1. ABSTRACT

Glutamine has many important functions in mammalian cells, and glutamine transport across cell membranes has accordingly been extensively studied. In the past few years a number of important glutamine transport proteins have been sequenced and their molecular properties have been characterised. In general, four major transporters are important physiologically. These are known as (i) SNAT3 (System N) which is important in glutamine uptake in periportal cells in liver and in across the basolateral membrane of renal proximal tubule cells and is also involved in glutamine release by liver perivenous cells and by astrocytes; a variant of this protein catalyses glutamine release from skeletal muscle. (ii) SNAT1 (a specific System A sub-type) which is important in glutamine uptake by neuronal cells (iii) ASCT2 which is essential for glutamine uptake by rapidly growing epithelial cells and tumour cells in culture and (iv) the recently discovered brush border membrane transporter B0AT1 (SLC6A19). Recent studies considered both the importance of ASCT2 in tumour cell growth and the regulation of ASCT2 expression. In SK-Hep hepatoma cells, knockdown of ASCT2 using antisense mRNA has been shown to cause apoptosis. Expression of the ASCT2 transporter in HepG2 hepatoma cells is stimulated by glutamine by a pathway involving the promoter element AGGTGAATGACTT which binds FXR/RXR dimers.

2. INTRODUCTION

Glutamine has a variety of different functions in mammalian organisms. It is a precursor for protein synthesis and a source of nitrogen for many important compounds including nucleotides, haem, amino sugars and glutathione. It is essential for the maintenance of function of many organs and cells. It is also essential for the growth of cells in tissue culture. Some dietary glutamine is absorbed in the intestine, but glutamine is produced mainly in skeletal muscle via the glutamine synthetase reaction and is metabolised particularly in the liver, intestine and kidney. Glutamine metabolism is particularly important in liver nitrogen metabolism, in kidney ammoniagenesis especially in metabolic acidosis, and in the glutamine-glutamate cycle in brain involving the function of glutamate as a neurotransmitter. Glutamine is also an important energy substrate for the intestinal epithelial cells and for other rapidly growing cells, especially tumour cells. In addition to these functions which are well established, glutamine has now been shown to regulate the expression of a number of genes. This area has been reviewed extensively (see e.g.(1)).

Glutamine transport into cells and tissues has been studied extensively since the early 1980s. The study of glutamine transport falls into two phases. The early work concentrated on the characterisation of transport proteins...
Recently there has been a concerted effort to rationalise the nomenclature of all transporter proteins and their genes based on sequence similarities and the elucidation of gene and protein families. Table 1 clarifies the terminology used in this article.

4. GLUTAMINE TRANSPORT IN LIVER: RELATION TO AMMONIA DETOXIFICATION

Glutamine has a number of important functions in the liver. After uptake across the plasma membrane of perportal hepatocytes, glutamine enters the mitochondria where it is hydrolysed to glutamate and ammonium ions by phosphate-dependent glutaminase. Under some conditions the glutamate formed is a major substrate for liver gluconeogenesis, while the ammonium ions are converted to urea in the urea cycle and urea is excreted. In the mitochondria, glutamate also undergoes reaction with acetyl CoA forming N-acetylglutamate which is an obligatory activator for carbamoyl phosphate synthase, the major regulatory enzyme in urea synthesis. Thus glutamine acts not only as a metabolic substrate but also as an indirect regulator of ammonia metabolism in perportal hepatocytes (see (7)).

Glutamine is accumulated into isolated hepatocytes mainly by a Na⁺-dependent mechanism involving Na⁺-glutamate cotransport, together with a small Na⁺-independent component. The transporter responsible for Na⁺-dependent glutamine uptake was first characterised in 1980 and shown to be specific for glutamine, asparagine and histidine (8). This transporter was termed System N. Li⁺ was able to substitute for Na⁺, and the transport was pH sensitive, with reduced activity at acid pH. The transporter did not interact with the artificial substrate α-aminoisobutyrate, thus discriminating it from System A. In isolated hepatocytes the transport process is driven by the Na⁺ gradient, thus allowing the cells to accumulate glutamine; the intracellular glutamine concentration in liver has been shown to be 10 times higher than the plasma concentration of approximately 0.5 mM (9).

