (AARE) in CHOP gene [2] or nutrient-sensing response element-1 (NSRE-1) in AS gene [3], respectively, can regulate the induction of these genes in response to deprivation of several individual amino acids. These two elements share nucleotide sequences and functional similarities [4]. Several transcription factors such as activating transcription factor 2 (ATF2),

☆ *Abbreviations:* AARE, amino acid response element; AS, asparagine synthetase; CHOP, CCAAT/enhancer-binding protein homologous protein; NSRE-1, nutrient-sensing response element-1; SNAT2, sodium-coupled neutral amino acid transporter-2; ATF, activating transcription factor; Cat-1, cationic amino acid transporter-1; DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum.

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ATF3, ATF4, ATF7, C/EBPα, C/EBPβ, C/EBPγ, and C/EBPδ have been demonstrated to interact with these elements in vitro [5] and could regulate the expression of CHOP and AS genes.

Recently, it has been shown that AARE is involved in the expression of two amino acid transporters, cationic amino acid transporter, Cat-1 [6], and sodium-coupled neutral amino acid transporter, SNAT2 [7]. AARE of Cat-1 is localized within the first exon of the cat-1 gene, and that of SNAT2 is in the first intron of the gene. It has been shown that these AAREs transcriptionally regulate the expression of these genes in response to amino acid deprivation.

Transport system x_c^- is an exchange agency for anionic amino acids with high specificity for anionic form of cystine and glutamate [8]. This transporter is known to contribute to the maintenance of intracellular GSH levels in many types of mammalian cells in culture [9]. System x_c^- consists of two protein components, xCT and the heavy chain of 4F2 antigen (4F2hc) [10], and the transport activity is thought to be mediated by xCT. The activity of system x_c^- is induced by various stimuli, including electrophilic agents like diethyl maleate [11], oxygen [12], bacterial lipopolysaccharide [13], and cystine deprivation [11]. We have demonstrated that the induction of xCT mRNA by diethyl maleate is mediated by electrophile response element located in the 5′-flanking region of xCT gene and that the transcription factor Nrf2 binds to this element to activate the transcription of xCT gene [14]. However, little progress was made toward understanding the molecular mechanism underlying the induction of xCT by cystine deprivation.

In the 5′-flanking region of xCT gene, we have found sequences similar to AARE. This region contains two AARE-like sequences each located in the opposite direction with an intervening sequence. In the present study, we have investigated the function of this region in the transcriptional regulation of xCT mRNA by amino acid deprivation. Our data indicate that xCT falls into the category of the genes whose expression is mediated by AARE in response to amino acid deprivation.

Materials and methods

Cell culture and cystine uptake. Mouse NIH3T3 cells were cultured routinely in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum at 37 °C in 5% CO_2 and 95% air. Cells were plated at 2×10^5 cells/35-mm diameter dish and cultured for one day. Then, the medium was replaced with DMEM lacking a single amino acid and containing 5% dialyzed fetal bovine serum, and after 24 h the activity of cystine transport was measured as described previously [13]. Briefly, cells were rinsed three times in warmed PBSG [10 mM phosphate-buffered saline (137 mM NaCl, 3 mM KCl), pH 7.4, containing 0.01% $CaCl_2$, 0.01% $MgCl_2 \cdot 6H_2O$ and 0.1% glucose], and then incubated in 0.5 ml of the warmed uptake medium at 37 °C for specified time periods. The uptake medium was PBSG containing L-[^{14}C]cystine (Perkin–Elmer Life Sciences) (0.05 mM and 0.1 μCi/0.5 ml). Uptake was terminated by rapidly rinsing the cells three times with ice-cold PBS, and radioactivity in the cells was determined. Cystine uptake was determined under conditions approaching initial rates of uptake, i.e., measuring uptake for cystine at 120 s. The uptake of cystine increased linearly during this incubation interval.

Construction of 5′-deletion mutants, cell culture, and transfection into cells. Constructs used in this study were prepared as described in the previous report [14]. The constructs containing deletion of AARE sequences were produced by the method described by Imai et al. [15]. The sequences of the final constructs were verified by dideoxynucleotide sequencing. Cells were plated at 2×10^5 cells/35-mm diameter dish, cultured for 24 h, and transfected with the constructs using Lipofectamine and PLUS reagents (Invitrogen) by following the manufacturer's instructions. Cells were washed once with OPTI-MEM I (Invitrogen) and incubated at 37 °C for 3 h in 1 ml OPTI-MEM containing 1 μg DNA. Then, the cells were cultured routinely in fresh DMEM containing 5% fetal bovine serum for 24 h, and the medium was replaced with fresh DMEM lacking a single amino acid and containing 5% dialyzed fetal bovine serum. After culturing for further 24 or 48 h, the activity of luciferase was measured. The maximum luciferase activity was observed in 24–48 h after replacing the medium with the DMEM lacking the amino acid (data not shown). To correct for transfection efficiency, 0.1 μg of the reporter plasmid pCMVβ (Clontech) containing the *lac*Z gene encoding β-galactosidase was co-transfected with each construct. For cell harvest, transfectants were washed twice with PBS and incubated at room temperature for 15 min in 160 μl of reporter lysis buffer. Cells were then scraped from the plates and the resulting lysates were vortexed, frozen in liquid nitrogen, thawed at room temperature, vortexed again, and centrifuged at 12,000*g* for 15 s at 4 °C. The supernatants were stored at −80 °C until measuring the activities of luciferase and β-galactosidase.

Measurement of luciferase and β-galactosidase activities. Luciferase activity was measured using the luciferase assay system with reporter lysis buffer (Promega) following the manufacturer's instruction. β-Galactosidase activity was determined by the coloration assay using *o*-nitrophenyl-β-D-galactopyranoside as a substrate. The relative luciferase activity was normalized for transfection efficiency on the basis of β-galactosidase activity and expressed as arbitrary units.

Northern blot analysis. The RNA probes for mouse xCT, mouse 4F2hc, and mouse β-actin were digoxigenin (DIG)-labeled by transcription from the linearized plasmids using RNA-labeling mix (Roche) and T3/T7 RNA polymerase (Stratagene). RNA was electrophoresed on a 1% agarose gel in the presence of 2.2 M formaldehyde, transferred onto positively charged nylon membrane (Roche), and hybridized with the DIG-labeled RNA probes in DIG Easy Hyb (Roche) for 16 h at 68 °C. The membranes were washed twice for 5 min at room temperature with 1× SSC, 0.1% SDS and then washed twice for 15 min at 68 °C with 0.1× SSC, 0.1% SDS. The hybridized bands were visualized using CDP-Star (Roche).

Electrophoretic mobility shift assay. Cells were plated at 1×10^6 cells/100-mm diameter dish and cultured for 24 h. Then, the medium was replaced with the complete medium or with DMEM lacking cystine and containing 5% dialyzed fetal bovine serum. After culturing for 12 h, the nuclear extract was prepared using Nu-Clear Extraction kit (Sigma) by following the manufacturer's instruction and total protein content was determined using the BCA protein assay reagents (Pierce). Double-strand oligonucleotides containing the sequence of the xCT proximal promoter (5′-GTGGCTGATGCAAACCTG-3′, nt −99 to −82) were radiolabeled using T4 polynucleotide kinase in the presence of [γ-^{32}P]dATP. For each binding reaction, 5 μl of nuclear extract protein was incubated with 20% (v/v) glycerol, 5 mM $MgCl_2$, 2.5 mM EDTA, 250 mM NaCl, 50 mM Tris–HCl (pH 6.5), and poly(dI · dC) 0.5 μg for 1 h at 4 °C. Electrophoretic mobility shift assay was performed as described previously [16]. To test the effect of anti-ATF2 and anti-ATF4 antibodies, 2 μg of each antibody (Santa Cruz Biotechnology) was added to the incubation mixture at room temperature 1 h prior to addition of the labeled probe.