Glutamine uptake via this transporter can be stimulated by other amino acids which are accumulated into hepatocytes via other transport systems. This effect is mimicked by exposure of cells to hypotonic medium and has been shown to be due to cell swelling. (10). Uptake of glutamine itself also increases cellular hydration and this has been shown to stimulate a number of signalling pathways involving MAP-kinases (11).

Table 1. Major proteins involved in mammalian glutamine transport

<table>
<thead>
<tr>
<th>Original designation</th>
<th>Systematic Protein name</th>
<th>Systematic name of human gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>System N, SN1 subtype</td>
<td>SNAT3</td>
<td>SLC38A3</td>
</tr>
<tr>
<td>System N SN2 subtype</td>
<td>SNAT5</td>
<td>SLC38A5</td>
</tr>
<tr>
<td>System A ATA1 subtype</td>
<td>SNAT1</td>
<td>SLC38A1</td>
</tr>
<tr>
<td>ASCT2 or B⁰ or ATB⁰</td>
<td>ASCT2</td>
<td>SLC1A5</td>
</tr>
<tr>
<td>Brush border broad specificity</td>
<td>B⁰AT</td>
<td>SLC6A19</td>
</tr>
</tbody>
</table>

References to the cloning of these transporters and their molecular properties are given in the text and in (4,6).

The study of glutamine transport is not without methodological challenges and these were not always recognised, particularly in earlier work. In particular, glutamine is rapidly metabolised by many cells, and if radioactive glutamine is to be used as an externally added transport substrate, the identity of the intracellular labelled compound must be established to interpret the results correctly. Secondly, there is no transporter which is absolutely specific for glutamine, and in each case glutamine is only one of the amino acids recognised by a particular transporter. Often, the specificity of a particular transporter is characterised by competition of glutamine uptake by other amino acids. In this case a rigorous conclusion that glutamine and another amino acid are transported via the same carrier protein depends on the demonstration that transport of the two amino acids are mutually competitive (see e.g. (5)). Finally, recently much information about the molecular mechanisms of glutamine transport has been obtained by expressing the transport proteins heterologously in Xenopus Oocytes. It is however possible that some of the properties of transport proteins when expressed in Oocytes may differ from those as they occur in normal cells.

This article will first review the importance of glutamine transport in liver, kidney, brain and rapidly growing cells, focussing mainly on recent findings on the identification, mechanism and expression of the transport proteins involved. A subsequent section will review the importance of the ASCT2 transporter in tumour cell growth and survival and will present a possible mechanism by which glutamine regulates the expression of the transporter ASCT2 in a human hepatoma cell line.

3. NOMENCLATURE OF GLUTAMINE TRANSPORTER PROTEINS AND THE GENES ENCODING THEM

Originally, amino acid transporters were given non-systematic names e.g. System A, System N based on substrate specificity. When these transporters were cloned, the cDNAs were also initially given non-systematic names. In terms of kinetics and specificity. Little was known about the molecular properties of plasma membrane glutamine transporters until cDNA for the first such transporter was cloned in 1996 (2). Since that time there has been a large expansion in knowledge of the molecular identity and expression of various glutamine transporters in different tissues, although it should be noted that the cDNA for some of the transporters involved was cloned only in the last two or three years. Two recent reviews on glutamine transport are of particular importance and amplify the material discussed in present article (3,4).

Glutamine has a number of important functions in the liver. After uptake across the plasma membrane of perportal hepatocytes, glutamine enters the mitochondria where it is hydrolysed to glutamate and ammonium ions by phosphate-dependent glutaminase. Under some conditions the glutamate formed is a major substrate for liver gluconeogenesis, while the ammonium ions are converted to urea in the urea cycle and urea is excreted. In the mitochondria, glutamate also undergoes reaction with acetyl CoA forming N-acetylglutamate which is an obligatory activator for carbamoyl phosphate synthase, the major regulatory enzyme in urea synthesis. Thus glutamine acts not only as a metabolic substrate but also as an indirect regulator of ammonia metabolism in perportal hepatocytes (see (7)).

Glutamine uptake via this transporter can be stimulated by other amino acids which are accumulated into hepatocytes via other transport systems. This effect is mimicked by exposure of cells to hypotonic medium and has been shown to be due to cell swelling. (10). Uptake of glutamine itself also increases cellular hydration and this has been shown to stimulate a number of signalling pathways involving MAP-kinases (11).

cDNA encoding System N was cloned only in 1999 from a rat brain cDNA library on the basis of its
Glutamine Transport

Similarity to brain vesicular glutamine transporters (12) and was termed SN1 (now SNAT3). A report of cloning of SNAT3 cDNA from a rat kidney cDNA library followed (13), and human SNAT3 was cloned and sequenced in 2000 (14). Human SNAT3 encodes a 504 amino acid protein. When SNAT3 was expressed in Xenopus oocytes the substrate and ion specificity and pH dependence of the induced transport was similar to that of System N. Analysis of substrate-induced currents in Oocytes expressing SNAT3 indicated that the uptake of one glutamine was accompanied by the uptake of 2 Na+ ions and the efflux of 1 H+, suggesting that the uptake is electrogenic and accounting for the pH dependence of the uptake process (14). However, other investigators studying SNAT3 expressed in oocytes have concluded that the uptake of glutamine is electroneutral with 1 Na+ being transported with one glutamine and 1H+ moving in the opposite direction and that inward currents observed during glutamine uptake were in part due to pH regulated endogenous oocyte cation channels or other uncoupled H+ movements (15,16). An important observation in all these oocyte studies was that glutamine could move across the membrane in either direction depending on factors such as the glutamine concentration gradient, the Na+ and pH gradient and the membrane potential, and that therefore in principle SNAT3 could be responsible for the physiological efflux of glutamine in some cell types.

The liver expresses high activities of both glutaminase and glutamine synthetase, and for a long time this appeared paradoxical in that simultaneous operation of both these enzyme reactions would lead to a futile cycle, dissipating ATP. This was resolved by the finding that glutamine synthetase is expressed only by the 10-15% of hepatocytes at the end of the acinus near the central vein – the perivenous or pericentral hepatocytes. These cells do not express the urea cycle enzymes or glutaminase. Perivenous hepatocytes take up any ammonia not metabolised by the perportal hepatocytes, and convert it to glutamine. The glutamine is then released from the liver and is metabolised in the kidney (17). It has been shown that isolated perivenous hepatocytes express high activities of System N transport (18) and it was shown by immunoblotting that SNAT3 is expressed strongly in the pericentral region of liver (13). It is therefore likely that SNAT3 is responsible both for glutamine uptake in perivenous cells and glutamine release in the perivenous cells.

A further member of this transporter family has been identified. SNAT5 was cloned from rat brain and, like SNAT3, catalyses uptake of glutamine with Na+ in exchange for H+, and tolerates Li+ for Na+ substitution. However, SNAT5 differs from SNAT3 in substrate specificity, catalysing the uptake of glycine, alanine and serine as well as glutamine and asparagine (19). Although SNAT5 mRNA is expressed most abundantly in liver, its physiological function is not yet clear, since alanine and serine uptake in isolated hepatocytes are not transported via System N. In a recent report, it was shown that both SNAT3 and SNAT5 when expressed in oocytes are able to catalyse influx and efflux of glutamine. SNAT5 mRNA was expressed more in the perportal region of rat liver, while SNAT3 was preferentially expressed in the pericentral region suggesting a possible role for SNAT5 in glutamine uptake by perportal hepatocytes (20).

As stated above, glutamine taken up into perportal hepatocytes then has to cross the inner mitochondrial membrane before it can be metabolised. Due to the difficulty in measuring the very rapid equilibration of glutamine across the inner membrane of isolated liver mitochondria no liver mitochondrial glutamine transporter has been unambiguously identified either kinetically or by molecular methods.