Results

In NIH3T3 cells cultured in the complete medium, the activity of cystine transport was 0.15 ± 0.05 nmol/min/mg protein (Fig. 1A), and the activity was potently inhibited by glutamate (data not shown), indicating that cystine transport is mainly mediated by system x_c^- in these cells. We have measured the activity of cystine transport in these cells cultured for 24 h in media lacking a single amino acid (Fig. 1A). The activity of cystine transport was strongly induced by deprivation of cystine, or arginine, significantly induced by deprivation of histidine, tryptophan, serine and glycine, lysine, leucine, or threonine, but not induced by deprivation of methionine or phenylalanine. Fig. 1B shows the expression of xCT and 4F2hc mRNA in the cells cultured in media lacking single amino acids. The xCT mRNA was potently induced by deprivation of cystine or arginine, slightly induced by deprivation of leucine, but not by deprivation of methionine. On the other hand, 4F2hc mRNA was constantly induced by the deprivation of the amino acids tested. Cystine deprivation caused the decrease of intracellular GSH. Thus, we investigated the effect of buthione sulfoximine (BSO), the inhibitor of GSH synthesis, on the activity of cystine transport, and found that BSO had no effect on the induction of the activity of cystine transport, despite decreasing the intracellular GSH to less than 5% of the control (data not shown).

To identify *cis*-elements mediating the basal and inducible expression of xCT mRNA, a series of 5′-flanking region of xCT gene were cloned into a luciferase reporter vector and transiently transfected into NIH3T3 cells (Fig. 2). The transfected cells were cultured in the medium lacking cystine for 24 h and the luciferase activity was measured. The constructs containing the fragments of 4700–94 bp upstream from the transcription initiation site showed basal and inducible expression of the luciferase activity by cystine deprivation, whereas the construct containing the fragment of 93, or 87 bp showed almost no basal and inducible luciferase activity. The results of this 5′-deletion analysis indicated that the downstream of nt −94 in the xCT proximal promoter was essential for both basal and inducible expression of xCT gene. The sequence analysis revealed that the region from −94 to −86 had very high homology to AARE/NSRE-1 observed in AS and CHOP (reverse direction) genes (Fig. 3). Interestingly, another AARE-like sequence in the reverse orientation was found in 10 nucleotides downstream of the first AARE-like sequence of xCT gene. Thus, we made the constructs in which either the first (AARE F) or the second (AARE R) AARE-like sequence was deleted from pGL3-116, transfected into the cells, and measured the luciferase activity. Both deletion mutants showed neither the basal expression nor inducible expression under the cystine-deprived condition (Fig. 2). To further clarify the importance of these sequences, we constructed the point mutants by substituting one of the 9-bp core sequence nucleotides of AARE F and/or AARE R (Fig. 4). As shown by Bruhat et al. [4], these mutants in CHOP AARE lost the sensitivity to amino acid deprivation in the reporter gene. These constructs were transfected into the cells followed by culturing cystine-deprived medium (Fig. 4). Among all the mutants, M1, M2, M4, and M5, which contain a single substitution in either AARE F or AARE R, showed responsiveness to cystine deprivation, although the basal luciferase activity was significantly decreased (M1, M2, and M5) or increased (M4). By contrast, M3 and M6 did not respond at all to cystine deprivation.

To determine whether the increase in the transcriptional activity of xCT during deprivation of amino acid other than cystine is mediated by this element, the cells were transfected with pGL3-116. After culturing in the medium lacking the amino acid indicated, the luciferase

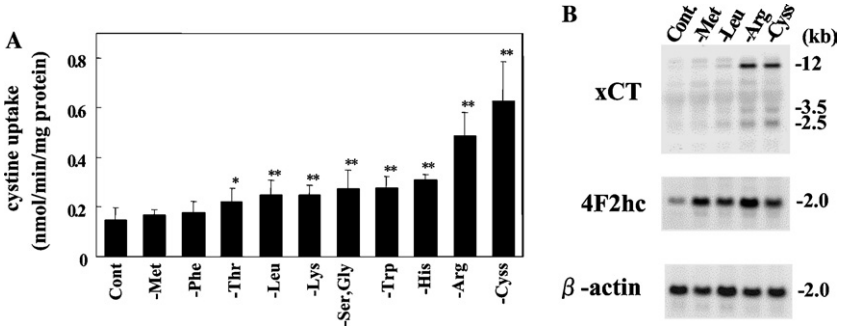


Fig. 1. The activity of cystine transport and the expression of xCT and 4F2hc mRNAs. (A) The rate of uptake of 0.05 mM L-[^{14}C]cystine was measured in NIH3T3 cultured for 24 h in the medium lacking the amino acid indicated. Values represent means ± SD ($n = 4$–6). *$p < 0.05$, **$p < 0.01$ (relative to control). (B) NIH3T3 cells were cultured in the medium lacking the amino acid indicated for 24 h. Then, total RNA was isolated and 2.5 μg each of the total RNA was loaded per lane. The hybridization was performed with digoxigenin-labeled cRNA of mouse xCT, 4F2hc, and β-actin.

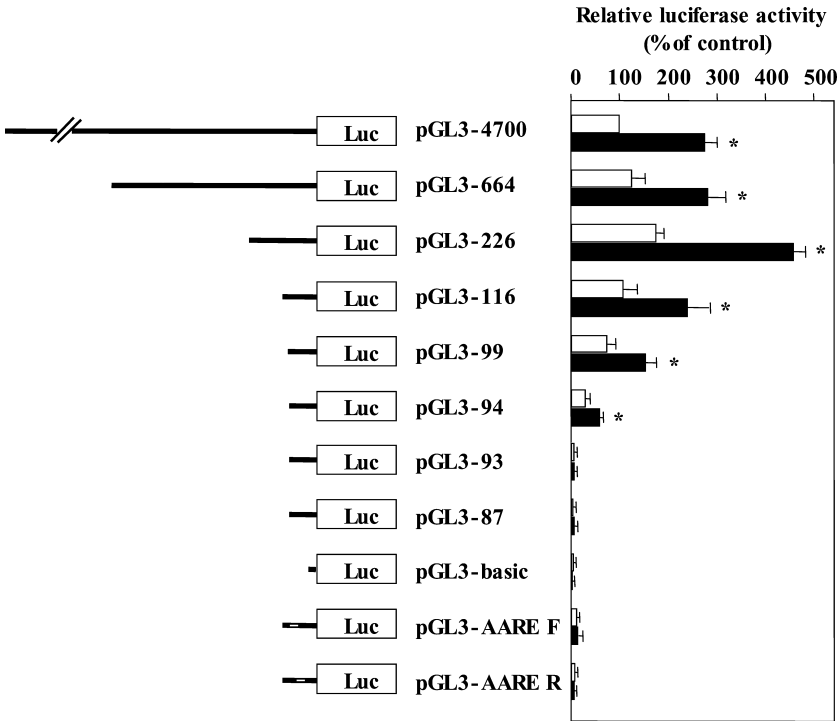


Fig. 2. Schematic illustration of xCT-luciferase chimeric genes and their luciferase activities in NIH3T3 cells. The size of the flanking insert in each 5′-deletion mutant is specified by the number of each plasmid (base pairs). Each construct was transfected into the cells, and the cells were cultured for 24 h in the medium with (open bar) or without cystine (filled bar), and the luciferase activities were determined as described under Materials and methods. pGL3-AARE F and pGL3-AARE R represent the pGL3-116 construct from which AARE F and AARE R were deleted, respectively. Values represent means ± SD ($n = 4$–6) and are expressed as a percentage of the luciferase activity of pGL3-4700 transfected cells cultured in the complete medium. *$p < 0.01$ (relative to the corresponding open bar).