5. GLUTAMINE TRANSPORT IN KIDNEY: RELATION TO AMMONIAGENESIS

Glutamine metabolism is of major importance in kidney in relation to acid/base homeostasis. Glutamine is transported into proximal tubule cells and then enters the mitochondria where it is hydrolysed to glutamate and ammonium ions by the kidney isoform of phosphate-dependent glutaminase. NH4+ ions are excreted and the glutamate is metabolised to glucose with the production of bicarbonate. In metabolic acidosis these processes are accelerated, hence generating more bicarbonate to compensate excess acid.(see (21)).

The mechanisms of glutamine transport into renal proximal tubule cells are not fully resolved. Glutamine may be reabsorbed across the brush border membrane from the glomerular filtrate. However, glutamine uptake from the plasma for metabolism in metabolic acidosis occurs also across the basolateral membrane. Isolated kidney brush border membrane vesicles have been shown to accumulate glutamine by cotransport with Na+ ions on a transport system of unusually wide specificity which also transports hydrophobic and aromatic neutral amino acids, but not charged amino acids (22). The molecular identity of this transporter has proved elusive, but was eventually elucidated in 2004. Genes in the 5p15 region of human chromosome 5 were examined for the presence open reading frames predicted to be transporter-like in terms of number of transmembrane helices, but which had no previously defined transporter function. The mouse homologue of one of these genes was amplified, expressed in oocytes and found to have properties identical to the kidney transporter.(23). This transporter was termed System B0AT1 and is expressed only in intestine, kidney and skin. The human gene for this transporter is classified as SLC6A19 and mutations in this gene have recently been shown to be responsible for Hartnup’s disease (24).

The System N transporter SNAT3 has been shown to be expressed in basolateral and brush border membrane vesicles in rats, and metabolic acidosis increased the SNAT3 mRNA level 10 fold. Increase in transport activity was observed mainly in basolateral membrane vesicles (25). In contrast a more recent report showed that SNAT3 was specifically targeted to the basolateral membranes of cortical tubule cells and again its expression increased in acidosis. (26). These findings suggest an
imported role for the SNAT3 transporter in pH regulation. Glutamine is also recognised as a substrate by a number of other transport systems in kidney tubule cells. These all act as Na’- independent amino acid exchangers. They include the heterodimeric transporters γL, System L (LAT1 and LAT2) and System b0H+. Molecular properties of these proteins have been reviewed in detail, (3, 27), but they appear to have no defined role in renal ammoniagenesis.

Direct measurement of glutamine transport across the kidney mitochondrial membrane has proved technically difficult, in particular because of the rapid metabolism of glutamine in the matrix space. In kidney submitochondrial particles, glutamine uptake was shown to be an electroneutral uniport process and the activity increased on reducing the pH for 7.5 to 6.5. (28) A glutamine transport protein from rat kidney mitochondria was isolated and reconstituted and defined in terms of its specificity and inhibition by sulphydryl reagents.(29) No sequence information is at present available. A number of early investigations suggested that mitochondrial glutamine transport may limit the rate of glutamine metabolism (reviewed in (29)) but the evidence for this is indirect and somewhat contradictory.

6. GLUTAMATE TRANSPORT IN BRAIN: RELATION TO GLUTAMATERGIC NEUROTRANSMISSION

Astrocytes take up the neurotransmitter glutamate from the synaptic cleft via high-affinity glutamate transporters. Glutamate is converted to glutamine in the astrocytes via glutamine synthetase, and the glutamine is released and taken up by the neurons where it is hydrolysed to glutamate and glutamate is stored in vesicles until released. Glutamine transport is important at two points in this cycle:- the release of glutamine from the astrocytes and its uptake by neurons.

It appears that the System N transporter SNAT3 is mainly responsible for glutamine release from astrocytes. As discussed previously this transporter is known to operate in either direction, and its immunochemical localisation makes it a good candidate for this role. Neurons express high levels of the System A-type transporter known as ATA1 or SNAT1. This transporter catalyses the electrogenic Na’-dependent uptake of glutamine, among other substrates, and is thought to be responsible for glutamine uptake into neurons. This appears to be a major physiological function of this transport protein. Expression of SNAT1 is relatively restricted. The System A transporter ATA2 (SNAT2) is much more widespread in other tissues, but has a relatively low affinity for glutamine. This area has been recently reviewed in detail (4), and will not be considered further here. Other transporter sub-types may also play a role in the glutamine-glutamate cycle in brain (30).