A

AARE F AARE R

−116 AGCTGGAGTTTTCTCATGTGGCTGATGCAAACCTGGAGAATTTGCATCAT

−66 CATGCAGCCCAGTAAGTTGGTGTGACAGGCGGTTGCTTAAATACGGAGCC

−16 TTCCACGAGGAAGCTGAGCT (+1)

B

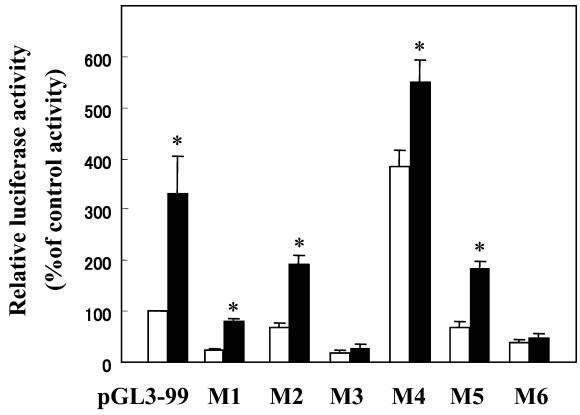
	AARE F	AARE R
xCT	−94 TGATGCAAA −86	−76 TTTGCATCA −68
AS	−68 TGATGAAAC −60	
Cat-1	+45 TGATGAAAC +53	
CHOP		−310 ATTGCATCA −302
SNAT2		+709 ATTGCATCA +717

Fig. 3. Comparison of xCT AARE with other AARE/NSRE sequences. (A) The sequence of the 5′-flanking region of mouse xCT gene is shown. The two AARE sequences are boxed. (B) The position of the AARE sequence in each gene is specified by the number of base pairs from their transcription initiation sites.

activity was measured (Fig. 5). The result showed that luciferase activity was significantly induced by the deprivation of the amino acids which induced the activity of system x_c^-.

AS is induced not only by amino acid deprivation but also by glucose deprivation or endoplasmic reticulum (ER) stress [3]. Thus, the effect of the ER stress on the expression of xCT gene was investigated. As shown in Fig. 6, glucose deprivation did not affect either the activity of system x_c^- or the expression of the reporter gene. On the other hand, treatment of the cells with tunicamycin, which causes ER stress by inhibiting glycosylation of protein, induced the activity of system x_c^- (Fig. 6).

To investigate protein–oligonucleotide complex formation in vitro, electrophoretic mobility shift assay was performed with the 18-bp oligonucleotide containing AARE F sequence (Fig. 7). A specific protein–oligonucleotide complex was detected and a significant



AARE F AARE R

pGL3-99	TGATGCAAA---------TTTGCATCA
M1	TG[T]TGCAAA---------TTTGCATCA
M2	TGATGCAAA---------TTTGCA[A]CA
M3	TG[T]TGCAAA---------TTTGCA[A]CA
M4	TGAT[T]CAAA---------TTTGCATCA
M5	TGATGCAAA---------TTTG[A]ATCA
M6	TGAT[T]CAAA---------TTTG[A]ATCA

Fig. 4. Effect of the mutation within the AAREs on the induction of xCT gene by cystine deprivation. Each construct was transfected into the cells, and the cells were cultured for 24 h in the medium with (open bars) or without (filled bars) cystine, and the luciferase activities were determined. Values represent means ± SD ($n = 6$–8) and are expressed as a percentage of the luciferase activity of pGL3-99 transfected cells cultured in the complete medium. *$p < 0.01$ (relative to the corresponding open bar). Mutagenesis within the AAREs of mouse xCT gene promoter is shown below. Boxed bases represent substitutions for each mutant. Each construct was made from pGL3-99.

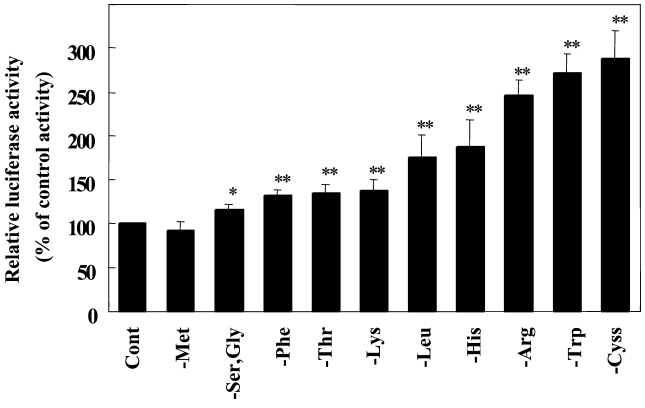


Fig. 5. Effect of amino acid deprivation on induction of luciferase activity in NIH3T3 cells transfected with the xCT/luciferase chimeric gene. Cells were transfected with pGL3-116 and cultured for 24 h in the medium lacking the amino acid indicated. Then, the luciferase activities were determined. Values represent means ± SD ($n = 6$–8) and are expressed as a percentage of the luciferase activity of the cells cultured in the complete medium (Cont). *$p < 0.05$, **$p < 0.01$ (relative to control).

increase in the binding was observed when the extract was prepared from cystine-deprived cells. To determine whether the observed complex contains ATF family proteins, antibodies against ATF2 and ATF4 were incubated with the complexes. ATF4 antibody caused a supershift of the complex, whereas the complex was not supershifted by ATF2 antibody (Fig. 7). We tried to use the probe containing both AARE F and AARE R. However, probably due to its palindrome structure, the fragment containing the two AARE sequences did not work properly as a probe for the electrophoretic mobility shift assay.

Discussion

It has been demonstrated that the activity of system x_c^- is induced by the cystine deprivation [11]. In the present study, we have shown that both the activity of cystine transport and the expression of xCT mRNA are induced not only by cystine deprivation but also by other amino acid deprivation, and that this induction is mediated by the two AARE elements located in the 5′-flanking region of mouse xCT gene. These two AARE elements are conserved in the human xCT gene [17]. Recent studies have demonstrated that AARE/NSRE-1 is involved in the expression of some genes, including CHOP and AS genes, in mammalian cells [1]. The present study indicates that xCT is a novel member of this gene family. The 9-bp AARE sequences of xCT gene differ from the core consensus sequence of CHOP gene ((R/C)TT(R/T)CRTCA) described by Bruhat et al. [4] by only one nucleotide (the T at position 1 of AARE R) (Fig. 3). However, when the construct containing the replacement of the A (at position 1 in AARE of CHOP) with T, which is identical with AARE R, was transfected, the significant sensitivity to amino acid deprivation was reserved in the expression of CHOP [4].

In the present study, it is suggested that ATF4, but not ATF2, is involved in the basal and inducible expression of xCT by amino acid deprivation. This is consistent with the findings that ATF4 is a mediator for modulating the transcription of the other AARE-mediated genes like CHOP, AS, and Cat-1 [5,6,18]. Several molecular mechanisms involved in the regulation of gene expression by amino acid deprivation have been described in yeast, including the general control process [19]. A signal pathway for regulating gene expression by amino acid deprivation in mammalian cells has been demonstrated to be similar to the general control process characterized in yeast [20]. Its components include the mammalian homologue of general control nondepressible (GCN) protein-2 kinase, eukaryotic initiation factor-2α (eIF2α), and ATF4. In this process, a single amino acid deprivation causes the accumulation of the free tRNA corresponding to the amino acid, resulting in the activation of the mammalian homologue of GCN protein-2 kinase. This protein phosphorylates eIF2α, and the translation of ATF4 is activated. These