7. GLUTAMINE TRANSPORT IN CULTURED EPITHELIAL AND TUMOUR CELL LINES

Glutamine is an essential substrate for cells growing in culture. The uptake of glutamine into cultured mammalian cells has been studied extensively. In various immortalised cell lines such as renal epithelial cells and in many tumour cells including hepatoma cells, choriocarcinoma cells breast tumour cells and colon carcinoma cells glutamine is accumulated with Na’ ions via a transport system of relatively broad specificity which accepts alanine, serine, threonine and cysteine as major substrates, but does not interact with N-methylaminoisobutyrate (see (3)). Similarly leucine and phenylalanine are not substrates. In an early study this transport system was characterised kinetically in the bovine renal epithelial cell line NBL-1 and was termed System B0 to indicate a broad-specificity transporter which did not transport charged amino acids. (31) Glutamine transport in various hepatoma cell lines has been extensively characterised and found to have generally similar properties to those in epithelial cells. (32)

Starting in 1996 a number of cDNA clones were isolated which, when expressed heterologously in Xenopus Oocytes or in mammalian cells induced transport activity with the general characteristics of System B0. These included ASCT2 from rat testis (33) ASCT2 from rat brain (34) B0 from NBL-1 cells, (35) ATB0 from rabbit intestine (36) B0 from the human choriocarcinoma cell line JAR (37) and ASCT2 from the rat hepatoma cell line H4 (38). The nomenclature used was arbitrary. Alignment of the sequences of these clones showed that they are all greater than 80% identical at the amino acid level and belong to the same protein family. Although minor differences in amino acid transport specificity between clones from various species do exist, the originally held view that B0 and ASCT2 clones represented different transporters is no longer valid. These broad-specificity transporters are now all commonly referred to as ASCT2 and the human gene encoding ASCT2 is termed SLC1A5. It should be noted that these transporters represent a different protein family from the kidney brush border transporter SLC6A19 which has broader specificity.

The mechanism of transport catalysed by ASCT2 expressed in Xenopus Oocytes is not altogether clear. Kekuda et al (1977) using the ATB0 clone from rabbit intestine detected inward currents evoked by substrates and proposed an electronegenic Na’-substrate uptake mechanism. (36) In a later paper, using the ATB0 clone from human small intestine it was proposed that ASCT2 catalysed an exchange of external for internal amino acids that was completely dependent on Na’ ions. (39) However, it was noted if such an exchange were not obligatory, Na’ plus glutamine uptake could still be electronegenic in the absence of internal amino acids. A study using mouse brain ASCT2 concluded that the amino acid exchange was obligatory and that exchange of Na’ ions also occurred, although this was not strictly coupled to amino acid movement. (40) Since all these studies were performed using clones of different origin it is not impossible that relatively small differences in amino acid sequence in these clones can produce different behaviour when expressed in oocytes. However, whatever the situation in oocyte expression experiments, glutamine uptake catalysed by ASCT2 in hepatoma cells is highly concentrative and...
very largely Na⁺ dependent. The ASCT2 protein has recently been purified and reconstituted into liposomes in an active form (41).

8. ROLE OF ASCT2 IN HEPATOMA CELL GROWTH AND VIABILITY

Transport of glutamine via the ASCT2 transporter appears to be important in a number of rapidly growing cell lines, and has been particularly studied in hepatoma cells. As discussed above, ASCT2 is not expressed in normal liver cells, which transport glutamine via the SNAT3 (System N) transporter. It has been suggested that expression of ASCT2 is essential for hepatoma growth and viability.

An important advance in this area was made using the rapidly growing human hepatoma SK-Hep cell line as a model, (42). These are aggressive and fast growing cells with a high rate of glutamine consumption, enhanced glutaminase activity and low expression of glutamine synthetase, similar to clinical human hepatocellular carcinoma (see (42)). Anti-sense RNA for ASCT2 was generated in SK-Hep cells by the inducible pSwitch system (invitrogen). Cells were transfected with anti-sense cDNA in the pGene5-HisA vector which will only switch on mRNA production when its promoter is activated by the product of a second transfected vector which is itself switched on by the inducer mifepristone (MFP). This system allows the isolation of stable cell lines transfected with both vectors which grow normally in the absence of inducer.