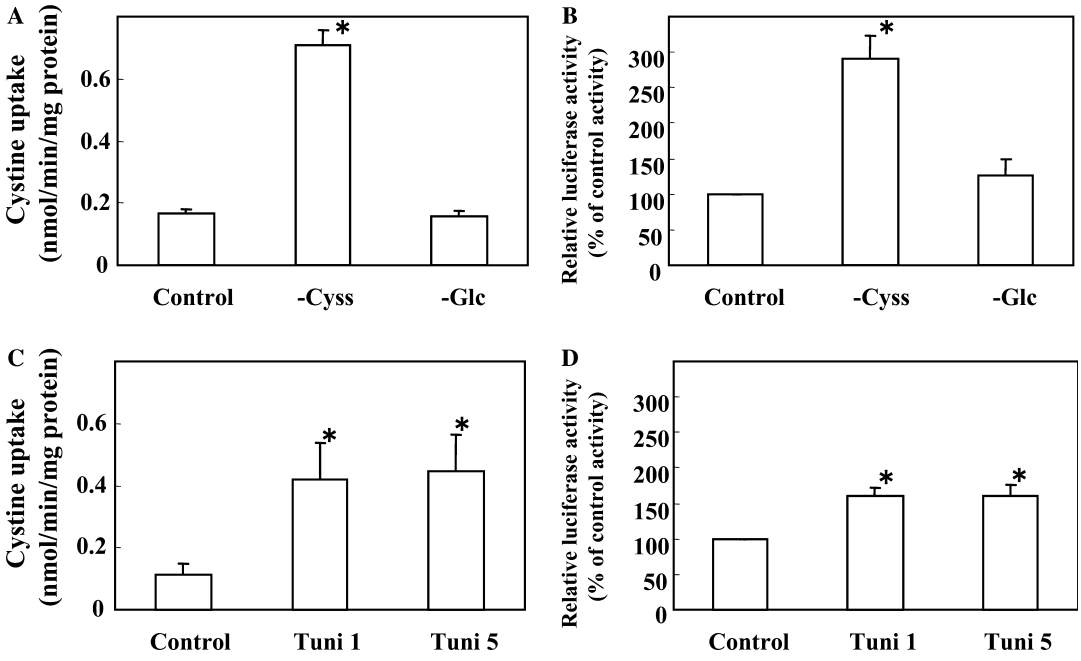


Fig. 6. Effect of glucose deprivation and tunicamycin exposure on the activity of cystine transport and the luciferase activity. (A) Cells were cultured in the medium with or without cystine or glucose for 24 h, and the rate of uptake of 0.05 mM L-[^{14}C]cystine was measured. (B) Cells were transfected with pGL3-116, followed by culturing in the medium with or without cystine or glucose for 24 h, and the luciferase activity was measured. (C) Cells were cultured containing 1 (Tuni 1) or 5 μg/ml (Tuni 5) tunicamycin for 24 h, and the rate of uptake of 0.05 mM L-[^{14}C]cystine was measured. (D) Cells were transfected with pGL3-116, followed by culturing in the medium containing 1 (Tuni 1) or 5 μg/ml (Tuni 5) tunicamycin for 24 h. Then, the luciferase activity was measured. Values of cystine uptake represent means ± SD ($n = 6$). Values of luciferase activity represent means ± SD ($n = 4$–6) and are expressed as a percentage of the luciferase activity of pGL3-116 transfected cells cultured in the complete medium. *$p < 0.01$ (relative to control).