When anti-sense mRNA production was switched on in these cells by the addition of MFP, normal ASCT2 mRNA decreased by 80% in 14 hours. The rate of glutamine transport via ASCT2 decreased by 50% in the same time interval. There was also a decrease in the rate of cell growth such that cell numbers were reduced by 17%, 77% and 98% respectively 14, 24 and 48 hours after induction.

Cell death after ASCT2 antisense expression was shown to be due to apoptosis characterised by cell blebbing and activation of caspases 2, 8 and 9. A major effect of transporter knockdown is cell deprivation of glutamine, and as a comparison, SK-Hep cells were grown in the absence of glutamine. This procedure also resulted in cell death by apoptosis, but in this case cell death was slower in onset and caspases 8 and 9 were not activated. It appears that apoptosis due to ASCT2 knockdown has features additional to those produced by glutamine deprivation. It was concluded that expression of the ASCT2 transporter is essential for the growth and viability of SK-Hep cells (42).

9. REGULATION OF ASCT2 EXPRESSION BY GLUTAMINE AVAILABILITY IN HEPATOMA CELLS

Glutamine uptake in the human hepatoma cell line Hep-G2 had been shown to be attributable to ASCT2 in previous investigations (3). The expression of ASCT2 was investigated in this cell line using a specific ASCT2 antibody (43) ASCT2 expression and cell growth rate were measured under several different conditions. It was found that cell growth rate and ASCT2 expression were both reduced by glutamine deprivation. However, cell growth could be increased by phorbol esters and reduced by novobiocin without corresponding changes in ASCT2 expression. On the basis of these and other observations, it was concluded that ASCT2 expression is stimulated specifically by glutamine in this cell line. Part of the 5'flanking region of the ASCT2 gene was isolated, cloned into the vacant promoter site of the luciferase reporter vector pGL3 (Promega) and transfected into HepG2 cells. It was found that this sequence exhibited promoter activity when cells were grown in normal medium. Promoter activity was lost when the cells were deprived of glutamine and was regained when glutamine was re-added to the growth medium. The effect was specific to glutamine and the promoter activity of a number of other genes was not affected. It was concluded that glutamine specifically stimulated ASCT2 expression in these cells by activating the ASCT2 promoter (43).

10. MECHANISMS OF TRANSCRIPTIONAL ACTIVATION OF ASCT2 EXPRESSION BY GLUTAMINE

In recent work we have extended these investigations (44). Successive deletions of a 703bp 5'flanking region construct were cloned into the PGL3 basic vector and transfected into HepG2 cells grown in the presence or absence of glutamine. Promoter activity was measured in each case. The constructs starting at −713 and at −653 (taking the transcription start site as 0) showed significant promoter activity which was greatly increased when cells were grown in the presence of glutamine. Constructs starting at −543 or nearer to the transcription start site showed much less activity, with no effect of glutamine. Thus the response element which is activated by glutamine lies between bases −543 and −653.

MatInspector software predicted putative binding sites for certain transcription factors in between −592 and −568 in this region. In particular, an inverted repeat separated by 1 base (IR-1 repeat site) with the sequence AGGTAATGACTT was identified to be of interest.

Electromobility Shift Assays (EMSA) were performed to identify proteins in nuclear extracts which bound to a 24bp synthetic oligonucleotide corresponding to bases −592 to −568 of the 5'flanking region of ASCT2. This nucleotide bound one major and one minor protein of different sizes, but the amount of protein bound was greater in cells grown in the presence of glutamine than in those grown in its absence.

Competition experiments with unlabelled nucleotides indicated that the major and larger protein required the entire intact IR-1 sequence for binding, whereas the smaller and minor protein required only a partial sequence. When cells were transfected with...
Glutamine Transport

Figure 1. Putative mechanism for the regulation of ASCT2 expression by glutamine. Details are given in the text. The link between ERK phosphorylation and stimulation of the FXR promoter is hypothetical at present. Abbreviations: FXR: farnesyl X receptor; RXR: retinoid X receptor.