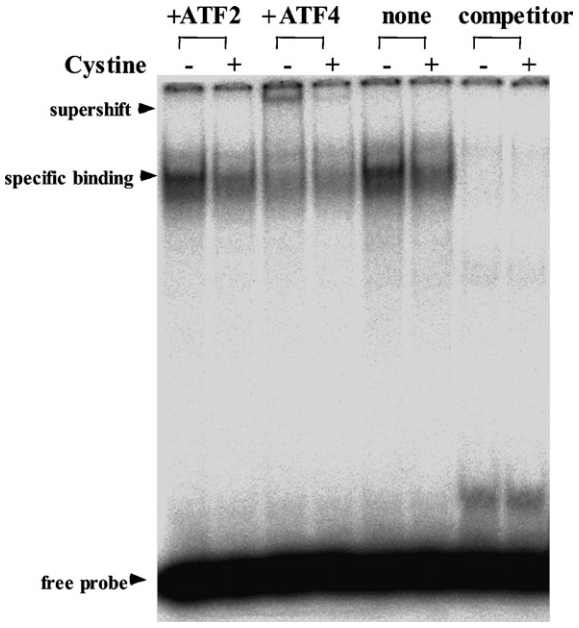


Fig. 7. Supershift assay using specific antibodies and nuclear extracts from NIH3T3 cells cultured in the medium with or without cystine. The nuclear extracts were prepared from the cells cultured in the medium with (+) or without (−) cystine for 24 h, and electromobility shift assay was performed using ^{32}P-labeled 18-bp probe containing AARE F sequence as described under Materials and methods. Antibodies to ATF2 (+ATF2) or ATF4 (+ATF4), or unlabeled probe (competitor) were added as indicated.

processes lead to the increase in the expression of genes like CHOP and AS. Because ATF4 is involved in the induction of xCT gene by amino acid deprivation, it is likely that the expression of xCT gene is activated by the similar signal pathway for the amino acid deprivation to that observed in the expression of CHOP and AS genes. Harding et al. [20] have recently investigated the genes whose expression can be regulated by ATF4 using ATF4-deficient cells. In these cells, the expression of various genes, including 4F2hc, glycine transporter, and cationic amino acid transporter, was significantly decreased. Interestingly, they have demonstrated that when ATF4-deficient cells are cultured, they require higher concentration of cyst(e)ine or supplementation of 2-mercaptoethanol to grow in the routinely used culture medium (DMEM with 10% fetal calf serum). Probably supply of cysteine is limited because of the impaired activity of system x_c^- in these cells. It has long been known that 2-mercaptoethanol enables the utilization of cystine in lymphoma cells lacking system x_c^- activity [21]. Thus, their data suggest that ATF4 is involved in the expression of xCT gene. As shown in Fig. 1B, the 4F2hc mRNA is induced by amino acid deprivation, agreeing with the result shown by Harding et al. [20]. 4F2hc is known to be the common component of not only system x_c^- but also other amino acid transporters such as systems L, y^+L, and asc [22]. By the database

analysis, a sequence (5′-ATTTCATCA-3′) which coincides with the AARE consensus sequence described above was found in approximately 1.6 kbp upstream of the 5′-flanking region of mouse 4F2hc gene. Although this sequence is located far from the transcription initiation site of 4F2hc gene, it might be functional for the induction of 4F2hc gene by the amino acid deprivation.

As shown in Fig. 4, a single mutation in AARE F or AARE R caused the decrease (M1, M2, and M5) or increase (M4) in the basal expression of the reporter gene, however, these constructs still persist in significant sensitivity to cystine deprivation. On the other hand, the induction was completely abolished by the constructs containing a mutation in both AAREs (M3 and M6), suggesting that two AAREs seem to work cooperatively. It should be noted that since a right-handed double helix of DNA has 10 nucleotide pairs per helical turn, and there is 9 bp intervening sequence between the two AAREs, they occur symmetrically on the double helix. This topology might be important for the induction of xCT gene by the amino acid deprivation.

As shown in Fig. 6, the activity of system x_c^- is not induced by glucose deprivation, but tunicamycin increased the activity of system x_c^-. Both treatments induce ER stress response mediated by the accumulation of malfolded proteins. However, tunicamycin also causes the accumulation of reactive oxygen species [20]. The expression of xCT is induced by oxidative stress like hydrogen peroxide [23]. Thus, it might be that oxidative stress caused by tunicamycin contributes to the induction of xCT gene by this agent. Responses to amino acid deprivation and to ER stress are distinct, although it appears clear that these two pathways are overlapping [24].

In conclusion, the expression of xCT gene is induced by amino acid deprivation and this induction is mediated by two AAREs. The present data suggest that the AAREs/ATF4 are involved in both basal and inducible expression of xCT. The two AAREs, each located in the opposite direction, in the regulatory region of xCT gene are quite unique among the genes whose expression is regulated by AARE and a possible interaction between these two elements remains to be investigated.

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