The IR-1 site binding the major protein is a consensus binding sequence for dimers comprising the transcription factors FXR and RXR. In order to test whether these transcription factors were involved in glutamine stimulation of the ASCT2 promoter, cells grown in the absence of glutamine were cotransfected with the ASCT2 promoter construct and with constitutively active expression vectors containing FXR and RXR cDNAs. Such cells exhibited increased activity of the ASCT2 promoter to the same level as non-transfected cells grown normally in the presence of glutamine. The effects of glutamine and FXR/RXR transfection were not additive, suggesting activation by the same mechanism.

Real time PCR experiments indicated that FXR mRNA levels were increased in cells grown in the presence of glutamine compared with those grown in its absence. The sequence of the 5' flanking sequence of the human FXR gene is known, and a 900bp fragment of this sequence was cloned into PGL3 basic vector and transfected into HepG2 cells. Activity of the FXR promoter was greatly increased in cells grown in the presence of glutamine.

These results suggest a mechanism whereby glutamine indirectly activates the FXR promoter leading to an increased level of FXR, activation of the ASCT2 promoter by increased levels of FXR/RXR dimers and consequent stimulation of ASCT2 mRNA and protein synthesis. The compound CDCA (chenodeoxycholic acid) is a ligand of FXR/RXR, the binding of which activates certain promoters containing FXR/RXR binding sites in genes involved in bile acid metabolism and transport. CDCA had no effect on the ASCT2 system. Further work is required to substantiate this proposed mechanism. Nevertheless, the positive identification of the IR-1 site in promoter stimulation by EMSA and mutagenesis approaches together with activation of the promoter by transfection of cells with FXR/RXR strongly implicates FXR/RXR in this mechanism. If this is the case, it represents a novel function for FXR which is independent of the presence of CDCA. Whether other endogenous activators are required remains to be determined.

Glutamine is known to activate protein kinase pathways in IEC-6 rat intestinal crypt cells. In glutamine-starved cells addition of glutamine stimulated cell proliferation and activated both ERKs and JNKs leading to an increase in AP-1 dependent gene transcription [15]. In order to investigate whether these pathways were involved in the activation of ASCT2 expression by glutamine, cells were incubated with a number of specific inhibitors of signalling pathways and the effects on ASCT2 protein expression were measured by Western Blotting. PD98059, a MEK1/2 inhibitor, prevented the increased ASCT2 protein expression in response to glutamine. Inhibitors of the JNK pathway (SP600125) and the p38 pathway (SB203580) did not block the stimulation by glutamine. These results implicate a signalling pathway involving extracellular regulated kinase (ERK). A time course of ERK phosphorylation in response to glutamine was performed using an antibody specific to the phosphorylated form of ERK. The results showed a progressive phosphorylation of ERK which was maximum after 60 minutes and remained at this level for at least a further 24 hours (C. Bungard and J. McGivan- unpublished work).

Figure 1 depicts a putative mechanism for the activation of ASCT2 protein expression by glutamine. The link between ERK phosphorylation and activation of the FXR promoter is hypothetical at present.

These results together with the ASCT2 antisense work described above confirm the essential role of ASCT2 expression in hepatoma cell viability, show that glutamine is acting as a cellular signal to maintain ASCT2 expression and define for the first time a detailed mechanism by which glutamine could be exerting this effect. A number of other tumour cells lines transport glutamine via the ASCT2 transporter. Whether similar mechanisms regulate ASCT2 expression in these cells remains to be determined.
Glutamine Transport

Table 2. Major glutamine transporters of physiological importance

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Cell type</th>
<th>Physiological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNAT3</td>
<td>Periportal hepatocytes</td>
<td>Glutamine accumulation</td>
</tr>
<tr>
<td></td>
<td>Basolateral membrane renal cells</td>
<td>Glutamine accumulation</td>
</tr>
<tr>
<td></td>
<td>Perivenuous hepatocytes</td>
<td>Glutamine release</td>
</tr>
<tr>
<td></td>
<td>Astrocytes</td>
<td>Glutamine release</td>
</tr>
<tr>
<td></td>
<td>Skeletal muscle</td>
<td>Glutamine release</td>
</tr>
<tr>
<td>SNAT1</td>
<td>Neurons</td>
<td>Glutamine accumulation</td>
</tr>
<tr>
<td>B0AT1</td>
<td>Brush border membranes</td>
<td>Glutamine accumulation</td>
</tr>
<tr>
<td>ASCT2</td>
<td>Rapidly growing cells in culture, Tumour cell lines</td>
<td>Glutamine accumulation</td>
</tr>
</tbody>
</table>

11. PERSPECTIVE

Glutamine transport has been recognised for many years to be an important function of mammalian cells. Substantial progress in the cloning of glutamine transporters since 1996 has now given an overall picture of the mechanisms involved. Although glutamine is a substrate for a considerable number of transport proteins (see (3)), four transporters appear to be of major physiological importance. (Table 2) The ASCT2 transporter is responsible for the accumulation of glutamine in rapidly growing cells in cultures especially epithelial cells and a number of tumour cell lines. SNAT3 has a different mechanism and catalyses the uptake of glutamine with Na⁺ ions in exchange for H⁺. The characteristics of this transporter enable it to work in either direction physiologically and SNAT3 is responsible for the uptake of glutamine by periportal cells of the liver and for the release of glutamine by the perivenous cells. It is also mainly important in the release of glutamine form the astrocytes in the brain, and a variant of this transporter catalyses release of glutamine from skeletal muscle. SNAT3 may also be important in the uptake of glutamine across the basolateral membrane of renal tubule cells for ammoniagenesis and gluconeogenesis.

The System A variant SNAT1 (ATA1) catalyses the electrogenic uptake of glutamine with 1 Na⁺. This transporter is of limited distribution and is important mainly in the uptake of glutamine by neurons. Finally, the recently cloned broad-specificity transport B0AT1 (SLC 16 A19) is responsible for the uptake of glutamine across the kidney and intestinal brush border. This transporter has high activity and low amino acid specificity. Mitochondrial transport of glutamine is also of importance but mitochondrial glutamine transporters are poorly characterised at the molecular level.

In general there is little direct and convincing evidence that plasma membrane transport of glutamine is a rate-limiting step in glutamine metabolism. The relationship between expression of the mRNA for a transport protein and the level of functional protein itself in a particular tissue is also not always clear, and whether transporter expression may be controlled at a translational level remains to be determined. So far there are few reports of differences in expression of glutamine transport proteins in pathological conditions with the exception of Hartnup’s disease discussed above.

Two particular problems are now of importance. Firstly, what mechanisms determine the level of expression of glutamine transporters under different conditions? The recent results reported here on the promoter analysis of ASCT2 are a step in this direction, and are another example of how glutamine can act as regulator of gene expression. Secondly, is interference with transporter activity or expression a feasible approach to inhibition of tumour cell growth?. The demonstration of expression of the importance of expression of ASCT2 transporter in the growth of hepatoma cells suggests that this may be a line of investigation worth pursuing.

12. ACKNOWLEDGEMENTS

Work in the authors laboratory was funded in part by BBSRC and the Medical Research Council.

13. REFERENCES


8. Kilberg, M. S., M. E. Handlogten & H. N. Christensen: Characteristics of an amino acid transport system in rat
Glutamine Transport


Glutamine Transport

31. Doyle, F. A. & J. D. McGivan: The bovine renal epithelial cell line NBL-1 expresses a broad specificity Na(+) dependent neutral amino acid transport system (System B0) similar to that in bovine renal brush border membrane vesicles. *Biochim Biophys Acta* 1104, 55-62 (1992)


**Abbreviations:** FXR: farnesyl X receptor; RXR: retinoid X receptor

**Key Words:** Ammonia detoxification, ASCT2, Glutamate, Glutamatergic neurotransmission, Hepatoma cells, Review

**Send correspondence to:** Dr. J.D.McGivan, Department of Biochemistry, School of Medical Sciences, University Walk, Bristol BS8 1TD, UK, Tel: 44-1179287435, Fax: 44-1179288274, E-mail: j.mcgivan@bris.ac.uk

http://www.bioscience.org/current/vol12.htm